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Predicting the product specificity and coupling of cytochrome P450cam

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SUMMARY

We present an analysis of several molecular dynamics trajectories of substrate-bound cytochrome P450cam. Trajectories were calculated for the native substrate, camphor, as well as for the alternative substrates, norcamphor and thiocamphor. The system modeled consisted of the crystallographically resolved amino acids, the heme group with a single oxygen atom as the distal ligand, the bound substrate, and the crystallographic waters. These trajectories of the presumptive ferryl oxygen intermediate were used to predict regiospecificity of hydroxylation and coupling between NADH consumption and product formation. Simple geometric criteria in combination with electronic considerations were used to calculate the probability of hydroxylation at specific sites on the substrate. We found that for all the cases examined, the predicted product ratios were in good agreement with the experimentally observed values. We also determined that these simple geometric criteria can be used to predict the degree of coupling between NADH consumption and product formation for a given substrate, which was in good agreement with the experimental values.

INTRODUCTION

The cytochromes P450 are a superfamily of heme enzymes that activate molecular oxygen and subsequently catalyze a wide variety of reactions, including hydroxylation of carbon and heteroatoms, dealkylation of amines and ethers, epoxidation of double bonds, and reductive dehalogenation [1]. They are involved in steroid biosynthesis, fatty acid metabolism, and detoxification of a wide variety of xenobiotics. The understanding, prediction, and control of P450–substrate interactions is therefore of paramount interest for engineering cytochromes P450 as biotechnological tools or for designing therapeutic inhibitors.

The best studied isozyme is cytochrome P450cam from the soil bacteria *Pseudomonas putida* [2,3,4]. It is also the only member of the superfamily for which a detailed 3-dimensional structure has been determined. Structures have been determined for both the camphor-bound and cam-

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phor-free structures as well as for numerous alternative substrates and inhibitors [5,6]. The product specificity of the hydroxylation reaction has been experimentally determined both for the native substrate, camphor, and for a number of substrate analogs including norcamphor and thiocamphor [7,8,9]. Site-directed mutagenesis has revealed a wealth of information on the relative importance of active-site residues to the binding properties of P450cam [10]. We have used this set of experimental data to test our ability to predict the product specificity of cytochrome P450cam as an aid in understanding how to redesign P450cam for novel substrates.

The native substrate, camphor, is hydroxylated by P450cam with 100% regiospecificity at the C5 position. This is in contrast to the nonenzymatic hydroxylation of camphor in which the C3 alcohol is the major product [11]. An examination of the camphor-bound crystal structure clearly indicates the reason for the observed regiospecificity. Camphor is locked into an orientation that leaves the C5 position closest to the heme iron. However, hydroxylation of camphor analogs results in multiple products of varying proportions [7,8,9]. This implies that these analogs, when bound to P450cam, are sampling a number of conformations that are not available to camphor and are not observed in the static crystal structure of these analogs. Molecular dynamics simula-

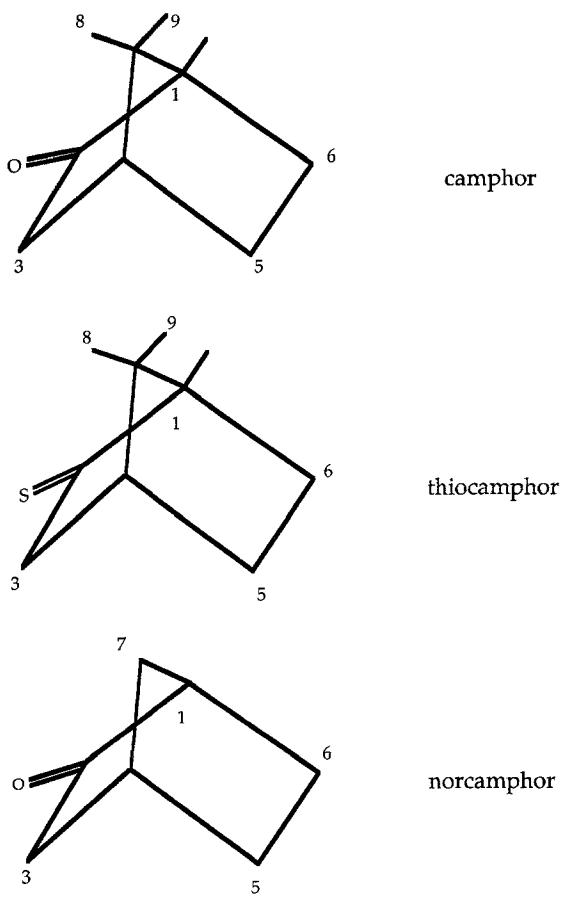

Fig. 1. The structures of camphor, norcamphor, and thiocamphor along with the numbering scheme used in the paper.

