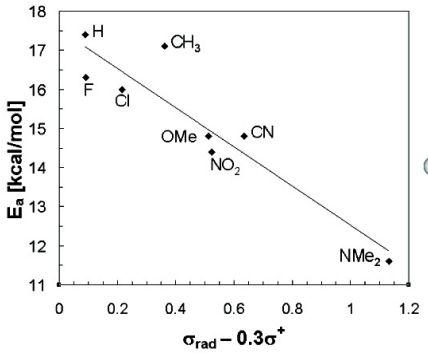
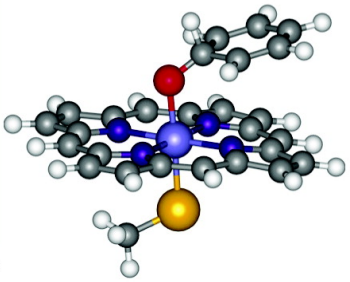
Communication

Aromatic Hydroxylation by Cytochrome P450: Model Calculations of Mechanism and Substituent Effects

Christine M. Bathelt, Lars Ridder, Adrian J. Mulholland, and Jeremy N. Harvey

J. Am. Chem. Soc., **2003**, 125 (49), 15004-15005• DOI: 10.1021/ja035590q • Publication Date (Web): 15 November 2003





More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 11/15/2003

Aromatic Hydroxylation by Cytochrome P450: Model Calculations of Mechanism and Substituent Effects

Christine M. Bathelt, Lars Ridder,† Adrian J. Mulholland, and Jeremy N. Harvey*

School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK

Received April 11, 2003; E-mail: jeremy.harvey@bris.ac.uk

Hydroxylation by cytochrome P450 enzymes is one of the most important processes in drug metabolism.[1,2] A detailed understanding of its mechanism is vital for predicting biotransformations of pharmaceuticals and other xenobiotics, and hence for drug development. Following our previous studies of mechanisms and substituent effects in flavin-based aromatic hydroxylation,[3] we here consider P450 hydroxylation of aromatic compounds on the basis of accurate density functional theory (DFT).

The rate and selectivity of P450 substrate oxidation is determined by many factors, such as the nature of the rate-limiting step, docking-related steric effects[4] and intrinsic electronic reactivity.[5] There has been intense experimental[6] and theoretical[7] work on the mechanism of aliphatic P450-mediated hydroxylation, but aromatic hydroxylation is less well understood. The first step in the mechanism is addition of the active iron-oxo species ("compound I") to a substrate carbon to give a tetrahedral intermediate.[8] Subsequent rearrangement to the phenol product can proceed directly or via an epoxide. While aliphatic hydroxylation proceeds via radical intermediates, it is not clear whether the tetrahedral intermediate in aromatic hydroxylation has radical or cationic character. Previous semiempirical[9] and local density approximation (LDA)[10] calculations on the reaction mechanism provide only qualitative insight into these questions.

Our DFT computations[11] use the B3LYP functional, which gives accurate results in studies of aliphatic P450 reactivity,[7] as well as for other porphyrin[12] and bioinorganic systems.[13] We address first the reaction pathway for hydroxylation of benzene itself. Compound I has several close-lying electronic states,[14] with the ground state a quasi-degenerate pair of triradicaloid states, labeled $^2A_{2u}$ and $^4A_{2u}$, which differ only in the spin coupling of their three unpaired electrons, two of which reside on the iron-oxo moiety, the other residing in a porphyrin π orbital. We optimized the geometry of the model system at a set of fixed O−C distances between benzene and the oxygen atom of compound I (in different electronic states). The lowest-energy pathway (3 kcal/mol below the next lowest), was found to be an electrophilic addition leading from the $^2A_{2u}$ state of compound I to a purely cationic tetrahedral intermediate (Figure 1). The corresponding transition state was then optimized and confirmed by frequency analysis.

