Isotopes—Terminology, Definitions and Properties

Book Chapter

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Chapter 8
Isotopes—Terminology, Definitions and Properties

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Abstract The intention of this chapter is to give insight into the properties and peculiarities of the stable isotopes of the bioelements. Following an overview about the terminology and 'technical jargon' used in stable isotope sciences, methods to calculate and express isotopic abundances are presented. Subsequently, a short description of the physicochemical basis of equilibrium and kinetic (mass-dependent) isotope effects (EIEs and KIEs) as origin of isotope fractionation in chemical and biological systems is given. Further, measures for calculation and presentation of isotope fractionation are introduced and the corresponding properties of these quantities are critically discussed. Finally, examples for equilibrium and kinetic isotope fractionation in biochemical reactions are presented in more details and subsequent effects and consequences including the relationship between EIEs and KIEs are reviewed.

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8.1 Introduction

The bioelements H, C, N, O and S occur in nature as mixtures of stable isotopes.[1] As a consequence, all organic material also consists of a mixture of stable isotopes. With the exception of sulfur, the natural mean global abundance of the heavy stable isotopes of the bioelements is around one percent or less. The isotopic composition of organic compounds is primarily dependent on the isotopic composition of the precursor molecules and on isotope fractionation during (bio)synthesis or (bio)degradation reactions. The extent of the observable isotope fractionation is also dependent on environmental and climatic variables and their impact on the source organism producing the biomass. Plants record these isotope fractionations annually e.g. in tree rings. Therefore, with a proper understanding of the processes involving isotope fractionation, it will be possible to use the information behind the isotopic signatures of tree rings to reconstruct the climatic conditions (climate proxy) during tree ring growth. The principles and techniques of tree ring research will be explained in the following chapters of this book. The aim of the present chapter is to provide key knowledge on isotope terminology and related 'technical jargon', as well as on properties and behavior of stable isotopes in biochemical reactions and/or physical processes. The chapter gives definitions, thereby explaining terms in simple examples with reference to mono-, disaccharides and cellulose (and other wood ingredients) where possible.

8.2 Terminology

8.2.1 Isotopes

Atoms consist of a nucleus and an electron shell; the mass of an atom is concentrated mainly in the nucleus. The nucleus contains nucleons (positively charged protons and neutrons without charge). The much lighter electrons with negative charge "orbit"[2]

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[1] In this chapter the authors follow in principle below listed recommendations with the following supplement: the terms 'isotope fractionation (factor)' and 'isotopic fractionation (factor)' are used synonymously.

Official terms used in "isotope chemistry" according to the IUPAC Compendium of Chemical Terminology (Gold Book) and related glossary recommendations (Muller 1994; Coplen 2011; Brand et al. 2014) are:

(1) Isotope effect (IE -> equilibrium, heavy atom, intramolecular, inverse, kinetic, normal, primary, secondary, solvent, thermodynamic IE), isotope exchange, isotope ratio, isotope scale.

(2) Isotopic abundance, isotopic atom, isotopic composition, isotopic enrichment (factor), isotopic exchange, isotopic fractionation (factor), isotopic mass, isotopic separation, isotopic signature, isotopic substitution, isotopic tracer.

(3) Isotopically enriched, isotopically labelled, isotopically substituted.

[2] In quantum mechanics there is no orbiting motion of electrons, but only a probability to find an electron in a certain location in the electron shell.
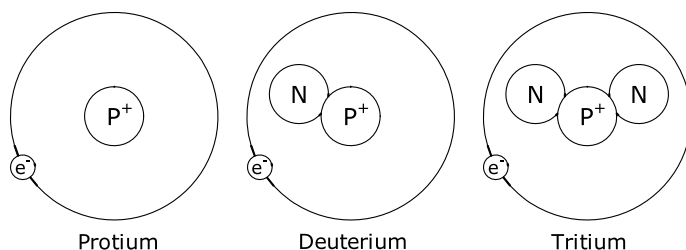
Fig. 8.1 Schematic representation of the three naturally occurring hydrogen isotopes. The origin of the names protium, deuterium and tritium are the ancient greek terms prôtos (πρῶτος—first), *deúteros* (δεύτερος—second) and *trítos* (τρίτος—third) Urey et al. (1933). The three hydrogen isotopes differ only in mass of the nucleus (atomic mass) and not in the arrangement of the electron(s)

around the central nucleus in the electron shell. Electrons and protons are mutually attracted to each other by electromagnetic force. The term nuclide is used to describe atomic species by the composition of the nucleus e.g. by the number of protons (Z) and the number of neutrons (N). Nuclides with the same number of protons are described as **isotope**s. In this context isobaric nuclides (**Isobar**s) have the same nominal mass while **isotone**s are nuclides having the same number of neutrons. The term 'isotope' was introduced by Nobel laureate Frederick Soddy (1923) through merging the Greek words for 'equal' ($\iota\sigma o$—iso) and 'place' ($\tau\acute{o}\pi o\varsigma$—topos) indicating that all isotopes of the same chemical element share the same position in the periodic table of the elements (IUPAC periodic table of the elements and isotopes (IPTEI), Holden et al. 2018). As an example, the isotopes with the simplest schematic structure are shown in Fig. 8.1. The most abundant hydrogen atom, 'protium' consists of a single proton and one electron. 'Deuterium' is the heavier stable hydrogen isotope possessing an additional neutron. 'Tritium' possessing two neutrons is the only naturally occurring, not stable, radioactive hydrogen isotope (Fig. 8.1).

Isotopes of the same element have different masses owing to the various number of neutrons at constant proton number in the nucleus. Universally, the mass number of nuclides (sum of protons and neutrons) is denoted on the left side of the atomic symbol as a superscript and the atomic number (number of protons) is noted as the left subscript (International Union of Pure and Applied Chemistry 1959). For example, $_{6}^{12}C$ and $_{6}^{13}C$ are the stable isotopes of carbon and $_{6}^{14}C$ is the naturally occurring radioactive carbon isotope with the masses (N + Z) changing from 12 to 14, respectively. By definition, all of them have six protons (Z = 6); the number of neutrons varies from six (^{12}C; N= 6) to eight (^{14}C; N = 8) for the naturally occurring carbon isotopes. Additionally, other radioactive carbon isotopes like $_{6}^{11}C$ (N = 5) can be artificially produced, but are highly unstable (very short half-life time). The atomic weight of the elements is not considered as a constant of nature (Coplen and Holden 2011; Brand 2013); it could be calculated from the number of stable isotopes and the corresponding global mean natural abundances (with the natural isotopes of the bioelements as example). In the case of carbon, about 98.93% ^{12}C and 1.07% ^{13}C (with negligible amounts of ^{14}C) are occurring naturally. Multiplying these numbers

Table 8.1 Natural abundance of the stable isotopes of the bioelements. The standard atomic weight of these elements is derived from best estimates by the IUPAC of atomic weights occurring in terrestrial materials (Meija et al. 2016; IUPAC 2018)

Element	Atomic mass	Range of atomic mass	Mean natural abundance
Hydrogen	1.008	1.00784–1.00811	99.9885% ^{1}H
			0.0115% ^{2}H
Carbon	12.011	12.0096–12.0116	98.93% ^{12}C
			1.07% ^{13}C
Nitrogen	14.007	14.00643–14.00728	99.636% ^{14}N
			0.364% ^{15}N
Oxygen	15.999	15.99903–15.99977	99.757% ^{16}O
			0.038% ^{17}O
			0.205% ^{18}O
Sulfur	32.06	32.059–32.076	94.99% ^{32}S
			0.75% ^{33}S
			4.25% ^{34}S
			0.01% ^{36}S

with the corresponding mass numbers (12 Da[3] for ^{12}C and 13.003354835 Da for ^{13}C; Meija et al. 2016, IUPAC 2018) will result in an average atomic weight of 12.011 Da for the element carbon (IUPAC 2018).[4]

As mentioned above, isotopes of an element are either stable or radioactive. In the following we will focus on the stable isotopes of the bioelements H, C, N, O and S. The natural abundance of the primordial isotopes (or primordial nuclides) on Earth, including the stable and the long-lived radionuclides have been determined during the formation of our solar system (Chown 2001).

Table 8.1 lists the atomic weight in intervals because the natural abundance of the isotopes of the bioelements on Earth varies compound-wise on spatial and temporal scales.

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[3] The mass of nuclides (and nucleons) can be expressed in dalton Da or in unified atomic mass units u (IUPAC Green book: Quantities, Units and Symbols in Physical Chemistry, 3rd ed.; https://doi.org/10.1039/9781847557889 (February 2022). Da and u both are defined as exactly 1/12 of the mass of a single ^{12}C atom (BIPM 2019).

[4] Please refer to the web site of the Commission on Isotopic Abundances and Atomic Weights (CIAAW) of the International Union of Pure and Applied Chemistry (IUPAC) for relevant and recent authoritative literature: https://www.ciaaw.org (December 2021).

8.2.2 Isotopocule, Isotopologue and Isotopomer

The umbrella term used for molecules having identical chemical constitution, but differing in isotopic composition and different number of isotopic substituents or different chemical positions of the specific isotope substituent(s) in these molecules is **isotopocule**s (Kaiser and Röckmann 2008). The term is a short form of the description "all *isotopically* substituted species of a m*olecule*". Molecules that differ only in the number of isotope substituents are designated as **isotopologue**s (derived from contracting "*isotop*ic homol*ogues*"; Seeman et al. 1992; Seeman and Paine 1992). Isotopologues can have different masses. However, in some cases also isobaric isotopologues are possible (e.g. $^{14}N^{15}N^{16}O$ and $^{14}N^{14}N^{17}O$ in case of nitrous oxide). Molecules differing in the chemical positions of isotopic substituents (and not in bulk isotopic composition) are named **isotopomer**s (abbreviated from "*isotop*ic is*omer*"; Seeman et al. 1992; Seeman and Paine 1992). These isotopomer molecules do have identical mass.

