

MOLECULAR DESIGN OF SUBSTRATE BINDING SITES

John A. Shelnut, J. David Hobbs

Fuel Science Division 6211
Sandia National Laboratories
Albuquerque, NM 87185

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ABSTRACT

Computer-aided molecular design methods were used to tailor binding sites for small substrate molecules, including CO₂ and methane. The goal is to design a cavity, adjacent to a catalytic metal center, into which the substrate will selectively bind through only non-bonding interactions with the groups lining the binding pocket. Porphyrins are used as a basic molecular structure, with various substituents added to construct the binding pocket. The conformations of these highly-substituted porphyrins are predicted using molecular mechanics calculations with a force field that gives accurate predictions for metalloporphyrins (Shelnutt, *et al.*, *J. Am. Chem. Soc.* **1991**, *113*, 4077). Dynamics and energy-minimization calculations of substrate molecules bound to the cavity indicate high substrate binding affinity. The size, shape, and charge-distribution of groups surrounding the cavity provide molecular selectivity. Specifically, calculated binding energies of methane, benzene, dichloromethane, CO₂, and chloroform vary by about 10 kcal/mol for metal octaethyl-tetraphenylporphyrins (OETPPs) with chloroform, dichloromethane, and CO₂ having the highest affinities and with methane and benzene having the lowest. Significantly, a solvent molecule is found in the cavity in the X-ray structures of Co- and CuOETPP crystals obtained from dichloromethane.

INTRODUCTION

We have set for ourselves the ambitious goal of designing and synthesizing molecules with a high affinity binding site for a given substrate molecule. Preferably, the molecule containing the substrate binding cavity would also possess some additional chemical functionality, such as a catalytic center at which the substrate would be converted to a useful product. Because over 70 metals in various oxidation states can be incorporated into the porphyrin macrocycle and because of the high degree of variability in the peripheral substituents, we have chosen the metalloporphyrins as a foundation for construction of the required binding site. By using the porphyrin ligand, we gain considerable freedom both in the choice of catalytic metal at its center and in the selection of peripheral substituents used to construct the binding cavity of the chosen substrate molecule.

Our initial goal is to construct a porphyrin with a substrate binding pocket for which only non-bonding forces contribute to the selective binding of the substrate to the active site. The inclusion of such a cavity gives several potential benefits. These benefits include (1) increased affinity for the substrate and decreased product affinity, (2) enhanced selectivity for a particular substrate molecule, (3) enhanced regioselectivity of the reaction, (4) lower catalyst self-destruction rates and increased catalyst stability, (5) product slate selectivity resulting from trapping of radical intermediates for recombination, and (6) enhanced reactivity from molecular orientational and conformational lowering of transition states, to name a few. All of these benefits of a substrate binding pocket are realized to one degree or another in naturally occurring biological catalysis called enzymes, particularly for the metalloporphyrin-containing enzymes. These enzymes, for example cytochrome P₄₅₀, methylreductase, and the photosynthetic reaction center, catalyze many important energy-related chemical or photochemical reactions. Thus, computer-modeling studies of these enzymes give many important clues to the structural features that might be engineered into our designed catalyst. These features include (1) a catalytic metal ion, (2) appropriate electronic properties for the ligands of the metal, (3) complementary size and shape for the substrate binding pocket, (4) suitable properties like hydrophobicity and charge distribution for the groups lining the pocket, and (5) molecular rigidity of the cavity.

The molecular rigidity of the cavity results in enhanced substrate binding affinity, an

especially important asset for catalytic conversion of small gaseous substrate molecules like CO₂ and methane. Because the rigid binding pocket is present even in the absence of the substrate molecule, enzyme studies show that the 'hole' in the catalyst contributes (about 2 kcal/mol) to the binding energy (for each methylene-group-sized unit) of the substrate molecule through the dispersion energy contribution to the interaction with the bound substrate. In the example of an enzyme and its alkane substrate in water, two unfavorable hydrophobic interfaces result, one from the water molecules filling the hydrophobic pocket and another from the alkane dissolved in the solvent; these unfavorable hydrophobic interactions are removed when the substrate binds to the preformed pocket, accounting for about 3 kcal/mol for a methylene-sized molecular unit. Electrostatic forces also enhance the binding energy. After the rigidity of the cavity is insured, energy contributions to the binding energy of the substrate molecule can be optimized by careful design of the binding cavity of our tailored enzyme analog.

