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Inclusion Chemistry for the Modeling of Heme Proteins

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Abstract. Early attention to the modeling of heme proteins is enhancing the understanding of biochemistry. Those studies are also contributing to the development of techniques for the modeling of still more intricate, multifunctional, variously selective natural systems. Selectivity in simple systems may involve the molecular capability to bind only one of a family of related species or it may mean the ability to select and control one of a number of possible functions of a given bound species. Complicated systems simultaneously combine the two kinds of simple selectivities for two or more different classes of guest, often with synergistic interrelationships. The subject is developed around examples of binary, tertiary, and quarternary complexes designed to model the behavior of monooxygenases.

Key words. Selectivity, hemoglobin, myoglobin, O_2 transport, O_2 binding, O_2 carrier, cytochrome P450, synthetic enzymes, biomimics, molecular cavity, cyclidene, superstructure, lacuna, molecular design, host/guest complex, quarternary complex.

1. Introduction

Nature manages the intricate choreography of life processes through multilayers of variously coupled, highly selective molecular events. The chemist is presently learning to produce a plodding burlesque of that elegant molecular selectively. The active sites of heme proteins provide useful motivations for early learning experiences of this kind because of the relatively obvious and simple character of some of the selectivities involved. O_2 transport and storage and electron transport are the sole functions of the heme proteins hemoglobin, myoglobin and cytochrome c and these functions represent single specific chemical processes. Consequently, their biomimics constitute relatively straightforward synthetic goals. In contrast, enzymes such as cytochromes P450, and cytochrome c oxidase, the terminal member of the mitochrondrial electron transport chain, involve sequences of separately identifiable chemical processes, making duplication of their primary chemistry a much more complicated endeavor.

1.1. SELECTIVITY IN SIMPLE SYSTEMS

The work summarized here fits into the broad subject of molecular inclusion chemistry [1–5]. Cavities in molecules are tailored electronically and geometrically to the chemical purpose at hand. In simple systems, the goal might be selective coordination of metal ions or selective binding of small ligands to previously coordinated metal ions.

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Selective coordination of metal ions is most often understood to mean the action of the ligand to bind to one metal ion in preference to some other metal ion [6–14]. Such selectivity is important in separations chemistry [13–17] and in nature [18–20] and is fundamental to coordination chemistry [6, 10, 11, 17].

An equally important but different kind of selective coordination is emphasized here. It involves providing those ligand characteristics that *select the capabilities* of a particular metal ion when it is bound to the ligand [21]. This constitutes one aspect in the design of metal complexes capable of combining selectivity with such small ligand molecules as O_2, CO, H_2S, or CO_2. We discuss the requirements for O_2 carriers to illustrate these general principles.

1.2. SELECTIVITY IN COMPLICATED SYSTEMS

Complicated natural systems typify the need to implement the simultaneous selective binding of more than one species. The example of small molecule binding to previously bound metal ions constitutes the first step in that direction. Enzyme/substrate complexes illustrate the need to study the simultaneous, variously dependent or independent, binding of both metal ions and substrates within more complicated ligand systems. Homogeneous catalysis is a vast area in need of the implicit ability to organize molecules in a single multicomponent, yet specifically arranged complex. Other likely areas of interest are intricate biomimics, synthetic enzymes, molecular machines, and molecular switches.

1.3. CLASSES OF CAVITY EFFECTS

Figure 1 attempts to summarize the general concepts associated with the use of inclusion chemistry to produce selective binding for various chemical purposes. The