This terminology is best explained with a simple molecule like nitrous oxide (N_2O) having both isotopologues and isotopomeric variants. Nitrous oxide is a linear asymmetric molecule; each nitrogen atom has different chemical bonding partners (Fig. 8.2 left side). The most abundant 'isotopic' nitrous oxide molecule is $^{14}N_2^{16}O$. With two stable nitrogen isotopes and three stable oxygen isotopes a total of twelve stable nitrous oxide isotopocules are possible with masses from 44 ($^{14}N_2^{16}O$) to 48 ($^{15}N_2^{18}O$) (Fig. 8.2 right side; Magyar et al. 2016). Among them, nine isotopologues and six isotopomers (corresponding to three 'isotopomer pairs') can occur. Isotopomer molecules have the same mass but the heavy isotope(s) are located at different chemical positions in the molecule. In case of N_2O, the two nitrogen atoms have different bonding partners allowing isotopomer molecules with one ^{14}N and one ^{15}N atom. The ^{15}N can be either positioned as terminal or central N atom (Fig. 8.2).
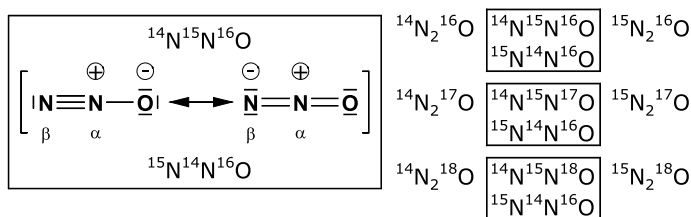

Fig. 8.2 Left side: The chemical bonding inside the nitrous oxide molecule can be described by two stable resonance structures with a delocalized electron system. Nitrous oxide molecules with one ^{14}N and one ^{15}N atom can have two 'constitutional isotopomers'. ^{15}N can be either in the central (α) or terminal (β) N position. The concept of 'site preference' with α and β denotation of the N atoms in N_2O was introduced by Toyoda and Yoshida (1999). Right side: Twelve stable isotopocule molecules of nitrous oxide. The three isotopomer pairs are marked with rectangular boxes. Only one member of each isotopomer part can be counted as isotopologue; giving in total nine isotopologues
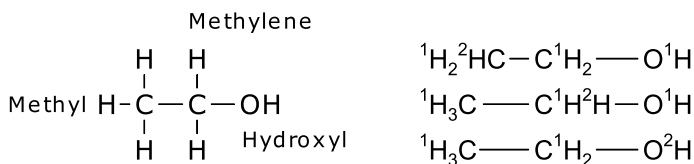
Fig. 8.3 Left side: Ethanol—structural formula and functional groups. Right side: Three different 'constitutional isotopomers' of ethanol molecules labelled with one ^{2}H atom. This ^{2}H atom can be positioned in either the methyl, the methylene or the hydroxyl group of ethanol

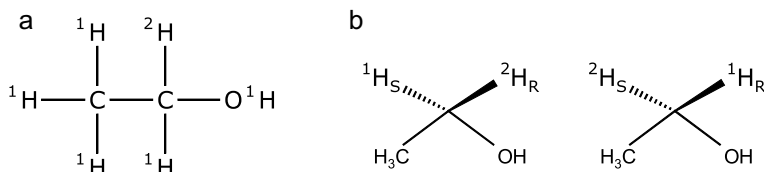**Fig. 8.4 a** Structural formula of ethanol. **b** Fischer projections of the two optical 'stereo isotopomers' of ethanol position-specifically labelled with one ^{2}H atom ('isotopically labelled') in the methylene group. The ^{2}H atom can be either in (R)- or (S)-position[5]

Isotopomers can be either constitutional isotopomers ('constitutional isotopomer', see $^{14}N^{15}N^{16}O$ and $^{15}N^{14}N^{16}O$ molecules above) or isotopic stereo isomers ('stereoisotopomer'). Constitutional isotopomers have the same molecular formula and same isotopic mass, but a different spatial arrangement of the atoms and a different chemical connectivity inside the molecule.

As an example, the three possible constitutional ^{2}H isotopomers of ethanol are shown in Fig. 8.3 (right side). ^{2}H could be found either in the methyl, or in the methylene or the hydroxyl group of the molecule. All three hydrogen atoms in the methyl group of ethanol are chemically indistinguishable, but the H atoms in the methylene group can be differentiated as *pro-R-* and *pro-S-H* atoms (prochiral ethanol molecule[5]; Zhang and Pionnier 2002) allowing two possible enantiomeric optical stereo isotopomer molecules of 1-(^{2}H)-ethanol (Fig. 8.4).

The two hydrogen atoms bound to position C-6 of glucose can be differentiated as (R)- and (S)-position (Zhang et al. 2002). The position-specific hydrogen isotopic signature in these two positions of glucose hydrolyzed from plant cellulose bears also information on the photorespiration/ photosynthesis ratio of the respective plant (Ehlers et al. 2015).

Geometric stereo isotopomers show a usually small restricted rotation of two different isotopic substituents around a carbon–carbon double bond allowing a

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[5] Prochiral molecules can be converted to chiral molecules by a single desymmetrization step; the two hydrogen atoms in the methylene group of ethanol are enantiotopic, but can be differentiated in an asymmetric environment (e.g. by enzymes; Smith 2013, pp. 170–171). Substitution of the two hydrogen atoms in the CH_2 position-specifically with ^{1}H and ^{2}H produces monodeuterated isotopic enantiomers → chiral isotopomers (see e.g. Zhang and Pionnier 2002).

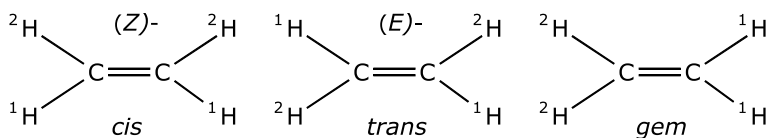**Fig. 8.5** Lewis structures of three isotopomers of dideuteroethene: *cis*-1,2-$^2H_2$ ethene and *trans*-1,2-$^2H_2$ ethene are diasteromeric isotopomers. Meanwhile IUPAC prefers the *(Z)-/(E)*-notation to describe the absolute stereochemistry for alkenes. In contrast 1,1-dideuteroethene ("*gem*-dideuteroethene") is a constitutional isotopomer. All of them have different physical properties (Bigeleisen et al. 1977)

different spatial positioning of the two different isotopic substituents relative to the double bond. This is best explained with e.g. ethene molecules consisting of two ^1H and two ^2H atoms (Fig. 8.5). The two ^1H (and the two ^2H) atoms can be oriented on the same side of the carbon double bond (*cis*) or on the opposite side (*trans*). The resulting diasteromeric isotopomer molecules are termed *cis*- and *trans*-1,2-$^2H_2$ ethene or *(Z)*- and *(E)*-1,2-$^2H_2$ ethene (latter terms now preferred IUPAC notation for alkenes).

In contrast 1,1-dideuteroethene with the two ^2H atoms as substituents at the same carbon atom ("*gem*-dideuteroethene") is a constitutional isotopomer.

Isotope ratio analysis of tree ring cellulose has been used since the 1970s to reconstruct past climate conditions. The site-specific isotopic signature (isotopomer analysis) of glucose hydrolyzed from the extracted cellulose fraction shows high intramolecular differences for ^2H (seven carbon-bound hydrogen isotopomers[6] of glucose; Augusti et al. 2006, Betson et al. 2006) and ^{13}C (six carbon isotopomers of glucose; Wieloch et al. 2018, 2021). This site-specific isotopic signature has its origin in position-specific isotope effects on enzyme reactions during glucose and/or cellulose biosynthesis and might help to elucidate the mechanism of biochemical reactions causing plant-specific adaption to environmental and climatic changes. A corresponding site-specific oxygen isotopic signature (^{18}O isotopomers) of glucose (from soluble sugars and cellulose monomers) has been proven (c.f. Schmidt et al. 2001; Sternberg 2009; Ma et al. 2018) with six possible oxygen isotopomers of glucose. Recently the possible application of the analysis of lignin isotopomers (i.e. carbon and hydrogen isomers in the methoxy groups of lignin) isolated from tree ring wood in reconstructing paleoclimate has been described by Gori et al. (2013) and Mischel et al. (2015). Wang et al. (2021) analyzed the position-specific oxygen isotopic signature of lignin monomeric units.

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[6] Hydrogen atoms bound to heteroatoms like O, N or S are easily exchangeable with hydrogen atoms from (polar) solvents (Bonhoeffer and Brown 1933; Gold and Satchell 1955). Carbon-bound hydrogen atoms preserve the isotopic signature originally imprinted during synthesis reactions with the exception of H atoms bound to enol tautomers (see e.g. Zhang et al. 1993).

Table 8.2 Masses and abundances of the twelve isotopocules of nitrous oxide assuming random distribution (without mass selective processes). Proportional abundances of the isotopocule N_2O molecules have been calculated using the natural abundances of the nitrogen (^{14}N 99.636 and ^{15}N 0.0364%) and oxygen isotopes (^{16}O 0.99757, ^{17}O 0.00038 and ^{18}O 0.00205%) given by IUPAC (2018)

Mass	Isotopocule	Proportional abundance
44	$^{14}N^{14}N^{16}O$	9.90×10^{-1}
45	$^{14}N^{15}N^{16}O$	3.62×10^{-3}
45	$^{15}N^{14}N^{16}O$	3.62×10^{-3}
45	$^{14}N^{14}N^{17}O$	3.77×10^{-4}
46	$^{15}N^{15}N^{16}O$	1.32×10^{-5}
46	$^{14}N^{15}N^{17}O$	1.38×10^{-6}
46	$^{15}N^{14}N^{17}O$	1.38×10^{-6}
46	$^{14}N^{14}N^{18}O$	2.04×10^{-3}
47	$^{15}N^{15}N^{17}O$	5.03×10^{-9}
47	$^{14}N^{15}N^{18}O$	7.43×10^{-6}
47	$^{15}N^{14}N^{18}O$	7.43×10^{-6}
48	$^{15}N^{15}N^{18}O$	2.72×10^{-8}

8.2.3 Clumped Isotopes

Molecules containing more than one heavy isotope (multiply-substituted isotopocules) are labeled as showing the "clumped isotopes" effect. For molecules with "clumped isotopes" the chemical bonds between the heavy isotopes are the focus. Due to their often low isotopic abundances, these molecules usually are difficult to observe in tree rings. Interestingly, the analysis of clumped isotopes in methoxy groups extracted from organic material was recently shown by Lloyd et al. (2018, 2021). This opens a door for possible applications of "clumped isotopes" in methoxy groups of lignin extracted from tree rings.

The stochastic proportional abundance (Table 8.2) of the 'clumped isotopocules' of nitrous oxide can be calculated using the known numbers for the natural abundance of the nitrogen and oxygen isotopes shown in Table 8.1. In nature, the isotopocule molecules of nitrous oxide do not exhibit a stochastic distribution because of equilibrium and kinetic isotope effects influencing thermodynamic stability and/ or kinetic reaction rates of the involved synthesis or degradation reactions of each isotopocule showing "clumped isotopes".

8.3 Notation and Measurement Units

8.3.1 Atom Fraction

Abundances of isotopes should be reported in terms of atom fraction (x). The new term atom fraction is compatible with the International System of Units (SI) and should replace the old term atom percent (atom %). For example, the ^{13}C fraction of

a substance A is given by:

$$x\left(^{13}C\right)_A = \frac{n\left(^{13}C\right)_A}{n\left(^{13}C\right)_A + n\left(^{12}C\right)_A} \tag{8.1}$$

where n = amount of ^{13}C or ^{12}C atoms in substance A[7]. Atom fraction is a dimensionless quantity (Coplen 2011); therefore, a specification of results in form of a relative indication in per cent[8] is possible (see Table 8.1 with estimates of the natural isotopic abundances given in relative form in per cent).

8.3.2 Isotope Delta

The measurement of the absolute isotopic abundances of the bioelements is too labor-intensive, error-prone and costly relative to used methods and techniques to perform it on a daily basis (Nier 1946; De Bièvre et al. 1996). In contrast, the "simultaneous" measurement of isotope ratios[9] (e.g. $^{13}C/^{12}C$) of a sample relative to that of a standard with an isotope ratio mass spectrometer allows a high-precision determination (error cancelling; Nier 1946; Werner and Brand 2001; Brand 2004).

