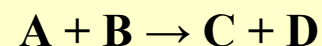


Chemistry BC3252: Special Topics

Kinetic Isotope Effects

A powerful tool used to investigate mechanisms in chemistry and biochemistry

1



Measure the rate constant k

Suppose molecule A contains an H atom, and we replace H with D

The ratio of the rate constants, k_H/k_D , is called the **kinetic isotope effect (KIE)**.

If the bond to the H atom is being broken, this is a **primary KIE**.



Typically, $k_H/k_D > 1$ (**normal KIE**). Why?

2

Origins of kinetic isotope effects

Forces between atoms are governed by electrons, so these are independent of isotope.

The potential energy function is isotope independent.

Picture an R-H bond:

Near the bottom of the well, nearly parabolic: $V = \frac{1}{2} a(r - r_0)^2$

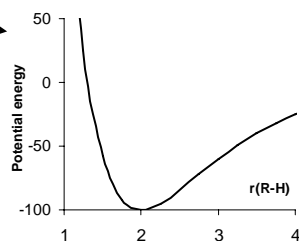
a is the harmonic force constant (using a to avoid confusion with rate constant)

The frequency of the vibration

$$\text{is } \nu = (a/\mu)^{1/2}/2\pi$$

where μ is the reduced mass.

$\mu = m_A m_B / (m_A + m_B)$. Note that if $m_A \gg m_B$, $\mu \approx m_B$.



3

Origins of kinetic isotope effects: quantized vibrational motion

According to quantum mechanics, vibrational energy is **quantized**:

It must have energy $E_{\text{vib}} = h\nu(v + \frac{1}{2})$ where v is an integer, $v = 0, 1, 2, \dots$

The minimum energy in vibration is $E(v=0) = \frac{1}{2}h\nu =$ **zero point energy**

For a typical R-H bond, $\nu = 7.8 \times 10^{13} \text{ sec}^{-1}$ (2600 cm^{-1})

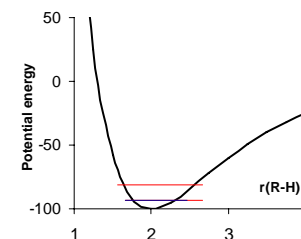
$$E_H(v=0) = \frac{1}{2}(6.6256 \times 10^{-34} \text{ J-s})(7.8 \times 10^{13} \text{ s}^{-1})$$

$$= 2.6 \times 10^{-20} \text{ J (per molecule)}$$

$$\text{times } N_A \rightarrow \text{ZPE}_H = 15.7 \text{ kJ/mole.}$$

$$\nu_D/\nu_H = (\mu_H/\mu_D)^{1/2} = (m_H/m_D)^{1/2} = 1/2^{1/2} = 1/1.414 = 0.707$$

$$\text{ZPE}_D = 11.1 \text{ kJ/mole.}$$



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Primary kinetic isotope effects

If we assume that the R–H bond in the transition state is **not** quantized (since it is breaking)
Then E_a for breaking an R–D bond is 4.6 kJ/mole greater than E_a for breaking R–H.

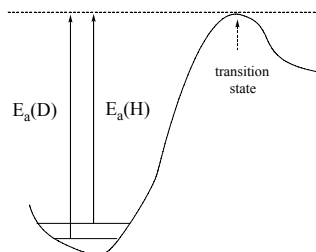
So $k_D < k_H$

If $k = A e^{-E_a/RT}$,

$$\begin{aligned} \ln(k^H/k^D) &= -(E_a^H - E_a^D)/RT \\ &= (4.6 \text{ kJ/mole})/(2.48 \text{ kJ/mole}) \\ &= 1.85 \end{aligned}$$

$$k_H/k_D = \exp[1.85] = 6.4$$

It is the zero-point energy (ZPE) of reactants that differs.



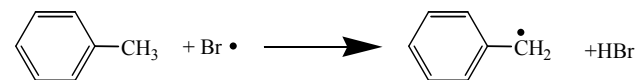
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Primary kinetic isotope effects

Predicted $k_H/k_D = 6.4$ at 25 C

Primary H/D KIE's of this magnitude are quite common.

Example:



$$k_H/k_D = 6.1$$

Tells us that the R–H bond is broken in the transition state.

Isn't this obvious, since it is ultimately broken? No..

It is possible that it might be broken after the transition state.

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Secondary kinetic isotope effects

k may differ even when the D/H substitution is **not** at the bond that breaks. However, it is usually quite close by.

Secondary KIE's are often called α and β , depending on whether the H is on the same atom or an adjacent one to the atom which is attached to the bond being cleaved.