The energy profile for this addition is shown in Figure 1, which also illustrates the model system used (neutral $FeC_{27}N_4H_{21}O$ in the benzene case), with the protein cysteinato side chain described by a methyl mercaptide group, and the porphyrin devoid of side chains. During addition, two electrons of the substrate are transferred into two singly occupied orbitals of compound I, the porphyrin a_{2u} orbital and the Π_{xz}* orbital on the FeO moiety. Charge and spin density analysis demonstrate a wholly cationic nature for the final tetrahedral adduct, but the electronic structure of the transition state is of a mixed cationic and radical nature, with considerable spin

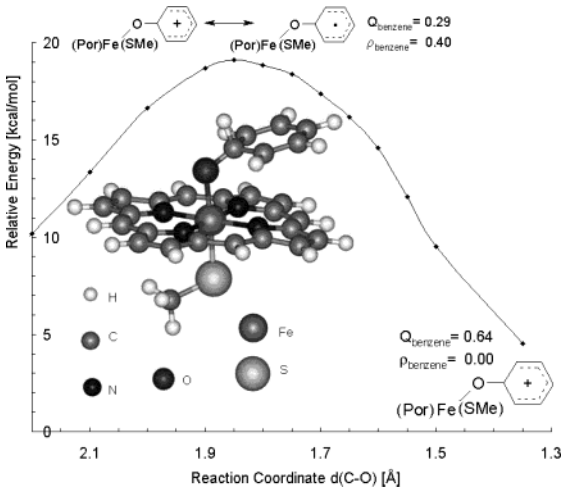


Figure 1. UB3LYP/BS I[11] energy profile for the addition of compound I to benzene, showing the model system, the transition-state structure and product structure. Energies are relative to the separated reactants. Group charge densities (Q) and spin densities (ρ) for benzene are shown.

and charge transferred to the substrate. The activation energies presented here are similar to those computed for the epoxidation of ethene[7c] and slightly lower than for hydroxylation of C−H bonds[7a,b,e] in alkanes.

We also considered further reactivity by locating transition states for rearrangement of the tetrahedral adduct to a complex of the ferric P450 system with benzene epoxide or the ketone tautomer of phenol (this is the "NIH shift"[15] product). These very low barriers to product formation lie only 8.0 and 2.4 kcal/mol above the tetrahedral intermediate, respectively, so that C−O bond formation between compound I and the substrate is predicted to be the rate- and selectivity-determining step.

We next addressed substituent effects by considering first addition to the meta and para positions of a series of monosubstituted aromatics. For some of these substrates, the mechanism studied here may not be metabolically significant as P450 oxidation may have a different rate-limiting step or follow a different route (e.g., side-chain oxidation). However, the present preliminary set of compounds does span a very broad range of electronic properties and thereby provides useful insight. The lowest route again goes through a transition state with mixed radical/electrophilic properties and leads to cationic adducts, although in the case of the electron-withdrawing substituents, a radical-like state of the adduct lies slightly lower in energy than the cationic one. Whereas the barrier heights with substituents in the meta position are all very similar to that in benzene, both electron-withdrawing and -donating groups decrease the barrier height for addition in the para position.

Finding correlations between our computed energy barriers and known substituent properties[16] is a first step toward developing a quantitative structure−activity relationship.[17] Despite the radical-

† Current address: Molecular Design & Informatics, N.V. Organon, P.O. Box 20, 5430 Oss, The Netherlands.

10.1021/ja035590q CCC: $25.00

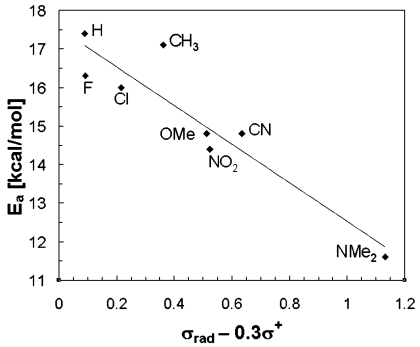


Figure 2. UB3LYP/BS II[11] activation energies for para addition to monosubstituted benzenes, versus a combination of the substituent radical (σ_{rad}) and cationic (σ^+) Hammett parameters.

like pattern of behavior found above, the purely radical[18] Hammett σ-scale does not give a good linear correlation with the computed barrier heights. Instead, the dual radical and cationic nature of the transition states computed here suggests that a combination of radical and cationic[19] σ-scales would provide a better correlation. The best such equation that we have found so far ($R^2 = 0.86$), $E_a = 17.53-5.01(\sigma_{rad} - 0.3\sigma^+)$ kcal/mol, is shown in Figure 2. This also agrees with the small meta substituent effects, as σ parameters are small for meta substituents.