Changes of isotope ratios through natural processes are very small and the isotope ratios generally vary only at the third decimal place or beyond. To better illustrate changes in isotope ratios caused by natural processes, the term 'isotope delta' (relative isotope-ratio difference; Coplen 2011) has been accepted showing the difference of the sample isotope ratio from the standard one. The isotope delta was first formally defined by McKinney et al. (1950). Meanwhile the definition of the isotope delta was made compatible (see Coplen 2011) to the requirements of the International System of Units (SI). Equations (8.2a and 8.2b) show the isotope delta formula (in the two common notations) applied for stable carbon isotopes (i.e. $\delta^{13}C$).

$$\delta^{13}C_{VPDB} = \frac{^{13}R_{Sample} - {}^{13}R_{VPDB}}{^{13}R_{VPDB}} \tag{8.2a}$$

$$\delta^{13}C_{VPDB} = \frac{^{13}R_{Sample}}{^{13}R_{VPDB}} - 1 \tag{8.2b}$$

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[7] ^{14}C is neglected due to very low natural abundance.

[8] It is recommended to use 'per cent' in the text of manuscripts and the '%' sign only in tables and figures or behind numbers in the text (Coplen 2011).

[9] By convention the ratio is always determined as the ratio of the heavy to the light isotope (O'Neil 1986b).

$^{13}R_{\text{Sample}}$ represents the ^{13}C/^{12}C ratio of the sample. $^{13}R_{\text{VPDB}}$ is the ^{13}C/^{12}C ratio of the international VPDB (Vienna Peedee Belemnite) 'standard'[10] defining the carbon isotope scale (c.f. Werner and Brand 2001; Brooks et al. Chap. 6); corresponding scale defining standards and reference materials for the bioelements can be found in Chap. 6 (Table 6.3). Up to few years ago, this equation contained an additional multiplying factor 1000 and the resulting δ^{13}C values were written in per mill (‰) "units".[11] To ensure comparability of the old and the actual valid definition of isotope delta values, it is recommended to avoid ‰ or the factor 1000 when including the isotope delta equation in manuscripts. A δ^{13}C value of '−0.010' according to Eqs. (2a and 2b) can be also expressed as '−10 ‰'. According to Coplen (2011), it is recommended to replace the old "traditional" ‰ sign by e.g. '10^3 δ^{13}C' (here exemplarily shown for carbon isotopes) in tables and figures of articles and presentations. To prevent possible confusion with two otherwise identical equations differing only by multiplication with a factor of 1000, Brand and Coplen (2012) suggested the new unit 'milli-Urey' (mUr) replacing the old ‰.[12] The use of the ‰ symbol is not encouraged any further by IUPAC. Consequently, all δ values in this chapter will be expressed in the unit 'mUr'.[13]

8.3.3 Isotope phi

Isotope delta values can be added or subtracted linearly allowing the use in mass balance equations or correction procedures for (analytical) blanks. This is strictly valid only in the natural isotopic abundance range of ^{13}C, ^{15}N, ^{18}O (and ^{34}S). For hydrogen isotopes with a wide natural range of δ^2H values from −500 to +300 mUr and applications of artificially heavy isotope enriched tracer of the other isotopes ('isotopic tracer') it is recommended to use the phi notation in mass balance equations (Φ defined in Eq. 8.3 exemplarily shown for carbon isotopes) instead of δ values (Corso and Brenna 1997) because the isotope delta is not linearly connected to the isotopic abundance. Brand and Coplen (2012); Coplen (2011).

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[10] VPDB is not a physical existing standard; the VPDB scale can be traced back to the now exhausted carbonate standard PDB and the ^{13}C/^{12}C and ^{18}O/^{16}O ratios of VPDB are now defined via NBS 19 reference material.

[11] The official diction for '‰' is 'per mill' and not 'per mil', 'permil' or 'per mille' (International Organization for Standardization 1992). But keep in mind, 'per mill' is a fraction or comparator sign and not a true unit. The extraneous factor 1000 is not permitted in quantity equations (Coplen 2011).

[12] Especially as the original isotope delta equation with the factor 1000 was introduced to avoid giving results with several decimal places.

[13] The term 'mUr' can be used for all isotope scales of the bioelements. A δ value of 1 ‰ in the old form (McKinney et al. 1950) corresponds to 0.001 ("modern" δ equation according to Coplen 2011) and 1 mUr (Brand and Coplen 2012).
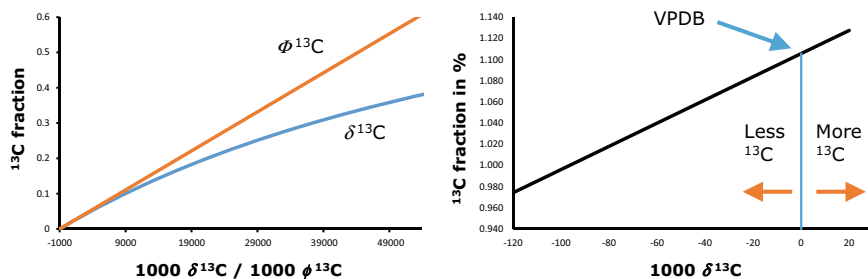
Fig. 8.6 Left side: $\delta^{13}C$ versus $\phi^{13}C$ values in dependence of ^{13}C atom fraction: $\phi^{13}C$ values show a perfectly linear connection to ^{13}C atom fraction whereas $\delta^{13}C$ values do not. Right side: For carbon isotope ratio measurements the international scale-defining standard is VPDB with an accepted $^{13}C/^{12}C$ ratio of 11180.2×10^{-6} (Zhang and Li 1990 cited in Werner and Brand 2001). VPDB is a virtual standard (based on exhausted carbonate standard PDB) and defined via NBS 19 reference material

$$\Phi^{13}C = \frac{\delta^{13}C}{^{13}R_{Sample} + 1} \tag{8.3}$$

The $^{13}R_{Sample}$ needed to calculate $\Phi^{13}C$ values can be derived from Eq. 8.2b.

In Fig. 8.6 the relationship between 'isotope delta' values, 'isotope phi' values and corresponding 'atom fraction' values is exemplarily shown for carbon isotopes.

A sample that contains relatively more ^{13}C than the standard, is enriched in ^{13}C relative to the standard and will therefore show more positive or higher $\delta^{13}C$ values relative to the standard. In contrast samples with relatively less ^{13}C as compared to the standard will be depleted in ^{13}C relative to the standard and correspondingly will have more negative or lower $\delta^{13}C$ values than the standard (Fig. 8.6 right side). The isotope delta value of the scale-defining standard is per definition set to zero (0.000 = 0 ‰ = 0 mUr) on all the corresponding isotope scales. Per definition (see Eqs. 8.2a and 8.2b) the minimum $\delta^{13}C$ value is -1000 mUr. A special feature of the international carbon isotope scale with the virtual VPDB standard is its relatively high $^{13}C/^{12}C$ ratio with the consequence that nearly all natural organic carbonaceous material is ^{13}C depleted relative to VPDB and will therefore show negative $\delta^{13}C$ values.

Table 8.3 Selected properties of the water isotopologue ^2H_2O in relation to H_2O. Data from Krumbiegel (1970)[15]

Property	H_2O	^2H_2O	Difference (%)
Molecular weight in g/mol	18.01558	20.02823	11.2
Melting point in °C, 1013.25 hPa	0	3.813	1.4[a]
Boiling point in °C, 1013.25 hPa	100	101.43	0.4[a]
Vapor pressure in hPa, 20 °C	23.38	20.32	−13.1
Vapor pressure in hPa, 100 °C	1013.25	962.05	−5.1
Density in g/mL 20 °C	0.998232	1.10536	10.7
Ionic product 25 °C in mol^2/L^2	1.01×10^{-14}	1.38×10^{-15}	−86.3
Solubility of NaCl, 25 °C in g salt / 100 g water	35.92	30.56	−14.9

[a]Relative to 0 K (−273.15 °C)

8.4 Properties of Isotopes

The term mass-dependent[14] 'isotope effect' in a wider sense describes small changes in physical and/ or chemical properties of isotopic atoms of elements and/ or of isotopocules caused by differences in nuclear mass. The following chapters deal exclusively with mass-dependent isotope effects.

8.4.1 Isotope Effects—Physical Effects

The mass variance of isotopic atoms and/or isotopocule molecules can influence their physical properties. Isotopes of the same element may be separated using physical processes like diffusion, fractional distillation, centrifugation, electrolysis etc. The large relative mass difference of hydrogen isotopes in the water molecule as an example is responsible for the considerable differences in vapor pressure, melting and boiling points of normal H_2O and ^2H_2O (Table 8.3), but obviously has no significant influence on the organoleptic tasting of both water samples (Urey and Failla 1935). The different water isotopologues also have different molar volumes ($^2H_2O > H_2{}^{16}O > H_2{}^{18}O$; Jancsó 2011). The crossover of the lighter water molecules from liquid to gaseous phase is facilitated in contrast to the heavier ones. Physical processes with

[14] Mass dependent isotope effects on physical processes and/or (bio)chemical reactions lead to changes in isotope ratios between reactant(s) and product(s) with the size of the change being proportional to the relative difference in mass between the isotopes. Non-mass-dependent isotope fractionation (e.g. Thiemens 2006) often occurs in high energy systems (e.g. ozone formation by photolysis or electrical discharge).

[15] See similar data collection by Horita and Cole (2004), Jancsó (2011), Brand and Coplen (2012) and Haynes et al. (2014) and https://water.lsbu.ac.uk/water/water_properties.html (December 2021).

phase transitions (e.g. evaporation or condensation of water molecules) are known to be (partly) 'isotope-selective' and can be used for example to enrich heavier water isotopocules in the liquid phase. Similarly, heavier isotopocule molecules will be enriched in the solid phase ('isotopically enriched') during a phase transition between solid and liquid phase.

Other examples for 'isotope-selective' physical processes are a.o. diffusion and deflection in magnetic fields. Having the same kinetic energy E_{kin} (in the gas phase at a given temperature, Eqs. 8.4 and 8.5), heavier isotopologues (with m_h) will show a smaller average velocity v (White 2015) in contrast to lighter isotopologues (with m_l) when diffusing along a concentration gradient into a low-pressure system (Young et al. 2002). The basis for the measurement of isotope ratios by isotope ratio mass spectrometry (IRMS) is also the behavior of ionized isotopologue molecules in a magnetic field. The radius of deflection (r) of the accelerated ions generated from the isotopologue molecules in the magnetic field can be described by the Eq. (8.6) with m = mass of the ion, e = electric charge unit, B = magnetic field strength and U = acceleration voltage (e.g. Ardenne 1944; Love 1973).

$$E_{kin} = 1/2 m_l v_l^2 = 1/2 m_h v_h^2 \tag{8.4}$$

$$\frac{v_l}{v_h} = \sqrt{\frac{m_h}{m_l}} \tag{8.5}$$

$$r = \frac{1}{B}\sqrt{\frac{2mU}{e}} \tag{8.6}$$

The mass of the ion is proportional to the square radius of deflection of the ion in the magnetic field of an isotope ratio mass spectrometer.[16]

8.4.2 Isotope Effects—Chemical Effects

Chemistry textbooks teach that isotopes of an element have identical chemical properties because the chemical 'reactivity' of atoms and molecules is determined by the electrons in the outermost shell (i.e. valence electrons) of the atom(s). These electrons participate in the formation (or cleavage) of chemical bonds between different atoms. Isotopes of the same element have the same number of electrons in the same electron shell; therefore also isotopocules have the same electron bonding system. But isotopes of an element do not behave like identical twins. The type of the chemical 'behavior' is nearly identical, but the rate of kinetic reactions and/or the position of the chemical equilibria of certain thermodynamic reactions can be 'isotope-selective'.

