

Supporting materials

Heterogeneous distribution of natural zinc isotopes in mice

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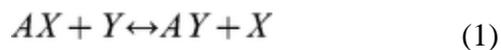
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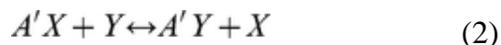
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Ab initio calculations:

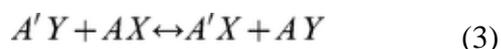
Isotopic exchange in chemical reactions can be represented by two half-reactions,



or



where A and A' are the heavy and light isotopes of the element A, and X and Y represent ligands. The difference between half-reactions 1 and 2 corresponds to a reaction of isotopic exchange between AX and AY:



The isotope separation factor α between AX and AY is defined as

$$\alpha = \frac{([A]/[A'])_Y}{([A]/[A'])_X} \quad (4)$$

where $([A]/[A'])_X$ and $([A]/[A'])_Y$ are the isotopic ratios A/A' measured in the complexes AX (and A'X) and AY (and A'Y), respectively. The isotope enrichment factor is defined as $\alpha_m - 1$. Since α is close to 1, $\alpha - 1$ can be approximated as $\ln \alpha$.

Deviations of isotopic ratios from a reference value in parts per 1000 are conventionally defined as

$$\delta = \left[\frac{([A]/[A'])_{species}}{([A]/[A'])_{reference}} - 1 \right] \times 1000 \quad (5)$$

If AX (and A'X) is the major component in the system, $\Sigma[A]/\Sigma[A']$ is approximated to be $([A]/[A'])_X$ such that an approximation expression $\delta \approx 10^3 \ln \alpha$ is suitable.

The standard theory of chemical isotope fractionation is based on mass-dependent isotopic differences in vibrational energies of isotopologues^{1,2}. The isotope enrichment factor is proportional to

$$\left(\frac{1}{m'} - \frac{1}{m} \right) \quad (6)$$

with m and m' the masses of the heavy isotope and the light isotope, respectively.

The isotope enrichment $\ln \alpha$ due to intramolecular vibrations can be evaluated from the reduced partition function ratio (RPFR) $\beta = (s/s')f$ defined as

$$\ln \frac{s}{s'} f = \ln \beta = \sum [\ln b(u_i') - \ln b(u_i)] \quad (7)$$

where the sum extends over all the molecular vibrational level with primed variables referring to the light isotopologue and

$$\ln b(u_i) = -\ln u_i + \frac{u_i}{2} + \ln(1 - e^{-u_i}) \quad (8)$$

In this equation, ν_i stands for vibrational frequencies, s for the symmetry number of the molecule, and $u_i = h\nu_i/kT$. The isotope enrichment factor due to the molecular vibration can be evaluated from the frequencies summed over all the different modes. The partition function ratio $(s/s')f$ for isotopologues A'X and AX (A'Y and AY, respectively) is noted β_X (β_Y , respectively). In the isotopic exchange reaction 3, isotope fractionation can be estimated from the relation $\ln \alpha \approx \ln \beta_Y - \ln \beta_X$. An adequate approximation of fractionation factors between different Zn species may be obtained by the conventional mass-dependent theory. All the calculations were made for the $^{66}\text{Zn}/^{64}\text{Zn}$ ratio.

In the present study, the optimized structures of Zn species were first determined for ^{64}Zn . The intramolecular vibrational frequencies ν_i were calculated for each complex. $\ln b(u_i')$ was determined by substituting ν_i into Eq. (7). Then ^{64}Zn was replaced by ^{66}Zn

and the vibrational frequencies were calculated again for the same molecular structures to obtain $\ln b(u_i)$, from which $\ln \beta$ was then determined.

Computational details

Orbital geometries and vibrational frequencies of Zn-cysteine and Zn-histidine species were computed using density functional theory (DFT) as implemented by the Gaussian09 code^{3,4}. The DFT method employed here is a hybrid density functional consisting of Becke's three-parameter non-local hybrid exchange potential (B3)⁵ with Lee-Yang and Parr (LYP)⁶ non-local functionals. In a quantum chemical study, the convergence of the reaction energies of Zn(II) species is excellent in 6-311+G(d,p) or higher basis sets⁷. Hence, the 6-311+G(d,p) basis set, which is an all-electron basis set, was chosen for H, C, N, O, S, and Zn. An "ultrafine" numerical integration grid was used and the SCF convergence criterion was set to 10^{-8} . Zn-binding modes of the two amino acids were reproduced from the literature⁸. Hydration and/or coordination of anions to unoccupied coordination positions of Zn^{2+} were neglected.

Table S1: Zn isotopic composition of the different mouse organs. RBCs: red blood cells; PLN: peripheral lymph nodes; MLN: mesenteric lymph nodes. Each sample has been analysed one time. The analytical uncertainty is evaluated from replicate analyses of the same sample processed through the full procedure and is $\pm 0.09\%$ (2σ) for $\delta^{66}\text{Zn}$ and 0.23% for $\delta^{68}\text{Zn}$ ³².

Table S2: Optimized structure Cartesian coordinates of Zn-cysteine and Zn-histidine species.

Figure S1: $\delta^{68}\text{Zn}$ vs $\delta^{66}\text{Zn}$ for all the mouse organs analysed. The slope of the correlation is 1.94 ± 0.06 , which correspond to mass-dependent isotopic fractionation.

Figure S2: Molecular structure of Zn-cysteine and Zn-histidine species. (a) $[\text{Zn-Cys-H}_1]^+$: H bound to S was substituted by Zn(II). (b) $[\text{Zn-Cys}]^{2+}$: H bound to S was substituted by Zn(II) and $-\text{NH}_2$ was protonated by the dissociated H^+ . (c) $[\text{Zn-His-H}_1]^+$: H bound to N in the imidazole group was substituted by Zn(II). (d)

[Zn-His]²⁺: H bound to N in the imidazole group was substituted by Zn(II) and another N in the group was protonated by the dissociated H⁺.

References

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