The explanation is similar.

The isotopically substituted bond is not broken in the transition state, but **is altered**.

It is often weakened, but sometimes strengthened.

Again use ZPE arguments to interpret, but now consider ZPE of both reactants and the transition state.

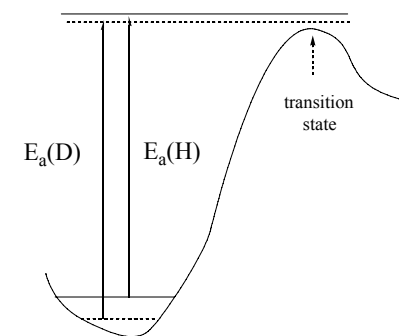
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Secondary kinetic isotope effects

If the isotopically substituted bond is **weaker in the transition state**, then the TS frequencies (ν_H and ν_D) are both less.

E_a^H is still less than E_a^D , but not by as much.

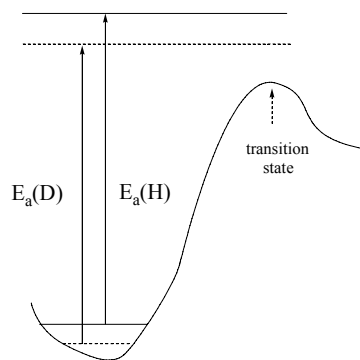
This gives a **normal KIE** (ratio greater than 1, but less than 6).



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Secondary kinetic isotope effects

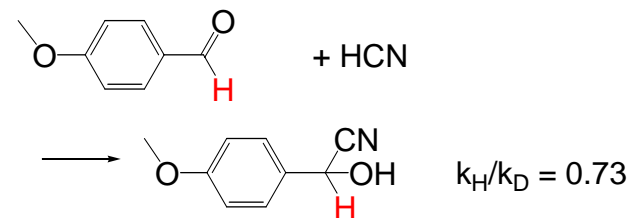
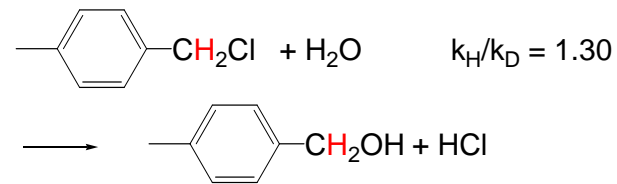
If the isotopically substituted bond is **stronger in the transition state**, then the TS frequencies (ν_H and ν_D) are both larger. E_a^H is now greater than E_a^D . This gives an **inverse KIE** (ratio < 1).



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Secondary kinetic isotope effects

Examples



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Inverse Primary KIE's too!

These arguments for secondary KIE's also apply to primary KIE's

If the bond being broken is only partially weakened in the transition state, then the D/H primary KIE's are less than ~6.

This is often observed.

Similarly, primary KIE's can also be inverse.

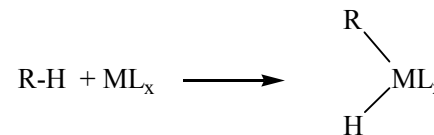
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Primary kinetic isotope effects

An important class of reactions of great interest today: **C-H bond activation** using metal catalysts.

A critical step in many important syntheses.

First step is typically **oxidative addition**:



With a number of different catalysts ML_x an **inverse** primary KIE is observed: $k_H/k_D \sim 0.7$

This suggests that in the transition state the H(D) atom is more strongly bound than in the reactants.

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Other isotopes

D/H kinetic isotope effects are the largest.

Nevertheless, there is much literature exploring KIE's of **N** (^{15}N vs. ^{14}N), **O** (^{18}O vs. ^{16}O), and **C** (^{13}C vs. ^{12}C).

With mass ratios much closer to 1, the effects are smaller.

Example: an O atom KIE in a reaction catalyzed by methane monooxygenase was measured as 1.0152 ± 0.0007 .

Rate constants are generally hard to measure accurately, so it is difficult to measure k 's *separately* with sufficient precision to get reliable results for ratios.

Use **competitive experiments** (k_{light} and k_{heavy} measured in the same reaction, often using **naturally abundant** isotopes).

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Two complications to this simple (?) story: (1) Kinetic Complexity

Consider the mechanism



If step 2 is slow (rate-limiting), then

$$v = d[\text{products}]/dt = k_2 K[A][B]$$

So $k_{\text{obs}} = k_2 K$.

If we measure an isotope effect on k_{obs} ,

is this the KIE for the rate determining step 2?

Can we use the observed KIE to learn about the transition state for step 2?