The molecular rigidity of the binding pocket possessed by the enzymes has been one of the most difficult structural features to mimic in our metalloporphyrin-based enzyme analogs. However, by using so-called highly substituted porphyrins we have been able to engineer the required molecular rigidity into our designed catalysts. Using a molecular model based on a porphyrin normal coordinate analysis, we found that the porphyrin ring, when maximally substituted at the periphery with non-hydrogen substituent groups, was rigidly distorted into a saddle shaped conformation. Furthermore, in this distorted geometry the substituents at the β -pyrrole carbons form a binding pocket of a size and shape suitable for binding small molecules like CO₂ and light alkanes. And, by lining the pocket with groups with properties complementary to the substrate, we could promote substrate binding to the pocket for times on the order of nanoseconds (in molecular dynamics calculations at 300 °K in vacuum).

Many of the designed catalysts have been synthesized and subjected to experimental structural studies. The calculated conformations of the designed catalysts have been verified by X-ray crystallography, NMR spectroscopy, UV-visible absorption spectroscopy, and resonance Raman spectroscopy.¹⁻³

Evidence of substrate binding is found in the X-ray crystal structures of the cobalt and copper derivatives of octaethyl-tetraphenylporphyrin. As described below, small solvent molecules are observed in the substrate binding cavity in X-ray crystal structures of this highly-substituted metalloporphyrin.

MATERIALS AND METHODS

Cobalt(II) and copper(II) octaethyltetraphenylporphyrin (OETPP) were synthesized as described previously.² The X-ray crystal structures were reported previously,² however the location of solvent molecules in the crystals was not discussed. Views of the packing of porphyrin molecules in the crystal are shown in Figures and were generated on an Evans&Sutherland PS390 graphics workstation. One of two crystallographically distinct solvent molecules is located in the cavity as shown (Figures 2 and 3).

Molecular modeling calculations were performed on a Personal Iris 4D35 workstation using BIOGRAF software from Molecular Simulations, Inc. The calculations were carried out as described previously for a series of nickel porphyrins.² The force field is the same as that reported earlier for nickel(II) porphyrins,² but extended to include other metal ions including Co(II) and Cu(II).³ This force field has been successful in predicting porphyrin conformations that agree well with X-ray crystal structures.^{2,3} Parameters for the oxygen atoms in CO₂ and for the Cl atoms in the halogenated methanes were taken from the DREIDING force field.⁴ Partial atomic charges were assigned to the porphyrin and solvent molecules by the method of charge equilibration.^{4,5} Minimizations were carried out for several initial orientations of the substrate molecule, because of the many local minima resulting from different substrate orientations in the binding pocket.

RESULTS AND DISCUSSION

Figure 1 shows the energy-minimized structure of nickel(II) octapropyl-tetraphenylporphyrin (NiOPTPP) with CO₂ bound in one of the grooves generated by four of the quasi-axial β -pyrrole ethyl substituents. Clearly, CO₂ fits nicely in the elongated cavity of the catalyst, so we decided to evaluate the selectivity of the cavity for a variety of small substrate molecules.

