

MNM Questions for 3-30-9

“Kinetic Isotope Effects in Hydroxylation Reactions Effected by Cytochrome P450 Compounds I Implicate Multiple Electrophilic Oxidants for P450-Catalyzed Oxidations”

These questions, I hope, will help focus our discussion of the MNM paper for this week. It would help me if you (the students) looked through these questions and make a small effort in thinking about these so that we might have a discussion rather than just listening to me talk.

Questions are in no particular order. Representatives of particular labs may be called on to provide expertise on certain questions if the need arises.

1. Primary KIEs originate from the fact that in some reaction mechanisms, C-D bonds behave as if they are stronger than C-H bonds. A more precise analysis indicates that isotope effects manifest themselves when there is a difference in activation energies between isotopomers in the rate-determining step on a reaction coordinate. This is caused by changes in zero point energy (ZPE) between the reactant and the transition state (TS). Remember that activated complexes can have zero point energies too! Be able to draw a reaction coordinate with no isotope effect and one with a high isotope effect. Be specific about the ZPE of the TS.
2. Secondary KIEs are less satisfying to ones chemical intuition. But remember, it's all about differences in vibrational zero point energy between the reactant and the transition state, and the affect that isotope substitution has on each. Secondary KIEs typically involve changes in bond hybridization or hyperconjugation. The authors report a small normal secondary KIE ($k_H/k_D = 1.05$). What does this tell you about the geometry at the transition state for the putative methyl radical? (planer or tetrahedral?) If the secondary KIE was $k_H/k_D = 1.4$ (the theoretical maximum) what would that tell you? Would you expect to get racemic alcohol if the participating carbon was chiral? Does the “caged radical” hypothesis come into play here? (Kunze and Atkins Lab question)
3. The theoretical maximum for a primary KIE is $k_H/k_D \approx 6.5$. Researchers routinely measure KIEs higher than this value, and they often give tunneling as a mechanistic justification for the high KIE. Try not to worry about the fact that their primary KIE values are higher than 6.5. Remember that a baseball will behave like a wave if it approaches the speed of light, and hydrogen atoms are way way smaller than a baseball. (sorry no question here)
4. The authors do not report doing many control experiments. Typical P450 control experiments might include an anaerobic control without oxygen, or perhaps with CO saturation. Would you expect to see product formation using the authors' experimental methods with these controls? Or perhaps $^{18}\text{O}_2$ or ^{18}O -water. Would you see labeled oxygen in the product? (Rettie Lab question)

5. Peroxynitrite (PN) is a very deleterious endogenous agent of oxidative stress. Where does it come from and what is the mechanism for its formation? The authors site a previous synthetic route for PN. How does the synthetic PN generation differ from what happens in me when I have oxidative stress? (S. Nelson Lab question)

6. Can peptides undergo modification by peroxynitrite? What residues might be affected? Is there any previous work characterizing nitrated peptides? (Goodlett Lab question)

7. Do you think that the authors could have overlooked any inherent oxidative activity of Cpd II? Did they explicitly mention doing that control? Is it fishy that the authors do not state the % conversion of Cpd II \rightarrow Cpd I?

8. The authors calculate two constants, K_{bind} and k_{ox} from the rates of Compound I disappearance (correcting for the rate of Cpd I disappearance without substrate present). Is K_{bind} comparable with K_D ? K_M ? Would you expect the K_D and K_M to change between the isotopomers? (Atkins Lab question) What is k_{ox} ? (Kunze Lab question)

9. The authors state in the abstract:

“Large intermolecular isotope effects for [the $-\text{CH}_3$ and $-\text{CD}_3$ compounds] $k_{\text{H}}/k_{\text{D}} = 11.2$ and 9.8 for the two Compounds I contrast with small intermolecular KIEs obtained previously for the same substrate in P450-catalyzed oxidations; these differences suggest that a second electrophilic oxidant, presumably iron-complexed hydrogen peroxide, is important in cytochrome P450 oxidations under turnover conditions.”

Do you agree? If the overall rate limiting step in the oxidation of substrate occurs before hydrogen atom abstraction (e.g. second electron transfer), would you observe any isotope effect? What's all this about KIE masking?

10. Do you care what the active oxidant is in a P450 reaction? (We will vote and see if this is the last P450 mechanism paper I present for MNM.)