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[16] An isotope enrichment method by electromagnetic separation has been used for enrichment of ^{235}U for the Manhattan Project (development of the Hiroshima bomb), starting in 1942 in Oak Ridge, TN, USA (Groves 1962; Love 1973).

Chemical reactions of isotopocule molecules are qualitatively equal but can be quantitatively different (c.f. Hoefs 2018). Different nuclear mass (and increasing nuclear volumes) of the isotopes will influence the intramolecular binding forces and therefore lead to a shift of lines in e.g. Raman and IR spectra of isotopocule molecules due to affecting the frequencies of the vibrational modes of a molecule ("isotopic shift"). Molecules in which positions are substituted with heavy isotopes (isotopic substitution) are more stable and more energy ('bond-dissociation energy') is needed to cleave a covalent bond homolytically[17] between atoms when heavier isotopes are involved. This implies that covalent chemical bonds between ^{12}C-^1H are weaker and easier to split than between ^{13}C-^1H or ^{12}C-^2H (even more energy would be needed for ^{13}C-^2H). The cleavage reaction of bonds between lighter isotopes normally will also have a higher reaction velocity. Correspondingly, higher energy ('bond enthalpy') is released during bond formation with heavier isotopes.

According to the IUPAC Gold Book[18] the term 'isotope effect' (IE) is defined more scientifically as the "effect on the rate or equilibrium constant of two reactions that differ only in the isotopic composition of one or more of their otherwise chemically identical components". Effects on rate constants are known as 'kinetic isotope effects' (KIE); effects on equilibrium constants are designated as 'thermodynamic' or 'equilibrium isotope effects' (EIE). The isotope effect is mathematically expressed as the ratio of the rate constants (in case of KIE) or equilibrium constants (in case of EIE) of the respective reaction; both exemplarily shown for carbon isotopes (after Coplen 2011):

$$^{13}KIE = {}^{12}k/{}^{13}k \tag{8.7}$$

$$^{13}EIE = {}^{12}K/{}^{13}K \tag{8.8}$$

Cautionary note: in other science fields $^{13}KIE = {}^{13}k/{}^{12}k$ and $^{13}EIE = {}^{13}K/{}^{12}K$ is used rather than Eqs. (8.7) and (8.8). ^{12}k and ^{13}k are the rate constants and ^{12}K and ^{13}K the equilibrium constants for reactions involving ^{12}C and ^{13}C isotopocules, respectively. According to Eq. (8.7) KIEs larger than 1 are termed 'normal' and smaller than 1 are called 'inverse'. Isotope effects related to the atoms (as part of molecules) directly taking part in bond formation or cleavage are named 'primary IE'; IE related to more remote atoms or molecule positions are designated as 'secondary'. Furthermore, the bond formation or split can be in the rate-determining step (KIE) or pre-equilibrating step (EIE). Secondary IE are typically smaller than primary IE and the dimension is usually dependent also on the distance of the respective atom or molecule position from the site of bond formation or cleavage (secondary IE in α position > secondary IE in β position and so on, relative to the positions directly involved in bond cleavage or bond formation; Van Hook 2011). Isotope effects caused

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[17] During a homolytic bond cleavage the shared electrons of the chemical bond to be split will be divided equally between the binding partners.

[18] IUPAC Gold Book: Compendium of Chemical Terminology https://goldbook.iupac.org/ (December 2021).

by the isotopic substitution of elements heavier than hydrogen (^{1}H, ^{2}H, ^{3}H) can be named 'heavy atom isotope effects'. Substrates with equivalent molecule positions (two or more identical functional groups) can be subject to 'intramolecular IEs' (e.g. decarboxylation reaction of carboxylic acids with two or more carboxyl groups, e.g. Yankwich and Belford 1954). Isotope effects often influence only the isotope ratio of a specific position in a molecule ('position-specific' or 'site-specific' isotope effect); a corresponding site-specific isotope ratio analysis ("isotopomer analysis") can be performed by quantitative NMR or by IRMS coupled to chemical degradation of the chemical molecules in question. This has been shown by measuring site-specific δ^{13}C values of all six carbon positions of glucose with IRMS by Rossmann et al. (1991) and with NMR by Gilbert et al. (2012); c.f. a recent review by Gilbert (2021). EIEs can be larger or smaller than unity depending on the potential enrichment of the heavier isotope in reactant(s) or product(s). Solvent isotope effects [18] are either KIEs or EIEs caused by changes of the isotope ratio of the solvent [19].

In the following the physicochemical basics of the theory of (kinetic and equilibrium) isotope effects will be discussed briefly. A more detailed discussion of the theoretical background of isotope effects as origin of isotope fractionation has been provided by a.o. Bigeleisen and Goeppert-Mayer (1947), Urey (1947) and Bigeleisen (1965) for equilibrium isotope effects and by Bigeleisen and Wolfsberg (1958), Saunders (1966) and Wolfsberg et al. (2010) for kinetic isotope effects.

8.4.2.1 Fundamentals of Mass-Dependent Isotope Fractionation in Chemical Reactions

Atoms in molecules are in constant movement (in translational, rotational and vibrational mode). Translational and rotational motions are of minor relevance for chemical isotope effects (Hoefs 2018) as they have limited effect on intramolecular bonds. Only (intramolecular) vibrations are influencing the atomic distances of chemical bonds in molecules. The energy at a specific quantum level n (E_n) associated to these stretching and bending (deformation) vibration modes[20] of the bond in question according to the quadratic harmonic oscillator approximation (see text below

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[19] Solvent molecules can interact in(bio)chemical reactions mostly in three ways: (1) Solvent molecules can be reaction partners (direct participation of water molecules, e.g. as H_2O or H_3O^+ in hydrolysis reactions interacting with reactant state, transition state and/or product state of the reaction; Schowen 2007). (2) Protic solvents can modify properties of involved reaction partners by exchanging (hydrogen) isotopes with reactants, products and/or involved enzymes possibly affecting enzyme activity and structure (Fitzpatrick 2015). (3) Solvent–solute interactions are known to stabilize the transition state of a reaction by influencing both, the free energy of the transition state and the activation energy of the specific reaction. The extent and nature of these interactions can be influenced by the isotope ratio of the used solvents (Smith 2013, p. 288).

[20] Depending on the degrees of freedom polyatomic molecules can show vibration modes influencing the length of a specific chemical bond (stretching mode) in the molecule or vibration modes that are related to the change in the angle between two bonds in the molecule. All these vibration modes influence the inner energy of the molecule. Asymmetric or symmetric stretching is important for the splitting (and formation) of that specific bond.

and Fig. 8.7) is:

$$E_n = (n + 1/2)h\nu \tag{8.9}$$

where h is the Planck constant, n = vibrational quantum number (0 plus positive integers) and v the vibrational frequency. The origin of isotope effects can be attributed to the dependence of the vibration frequency on the reduced mass (μ) of the binding partners and a molecule-specific force constant k of the bond involved (Eq. (8.10), analogous to the spring constant in Hooke's law). The force constant is identical for isotopologue molecules (Fujii et al. 2009) and the potential energy surface (Fig. 8.7)
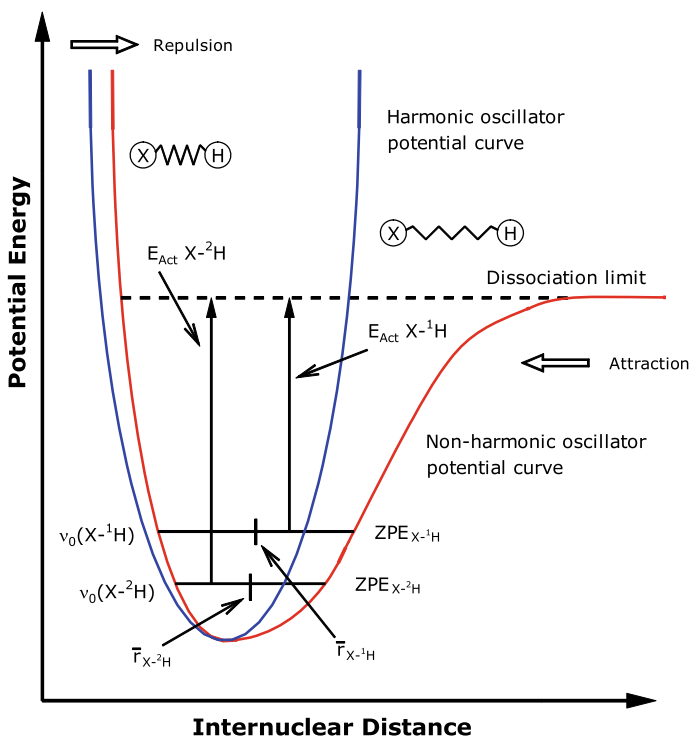
Fig. 8.7 Schematics of a potential energy curve for the diatomic molecule X–H, adapted and modified from Vogel and Houk (2019). Blue line represents the quantum harmonic oscillator energy well; whereas the red line shows the more realistic anharmonic oscillator (Morse potential). E_{Act} = activation energy needed to drive the corresponding bond to the dissociation limit (splitting of the chemical bond). ZPE = zero-point energy, corresponds to the energy at the lowest vibrational level ν_0. The anharmonic oscillator shows the minimum and maximum position (left and right side of the red line) of the H atom relative to the X atom positioned at 0 distance symbolized by the "two balls - spring model". Consequently, the difference from the X atom to the $_{X-}{}^1H$ and $_{X-}{}^2H$ marks in the horizontal ZPE lines corresponds to the average bond lengths of the $X-{}^1H$ or $X-{}^2H$ bond. The bond involving ${}^2H$ is shorter than the bond with ${}^1H$ (Vogel and Houk 2019)

is then essentially independent from isotopic substitution (Bennet 2012).

$$v = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \qquad (8.10)$$

Key influencing factor on the vibrational frequency is then the reduced mass μ (Eq. 8.11) of the two bonded atoms. Increasing reduced mass μ lowers the vibration frequency v. According to Young et al. (2002) the reduced mass is relevant for bond-breaking reactions, whereas molecular or atomic mass is relevant for transport processes.