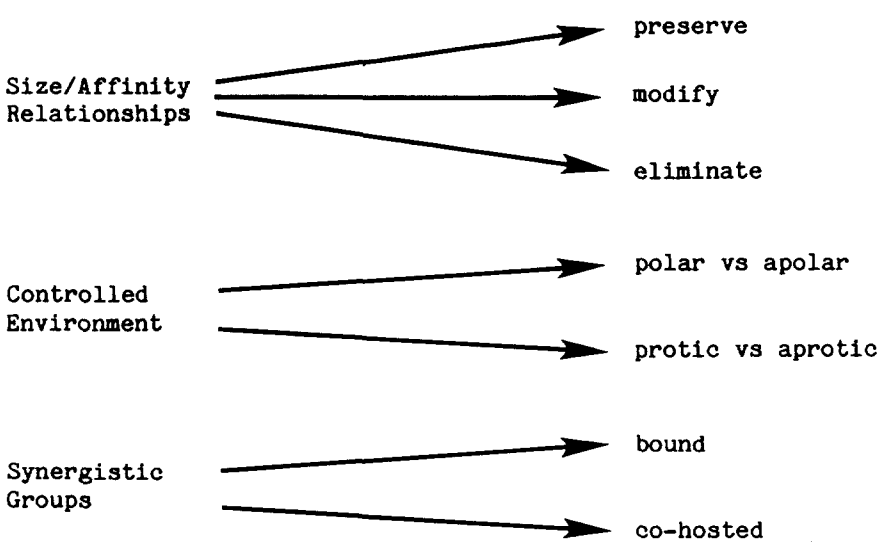

Fig. 1. Classes of cavity effects.

affinity a given host molecule displays toward individuals within a family of guest species will depend on size, as well as other, relationships. The affinity may be preserved without change, modified because of crowding or the advantages of well placed attracting groups, or the affinity might even be eliminated. The interior of a cavity may be polar or apolar and this may have a profound effect on the affinity, the stability, or the reactivity of a particular guest. Specific groups within the cavity may exert synergistic effects and such groups may be moieties bound to the cavity wall or they may be co-hosted molecular entities. Such groups may bind directly to a guest species (e.g., hydrogen bond to bound O_2), or they might deliver or extract protons to/from a guest.

2. Molecular Recognition in Transition Metal Systems

2.1. SMALL LIGAND BINDING

As pointed out by others [22–24] and emphasized in our work [25, 26], electronic criteria can be specified for the ability of cobalt(II) or iron(II) complexes to bind to O_2. In the case of iron, this is indicated by a proximity of the electrode potential for the iron(III)/iron(II) couple of the complex in question to that of the natural oxygen carriers [27, 28]. For cobalt, oxygen affinities have been related to the potential of the cobalt(III)/cobalt(II) couple [23]. Well established families of oxygen carriers are illustrated in Figure 2. The obvious topological requirement of such a ligand is to leave a site available for the binding of O_2 while providing a hospitable electronic environment. For both iron(II) and cobalt(II) derivatives [29, 30], it has been shown that coordination of a competing sixth ligand can prevent O_2 binding.

The traditional role of ligands has been to bind to the metal ion and control its electronic and topological properties, spin state, coordination number, stereochemistry, and extent of coordination saturation [31–34]. Modern research expands the role of the ligand by appending additional moieties to the ligand [21, 35]. The added structural components are described collectively as *superstructure* [35]. For small ligand binding, the appended superstructure is used to provide a protected cavity, called a *lacuna* [36], within which the small target molecule can coordinate to the metal ion. Lacunar porphyrins [2, 37–43] present a fascinating array of structures as shown in Figure 3. These lacuna promote selective binding of the ligand of choice. Further, they serve to determine the immediate environment of the bound ligand (O_2) and to limit the interactions between the bound ligand and other molecular species. This, in turn, can have profound effects on the reactions of the bound group.

The early examples of superstructured porphyrins, the capped [40] and picket fence porphyrins [44, 45], showed that superstructures can select against the binding of large base molecules [46–48], leaving the cavity available for binding to small molecules. It has long been believed that the lesser selectivity of hemoglobin and myoglobin for CO over O_2, when compared to free porphyrins, derives from the relative steric suitability for O_2 of the vacant space within which the small ligand must bind (*vide infra*) [49, 50].