tions can be used to sample more effectively the range of conformations available to the bound substrate, revealing minor conformations that are missed in constructing a 3-dimensional model from X-ray diffraction data but that may be important in determining product specificity. We present an analysis of several trajectories of cytochrome P450cam with different substrates. The trajectories are used to predict the product regiospecificity of the hydroxylation reaction. The underlying assumption is that a combination of electronic factors and geometry with respect to the reactive ferryl oxygen intermediate will control the regiospecificity of the hydroxylation reaction. This assumption has been used in several recent theoretical studies of the P450cam system [12,13,14]. In addition to determining product ratios, we were also interested in determining whether the dynamic behavior of the substrate could be correlated with the degree of coupling between NADH consumption and product formation. While the hydroxylation of camphor is 100% coupled to NADH consumption, the hydroxylation of several of the substrate analogs is uncoupled from NADH consumption, with the excess electrons being used in the production of water and hydrogen peroxide. It has been suggested previously that the decreased coupling must be related to the increased mobility of the camphor analogs [15]. We found that simple geometric criteria in combination with an assessment of the inherent reactivity of different carbon centers can be successfully used to predict both the regiospecificity and coupling for the hydroxylation of a variety of substrates by cytochrome P450cam.

METHODS

The system for which trajectories were calculated consisted of residues 10–414 of cytochrome P450cam, the heme moiety at the active site with atomic oxygen as an axial ligand, a bound substrate (either camphor, norcamphor, or thiocamphor), and 240 crystallographic waters. The structures of the 3 substrates modeled in this study are shown in Fig. 1 along with the numbering scheme used. Water 515, thought to be the cation that activates this enzyme [16], was modeled as a sodium ion. The crystallographic structures determined by Poulos and coworkers [6,16,17] and those obtained from the Protein Data Bank [18] served as the starting point for the simulations.

The simulations were done using an all-hydrogen model, and a distance-dependent dielectric. Side-chain polar hydrogens were positioned so as to maximize intramolecular hydrogen bonding, using a previously described automated method [19]. Although only a small number of explicit waters were included, this should have a small effect on the predictions made since we are focusing on the dynamics of the buried active site. The dynamic behavior of surface residues in the protein will be most susceptible to the method of modeling bulk solvent, while the interior of the protein will be much less affected by the method of modeling bulk solvent. The Discover simulation package (Biosym Technologies v2.7) with the consistent valence forcefield of Hagler and coworkers was used [20,21]. The parameters for the heme were identical to those described previously [22]. The valence and nonbonded parameters for the ferryl oxygen are summarized in Table 1.

For each simulation, the system was first minimized with all heavy atoms fixed to relax the added hydrogens and then 500 steps each of steepest descents and conjugate gradients were performed with all atoms free to move to allow any remaining hotspots to relax. The atomic velocities were initialized using a Maxwellian distribution at 50 K, and the system was gradually warmed to 300 K over a period of 10 ps. After this, the various simulations were continued at 300 K for 100 ps or more using 1.0 fs timesteps. Nonbonded interactions were evaluated using a group-

TABLE I
FORCEFIELD PARAMETERS FOR THE IRON-OXO COMPLEX

Partial atomic charges in ferryl intermediate

Atom	Charge
Fe	1.28
S	−0.10
O	−0.50

Nonbonded parameters for ferryl intermediate

Atom	A (kcal/A^{12})	B (kcal/A^{6})
S	365906.4	250.8
O	272894.78	498.88
Fe	3355443.0	1638.4

Bond parameters for ferryl intermediate

Bond	r_e (A)	K (kcal/mol)
Fe–S	2.20	120.0
Fe–O	1.78	300.0

Angle parameters for ferryl intermediate

Angle	θ (degrees)	K (kcal/degree)
N–Fe–O	90.0	50.0
S–Fe–O	180.0	10.0

based switching function between 9.5 and 11.5 Å and the nonbonded pair list was updated every 20 steps.