$$\mu = \frac{m_1 \times m_2}{m_1 + m_2} \qquad (8.11)$$

The reduced mass μ is increasing with increasing mass m. Substituting one of the bonding partners in a molecule with a heavy isotope will change neither nuclear charge, nor electron distribution in the molecule nor the potential energy curve of the "isotopic" molecule. But the vibrational frequency of a bond and the average bond length will be influenced (Vogel and Houk 2019). This is best shown schematically in Fig. 8.7 by drawing the potential energy of the most simple example, a diatomic molecule (X–H) in dependence to the internuclear distance in the molecule (potential energy surface). This course of the potential energy relative to the internuclear distance can be either modeled by the quantum harmonic oscillator (blue line in Fig. 8.7) or by the more realistic Morse potential (red line in Fig. 8.7) that includes a.o. corrections for anharmonicity of real chemical bonds. The anharmonic oscillator postulates different average bond lengths for e.g. X-^{1}H and X-^{2}H bonds (Vogel and Houk 2019). The two bonded atoms influence each other by repulsive and attractive forces, building an average bond length between them. In Fig. 8.7 the X atom would be near the vertical axis and the bonded H atom then would vibrate following the black horizontal lines (either at v_0(X-^{1}H) or v_0(X-^{2}H) depending on the isotopic mass of the H atom) represented by the two end positions of the "two balls—spring model" as an analogue for the chemical bond in the diatomic molecule shown in Fig. 8.7 (Mook 2001). The red line models the minimum potential energy in dependence of the internuclear distance (*energy well* or *potential well*) between the two atoms. The energy of such a system at the lowest vibrational energy level (ground state n = 0; at room temperature the majority of the molecules are in vibrational ground state) is defined as zero-point energy (ZPE) corresponding to the energy of the respective system at 0 K (−273.15 °C). The energy level of the atom in the energy well (the bottom of the well will not be reached even at 0 K) is then depending on its mass and is labelled as $ZPE_{X\text{-}^1H}$ and $ZPE_{X\text{-}^2H}$ in Fig. 8.7. To reach the dissociation limit with the effect to split the X–H bond, a certain amount of (kinetic) activation energy (E_{Act}) has to be applied. Correspondingly, this energy ('bond enthalpy') will be released during the bond formation process. Bonds with heavier isotopes show lower vibrational frequencies and therefore will have a lower ZPE. The heavier hydrogen isotope

is located at a lower level in the energy well than the lighter hydrogen isotope and needs therefore more energy (higher activation energy) to leave the chemical bond, which means, that it has a higher bond enthalpy.

To summarize the above: Chemical bonds with heavier isotopes have a lower vibrational frequency as compared to lighter ones. Therefore chemical bonds with heavier isotopes have a lower ZPE. Consequently, more energy (higher activation energy E_{Act}) is needed to cleave bonds with heavier isotopes (Fig. 8.7), and formation of bonds with heavier isotopes will then also release more energy (bond enthalpy) and are stronger. Therefore, heavier isotopocules are more stable than light isotopocules.

8.4.2.2   Thermodynamic or Equilibrium Isotope Effects for Reversible Reactions at Equilibrium

Reversible chemical reactions approach equilibrium with the consequence that forward and backward reaction then occur simultaneously and at chemical equilibrium also with equal rates. The concentration of reactants and products will change until a chemical equilibrium is established. Such reversible reactions can be described with equilibrium constants. In many cases also the position of the equilibrium in such reversible reactions is 'isotope-selective'. Reactants and products are then also 'isotopically' different. Such isotope exchange reactions tend to reach additionally an isotopic equilibrium. In these isotopic exchange reactions two different chemical structures in which the isotopes of the element in question are bonded in different ways are connected to each other. The distribution of the isotopes between the two structures in this system will occur ensuring that the free energy of the total system is at the minimum in equilibrium conditions (White 2015).

It can then be deduced that in a chemical and isotopic equilibrium[21] the heavier isotope will concentrate at the molecular site where it is bonded stronger (Fig. 8.8). A measure for the bond strength is the force constant k from Eq. (8.10). The force constant k in the harmonic oscillator model is independent from the nuclear masses of the isotopic bond partner (Fujii et al. 2009) and is directly proportional to the strength of the vibrating chemical bond. The small difference in ZPEs between the isotopologue molecules drives the isotopic exchange reaction; the size of ΔZPE is controlling the isotope effect. The extent of equilibrium isotope fractionation is mostly decreasing with increasing temperature (c.f. O'Neil 1986a; Hoefs 2018). The heavy isotope is usually concentrated in the molecule or ion with the more constrained site (O'Leary et al. 1992), the chemical structure in which the element in question is "bound most strongly" (Bigeleisen 1965). The heavy isotopes can preferentially be found in chemical structures connected to strong and short chemical

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[21] The free energy change (standard Gibbs energy change ΔG^0) for such an isotope exchange reaction typically is relative small (e.g. ~−5 J/mole for the oxygen isotope equilibrium between H_2CO_3/H_2O at pH 0, whereas the free energy of the hydration of CO_2 at same pH is around−17 kJ/mole; Usdowski and Hoefs 1993). Therefore it can be assumed that chemical systems in isotopic equilibrium are also in chemical equilibrium. But see Schimerlik et al. (1975) and footnote 26.
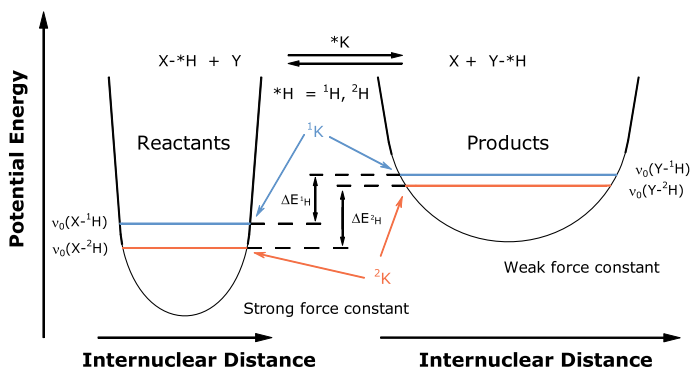
Fig. 8.8 Schematics of the potential energy curves (illustrating the harmonic oscillator curves only) for a reversible reaction between reactants(s) and product(s), approaching isotopic (and chemical) equilibrium (adapted and modified from Vogel and Houk 2019). A strong (stiff) X–H bond in the reactant is exchanged with a weak Y-H bond in the product. The difference ΔE^1_H ($ZPE_{Y\text{-}}{}^1_H$—$ZPE_{X\text{-}}{}^1_H$) is smaller than the difference ΔE^2_H ($ZPE_{Y\text{-}}{}^2_H$—$ZPE_{X\text{-}}{}^2_H$). This will lead to a normal equilibrium isotope effect with $^1K > {}^2K$ and following $^1K/^2K > 1$ (Vogel and Houk 2019)

bonds showing higher bond enthalpy (higher bond stiffness—the oscillator with the highest frequency; Bigeleisen 1965; Vogel and Houk 2019; Fig. 8.8). The bond stiffness is related to the force constant k in Eq. (8.10). A strong bond corresponds to a force constant with a high value and vice versa. In summary, heavy isotopes are concentrated in chemical structures which have the more constrained environment with bonds with the highest stiffness (low-energy systems having most stable bonds). Usually for carbon isotopes, the chemical state with four bonds is preferred under equilibrium conditions: Bicarbonate is ^{13}C enriched relative to CO_2 and carboxyl groups in organic acids are enriched in ^{13}C relative to CO_2 (O'Leary et al. 1992). In the review article by Rishavy and Cleland (1999) many isotope fractionation factors for (organic) molecules are tabulated.

8.4.2.3 Kinetic Isotope Effects on Unidirectional (One-Way) or Irreversible Reactions

The progress of a kinetically controlled chemical reaction can be characterized by showing the energy profile along the reaction coordinate[22] during transformation of reactant(s) to product(s). The reaction coordinate connects the reactant(s) and product(s), both at local energy minima via an intermediary transition state at maximum energy (Fig. 8.9). Reactants are separated from the products by a potential wall; the transition state correspond to the highest potential energy on the reaction

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[22] According to Van Hook (2011) the reaction coordinate describes the progress through the respective chemical reaction. The reaction coordinate thereby displays the reaction path at minimum energy level between the reactant(s) and product(s), both shown as (local) energy minima.
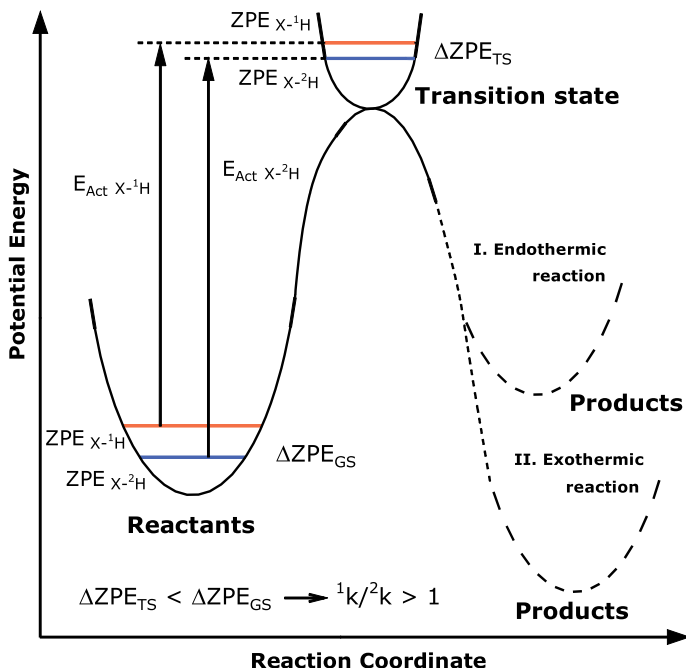
Fig. 8.9 Schematics of a potential energy diagram (potential energy following the reaction coordinate[22]) of a hypothetical reaction (X-H cleavage) from the ground state (GS) via the transition state (TS) to the product state. The activation energy (E_{Act}) of the $X-^2H$ bond between GS and TS is higher than the corresponding activation energy of the $X-^1H$ bond describing a reaction with normal kinetic isotope effect. The reaction can be either endothermic or exothermic. Scheme modified after Bennet (2012)

coordinate. Ground state (reactants) and activated transition state (TS) are in equilibrium, whereas the further reaction path from transition state to the product(s) is irreversible. The total reaction can be exothermic thereby releasing energy from the system to the environment during the reaction or endothermic with the need to absorb energy from the environment. Figure 8.9 illustrates the change of the potential energy surface of a homolysis[17] reaction (X–H bond) from the initial (ground) state via the transition state towards the products along the reaction coordinate. Both ground state and transition state of a reaction can be characterized by vibrational modes with associated ZPEs. The corresponding activation energies of the reactants are primarily determining the extent of the kinetic isotope effect. As stated above, chemical bonds with heavier isotopes have lower vibrational frequencies and a lower ZPE than bonds in the lighter isotopologues. These bonds are therefore located deeper in the potential energy surface (as can be seen in Fig. 8.7). Consequently, a higher activation energy is needed to reach the transition state (or the dissociation limit) of the respective chemical bond. This corresponds also to a slower reaction rate. In case of a 'normal' hydrogen isotope effect the difference in ZPEs of the isotopologue

molecules in the transition state is lower than the corresponding difference in ground state ($\Delta ZPE_{TS} < \Delta ZPE_{GS}$; Wolfsberg et al. 2010) because of and due to the different activation energies between the C-^{1}H and C-^{2}H bond.