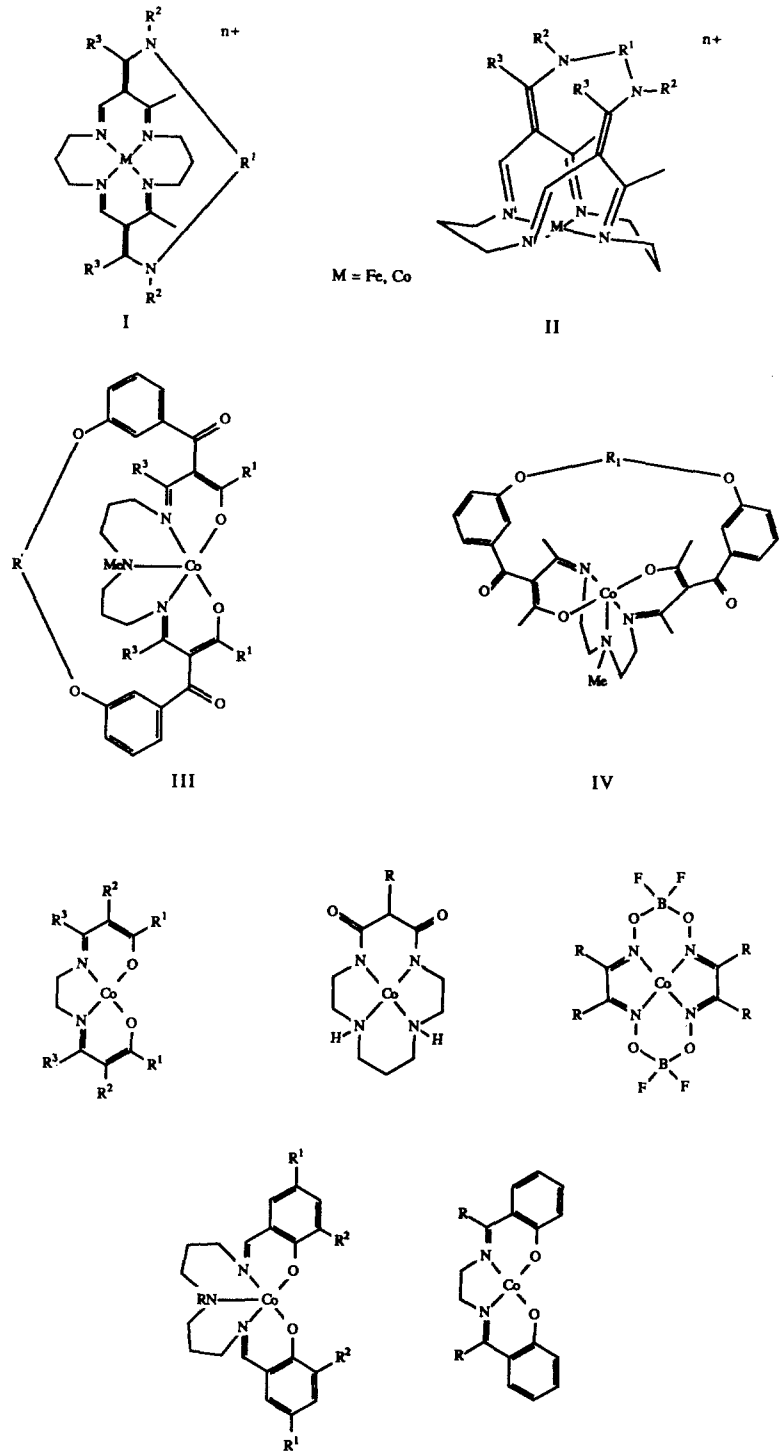

Fig. 2. Some transition metal complexes which bind dioxygen reversibly.