RESULTS AND DISCUSSION

Trajectories were calculated for three different substrates bound to cytochrome P450cam (camphor, norcamphor, and thiocamphor) for times ranging between 160 and 175 ps. Structures were saved every 0.5 ps to provide an ensemble of possible substrate orientations. The distances between the ferryl oxygen and substrate hydrogen atoms, which were likely to be abstracted based on electronic considerations, were determined for each of the collected conformations. In addition, the ferryl oxygen-substrate hydrogen-substrate carbon angle was monitored for each of the presumed reactive hydrogens. It was assumed that a nearly linear angle was the most favorable for hydrogen abstraction. It should be noted that no special terms were included in the potential energy function to promote a nearly linear abstraction angle. Thus any preferences in abstraction angle will be determined by the sum of all protein-substrate interactions rather than by the specific interactions of the ferryl oxygen with the presumptive reactive hydrogen.

A 170-ps simulation of camphor-bound cytochrome P450cam was used as a baseline for determining the best geometric constraints to use in analyzing the remaining trajectories. The mean distance between the ferryl oxygen and the 5-*exo* hydrogen of camphor was 2.83 $\pm$ 0.33 Å in this simulation. Conversely, the mean distances to the closest hydrogen on the 3 and 6 carbons of camphor were 6.28 $\pm$ 0.38 Å and 4.16 $\pm$ 0.49 Å, respectively. The fluctuations in the carbon-oxygen distances for the native substrate, camphor, are significantly smaller than those seen for smaller substrate analogs such as norcamphor (see below). This reflects the fact that camphor samples only a single orientation during the time course of the simulation. However, as the hydroxylation reaction is known to proceed with 100% regiospecificity, there is no reason to believe there is more than one catalytically important substrate orientation.

We were looking for geometric constraints that would correctly distinguish the high regiospecificity of camphor hydroxylation while at the same time be consistent with the high degree of coupling between NADH consumption and product formation. If the criterion was too generous, little specificity of hydroxylation would be predicted relative to the experimental observation, while if is was too restrictive, most conformations would be considered unreactive, which we would take as implying uncoupling of NADH consumption from product formation, again in disagreement with the experimental observation for camphor.

For the native substrate, the task was to distinguish between 5- and 6-hydroxylation. For no reasonable cutoff in the ferryl oxygen-substrate-hydrogen distance was 3-hydroxylation predicted. A plot of the number of configurations with a given oxygen-hydrogen distance versus that distance is shown in Fig. 2 for the 5-*exo* and 6-*exo* hydrogens. While the peaks in the distributions of the 5-*exo* and 6-*exo* hydrogen distances are well separated, there is a significant overlap in the tails of the two distributions. A cutoff of 3.5 Å is approximately two standard deviations above the mean of the 5-*exo*-hydrogen to oxygen distance and results in a prediction of 91% C5 hydroxylation. It was found that a slightly shorter distance criterion of 3.25 Å predicted 95% C5 alcohol, while still retaining a large percentage of active conformations, which we take to imply a high degree of coupling between product yield and NADH consumption. Distance cutoffs shorter than this result in a rapidly increasing number of unreactive conformations and are therefore inconsistent with the experimental observation of high coupling. In determining the number of reactive conformations, a single structure was counted multiple times if more than one hydrogen met the cutoff criteria. In addition, for comparison with experiments, the number of reactive conformations for a particular carbon was a sum of all reactive hydrogens bonded to that carbon. No attempt was made to predict the stereochemistry of hydroxylation for these substrates since the available experimental evidence demonstrates that for camphor the 5-*exo* product is formed regardless of whether the 5-*endo* or 5-*exo* hydrogen is abstracted [23].

