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Stable isotope fractionation concepts for characterizing biotransformation of organohalides

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Recent development allows multi-element compound-specific isotope analysis of D, ^{13}C, ^{37}Cl and ^{81}Br for characterizing the degradation pathways of halogenated organic substances in the environment. The apparent kinetic isotope effect (AKIE) obtained in (bio)degradation experiments yields information on the chemical mechanism of the bond cleavage. In biochemical reactions, rate limitation such as uptake into cells, substrate transport in cells and binding to enzymes as well as equilibrium isotope effects (EIE) are modifying the observed isotope fractionation associated with the bond cleavage reactions, thus, complicating mechanistic interpretation of isotope effect. One way for improved analysis of bond cleavage reactions by isotope effects reactions is the combination of isotope effects from two or more elements forming the reactive organic moieties where bond change reaction takes place as indicator. A further option is the combination of enantiomeric with isotope fractionation as complementary indicator for enzymatic bond cleavage reaction governing biodegradation. Finally an interesting concept with large potential for elucidation of the biochemical reaction mechanisms of biological dehalogenation may be quantum mechanical/molecular mechanical (QM/MM) modelling in combination with experimental multi-element isotope analysis, thus merging quantum chemical theory with experimental observation.

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Introduction and definition of isotope effects

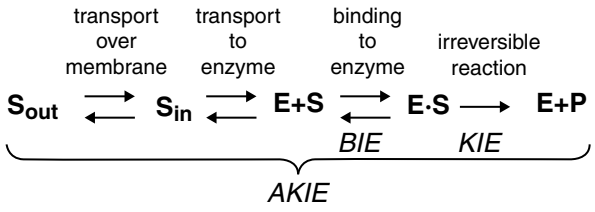
Stable isotope fractionation has been used to characterize the fate of organic contaminants in the environment, particularly halogenated organic chemicals, since more than one decade [1,2]. The ultimate goal of this approach is to interpret stable isotope fractionation patterns to understand degradation of organohalides. The concept for applying 'compound-specific stable isotope analysis' (CSIA) for characterizing degradation in the environment has been subject of several previous reviews [3,4] and recently summarized for the *in situ* degradation of hydrocarbons (Vogt *et al.*, current issue COB). For this work we summarize recent trends in employing isotope fractionation approaches for analyzing mechanisms of biological degradation reactions governing the fate of organohalides in the environment.

The theoretical background and definition of isotope effects in biological reactions have been recently summarized [5••]. The kinetic isotope effect (KIE) is based on the difference in kinetic rates during chemical bond change reaction of isotopologues in a unidirectional reaction, reflecting the relative stability of bonds formed by the heavy vs. light stable isotopes of an element. A primary kinetic isotope effect can be substantial in reactions when an isotopologue substituted chemical bond is cleaved or formed [6,7•]. A secondary kinetic isotope effect (SKIE) is observed when an adjacent isotopically substituted bond affects the rate of bond change in the rate-determining step of a reaction but is not directly involved in bond change [8]. SKIE tend to be much smaller than primary kinetic isotope effects. Larger SKIE effects are observed for hydrogen [9] and, more recently, for chlorine [10–12].

The concept for mechanistic interpretations of isotope effects in biological reactions has been established a long time ago making use of transition state theory and considerations for rate limitations in biological reactions [13]. The experimentally determined apparent kinetic isotope fractionation (AKIE) in biological system is considered to contain information of isotope sensitive mode of bond cleavage and all steps prior commitment of catalysis which need to be taken into account when interpreting isotope fractionation pattern (see Scheme 1).

In chemical homogeneous reactions the AKIE characterizes the mode of bond change in transition state providing full expression of the kinetic isotope effect (KIE) in many cases. In heterogeneous chemical reaction, such as reaction of mineral surfaces transport can become rate limiting to a significant extent lowering the AKIE compared to the expected KIE. In contrast, in complex enzymatic reactions the AKIE can be affected by rate limitation prior catalysis leading to masking of isotope effects, which can result in a reduction of the observed

Scheme 1



Simplified model of steps governing rate limitations in biological reactions such as uptake into the cell and transport to the enzyme, binding to the enzyme and catalysis and product formation (modified after Northrop [13]). S_{out} = substrate on the outside of the cell, S_{in} = substrate inside the cell. E = Enzyme, P = product. BIE = binding isotope effect, KIE = kinetic isotope effect, AKIE = apparent kinetic isotope effect.

isotope fractionation and the AKIE is smaller than the KIE [13].