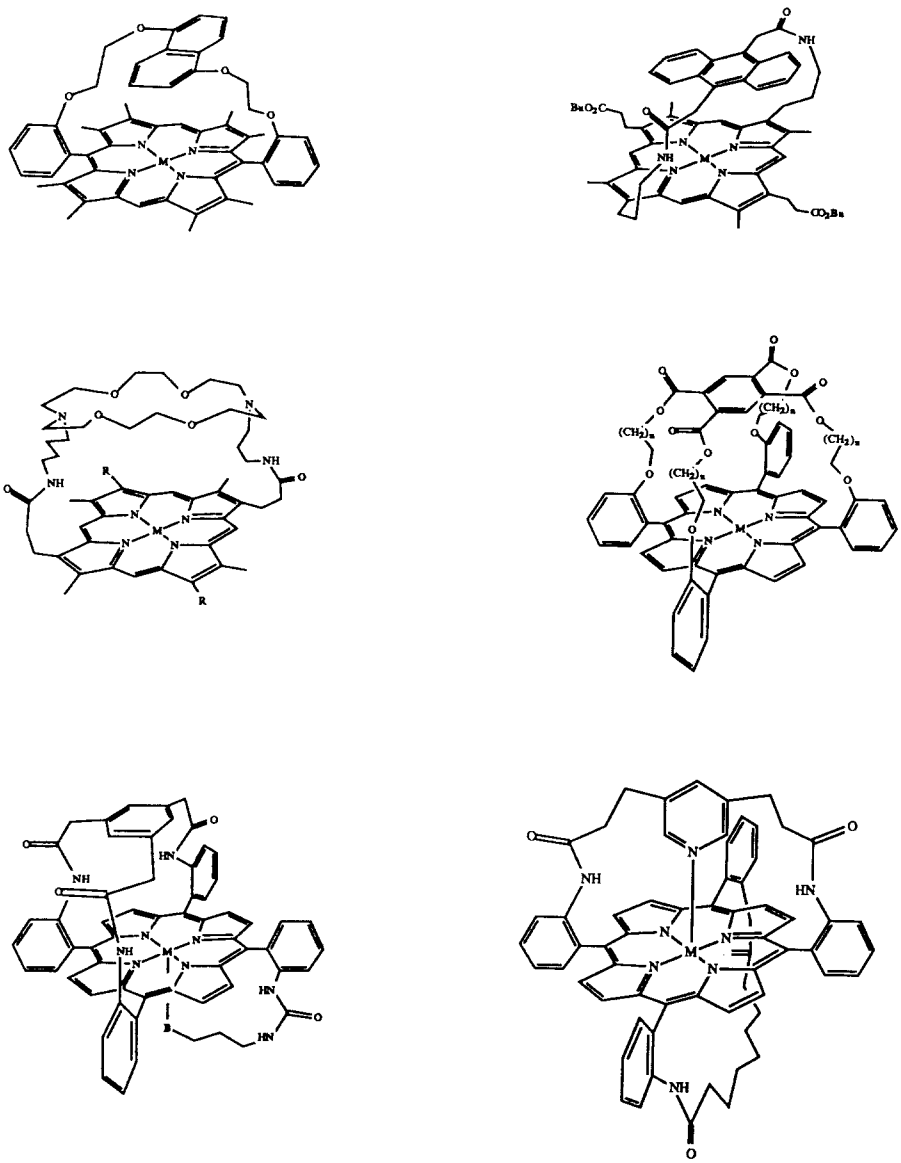

Fig. 3. Superstructured porphyrin complexes.

Many possible uses have been proposed for transition metal O_2 carriers [25, 26]. These applications require the capability of controlling certain critical properties [51], call them engineering parameters, of the oxygen carrier. For example, the partial pressures of O_2 at which the oxygen binds to or is released by the transition metal atom depends on the equilibrium constant for the binding process. Further, the rate of oxygen release from the metal complex is important in order to maximize the oxygen flux in a gaseous stream when an oxygen carrier is used to separate oxygen from air.