One interesting result of this analysis was that the C9 position was almost always at a favorable distance for hydrogen abstraction. There was no distance cutoff that would distinguish between C5 and C9 abstraction. The 3.25 Å cutoff, which distinguished well between the 5 and 6 carbons, still resulted in only a 3:1 ratio of C5 to C9 hydroxylation. However, abstraction at a secondary carbon is sufficiently more favorable than abstraction at a primary carbon that only C5 hydroxylation is seen experimentally. If the C5 position is blocked, for instance in 5,5-diflorocamphor, the major reaction is hydroxylation at the C9 position rather than at the C3 or C6 methylene carbons [24], which is consistent with our observation that C9 is consistently in a better abstraction orientation than the C6 position of camphor. We also predict that a measurable decrease in coupling

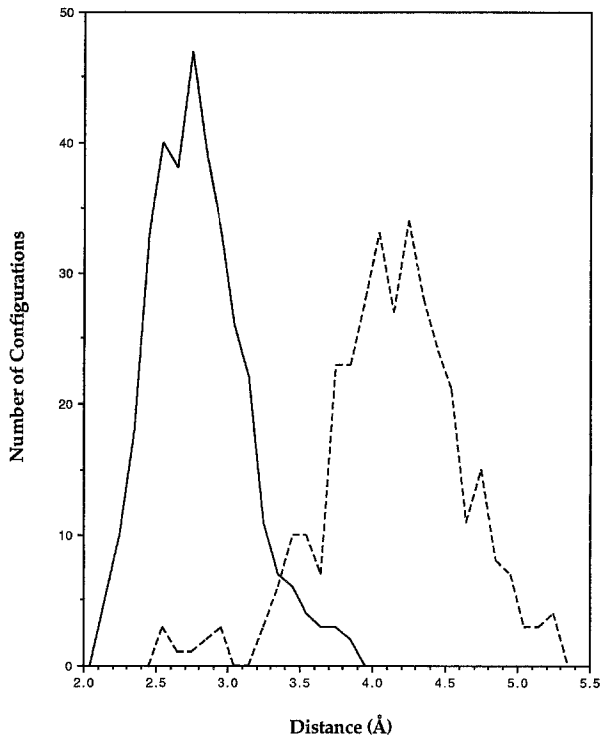

Fig. 2. The number of configurations with a given oxygen-hydrogen distance is plotted versus the distance for the 5-*exo* (solid line) and 6-*exo* (dashed line) hydrogens of camphor.

should be seen for the diflouro analog since a much larger fraction of the generated structures would have no hydrogens in a favorable geometry.

In addition to distance constraints, we also examined angular constraints to see if they were also good predictors of specificity. We used the same philosophy in determining an angular constraint as we did for the distance constraint. For the camphor-bound trajectory, the angular constraint should be able to distinguish between the 3, 5, and 6 carbon positions while counting a large percentage of the configurations as reactive. A plot of the number of configurations with a given angle versus the hydrogen-oxygen-carbon abstraction angle is shown in Fig. 3 for the 5-*exo* and 6-*exo* hydrogens. The mean oxygen-hydrogen-carbon angle for the 5-*exo* position on camphor was 154 $\pm$ 13°. This is consistent with the assumption that a nearly linear angle will result in the best orbital overlap for abstraction of the hydrogen by the oxygen radical. On the other hand, the mean angle for the 6-*exo* position was 106 $\pm$ 12°. As with the distance histograms, the peaks in the distributions were well resolved, but there was significant overlap in the tails of the distributions. We found that requiring an oxygen-hydrogen-carbon angle of 180 $\pm$ 45° for a conformation to be counted as reactive resulted in a prediction of 98% C5 product, while still retaining a large number of reactive conformations. As with the distance criterion, the angle criterion did not distinguish between C5 and C9.