Gas-phase barrier heights provide only indirect information on enzyme reactivity, which can be affected by steric and electrostatic effects. To assess the latter, single-point energies were computed for several substrates and electronic states using an electrostatic continuum model ($\epsilon = 4.0$, probe radius 2.60 Å). This raises the barriers for C−O bond formation by 4−5 kcal/mol without affecting their relative ordering and also lowers the energy of the cationic tetrahedral intermediates relative to alternative radical-like electronic states.

There is little systematic experimental data for the kinetics of hydroxylation of aromatic compounds catalyzed by cytochrome P450 enzymes. Burka et al.[20] investigated the hydroxylation kinetics in microsomes for monohalobenzenes and found a correlation of the overall reaction rates of this narrow range of substrates with the σ^+ Hammett constant. Rietjens et al.[21] have presented reactivity and regioselectivity data for a set of fluorobenzenes and anilines. Safari et al. studied oxidation of several aromatics by a P450-model system,[22] and found an experimental trend very similar to that described here, i.e., both electron-withdrawing and -donating groups increase reactivity.

On the computational side, Jones et al.[23] used the methoxy radical as a model for compound I to study the barrier for addition to various aromatics at the AM1 semiempirical level and found a linear correlation between activation energy and heat of addition, a trend not reproduced by our results. Rietjens et al. have shown that reactivity and selectivity in arene hydroxylation of polyhalogenated substrates can be correlated with the properties of the highest occupied orbitals of the substrates.[24] This correlation study, however, did not include substrates with more typical electron-withdrawing substituents and does not predict the enhanced reactivity for the latter found in our calculations.

In contrast, the explicit DFT calculation of barrier heights shows that the intrinsic electronic contribution to selectivity and reactivity in arene oxidation by the compound I intermediate of P450 enzymes can be reasonably well reproduced by a dual parameter equation based on radical and cationic Hammett σ parameters. This mechanistic insight[25] and new structure−activity relation should be of great value to researchers interested in this crucial area of bioinorganic oxidation.

Acknowledgment. We thank the EU (support to C.B. and J.N.H. through the MCInet TMR network, and to L.R. through a Marie Curie Fellowship), the Royal Society, the EPSRC (Advanced Research Fellowship to A.J.M.), and the Wolfson Foundation.