Biological kinetic isotope fractionation has to be considered as a sum of processes which includes the discrimination of stable isotopes during a unidirectional reaction comprising bond cleavage at the irreversible step, and masking effects resulting from the rate limiting steps related to uptake into the cell, transport within the cell, binding to the enzyme (Scheme 1). In this simplified model of biological reactions the last step is the isotope sensitive bond change after which the reaction becomes irreversible (commitment to catalysis) and all rate limitations of previous reaction steps modify (mostly lower) the KIE of the bond change. However, biological reactions may not always be completely unidirectional in a strict sense [13] and backward reactions are possible to some extent favoring equilibrium isotope effects (EIEs). The EIE describes the equilibrium constants for isotopologues present in two phases as well as the reversible binding of a ligand to a receptor [5••]. EIEs are connected with any physical process or chemical reaction that reached equilibrium. EIEs could be relevant in reactions in which a ligand binds to the binding pocket of an enzyme and has then been termed binding isotope effects (BIE) [5••].

Today, the progress in quantum mechanical/molecular mechanical (QM/MM) modelling allows to model kinetic isotope effects of enzymatic catalysis at various levels of theory and this calculated isotope fractionation can be used for a quantitative interpretation of isotope effects during degradation of organohalides in biological systems. The comparison of experimental examined isotope effects with quantum chemical modelling of carbon, chlorine and hydrogen isotope effects thus offers a perspective for mechanistic interpretation of dehalogenation reaction in future studies [14,15]. Next steps for improvement of interpretation of isotope fractionation patterns for analyzing reaction mechanisms of dehalogenation reactions may be a combination of carbon, hydrogen and chlorine isotope fractionation and their evaluation by molecular modelling. QM/MM modelling may improve the interpretation of AKIEs of complex biological bond cleavage reactions as expected for the degradation of halogenated organic contaminants when using considerations from theoretical calculation for interpretation of experimental results.

Compound-specific stable isotope analysis for the evaluation of microbial reactions

Analytical techniques for determination of ^{2}H, ^{13}C, ^{37}Cl, ^{81}Br of organohalides

The progress in CSIA during the last decade allowed the evaluation of the origin and fate, particularly the *in situ* transformation pathways, of halogenated environmental contaminants [16–18]. Recent analytical developments for applying CSIA to Cl, Br and H isotopes of organohalides [19,20,21•,22–24] was subject of a recent review [25]. Whereas CSIA of carbon is routine for halogenated organics, methods for hydrogen, chlorine or bromine stable isotope analyses are recent and not routine yet. Determination of D/H isotope composition was recently made possible by the combination of pyrolysis with chromium reduction, preventing the formation of by-products containing hydrogen such as HCl, HBr, HCN, H_2S from heteroatom (N, Cl, Br, S) containing organics [21•,26]. Main bottleneck is the relatively high amount of analytes required. Several approaches to analyze chlorine stable isotope compositions were recently presented: one approach uses the direct analysis of the compound [19] requiring standards for each of the substances of interest with known isotope composition. Another, more universal, concept was presented where chlorinated compounds were pyrolyzed at high temperatures in an excess of hydrogen in the carrier gas to obtain HCl as analyte gas [20,27]. Though promising, due to instabilities and memory effects, this method cannot be applied routinely yet. The CSIA of bromine isotopes required an inductively coupled plasma (ICP)-multi collector-mass spectrometer (MC-MS) linked to a GC [23,24]. This technique is currently only sparsely applied, thus far, mainly due to its cost.

Understanding of isotope fractionation for evaluation of *in situ* biodegradation

The carbon isotope fractionation for a large variety of common halogenated groundwater contaminants was investigated in laboratory studies as a reference for the interpretation of isotope fractionation in field studies (for an overview see [28•]). By evaluation of the carbon isotope fractionation, the reductive and oxidative degradation of, for example, dichloroethene (DCE) could be distinguished even in complex model systems like constructed wetlands [29]. Providing knowledge of a groundwater flow path, hydrological conditions and a fractionation factor representing the mode of biodegradation of the microbial community, the extent of biodegradation may, furthermore, be estimated within an aquifer system [28•,30]. The use and