The predicted product ratio and predicted coupling based on a combination distance and angle criteria are summarized in Table 2. A combination distance and angle criteria ($\leqslant$ 3.25 Å and 180

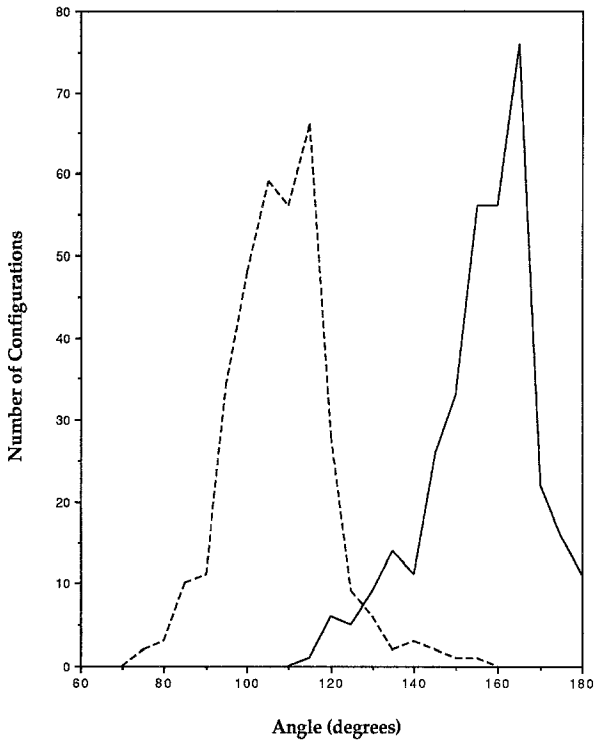

Fig. 3. The number of configurations with a given carbon-hydrogen-oxygen angle is plotted versus the angle for the 5-*exo* (solid line) and 6-*exo* (dashed line) hydrogens of camphor.

$\pm$ 45°) resulted in a prediction of 99% C5 hydroxylation and only 1% C6 hydroxylation, which is in excellent agreement with the experimental observation. If we assume that the fraction of total configurations that are counted as reactive configurations is correlated with the degree of coupling between NADH consumption and product formation, we would predict 86% coupling for the

TABLE 2
CAMPHOR HYDROXYLATION BY P450cam

	Calculation				Experiment
	Distance only[a]		Angle and distance[b]		
Atom	Hits	%	Hits	%	%
C3	0	0	0	0	0
C5	312	95	288	99	100
C6	15	5	4	1	0
Total hits	341		292		
Coupling [%]	96		86		100

[a] The distance criterion used was hydrogen-oxygen distance $\leq$ 3.25 Å.
[b] The angle criteria used was oxygen-hydrogen-carbon angles of 180 $\pm$ 45°.

hydroxylation of camphor. This is in qualitative agreement with the experimental observation of 100% coupling. Because of the overlap in the distributions of both the oxygen-hydrogen distances and the oxygen-hydrogen-carbon angles for the 5-*exo* and 6-*exo* positions, it was impossible to obtain a prediction of 100% coupling while still retaining high regiospecificity in the predicted product distribution. However, experimentally, the alternative substrates seem to be either highly coupled or highly uncoupled, and the distance and angle criteria can clearly distinguish these two possibilities.

We next used the constraints determined from the camphor trajectory to analyze two norcamphor trajectories. Norcamphor is a substrate analog in which the C8, C9, and C10 methyl groups have been replaced by hydrogens, resulting in a smaller, more spherical substrate. Experimentally, it is known that the hydroxylation reaction with norcamphor proceeds with a loss of regiospecificity relative to the native substrate, camphor. Collins and Loew found the calculated heats of formation of the 3, 5, and 6 radicals of norcamphor were similar and that rotation of norcamphor around its oxygen-carbon bond located a second minima that brought the 3 position in to closer proximity to the heme iron than it is in the crystal structure [12]. However, in their study, they did not make any estimate of relative residence times in the different conformational minima they located, and thus made no prediction of the relative amounts of the various alcohols produced.

