An Agostic Alternative to the P-450 Rebound Mechanism

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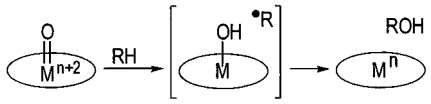
The catalytic oxygenation of hydrocarbons by the enzyme family, cytochromes P-450, is widely considered to proceed through a "rebound mechanism."[1] The overall rate-determining step in the enzyme cycle involves electron transfer eventually leading to the active oxidant, which is believed to be a high-valent iron oxo species.[2] In the subsequent, product-forming step, a hydrogen atom is thought to be extracted from the substrate C−H bond, forming a carbon radical that proceeds to the hydroxylated product in rapid successive electron-transfer and atom-transfer steps (Scheme 1). Considerable evidence has been accumulated which supports the existence of this putative carbon radical intermediate. For example, intramolecular isotope studies using substrates with CHD groups show primary isotope effects (k_H/k_D) of about 11.[3] Hydrocarbon hydroxylations exhibit C−H bond selectivities (tertiary > secondary > primary) which are similar to those of a radical reaction.[4] On the other hand, Newcomb has recently used very fast radical clocks to probe for this postulated radical intermediate;[5] his results are inconsistent with a long-lived free-radical intermediate in P-450-catalyzed hydroxylations. Further, P-450 and methane monooxygenase (MMO) hydroxylations show a regiochemical selectivity which differs dramatically from that of a *tert*-butoxyl radical.[6]

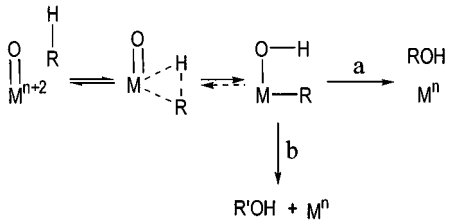
Newcomb's results prompted us to consider alternate mechanisms which are consistent with the experimental data. It is known that high-valent transition metal oxides are capable of reacting with dihydrogen, as demonstrated by the oxidation of H_2 by permanganate ion.[7] The kinetics of this reaction were reported to show clean second-order behavior.[8] This led us to speculate about the formation of an "agostic" substrate−catalyst complex[9] as a key intermediate, prior to the oxygen-transfer step.

We report here that hydrocarbon hydroxylation by models of cytochrome P-450 are inhibited by hydrogen and methane. Our proposed mechanism (Scheme 2) involves the reversible formation of such a "σ-complex" between the substrate and the high-valent iron oxo center. Because agostic interactions of this kind are known to increase the Brønsted acidity of the hydrogen,[9a] a logical second step would be an intramolecular acid−base reaction: the C−H bond could become polarized, prompting a proton to migrate to the basic oxo group, leaving the carbon σ-bonded to the metal.[10] This "insertion" would leave the oxidation state of the metal unchanged. Subsequent reductive elimination of the alkyl and hydroxyl groups would then lead to the hydroxylated product (path a). For some alkyls, the reductive elimination could be preceded by rearrangement to form a more stable alkyl complex, resulting in products similar to those seen with carbon radicals or cations (path b).

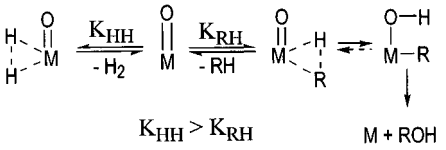
Scheme 1



Scheme 2



Scheme 3



The existence of these putative agostic complexes would have significant kinetic implications. Substrates which form strong agostic complexes should inhibit the oxidation of more weakly bound substrates by sequestering the active oxidant (Scheme 3). Furthermore, there should be inhibitors which complex strongly but are not oxygenated. Dihydrogen is known to form stronger agostic complexes than C−H bonds, even though the H−H bond strength is similar to that of the C−H bond in methane. This led us to examine the interaction between P-450 model complexes and H_2.