To summarize: Bonds with heavier isotopes vibrate at lower frequency and have therefore a lower ZPE. This implies a higher activation energy for the specific chemical bond in the heavy isotopologue to approach a transition state or the dissociation limit of the bond in question. Heavier isotopologues are more stable and correspondingly react more slowly as compared to lighter isotopologues (Elsner 2010). This results in lower turnover rates causing (normal) isotope fractionation in irreversible or one-way reactions without complete turnover of reactants to products (non-quantitative reaction). Kinetic isotope effects are depending on the mechanism of the involved (bio)chemical reaction and are therefore an effective instrument to get insights in (enzyme) reaction mechanisms (e.g. Elsner and Hofstetter 2011).

8.5 Isotope Fractionation

Isotope effects on (chemical) reactions are physical phenomena which cannot be directly measured by itself, but can lead to observable **isotope fractionation** (partial isotopic separation) between products and reactants in case of a non-quantitative turnover or a (quantitative) allocation of the isotopic composition of e.g. one reactant to two or more products. To get a KIE (or EIE) expressed in form of an isotope fractionation in reaction networks needs branching points involved (e.g. Hayes 2001; Schmidt et al. 2015). Isotope effects on enzyme reactions normally are position-specific (site-specific) thereby influencing the isotope ratio of one specific molecule position. The relative isotopic abundance (and the corresponding atom weight) of chemical compounds and biological materials are not precisely constant at a local scale. Isotope effects on (bio)chemical reactions and/ or physical processes will influence the isotope ratio of involved compounds by fractionating isotopes during the reaction and/or the process. Thereby, the corresponding isotope ratio of these compounds will be changed leading to an enrichment or depletion of the relative abundance of one isotope compared to the other(s) during the chemical reaction or physical process. In the following, isotope fractionation in (bio)chemical reactions on the way from the reactant(s) to the product(s) during establishing an equilibrium in reversible reactions or in an irreversible kinetically controlled reaction will be discussed. The change in isotope ratio is proportional to the size of the involved isotope effect.

Isotope fractionation can be caused by:

- Thermodynamic or equilibrium isotope effects on reversible reactions reaching a chemical (and isotopic) equilibrium.
- Kinetic isotope effects on (irreversible) unidirectional reactions and reactions not leading to (full) chemical equilibrium.

Often the situation is more complex; there are (bio)chemical reactions (and physical processes) that are not completely unidirectional, but also a full (chemical) equilibrium is not accomplished. In such systems the net isotope fractionation can vary between the kinetic isotope fractionation of e.g. the forward reaction and an isotope fractionation near the equilibrium isotope fractionation depending on the realized ratio of forward to backward flux in relation to the equilibrium fluxes (e.g. Sade and Halevy 2017). An example for such a (physical) system combining kinetic and equilibrium isotope fractionation would be the relationship between the isotope ratios of $H_2O(l)$ and $H_2O(g)$ in an open system with liquid and gaseous water. Kinetic isotope fractionation dominates the evaporative transition of $H_2O(l)$ to $H_2O(g)$ up to the point when the vapor phase above the liquid water gets saturated, but complete irreversibility of the evaporation cannot be ensured and the proportion of incipient condensation is difficult to measure (Mook 2001). With starting saturation the kinetic isotope ^{18}O depletion of $H_2O(g)$ reduces to the equilibrium one (see Chap. 10). Evaporation and condensation of $H_2O(g)$ in a closed system is then an equilibrium process (Chaps. 10 and 18). As consequence the extent of the ^{18}O depletion of the $H_2O(g)$ above $H_2O(l)$ depends on the degree of saturation in the system under observation (O'Neil 1986a; Mook 2001). A plant physiological example for the combined effect of kinetic and equilibrium isotope fractionation is the CO_2 exchange between leaves (stomata) and atmosphere: The oxygen isotopic information of CO_2 that is back diffusing from the stomata in leaves to the atmosphere is depending on the reaction conditions of the oxygen isotope exchange reaction between leaf water and CO_2 catalyzed by the enzyme carbonic anhydrase (CA) in plant leaves. Depending a.o. on the activity of CA the actual ^{18}O enrichment of the CO_2 from back diffusion out of the stomata can be lower as the theoretically expected equilibrium isotope enrichment in ^{18}O (Gillon and Yakir 2000).

Enzyme catalyzed reactions should show identical equilibrium isotope fractionation than the not catalyzed reaction as enzymes in fact take part in the reaction but are not altering the position of the chemical and isotopic equilibrium (see e.g. Uchikawa and Zeebe 2012). Isotope fractionation by equilibrium isotope effects in multi-reaction sequences can be additive to other isotope fractionation caused either by equilibrium or kinetic isotope effects. Enzyme-catalyzed reactions often show lower kinetic isotope fractionation than their non-catalyzed analogues (O'Leary et al. 1992). Isotope fractionation caused by irreversible or unidirectional kinetically controlled reactions is often larger than related equilibrium isotope fractionation (O'Leary et al. 1992). Kinetic isotope fractionation is often related to one rate-limiting step in a chemical reaction or reaction network.

8.5.1 Quantities to Express Isotope Fractionation

The distribution of the stable isotopes between two compounds A and B can be displayed in several mathematical forms. The following equations will be exemplary for carbon isotope fractionation.

(A) The isotope fractionation factor[1] α describes the isotopic relationship between compounds A and B that can either be in equilibrium (A < = > B or B < = > A) or in an one-way (irreversible) relationship (A –> B). The isotope fractionation factors for equilibrium reactions can be identified as $\alpha_{eq}{}^{13}C_{A/B}$ and for kinetic reactions as $\alpha_{kin}{}^{13}C_{A/B}$.

$$\alpha^{13}C_{A/B} = \frac{^{13}R_A}{^{13}R_B} = \frac{\delta^{13}C_A + 1}{\delta^{13}C_B + 1} \qquad (8.12)$$

The isotopic fractionation factor $\alpha^{13}C_{A/B}$ can be easily determined by measuring the corresponding $\delta^{13}C$ values of the compounds in question. It is common use to list isotope fractionation factors for isotope exchange reactions of the molecules or ions of interest relative to so called 'reference molecules' (Rishavy and Cleland 1999). The reference molecule for $^2H/^1H$ and $^{18}O/^{16}O$ systems is usually water. For $^{13}C/^{12}C$ often (aqueous) carbon dioxide is used (Rishavy and Cleland 1999). Corresponding α factors would be e.g. $\alpha_{eq}{}^{13}C_{HCO_3^-/CO_2}$ for the bicarbonate/carbon dioxide $^{13}C/^{12}C$ system and $\alpha_{eq}{}^{18}O_{CO_2/H_2O}$ for the carbon dioxide/water $^{18}O/^{16}O$ system. α_{eq} Values larger than unity indicate an enrichment of the heavier isotope in the molecule or ion of interest. Values smaller than unity would show an enrichment of the heavier isotope in the corresponding reference molecule (Rishavy and Cleland 1999).

The kinetic carbon isotope fractionation factor $\alpha_{kin}{}^{13}C$ is defined analogous to Eqs. (8.7) and (8.12) for the reaction A –> B; reactant A ('source') is converted to product B. This mode of designation for the quantities describing isotope fractionation, especially connected to ^{13}C fractionation during photosynthesis, is used by Farquhar et al. (1989), O'Leary et al. (1992), Hayes (2001), Fry (2006) and many others. Authors working in other scientific disciplines prefer a reciprocal diction of Eq. 8.12 with $\alpha_{kin}{}^{13}C_{B/A} = (\delta^{13}C_B + 1)/(\delta^{13}C_A + 1)$ as recommended by Coplen (2011). The latter way of designation is more commonly used with hydrogen isotopes (see Hayes 2001, Sachse et al. 2012, Cormier et al. 2018 and many others).

In equilibrium reactions the isotopic fractionation factor α is related to the equilibrium constant K:

$$\alpha = K^{1/n} \qquad (8.13)$$

with n describing the number of atoms exchanged in the reaction. Normally reactions are written for the case n = 1 (only one isotopic atom is exchanged) to keep the mathematics involved at low level (O'Neil 1986b). It is important to note that, the equilibrium constant of an isotope exchange reaction depends on how the chemical equation for the isotope exchange reaction is written (O'Neil 1986b).[23] This then

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[23] As an example, the equilibrium reaction between CO_2 and HCO_3^- can be written either as (i) $^{13}CO_2 + {}^{12}HCO_3^- <—> {}^{12}CO_2 + {}^{13}HCO_3^-$ or as (ii) $^{12}CO_2 + {}^{13}HCO_3^- <—> {}^{13}CO_2 + {}^{12}HCO_3^-$. The corresponding isotopic fractionation factor α for equilibrium (i) would be $\alpha_{eq}{}^{13}C_{HCO_3^-/CO_2}$ and that for equilibrium (ii) would be $\alpha_{eq}{}^{13}C_{CO_2/HCO_3^-}$. Isotope fractionation factor α from

affects the definition of EIE (Eq. (8.8)) as well as the isotope fractionation factor α (leading correspondingly to values $\alpha_{A/B} > 1$ or $\alpha_{B/A} < 1$, or vice versa).

(B) Isotope fractionation can also be described with the epsilon notation (previous name 'isotopic enrichment factor' is not biuniquely used see IUPAC Gold Book[18]).

$$\varepsilon^{13}C_{A/B} = \alpha^{13}C_{A/B} - 1 \qquad (8.14)$$

The factor epsilon should be named 'isotopic fractionation' (Coplen 2011) or 'isotope fractionation'[1]; a specification for equilibrium and kinetic reactions is possible. In principle α and ε values can be used to describe isotope fractionation in equilibrium systems and kinetic processes with irreversible reactions. ε values can be expressed as '$10^3 \varepsilon$' or like the δ values in mUr.