Whereas camphor has a fairly small B-factor and shows very little mobility in a molecular dynamics trajectory [25], previous simulations of the binary complex of norcamphor bound to P450cam have shown a much larger degree of mobility [26], which is consistent with the larger B-factor determined for norcamphor from the X-ray diffraction data. Unlike camphor, norcamphor undergoes multiple rotations during the time course of the simulation and from the analysis described below appears to sample all of the catalytically important orientations. However, because of its high mobility, the dynamics of norcamphor and the resulting prediction of product distribution are more likely to be sensitive to the initial velocities assigned at the start of the trajectory than the dynamics of the native substrate. For the pair of trajectories presented here, with the same starting conformation but different initial velocities, there were only slight differences between the predicted product ratios in each case. The results for the pair of trajectories are summarized in Table 3. For the combined data set, the distance criteria alone ($\leqslant$ 3.25 Å) resulted in a predicted product distribution of 73:26:1 for C5, C6, and C3 hydroxylation, respectively. The combination distance and angle criterion altered the distribution slightly to 63:37:0. This result is in reasonable agreement with the experimentally observed ratio of 45:47:8 [15]. The slight overprediction of C5 product probably reflects the fact that this is the product consistent with the crystal conformation that was the starting orientation for both trajectories, and it is possible that longer trajectories might alter the calculated product ratio by damping this bias towards the starting conformation. Conformations favorable for hydrogen abstraction at C3 are clearly rare events in simulations starting from the crystal conformation, and, as such, it may require significantly longer simulations to sample such minor conformations fairly.

The combination angle and distance criteria also predicted only 21% reactive conformations. This number is dramatically lower than the predicted coupling for camphor and is in good agreement with the experimental observation of 12% coupling. A comparison of the two separate trajectories suggests that the uncertainty in the predicted amount of C5-alcohol is in the order of 10%. Therefore, this type of analysis will probably not be useful for accurately predicting small changes in product distribution between either closely related analogs or active-site mutants. However, it

TABLE 3
NORCAMPHOR HYDROXYLATION BY P450cam

	Calculation				Experiment
	Distance only[a]		Angle and Distance[b]		
Atom	Hits	%	Hits	%	%
C3	5	1	0	0	8
C5	354	73	85	63	45
C6	127	26	49	37	47
Total hits	486		134		
Coupling [%]	76		21		12

[a] The distance criterion used was hydrogen-oxygen distance $\leqslant 3.25$ Å.
[b] The angle criteria used was oxygen-hydrogen-carbon angles of $180 \pm 45°$.

should be able to predict that the change in product specificity will in fact be small. Increasing the number of structures examined by doing more or longer simulations should, in principle, increase the precision of the calculated product yields.

One interesting difference between camphor and norcamphor is that norcamphor has an additional secondary carbon, C7, which could, in principle, be hydroxylated. In camphor, this position has no hydrogens available for abstraction but in norcamphor it is a methylene carbon, as are the 3, 5, and 6 positions. Calculations by Collins and Loew suggest that while the radical formed by abstraction of a hydrogen at the tertiary carbons C1 and C4 is significantly less stable than any of the secondary carbon radicals, the C7 radical has a stability that is only about 2 kcal/mol less stable than that of the other methylene carbons, and thus it is intermediate between the other three secondary carbons which cannot be distinguished on the basis of radical stability and the two tertiary carbons which can be clearly ruled out as potential hydroxylation sites based on the calculated stability of their radicals [12]. Collins and Loew did not find a substrate orientation consistent with the formation of the C7 alcohol and therefore predicted that only the C5, C6, and C3 alcohols would be formed. However, their study used a rigid active site, which could artificially limit the possible substrate orientations. Previous simulations of the binary complex of norcamphor and P450cam (that is, without an explicit distal ligand for the heme iron) suggest that in fact the C7 alcohol should be as prevalent as the C3 product based on geometric considerations alone [26]. On the other hand, the study by Atkins and Sligar reports hydroxylation only at the C3, C5, and C6 positions [15]. An analysis of the norcamphor trajectories presented here with an explicit distal ligand also shows a small number of conformations consistent with the formation of the C7 alcohol, in qualitative agreement with the previous binary simulations. Therefore, the presence of configurations favorable for C7 hydroxylation does not seem to be an artifact caused by the lack of an explicit distal heme ligand. These data suggest that the calculated difference in radical stability for C7 is sufficiently large to insure that experimentally none of the C7 alcohol is observed. Thus the data in Table 3 count orientations in which only C7 is in a favorable geometry as an unreactive configuration.

TABLE 4
THIOCAMPHOR HYDROXYLATION BY P450cam

	Calculation				Experiment
	Distance only[a]		Angle and Distance[b]		
Atom	Hits	%	Hits	%	%
C3	35	6	18	4	2
C5	378	67	315	70	64
C6	150	27	118	26	34
Total hits	563		451		
Coupling [%]	88		71		98

[a] The distance criterion used was hydrogen-oxygen distance $\leqslant 3.25$ Å.
[b] The angle criteria used was oxygen-hydrogen-carbon angles of $180 \pm 45°$.