Tables I-III summarize the results of molecular mechanics calculations for the binding of various small molecules, including CO₂, methane, benzene, dichloromethane, and chloroform, to the elongated cavity. The relative binding energies are seen to vary over several kcal/mole, depending

primarily on the size of the substrate and distribution of charge in the substrate molecule. In the model, van der Waals interactions and electrostatic interactions between the substrate molecule and the designed porphyrin catalyst are the only forces that contribute to the binding energy. Benzene binds most weakly because the partial charges on the atoms are small. The chlorinated methanes bind most strongly because the highly electronegative chlorine atoms result in large partial charges on the substrate atoms which interact strongly with complementary charges on the catalyst. In particular, the interactions between the electronegative oxygen (or chlorine) atoms of the substrate and the nearby hydrogen atoms of the ethyl groups of the catalyst are important. In our calculation the electrostatic energy falls off as $1/r^2$, making the interactions with the closest atoms most significant. The $1/r^2$ -dependence is used to mimic the presence of a solvent in a crude way. The dielectric constant of the solvent is assumed to be 1, represents an organic solvent.

Examination of Tables I and II, for catalysts with well defined cavities (NiOETPP and CuOETPP), shows that the order of binding energies is: $\text{CHCl}_3 > \text{CO}_2 \approx \text{CH}_2\text{Cl}_2 > \text{benzene} > \text{methane}$. The van der Waals contribution to the binding energy varies over the range from 6 to 13 kcal/mole for all substrates, lowest values being for the smallest substrates of the group. The electrostatic contribution is large (2-6 kcal/mole) for the substrates with electronegative atoms and small for the hydrocarbons. The internal energies (bond stretch, angle bend, etc.) adjust to some extent upon substrate binding, particularly the torsions. The cavity is slightly larger for CuOETPP (Table II) than for NiOETPP (Table I), and the larger cavity results in weaker binding of each substrate. This effect is even more in evidence when the ethyl groups are replaced by methyl groups (NiOMTPP, Table III), and the cavity is lost altogether. Then, the binding energies of all substrates (except benzene) decrease.

Clearly, the results in Tables I and II indicate that dichloromethane is expected to have one of the highest affinities for the cavity. Therefore, it is not surprising to find a dichloromethane molecule in the cavity in the X-ray crystal structure of the Co and Cu derivatives of OETPP. Figures 2 and 3 show orthogonal views of two of the CuOETPP molecules in the crystal. Also shown are two equivalent dichloromethane molecules that are located in the cavity. The dichloromethane molecules are not coordinated to the copper(II) ion. Copper is usually four-coordinate, square planar with the porphyrin occupying all four ligand sites. The lack of coordination of the dichloromethane is also clear from the greater than 3-Å distance between the metal and the heavy atoms of the substrate.

The binding site apparently does not have a strong preference for a particular orientation of the bound substrate molecule. We conclude this from the many orientations of the substrate molecule that give rise to structures with energies close to the lowest listed for each substrate in Table I. Two such structures are given for dichloromethane and CO_2 in the Tables. Here too, the X-ray data support a similar conclusion based on the thermal parameters for atoms of the dichloromethane located in the cavity. The isotropic displacement coefficient for the carbon atom of the dichloromethane molecule in the pocket is approximately twice as large as for the average carbon atom of the porphyrin. The isotropic parameters for the chlorines are about four times as large as the average carbon atom. This points to a disordered orientation of the dichloromethane molecule in the cavity. The second crystallographically distinct dichloromethane molecule, which is not located in the pocket, is even more disordered.

Efforts are in progress to measure $^{13}\text{CO}_2$ binding to the iron(III) derivatives of the designed catalysts using ^{13}C -NMR spectroscopy. On the basis of the molecular modeling results we have chosen to carry out the experiments in the solvent benzene, which is less likely to spend time in the cavity. We wish to measure the decrease in the ^{13}C relaxation time (T_1) due to the interaction with the paramagnetic Fe(III) center in FeOETPP, when the porphyrin is added to a solution of benzene saturated with $^{13}\text{CO}_2$. The relaxation time decreases depending on how much time the $^{13}\text{CO}_2$ stays in the vicinity of the paramagnetic center and how close to the center the CO_2 comes. Thus, we expect to find a much larger decrease for FeOETPP, which has a binding cavity for CO_2 , than for Fe octamethyl-tetraphenylporphyrin (FeOMTPP), which is structurally very similar to FeOETPP but lacks a cavity in which CO_2 can bind. From Table III, note that the ordering of substrate binding energies is different when the cavity is lost for CuOMTPP. In particular, the benzene solvent binds more strongly than CO_2 for OMTTPs. Preliminary experiments in collaboration with R. A. Assink support these conclusions.