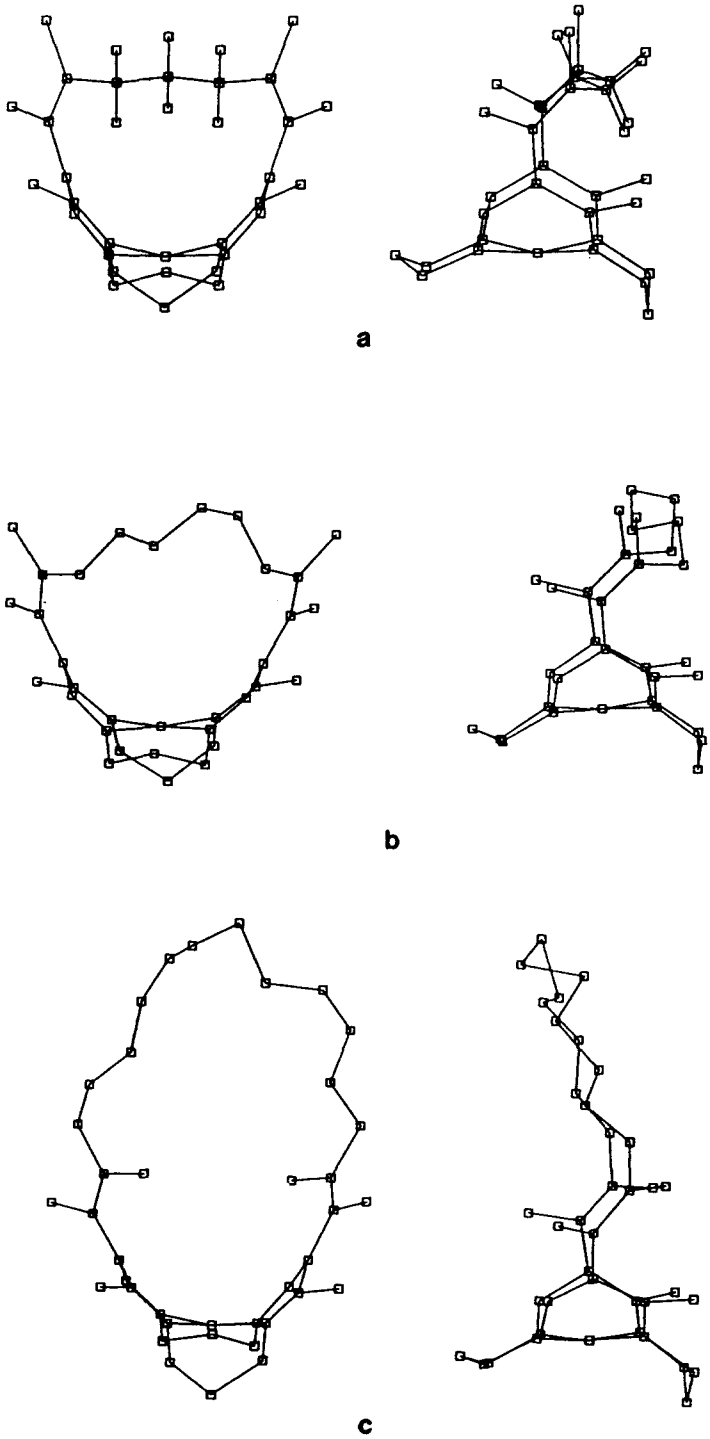

Fig. 4. Lacunar cyclidene complexes:
(a) $[Cu(Me,Me,C_3,cyclidene)]^{2+}$, (b) $[Co(Me,Me,C_6,cyclidene)]^{2+}$, (c) $[Ni(Me,Me,C_{12},cyclidene)]^{2+}$.

Both electronic and steric means can be used to modify and control the oxygen affinity of a metal complex. The lacunar cyclidene complexes [28, 52], structures **I** and **II** (Figure 2), bind O_2 in a cavity whose size is controlled by the size and orientation of a bridging group, R^1 (Figure 4) [25, 26, 28, 55]. The short trimethylene bridge so constricts the cavity that oxygen is not bound [54, 55]. Successively adding methylene groups from 4 through 7 produces a steady increase in O_2 affinity (Figure 5) [25, 53]. Thereafter, the O_2 affinity remains constant, indicating that the intrinsic affinity of the cyclidene ligands has been achieved; the smaller cavities decrease the affinity through steric constraint. Thus, it is possible to select the O_2 affinity as well as select against larger competing ligands. In fact, the cavity can be closed to all ligands, no matter how small.

Figure 6 shows the structures of lacunar complexes having small ligands in their cavities [56–59]. The O_2 adduct is easily formed because the cavity shape favors a small ligand that binds in an angular fashion (Figure 6b). In contrast, the thiocyanate ligand, that normally tends to be approximately linear when bound through nitrogen to cobalt(III), is forced into a distorted angular structure (Figure 6a).

The design characteristics of the lacunar cyclidene complexes can be incorporated into the structures of other better known oxygen carrier ligands. The Schiff base ligands derived from β-diketones [23, 60, 61] have been modified with the addition of a lacuna [62] and the optimal derivative shown in structures **III** and **IV**, Figure 2, also has a built-in axial ligand [63]. Special risers are built into the bridging group since this parent tetradentate ligand is basically planar.