A third substrate for which experimental data is available is thiocamphor, in which the keto oxygen of camphor has been replaced by a sulfur atom. Like norcamphor, this substrate has a hydroxylation pattern significantly different from camphor. It should therefore be a good test of our method of analysis. Unlike the other camphor analogs, which can be modeled well as binding to cytochrome P450cam in a single camphor-like orientation, although with increased mobility, thiocamphor binds in two different conformations, neither of which is similar to the camphor conformation [6]. In addition, neither conformation is consistent with the C5 alcohol being the major product, which is in fact observed experimentally [8]. We ran trajectories starting with both the major and minor X-ray crystal conformations of thiocamphor. The results for this pair of trajectories are summarized in Table 4. As in the case of camphor, only secondary carbons were considered as potential reactive sites, based on electronic considerations. For the major conformation, the distance criteria alone give a prediction of 93:2:5 for the ratio of C5:C6:C3 hydroxylation. Inclusion of the angle criteria altered the predicted ratio only slightly to 95:1:4. This prediction was not in particularly good agreement with the experimental values since it significantly overestimated the amount of C5 hydroxylation and underestimated the amount of C6 hydroxylation. Including the trajectory starting from the minor conformation dramatically improved the agreement between our prediction and the experimentally observed values. For the pair of trajectories, the predicted product ratio was 67:27:6 for C5:C6:C3 hydroxylation compared with experimental values of 64:34:2. The analysis of the thiocamphor trajectories predicted a high degree of coupling, 86%, which is in good agreement with the experimental observation of 98% [8]. Unlike the trajectories with norcamphor, with thiocamphor we were not able, starting from a single orientation, to sample the catalytically important configuration space properly. This problem is probably even more important with substrates that are even more dissimilar to camphor.

CONCLUSIONS

Product specificity in the hydroxylation of aliphatic compounds by the cytochromes P450 will

be controlled by a number of factors including both electronic effects and steric effects. Electronic factors will determine the inherent ease of hydrogen abstraction at a given site on the substrate and the subsequent stability of the radical intermediate produced. However, these electronic factors can be significantly modulated by geometric factors. In the cases of camphor, norcamphor, or thiocamphor, there is little at the electronic level to distinguish between hydrogen abstraction at the 3, 5, and 6 positions [12], but the steric restriction imposed by the active-site residues results in a significant skewing of the product distribution. Examination of the crystal structure will suggest which site will be the preferred hydroxylation site, but because it is a static picture, the crystal structure cannot be used to estimate the identity or yield of minor products, as for instance in norcamphor-bound P450. In addition, the favored conformation in a binary complex of substrate and P450 may not be the same as in the presumed reactive intermediate after O_2 binds to the heme iron, as appears to be the case for thiocamphor. To surmount these limitations, we developed a method for analyzing molecular dynamics trajectories of the presumed reactive intermediate in order to rank potential hydroxylation sites.

For the substrates shown here, the type of trajectory analysis we have presented is fairly successful in predicting product specificity. In addition, the angle criterion by itself or in combination with the distance criterion has been found to be an excellent predictor of coupling between NADH consumption and product yield. Accurate guesses about the expected coupling of a P450 reaction will be of some importance for predicting whether a naturally occurring or designed isozyme will be suitable for a biotechnological application, not only because reducing equivalents are costly, but also because uncoupling is usually related to auto-oxidation pathways and peroxide formation [27,28], which jeopardize enzyme activity. For some substrates and/or active-site mutants, especially those without hydrogen-bonding ability, the inclusion of a ferryl oxygen in the simulation gives a much better agreement with experimental data than a simulation without an axial ligand for the heme iron [in preparation]. We are now using this method to predict product yield and specificity for both novel substrates [in preparation] and active-site mutants [29]. Such an analysis can, in principle, prove to be much more difficult, since it will generally be done in the absence of detailed 3-dimensional information about the orientation of the novel substrate in the active-site pocket. Thus it is likely that multiple trajectories starting from several plausible binding orientations will be required in order to obtain accurate predictions.