In the absence of a suitable substrate, the iron porphyrin very slowly catalyzes the oxidation of H_2 to H_2O. This pathway, however, is negligible on the time scale of our inhibition experiments. In the presence of hydrogen, cyclohexane oxidation is inhibited. This inhibition presumably stems from the formation of a complex between H_2 and the catalyst. The fraction of H_2−catalyst complex increases as a function of H_2 partial pressure, thus lowering the concentration of catalyst free to oxidize cyclohexane. In turn, this decreases the observed rates of cyclohexanol production. From these rates (Figure 1), we can estimate the pressure at which the rate would be half that of the uninhibited[11] reaction; for dihydrogen, this pressure is 1.4 atm at 0 °C. When the temperature is decreased, the extent of inhibition

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(11) The reference reaction consists of a solution that is 0.5 mM in (5,-10,15,20-tetrakis(pentafluorophenyl)porphyrinato)iron(III) chloride, 25 mM in pyridine, and 0.5 M in cyclohexane, with a 100:1 iodosylbenzene: catalyst ratio, under 1 atm of Ar. α,α,α-Trifluorotoluene and 1,2,4-trichlorobenzene are employed as the solvent and internal standard, respectively. The major product is cyclohexanol; traces of cyclohexanone are also detected. Substrate oxidation does not occur in the absence of catalyst.

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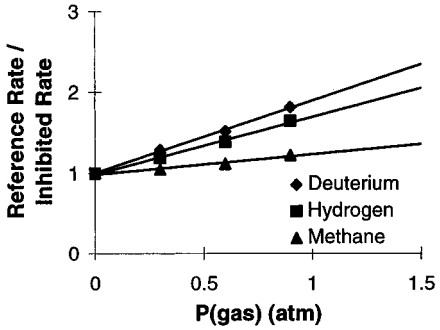

Figure 1. A plot of the rate of cyclohexanol formation versus the partial pressure of the inhibiting gas; rates are relative to that of a reference reaction under Ar.

increases. Upon removal of the H_2 from the reaction, the rate of cyclohexanol production returns to its original value, demonstrating that the inhibition is reversible. Interestingly, D_2 had a slightly stronger inhibitory effect than H_2, giving a $P_{1/2}(D_2)$ of 1.0 atm. This can be compared with the inverse isotope effect of 0.78 reported for H_2/D_2 binding to a tungsten complex.[12]

For alkyl complexes, it is known that methyl groups bind more strongly than methylene groups. Because of this reason and the steric constraints imposed by the porphyrin, we chose methane as a hydrocarbon inhibitor. Methane does not react under the reaction conditions employed, but it does inhibit cyclohexane oxygenation (though to a lesser extent than H_2): our data predict that 4.5 atm of methane should halve the rate of cyclohexanol production in our reference reaction.

The intramolecular proton transfer and reductive elimination steps follow logically from the initial agostic complex. Cis-disubstituted metalloporphyrins, such as would be required for the organometallic intermediate, are known.[13] Elimination of H_2O from the hydroxy/alkyl complex (Scheme 2) is a likely origin for carbene-derived byproducts. Reductive elimination would account for the many examples of retention of configuration observed during the oxygenation of hydrocarbons, not only by oxygenases[14] and their models,[4a] but also by simple high-valent metal oxides.[15]

We think it likely that the agostic interaction is occurring with the active high-valent metal oxo, since it can account for the observations mentioned above. Nonetheless, the possibility exists that catalyst is removed from the catalytic cycle by binding with the resting state Fe^{III} porphyrin. Complexation with the Fe^{III} would not be surprising, since coordination of an alkane to an Fe^{II} porphyrin has been reported.[16]

In summary, our present results are consistent with the reversible formation of an agostic complex between the substrate and a high-valent iron oxo group during the catalytic hydroxylation of hydrocarbons. This mechanism could accommodate the observed inhibition by H_2, D_2, and methane, in addition to the regioselectivity,[6] isotope effects,[17] and carbene-derived products. It is possible that some of the natural metal oxidases (both heme[18] and non-heme, *e.g.*, methane monooxygenase[19]) as well as several classic high-valent metal oxo reagents[20] and the Gif systems[9c] may involve a similar agostic mechanism. The present results are a platform from which many new experiments may be conducted.

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Supporting Information Available: Experimental details (2 pages). See any current masthead page for ordering and Internet access instructions.

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