Supporting Information Available: Computational details, additional calculated energies, and list of Cartesian coordinates and total energies for all species (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Guengerich, F. P. *Chem. Res. Toxicol*. **2001**, *14*, 611−650.
(2) Anzenbacher, P.; Anzenbacherova, E. *Cell. Mol. Life Sci*. **2001**, *58*, 737.
(3) Ridder, L.; Harvey, J. N.; Rietjens, I. M. C. M.; Vervoort, J.; Mulholland, A. J. *J. Phys. Chem. B* **2003**, *107*, 2118−2126 and references therein.
(4) See e.g. Bathelt, C.; Schmid, R. D.; Pleiss, J. *J. Mol. Model.* **2002**, *8*, 327.
(5) White, R. E.; McCarthy, M.; Egeberg, K. D.; Sligar, S. G. *Arch. Biochem. Biophys.* **1984**, *228*, 493−502.
(6) (a) Newcomb, M.; Hollenberg P. F.; Coon, M. J. *Arch. Biochem. Biophys.* **2003**, *409*, 72−79. (b) Manchester, J. I.; Dinnocenzo, J. P.; Higgins, L. A.; Jones, J. P. *J. Am. Chem. Soc.* **1997**, *119*, 5069−5070.
(7) (a) Ogliaro, F.; Harris, N.; Cohen, S.; Filatov, M.; de Visser, S. P.; Shaik, S. *J. Am. Chem. Soc*. **2000**, *122*, 8977−8989. (b) Yoshizawa, K.; Kamachi, T.; Shiota, Y. *J. Am. Chem. Soc*. **2001**, *123*, 9806−9816. (c) de Visser, S. P.; Ogliaro, F.; Harris, N.; Shaik, S. *J. Am. Chem. Soc.* **2001**, *123*, 3037−3047. (d) de Visser, S. P.; Ogliaro, F.; Sharma, P. K.; Shaik, S. *J. Am. Chem. Soc*. **2002**, *124*, 11809−11826. (e) Kamachi, T.; Yoshizawa, K. *J. Am. Chem. Soc*. **2003**, *125*, 4652−4661. (f) Guallar, V.; Baik, M.-H.; Lippard, S. J.; Friesner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6998.
(8) Korzekwa, K. R.; Swinney, D. C.; Trager, W. F. *Biochemistry* **1989**, *28*, 9019−9027.
(9) Korzekwa, K.; Trager, W.; Gouterman, M.; Spangler, D.; Loew, G. H. *J. Am. Chem. Soc*. **1985**, *107*, 4273−4279.
(10) (a) Zakharieva, O.; Grodzicki, M.; Trautwein, A. X.; Veeger, C.; Rietjens, I. M. C. M. *J. Biol. Inorg. Chem*. **1996**, *1*, 192−204. (b) Zakharieva, O.; Grodzicki, M.; Trautwein, A. X.; Veeger, C.; Rietjens, I. M. C. M. *Biophys. Chem*. **1998**, *73*, 189−203.
(11) Calculations were carried out with the Jaguar 4.2 software (Schrödinger, Inc., Portland, OR, 1991−2002) using the unrestricted hybrid density functional UB3LYP. Geometry optimizations were performed using the standard Los Alamos effective core potential and associated triple-ζ basis set on Fe (LACV3P basis), and the 6-31G* basis on all other atoms (BS I). Energies in Figure 2 are based on single-point calculations at optimized geometries performed with the same LACV3P basis on Fe, but the larger 6-311+G** basis on other atoms (BS II).
(12) Harvey, J. N. *J. Am. Chem. Soc*. **2000**, *122*, 12401−12402.
(13) Siegbahn, P. E. M. *Q. Rev. Biophys.* **2003**, *36*, 91−145. Siegbahn, P. E. M.; Blomberg M. R. A. *Chem. Rev.* **2000**, *100*, 421−437.
(14) (a) Harris, L. H.; Loew, G. H. *J. Am. Chem. Soc*. **1998**, *120*, 8941−8948. (b) Shaik, S.; de Visser, S. P.; Ogliaro, F.; Schwarz, H.; Schröder, D. *Curr. Opin. Chem. Biol*. **2002**, *6*, 556−567.
(15) (a) Guroff, G.; Daly, J. W.; Jerina, D. M.; Renson, J.; Witcop, B.; Udenfriend, S. *Science* **1967**, *157*, 1524−1530. (b) Koerts, J.; Soffers, A. E. M. F.; Vervoort, J.; De Jager, A.; Rietjens, I. M. C. M. *Chem. Res. Toxicol.* **1998**, *11*, 503−512.
(16) Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev*. **1991**, *91*, 165−195.
(17) Hansch, C.; Gao, H. *Chem. Rev*. **1997**, *97*, 2995−3059.
(18) Dinctuerk, S.; Jackson, R. A. *J. Chem. Soc., Perkin Trans. 2* **1981**, 1127−1131.
(19) Taylor, R. *Electrophilic aromatic substitution*; Wiley: Chichester, 1990; pp 458−463.
(20) Burka, L. T.; Plucinski, T. M.; MacDonald, T. L. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6680−6684.
(21) (a) Rietjens, I. M. C. M.; Soffers, A. E. M. F.; Veeger, C.; Vervoort, J. *Biochemistry* **1993**, *32*, 4801−4812. (b) Cnubben, N. H. P.; Peelen, S.; Borst, J.-W.; Vervoort, J.; Veeger, C.; Rietjens, I. M. C. M. *Chem. Res. Toxicol*. **1994**, *7*, 590−598.
(22) Safari, N.; Bahadoran F.; Mohammed, R. H.; Ghiasi R. *J. Porphyrins Phthalocyanines* **2000**, *4*, 285−291.
(23) (a) Higgins, L. A.; Korzekwa, K. R.; Rao, S.; Shou, M.; Jones, J. P. *Arch. Biochem. Biophys*. **2001**, 385, 220−230. (b) Jones, J. P.; Mysinger, M.; Korzekwa, K. R. *Drug Metab. Dispos*. **2002**, *30*, 7−12.
(24) Soffers, A. E. M. F.; Boersma, M. G.; Vaes, W. H. J.; Vervoort, J.; Tyrakowska, B.; Hermens, J. L. M.; Rietjens, I. M. C. M. *Toxicology in Vitro* **2001**, *15*, 539−551.
(25) After submission of this paper, a related study of the mechanism of oxidation of benzene appeared, with somewhat similar conclusions (de Visser, S. P.; Shaik, S. *J. Am. Chem. Soc*. **2003**, *125*, 7413−7424.)

JA035590Q