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REFERENCES

1. Medforth, C. J.; Berber, M. D.; Smith, K. M.; Shelnut, J. A., *Tetrahedron Letts.* **1990**, 31, 3719.
2. Shelnut, J. A.; Medforth, C. J.; Berber, M. D.; Barkigia, K. M.; Smith, K. M., *J. Am. Chem. Soc.* **1991**, 113, 4077.
3. Sparks, L. D.; Medforth, C. J.; Park, M.-S.; Chamberlain, J. R.; Ondrias, M. R.; Senge, M. O.; Smith, K. M.; Shelnut, J. A., *J. Am. Chem. Soc.*, 1992, submitted.
4. Mayo, S. L.; Olafson, B. D.; Goddard III, W. A., *J. Phys. Chem.* **1990**, 94, 8897.
5. Rappe, A. K.; Goddard III, W. A., *J. Phys. Chem.*, to be submitted.

TABLE I. Relative Substrate Binding Energies (kcal/mole) and Distance from Metal to Nearest Substrate Atom (Å) for Ni Octaethyl-Tetraphenylporphyrin.

Substrate	Total E	VDW	ES	Torsion	Angle	Bond	Invers.	Dist.*
methane	7.4	5.8	2.3	-1.9	0.3	0.7	0.0	3.88 C
benzene	10.4	9.6	0.0	0.5	0.2	0.0	0.0	3.37 C
CH ₂ Cl ₂	14.0	12.1	2.8	-2.1	0.0	1.2	0.0	3.17Cl
	12.7	9.7	4.4	-1.3	-0.5	0.4	0.0	3.73 C
CO ₂	14.3	8.9	5.7	-0.9	0.0	0.4	0.3	3.43 C
	13.9	9.1	6.1	-1.6	-0.1	0.4	0.0	3.20 C
CHCl ₃	17.1	13.0	5.1	-1.9	-0.2	1.2	0.0	3.23Cl

*Distance between the metal and the nearest heavy atom.

TABLE II. Relative Substrate Binding Energies (kcal/mole) and Distance from Metal to Nearest Substrate Atom (Å) for Cu Octaethyl-Tetraphenylporphyrin.

Substrate	Total E	VDW	ES	Torsion	Angle	Bond	Invers.	Dist.*
methane	5.5	4.6	2.0	-1.5	-0.2	0.7	-0.1	3.56 C
benzene	10.0	0.7	10.6	-1.6	-0.1	0.4	0.0	3.56 C
CH ₂ Cl ₂	12.5	7.0	3.8	1.6	-0.2	-0.2	0.4	3.65 C
	11.6	10.7	2.3	-1.3	-0.3	0.2	0.0	3.54Cl
CO ₂	12.8	6.7	5.4	-0.3	0.2	0.7	0.0	3.19 C
	12.7	9.8	7.2	-3.7	-0.8	0.5	-0.5	3.39 C
CHCl ₃	15.2	12.3	5.5	-2.6	-0.3	0.9	-0.6	3.29Cl

*Distance between the metal and the nearest heavy atom.

TABLE III. Relative Substrate Binding Energies (kcal/mole) and Distance from Metal to Nearest Substrate Atom (Å) for Ni Octamethyl-Tetraphenylporphyrin.