The cyclidene complexes, structures **I** and **II**, Figure 2, have provided the only well established examples of nonporphyrin iron(II) dioxygen carriers [26, 28, 52]. As the examples in Figure 7 show, depending on the substituent on the bridge

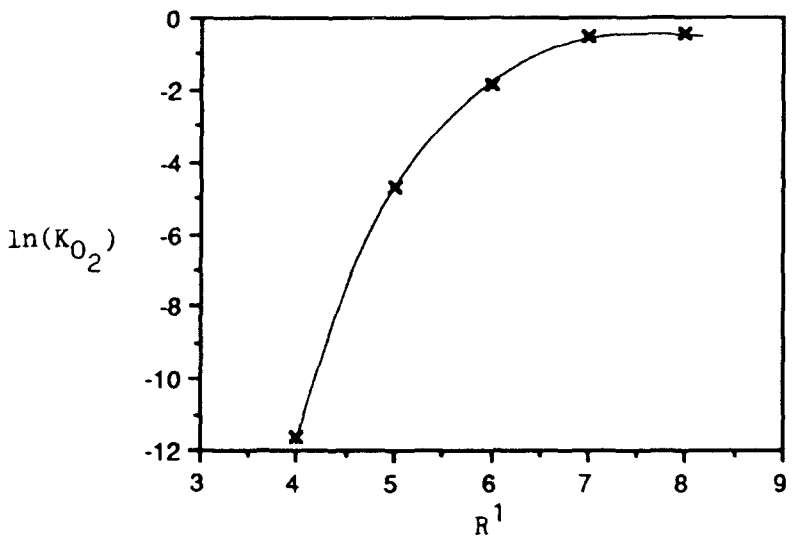

Fig. 5. Dependence of dioxygen affinity on bridge length for $[Co(Me,Me,R^1,cyclidene)]^{2+}$ at 20°C in acetonitrile containing 1.5 M 1-MeIm.

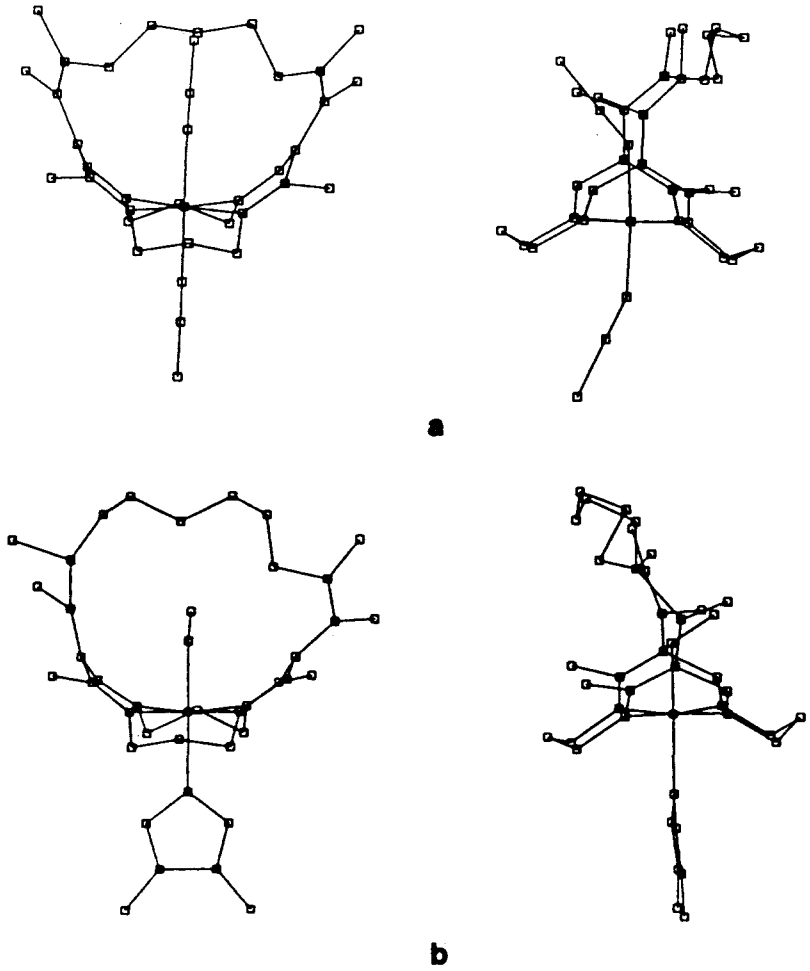

Fig. 6. Orientations of coordinated ligands for cobalt(II) cyclidene complexes: (a) $[Co(Me,Me,C_6,cyclidene)(NCS)_2]^+$, (b) $[Co(Me,Me,C_6,cyclidene)O_2MeIm]^{2+}$.