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REFERENCES

1 Porter, T.D. and Coon, M.J., J. Biol. Chem., 266 (1991) 13469.
2 Murray, R., Fisher, M., Debrunner, P. and Sligar, S., In Harrison, P. (Ed.) Metalloproteins, Part I: Metal Proteins with Redox Roles, MacMillan and Co., London 1985, pp. 157–206.
3 Sligar, S.G. and Murray, R.I., In Ortiz de Montellano, P.R. (Ed.) Cytochrome P450: Structure, Mechanism, and Biochemistry, Plenum, New York, 1986, pp. 429–503.
4 Gunsalus, I.C. and Sligar, S.G., Adv. Enzymol., 47 (1977) 1.
5 Poulos, T.M. and Raag, R., FASEB J., 6 (1992) 674.
6 Raag, R. and Poulos, T.L., Biochemistry, 30 (1991) 2674.
7 White, R.E., McCarthy, M.B., Egeberg, K.D. and Sligar, S.G., Arch. Biochem. Biophys., 228 (1984) 493.
8 Atkins, W.M. and Sligar, S.G., J. Biol. Chem., 263 (1988) 18842.
9 Atkins, W.M. and Sligar, S.G., J. Am. Chem. Soc., 111 (1989) 2715.
10 Sligar, S.G., Filipovic, D. and Stayton, P.S., Meth. Enzymol., 206 (1991) 31.
11 Lind, E.J. and Swallow, A.J., J. Chem. Soc. Faraday Trans. I, 75 (1979) 1849.
12 Collins, J.R. and Loew, G.H., J. Biol. Chem., 263 (1988) 3164.
13 Collins, J.R., Camper, D.L. and Loew, G.J., J. Am. Chem. Soc., 113 (1991) 2736.
14 Ortiz de Montellano, P.R., Fruetel, J.A., Collins, J.R., Camper, D.L. and Loew, G.H., J. Am. Chem. Soc., 113 (1991) 3195.
15 Atkins, W.M. and Sligar, S.G., J. Am. Chem. Soc., 109 (1987) 3754.
16 Poulos, T.L., Finzel, B.C. and Howard, A.J., J. Mol. Biol., 195 (1987) 687.
17 Raag, R. and Poulos, T.L., Biochemistry, 28 (1989) 917.
18 Bernstein, F.C., Koetzle, T.F., Willams, G.J.B., Meyer Jr., E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M., J. Mol. Biol., 112 (1977) 535.
19 Bass, M.B., Hopkins, D.F., Jaquysh, W.A.N. and Ornstein, R.L., Proteins, 12 (1992) 266.
20 Hagler, A.T., In Hruby, V.J. and Meienhofer, J. (Eds.) Conformation in Biology and Drug Design, The Peptides, Volume 7, Academic Press, New York, 1985, pp. 213–299.
21 Dauber-Osguthorpe, P., Roberts, V.A., Osguthorpe, D.J., Wolff, J., Genest, M. and Hagler, A.T., Proteins, 4 (1988) 31.
22 Paulsen, M.D. and Ornstein, R.L., Proteins, 11 (1991) 184.
23 Gelb, M.H., Heimbrook, D.C., Malkonen, P. and Sligar, S.G., Biochemistry, 21 (1982) 370.
24 Eble, K.S. and Dawson, J.H., J. Biol. Chem., 259 (1984) 14389.
25 Paulsen, M.D. and Ornstein, R.L., J. Biomol. Struc. and Dyn., 9 (1991) 187.
26 Bass, M.B., Paulsen, M.D. and Ornstein, R.L., Proteins, 13 (1992) 26.
27 Stayton, P.S. and Sligar, S.G., Biochemistry, 30 (1991) 1845.
28 Davies, M.D., Quin, L., Beck, J.L., Suslick, K.S., Koga, H.J., Horiuchi, T. and Sligar, S.G., J. Am. Chem. Soc., 112 (1990) 7396.
29 Bass, M.B., Filipovic, D., Sligar, S.G. and Ornstein, R.L., Prot. Eng., in press.