Substrate	Total E	VDW	ES	Torsion	Angle	Bond	Invers.	Dist.*
methane	4.4	3.2	1.0	0.1	0.3	-0.1	-0.1	3.48 C
benzene	10.9	10.2	0.1	0.1	0.4	0.2	-0.1	3.85 C
CH ₂ Cl ₂	10.1	8.5	2.2	-0.7	-0.3	0.5	-0.1	3.46 C
	10.1	8.6	2.5	-1.7	0.1	0.7	-0.1	3.57Cl
CO ₂	9.6	7.0	2.9	-0.2	-0.1	0.0	-0.1	3.13 O
	6.8	6.5	0.2	-0.9	-0.1	1.1	-0.1	3.24 C
CHCl ₃	11.9	9.2	3.8	-1.2	-0.4	0.5	-0.1	3.60Cl

*Distance between the metal and the nearest heavy atom.

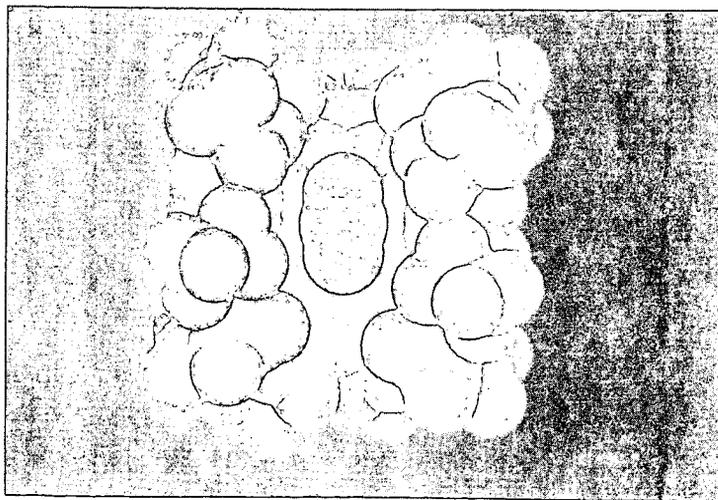
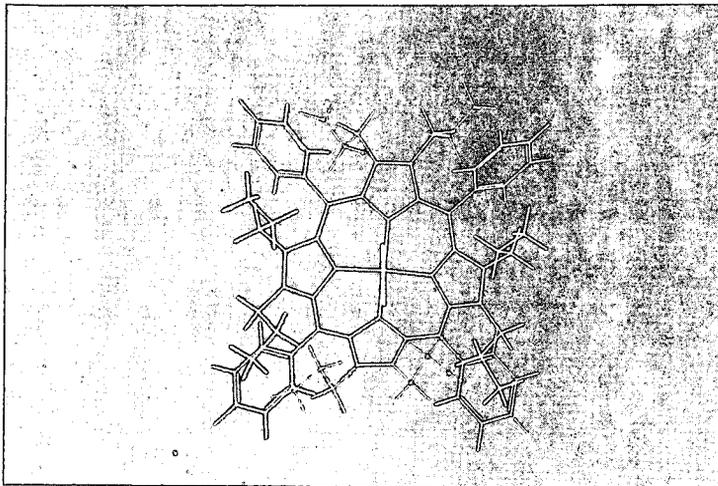
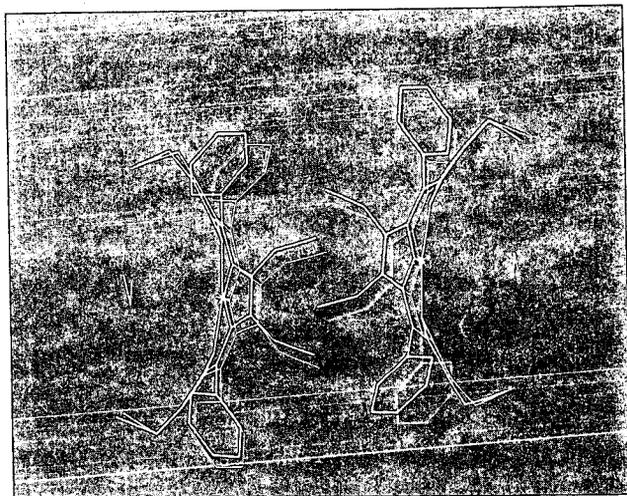
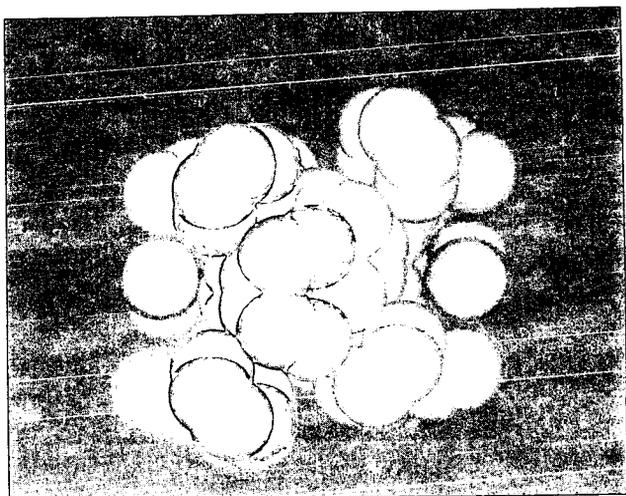