(C) The isotope fractionation between the two compounds A and B can be approximated as the difference of two (measurable) δ values (shown here for two $\delta^{13}C$ values).

$$\Delta^{13}C_{A/B} = \delta^{13}C_A - \delta^{13}C_B \qquad (8.15)$$

All these quantities are related to each other by the following equation.

$$\Delta^{13}C_{A/B} \approx \varepsilon^{13}C_{A/B} = \alpha^{13}C_{A/B} - 1 \qquad (8.16)$$

In geological studies "lnα values" are often used to relate (equilibrium) isotope fractionation between solids (e.g. minerals) to their formation temperature (Sharp and Kirschner 1994). The term '10^3 lnα' is indicated as 'per mill isotope fractionation'[11] in prominent geological literature (Friedman and O'Neil 1977; O'Neil 1986b). This 'per mill isotope fractionation' is often (depending on the temperature range) a linear function of T^{-1} (low temperature processes) or T^{-2} (high temperature processes) (O'Neil 1986a; Hoefs 2018). Such resulting isotope fractionation curves can then be applied in isotope thermometry (Clayton 1981). The interrelation between the isotopic difference Δ and lnα is shown (for ^{13}C) in equation

$$\Delta^{13}C_{A/B} \approx \ln\left(\alpha^{13}C_{A/B}\right) \qquad (8.17)$$

This is mostly valid for α values around 1, small numerical values for both $\delta^{13}C_A$ and $\delta^{13}C_B$ and for small differences between $\delta^{13}C_A$ and $\delta^{13}C_B$. O'Neil (1986b)

(i) is the inverse of α from (ii). $\alpha_{eq}^{13}C_{HCO_3^-/CO_2} > 1$ (e.g. Marlier and O'Leary 1984) and $\alpha_{eq}^{13}C_{CO_2/HCO_3^-} < 1$ (e.g. Mook et al. 1974), both describing the identical ^{13}C enrichment of $\alpha_{eq}^{13}C_{CO_2/HCO_3^-} < 1$ in equilibrium with CO_2. The equilibrium isotope effect (^{13}EIE in case of carbon isotopes) on an exchange reaction of one (carbon) atom as defined in eq. 8.8 is exactly the inverse of the equilibrium constant K (eq. 8.13; e.g. eq. 8.21 for the bicarbonate/carbon dioxide ^{13}C exchange) and correspondingly the equilibrium isotope fractionation factor ($\alpha_{eq}^{13}C$ in case of carbon isotopes) as defined in equation 8.12 (when the exchange reaction is written in the same direction, as discussed by Coplen 2011, p. 2549).

suggested a preferred use of the lnα values over Δ values when either the difference Δ or the involved $\delta^{13}C$ values of A and B are larger than 10 mUr (see Table 8.4).[24]

In the scientific literature, there are different definitions available for α, ε and Δ (e.g. Fry 2006). In disciplines like geochemistry and hydrology for example, it is more common to define $\alpha_{kin} = {}^hk/{}^lk$ with the heavy isotope in the numerator and the light one in the denominator (e.g. Mook 2001; Harris et al. 2012). To avoid any confusion, it is recommended on the one side to carefully verify how the particular values are given in the respective source article. On the other side, a clear definition and precise wording should be provided to the reader when writing new manuscripts.

In practice, one often needs to discuss the isotopic difference between two samples for which only δ values without information on turnover rates are available. The above mentioned quantities for isotope fractionation ε, α, lnα and Δ will be exemplified for the following arbitrary systems. The mode of designation for ^{13}C fractionation developed above (Eqs. 8.12–8.17) has been applied:

(I) A precursor material with a $\delta^{13}C_{Source}$ value of −8.00 mUr should be converted to a) product A with a $\delta^{13}C$ value of −14.00 mUr, b) product B with a $\delta^{13}C$ value of −28.00 mUr and c) product C with a $\delta^{13}C$ value of −90.00 mUr.

(II) (a) A precursor material A with a $\delta^{13}C$ value of −14.00 mUr, (b) product B with a $\delta^{13}C$ value of −28.00 mUr and (c) product C with a $\delta^{13}C$ value −90.00 mUr each should be converted to a product with a $\delta^{13}C$ value of −8.00 mUr (reversed case I).

The corresponding calculated values for Δ, lnα, α and ε for both scenarios are presented in Table 8.4. Simply subtracting the $\delta^{13}C$ values ($\Delta^{13}C$) delivers a good approximation to the involved isotope fractionation for case Ia) with a difference ($\delta^{13}C_{Source} - \delta^{13}C_{Product} < 10$ mUr). The difference of both $\Delta^{13}C_{Source/Product}$ and $ln\alpha^{13}C_{Source/Product}$ to $\varepsilon^{13}C_{Source/Product}$ is within the limits of the analytical error of a measurement of the $\delta^{13}C$ value (we assume here ±0.1 mUr for measurement with an elemental analyzer coupled to IRMS). For case Ib) with a difference of 20 mUr between $\delta^{13}C_{Source}$ and $\delta^{13}C_{Product}$, the difference between $\Delta^{13}C_{Source/Product}$ and $\varepsilon^{13}C_{Source/Product}$ increases quite considerably, whereas the difference between $ln\alpha^{13}C_{Source/Product}$ and $\varepsilon^{13}C_{Source/Product}$ would be still in the range of 2σ (analytical error). Nevertheless, the $\Delta^{13}C$ notation is in quite common use for estimating the photosynthetic isotope fractionation (Farquhar et al. 1989). In case Ic (with differences in $\delta^{13}C$ of >20 mUr between source and product and one of the involved $\delta^{13}C$ values more than 10 mUr distant from the 0 mUr of the standard) only $\alpha^{13}C$ values and/or the $\varepsilon^{13}C$ notation should be used. In case II, it is obvious that Δ and lnα show the same relation than in case I, except for the algebraic sign. In contrast $\alpha^{13}C$ and $\varepsilon^{13}C$ show always different numerical values. The $\varepsilon^{13}C$ values for case Ia and IIa still show similar mathematical absolute values, but with increasing mathematical distance from the 0 mUr of the standard (case Ib to IIb and Ic to IIc) the absolute $\varepsilon^{13}C$ values are getting more and more different (Table 8.4). The numerical values for

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[24] Therefore Δ values should not been used to describe hydrogen isotope fractionation.

Table 8.4 Comparison of the different measures for isotope fractionation (Δ, lnα, α and ϵ exemplarily shown for carbon stable isotopes) for two cases. Case II is the reversed form of case I. The δ^{13}C and Δ^{13}C values are given in 'mUr' corresponding to '10^3 δ values'. The calculated 'lnα values' are presented in form of 'ln$\alpha \times 10^3$'. Isotope fractionation ϵ values are given as '$\epsilon \times 10^3$'. In case II the sources and sinks have been exchanged relative to case I resulting in different values for α and ϵ, whereas both Δ and lnα values have the same values except for the algebraic sign

| $\delta^{13}C_{Source}$ in mUr	$\delta^{13}C_{Product}$ in mUr	$\Delta^{13}C_{Source/Product}$ in mUr	$\ln(\alpha^{13}C_{Source/Product})$ $\times 10^3$ or in mUr[25]	$\alpha^{13}C_{Source/Product}$	$\epsilon^{13}C_{Source/Product}$ $\times 10^3$ or in mUr
I					
8.00	−14.00	6.00	6.0668	1.0061	6.0852
−8.00	−28.00	20.00	20.3673	1.0206	20.5761
−8.00	−90.00	82.00	86.2785	1.0901	90.1099
II					
−14.00	−8.00	−6.00	-6.0668	0.9940	−6.0484
−28.00	−8.00	−20.00	−20.3673	0.9798	−20.1613
−90.00	−8.00	−82.00	-86.2785	0.9173	−82.6613

[25] lnα values of the examples presented in Table 8.4 (case I and case II (reversed case I) are identical except for the algebraic sign, although the α values of case I and case II are different. According to the 2nd law of logarithms: $\ln(x/y) = \ln(x) - \ln(y) = -[\ln(y) - \ln(x)] = -\ln(y/x)$.

$\alpha^{13}C$ and $\varepsilon^{13}C$ are depending on which mode of designation for isotope fractionation is used. $\alpha_{kin}^{13}C$ values >1 (case I) deliver positive values for $\Delta^{13}C$, $\ln\alpha^{13}C$ and ε^3C indicating that the products are depleted in ^{13}C, whereas $\alpha_{kin}^{13}C$ values <1 (case II) lead to negative values for $\Delta^{13}C$, $\ln\alpha^{13}C$ and $\varepsilon^{13}C$ showing an ^{13}C enrichment of the products.

In special cases, a combined use of the above described quantities can be applied in scientific literature: For instance, a simple subtraction (Δ) of different ε^2H values was recently used to compare the different hydrogen isotopic fractionations occurring during the biosynthesis of *n*-alkanes (i.e. ^2H-ε_{bio} quantifying the ^2H isotope fractionation between leaf water and synthesized leaf compounds) in plants growing under different environmental regimes implying different physiological plant metabolism (Cormier et al. 2018). In that context, this was expressed as the Δ^2H-ε_{bio} of *n*-alkanes between heterotrophic and autotrophic metabolisms, but the concept could be used to estimate other differences occurring in other complex isotope systems (e.g. systems encompassing many biochemical pathways).

8.5.2 Example for Equilibrium Isotope Effects

Reversible reactions aim at approaching a chemical equilibrium with forward and backward reactions then proceeding simultaneously at the same rate (principle of Le Chatelier). The concentrations of all reaction partners at this 'dynamic' equilibrium will be constant without a net change. The equilibrium constant of a reversible chemical reaction is related to the ratio of the stoichiometrically balanced product concentrations to reactant concentrations at equilibrium. In case of CO_2 hydration and HCO_3^- dehydration in water:

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \tag{8.18}$$
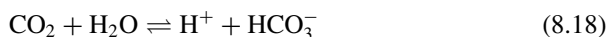
the equilibrium constant will be:

$$K_{eq} = \frac{\left[HCO_3^-\right]\left[H^+\right]}{\left[CO_2\right]} \tag{8.19}$$

Adding of reactant(s) will increase the forward reaction, by adding product(s) the backward reaction will be strengthened. A change of temperature will change the equilibrium concentration of reactants and products and correspondingly the equilibrium constant. The reversible reactions tend to approach both a chemical and an isotopic equilibrium (see Schimerlik et al. 1975; Rishavy and Cleland 1999).[26] Isotope exchange reactions are reversible reactions involving a redistribution of

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[26] Schimerlik et al. (1975) observed that a reversible system at chemical but not "isotopic equilibrium" is perturbed away from the (chemical) equilibrium after starting the reaction by adding the enzyme. Finally, a new equilibrium was achieved where both conditions are met. Consequently, a

isotopes between the reactants and products by substituting lighter isotopes in a chemical compound with a heavier one and vice versa. As an example the carbon isotope exchange between CO_2 and HCO_3^- will be discussed [23]:

$$^{13}CO_2(g) + H^{12}CO_3^-(aq) \rightleftharpoons {}^{12}CO_2(g) + H^{13}CO_3^-(aq) \tag{8.20}$$
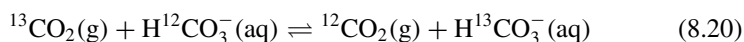
The equilibrium constant for that reaction is then:

$$K = \frac{[^{12}CO_2][H^{13}CO_3^-]}{[^{13}CO_2][H^{12}CO_3^-]} \tag{8.21}$$

The corresponding isotope fractionation factor $\alpha_{eq}{}^{13}C$ for the HCO_3^- / CO_2 system can be calculated according to Eq. 8.13:

$$\alpha^{13}C_{HCO_3^-/CO_2} = \frac{^{13}R_{HCO_3^-}}{^{13}R_{CO_2}} \tag{8.22}$$

The K values for the equilibrium constant are temperature dependent (Le Chatelier's principle). In addition, the EIE values for the isotope exchange reaction are temperature dependent and can be theoretically calculated using statistical mechanical methods on the basis of spectroscopic data (Urey and Greiff 1935; Urey 1947, Bigeleisen and Goeppert Mayer 1947). The K value for the equilibrium reaction between carbon dioxide and bicarbonate for example has been determined to be 1.0093 at 0 °C and 1.0068 at 30 °C (Friedman and O'Neil 1977). As a consequence, at 30 °C the bicarbonate is only by 6.8 mUr enriched in ^{13}C relative to the CO_2. In contrast, the ^{13}C equilibrium enrichment of bicarbonate at 0 °C is 9.3 mUr. Raising the temperature 'diminishes' the extent of ^{13}C enrichment by favoring the reaction towards the reactant(s) of the reaction because the (endothermic) back reaction is absorbing heat. As a rule of thumb one should keep in mind that the heavier isotopes prefer to concentrate in the more constrained chemical system with more and stronger chemical bonds (O'Leary 1978; O'Leary et al. 1992). In physical phase separations the lighter isotope is more 'volatile' with the effect that the heavier isotope will concentrate in the more condensed phase.