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Kinetic Complexity

$$v = d[\text{products}]/dt = k_2 K[A][B] \quad \text{so } k_{\text{obs}} = k_2 K_{\text{eq}}$$

Isotopes also affect **equilibrium constants**.

One must carefully sort these out.

If the equilibrium isotope effect on K_{eq} is properly taken into account, then we *can* find the KIE for step 2, and thus learn about its transition state.

There are examples in the literature where this may not have been taken into account properly

Jones (see references) discusses some examples.

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2) Quantum Mechanical Tunneling

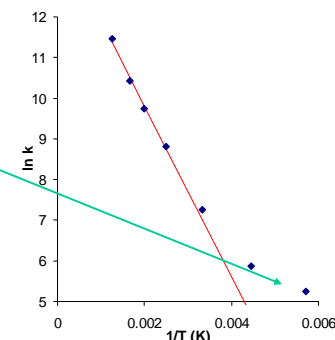
When some rate constants are measured down to very low temperatures, deviation from Arrhenius behavior at low T (large $1/T$) is often seen.

k does not fall as fast, but **levels off**.

Rate constants follow Arrhenius law due to the Boltzmann factor: the probability that a system at temperature T has energy E is proportional to $e^{-E/kT}$.

As T falls, fewer and fewer molecules have energy $E > E_a$ and can react.

But the data say otherwise..



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Tunneling: A curious fact from quantum mechanics

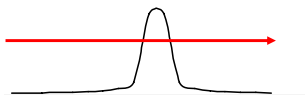
Quantum mechanics says that particles have wave-like properties, and waves do not have precise locations.

One weird consequence is that quantum mechanical particles are not confined like classical particles.

A classical particle with $E < E_a$ cannot pass over a barrier.

A quantum mechanical particle with $E < E_a$ has a finite (but small) probability of getting to the other side.

This is referred to as tunneling. The particle does not actually tunnel --- that is a classical concept --- but it is the language we use to express that fact that it got there.



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Tunneling

The tunneling probability depends on the **height** and **thickness** of the barrier, **and on the mass of the particle**.

H atoms are light enough that tunneling occurs fairly commonly (albeit slowly), but it is **much less likely** for D.

When the temperature is low enough that the thermally activated rate of passage over the barrier is sufficiently slow, tunneling effects emerge.

Tunneling rates are temperature independent.

Two quite different quantum mechanical factors contribute to D/H KIE's: **zero point energy effects** and **tunneling**.

Tunneling always favors H over D (normal KIE's), and is temperature independent.

ZPE KIE's can be normal or inverse, and depend on T.

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Biochemical example

Laboratory of **Judith Klinman** at **UC Berkeley**.

Her group has established that **tunneling plays a significant role in a number of enzymatic reactions**.

Studied KIE's for variety of enzymatic reactions.

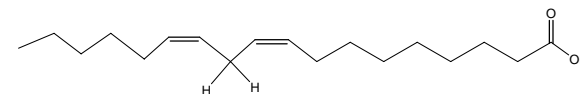
In some cases, they observed **primary D/H KIE's** for reactions involving R-H bond scission that are much greater than 6, suggesting a tunneling contribution.

By studying the temperature dependence of the KIE, they separated the T-dependent factor (ZPE's) from temperature independent (tunneling).

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Tunneling in Enzyme reactions Judith Klinman (UC Berkeley)

Example: Soybean lipoxygenase-1 (SLO-1) catalyzes hydroperoxidation of linoleic acid.



The primary H/D KIE for this reaction is $k_H/k_D \sim 55$.

They looked at mono- and di- D-substituted linoleic acid, carefully sorted out primary and secondary effects, and also did temperature studies.

It is a complicated system, and many different interpretations have been advanced. The kinetic evidence is pretty clear that tunneling is important.

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Selected references

- 1) "Isotope Effects in C–H Bond Activation Reactions by Transition Metals", W.D. Jones, *Acc. Chem. Res.* **36**, (2003) 140-146
- 2) "Oxygen Kinetic Isotope Effects in Soluble Methane Monooxygenase" S.S. Stahl, W.A. Francisco, M. Merckx, J.P. Klinman, and S.J. Lippard, *J. Biol. Chem.* **276** (2001) 4549-4553.
- 3) "Isotope Effects in the study of enzymatic phosphoryl transfer reactions" A.C. Hengge, *FEBS Letters* **501** (2001) 99-102.
- 4) "Enzyme Catalysis: Beyond Classical Paradigms", A. Kohen and J.P. Klinman, *Acc. Chem. Res.* **31**, (1998) 397-404