Figure 1. Energy-minimized structure of nickel(II) octapropyl-tetraphenylporphyrin (NiOPTP) showing CO₂ bound in the cavity formed by the porphyrin macrocycle and the quasi-axial ethyl groups. View looking down into the substrate binding cavity of NiOPTP where CO₂ is bound (center).



View of the X-ray crystal structure of copper(II) octaethyl-tetraethylporphyrin (CuOETTP) showing two molecules of CuOETTP and two dichloromethane molecules (top and bottom) in the binding cavity.

Figure 2.

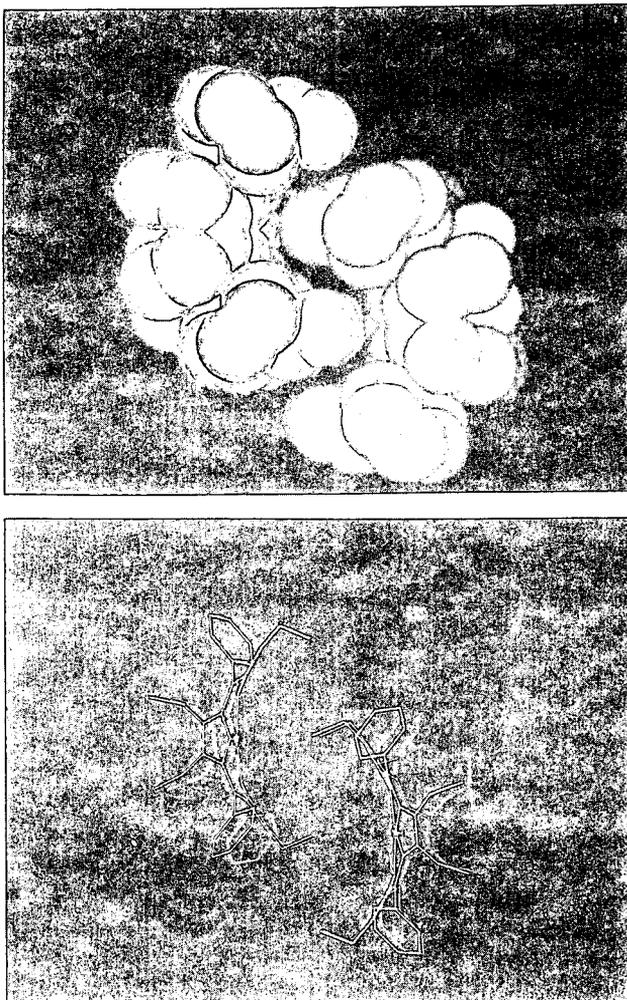


Figure 3. View of the X-ray crystal structure of copper(II) octaethyl-tetraphenylporphyrin (CuOETPP) showing two molecules of CuOETPP and two dichloromethane molecules (top and bottom) in the binding cavity. View is orthogonal to that of Figure 2.