Both the oxygen isotope exchange reaction between H_2O and CO_2 and the carbon isotope exchange reaction between CO_2 and HCO_3^- explain this very well. Oxygen in CO_2 is bonded with two double bonds in contrast to two single bonds in H_2O (Fig. 8.10). The carbon atom in HCO_3^- is connected to three oxygen atoms with double and single bonds in contrast to two double bonds between C and O in CO_2 (Fig. 8.10). This leads to the ^{13}C enrichment of the bicarbonate ion relative to CO_2 and the ^{18}O enrichment of CO_2 relative to H_2O under equilibrium conditions. Equilibrium isotope effects are temperature-dependent; the resulting isotope fractionation normally decreases with increasing temperature. Oxygen isotope exchange of

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system at isotopic equilibrium implies that the system is also at chemical equilibrium, but more time is needed to reach isotopic equilibrium (Rishavy and Cleland 1999).

Fig. 8.10 Structural formulas of H_2O, CO_2 and HCO_3^-

$$H_2O \qquad CO_2 \qquad HCO_3^-$$
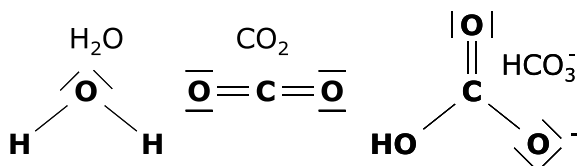
oxygen atoms in carbonyl groups of aldehydes and ketones with surrounding water is occurring under physiological conditions (c.f. Schmidt et al. 2001) during cellulose biosynthesis. This reversible hydration of carbonyl compounds easily establishes an equilibrium oxygen isotope enrichment of the oxygen function in the $C = O$ group relative to the oxygen in water via a *gem*-diol (HO-CR$_2$-OH) intermediate. This isotope exchange reaction of intermediates leads to the well-known ^{18}O enrichment of cellulose relative to the water present during biosynthesis of glucose/ cellulose by $+27 \pm 3$ mUr (Sternberg et al. 1986). Oxygen isotope analysis of cellulose in tree rings allows to infer climatic conditions during biosynthesis of cellulose in the trees via the oxygen isotopic composition of (leaf) water as long as the extent of ^{18}O enrichment is constant under physiological conditions.

8.5.3 Example for Kinetic Isotope Effects

Irreversible unidirectional reactions are reactions where only the forward reaction takes place without the possibility of a backward reaction. An irreversible reaction is characterized by a reaction rate that corresponds to the velocity of the conversion of reactant(s) to product(s). The reaction rate is directly proportional to the concentrations of the reactant(s) via the rate constant k. This rate constant k is related to the Gibbs free energy. Substituting the bond partners of a chemical bond with heavier isotopes will decrease the rate constant for the heavier isotopocule via the Gibbs free energy of the molecule. The KIE (kinetic isotope effect) of a reaction is defined as the ratio of the rate constants of the corresponding irreversible reaction according to Coplen (2011), exemplarily shown for ^{13}C in Eq. (8.7).

A kinetic isotope effect occurs when the rate of an irreversible chemical reaction is sensitive to the atomic mass. An example for a unidirectional reaction with kinetic isotope effect is the pyruvate dehydrogenase (PDH) reaction.

Pyruvate is decarboxylated to acetyl-CoA with gain of NADH. In plants the produced CO_2 can be either re-assimilated (during the day) or is respired (during night). The PDH catalyzed reaction is regulating an important interface between plant catabolic and anabolic metabolism (Tovar-Méndez et al. 2003) and PDH is a key enzyme in plant (dark) respiration (Werner et al. 2011). During the reaction the bond between C-1 and C-2 of pyruvate (Fig. 8.11) is cleaved. Melzer and Schmidt (1987) measured the KIEs on the PDH catalyzed reaction (PDH from *S. cerevisiae*) for all three carbon atoms of pyruvate; namely primary ^{13}KIEs on C-1 and C-2 and a secondary ^{13}KIE on C-3 of pyruvate.
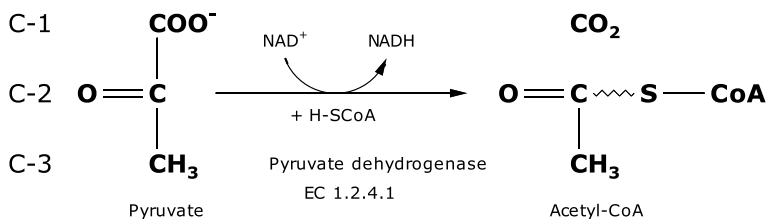
Fig. 8.11 Pyruvate dehydrogenase catalyzes the oxidative decarboxylation of pyruvate under production of acetyl-CoA and NADH. This reaction takes place in plant mitochondria and chloroplasts and is irreversible under physiological conditions

$$^{13}\text{KIE}_{C-1} = 1.0238 \pm 0.0013 \tag{8.23a}$$

$$^{13}\text{KIE}_{C-2} = 1.0254 \pm 0.0016 \tag{8.23b}$$

$$^{13}\text{KIE}_{C-3} = 1.0031 \pm 0.0003 \tag{8.23c}$$

The primary ^{13}KIEs on C-1 (1.0238) and C-2 (1.0254) of pyruvate are of a comparable dimension, whereas the ^{13}KIE on C-3 (1.0031) is one size smaller, typical for a secondary KIE on carbon atoms not involved in the bond cleavage (Melzer and Schmidt 1987). In case of a non-quantitative conversion of pyruvate to acetyl-CoA the C-1 and C-2 of pyruvate will be strongly enriched in ^{13}C. The produced acetyl-CoA will be depleted in ^{13}C leading to the prominent ^{13}C depletion of acetogenic lipids relative to their precursor glucose (see cited literature in Hayes 2001 and Hobbie and Werner 2004). Acetyl-CoA is ^{13}C depleted in C-1 (former C-2 of pyruvate) and enriched in ^{13}C in C-2 (former C-3 of pyruvate) relative to the ^{13}C/^{12}C ratio of the whole acetyl-CoA molecule. This alternating ^{13}C pattern can be again found in the ^{13}C pattern of fatty acids biosynthesized from acetyl-CoA units. The odd-numbered carbon positions in fatty acids (originating from C-1 of acetyl-CoA) are depleted in ^{13}C. In contrast, the even-numbered carbon positions (methyl position of acetyl-CoA acts as precursor) are enriched in ^{13}C relative to glucose (c.f. Hayes 2001).

The extent of isotope fractionation in natural systems is usually decreasing with increasing temperatures; approaching zero at very high (geological) temperatures (Hoefs 2018). Also heavy atom isotope effects per se tend to decrease with higher temperature (O'Leary 1978). Living organic systems are adapted to an environment with limited temperature and pH range ("physiological" temperature and pH values) that allows enzymes in reaction networks to function. As a consequence isotope effects in living organic systems are largely invariant with temperature (O'Leary 1978). For instance, the 27 ± 3 mUr ^{18}O enrichment of cellulose relative to water during its biosynthesis appears constant in the range of temperatures where trees can grow. However, the resulting isotope fractionation in organisms may nevertheless show some temperature effects. This can be the case because pool sizes, flow

rates, and enzymatic activities in these reaction networks may depend on temperature (O'Leary et al. 1992). Moreover, the environmental sources of reactants in these reaction networks (e.g. environmental water) may also be affected by temperature (e.g. the temperature of precipitation in rain and snow events will affect the isotopic composition of the water available to plants). As such, although isotope effects in living organic systems are largely invariant with temperature, a temperature signal may yet be recorded in the isotopic composition of organic material and used, if disentangled from other signals, to reconstruct temperatures at the moment of production.

8.5.4 Connection of EIE and KIE

Reversible chemical reactions approach chemical and isotopic equilibrium. The two involved (forward and backward) reactions can be associated with kinetic isotope effects (e.g. Jones 2003). If the reaction system is in chemical and isotopic equilibrium the fluxes of the forward and backward reactions are equal. As an example the hydration reaction of CO_2 and the dehydration of HCO_3^- is shown (Eq. (8.18)). Marlier and O'Leary (1984) measured the KIEs for the CO_2 hydration (forward) and the HCO_3^- dehydration (backward reaction) at 24 °C under conditions that secure the irreversibility of both reactions. The corresponding equilibrium isotope effect was calculated ($^{13}\text{EIE}_{HCO_3^-/CO_2} = {}^{13}\text{KIE}_{HCO_3^-/CO_2} / {}^{13}\text{KIE}_{/CO_2/HCO_3^-}$) and fits very well to experimentally determined ^{13}EIE values (Mook et al. 1974; c.f. Friedman and O'Neil 1977).

$$^{13}\text{KIE}_{CO_2/HCO_3^-} = 1.0069 \pm 0.0003 \tag{8.24a}$$

$$^{13}\text{KIE}_{HCO_3^-/CO_2} = 1.0147 \pm 0.0007 \tag{8.24b}$$

$$^{13}\text{EIE}_{HCO_3^-/CO_2} = 1.0077 \pm 0.0008 \tag{8.24c}$$

According to O'Leary et al. (1992) the equilibrium isotope fractionation can be calculated from the two kinetic isotope fractionations (on forward and backward reactions) via:

$$\Delta^{13}\text{C}_{(A/B)} = \Delta^{13}\text{C}_{(A \to B)} - \Delta^{13}\text{C}_{(B \to A)} \tag{8.25}$$

but compare with Table 8.4 and related text.

EIE reactions are sensitive to temperature but KIE reactions are not (at least not with the same level of "sensitivity"). These two facts are interconnected versus the Le Chatelier principle. The size of the equilibrium constant will change when the temperature is changing. This has as consequence that the ratio of the fluxes

of forward and backward reaction is changing but the values of the kinetic isotope effects on forward and backward reaction will not be influenced to the same extent by the temperature itself.

8.6 Conclusion

The measurement of stable isotope ratios of the bioelements is a technique used in many different scientific areas, and not all communities seem to use the same set of parameters and equations. While following the terminological recommendations by Coplen (2011), the present manuscript explains the basic principles and concepts underlying the use of stable isotopes in many diverse fields of study. It strongly suggests to thoroughly check the exact definitions and equations to derive specific parameters, e.g. to express isotope fractionation. Authors are encouraged to carefully select corresponding suitable parameters (cf. Table 8.4) and to use proper terminology and notation. They are also advised to provide a traceable derivation (e.g. in form of equations) in their texts to avoid confusion when reporting results of stable isotope measurements. This clarification should foster and grow the understanding and interpretation of stable isotope data, also across discipline borders.

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