nitrogen atoms, the cavity is either tall and narrow or short and wide. Effectively, the tall cavity accommodates the linearly coordinated CO molecule without change. However, the short, wide cavity must be greatly changed if CO is to be coordinated (Figure 8). The relative fits are reflected in the values of the equilibrium constants for CO binding to the two iron(II) complexes (Table I) [59, 64]. Obviously, the configuration of the cavity of these lacunar complexes can rearrange substantially in order to accept the guest species.

Examples discussed earlier in other contexts show how the cavity shape is changed upon entry of the small ligand (guest). The structure of the hexamethylene bridged cyclidene complex (Figure 4b) shows that the central two methylene groups of the chain fold back into the cavity, rather like the tail of a scorpion [57]. In contrast, as shown in Figure 6b, when a small ligand goes into the same cavity, this portion of the bridge swings up and away from the cavity into another favorable bridge conformation [57].

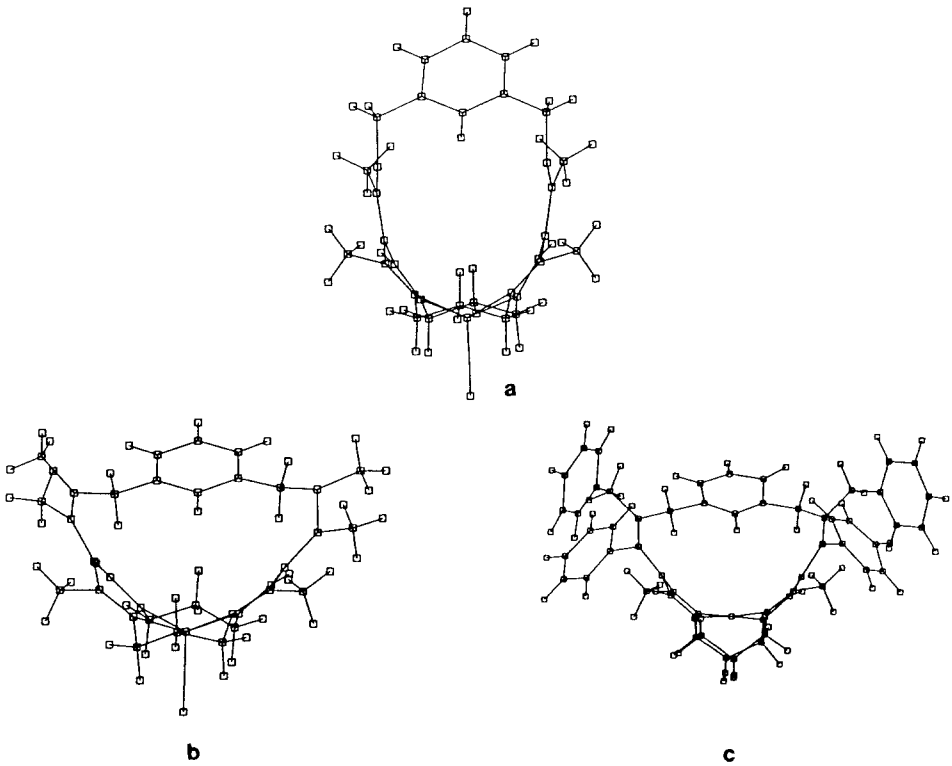

Fig. 7. Dependence of cavity shape on substituents for lacunar cyclidenes: (a) [Fe(Me,H,*m*-xyl,cyclidene)Cl]$^+$, (b) [Fe(Me,Me,*m*-xyl,cyclidene)Cl]$^+$, (c) [Ni(Ph,Bz,*m*-xyl,cyclidene)]$^{2+}$.

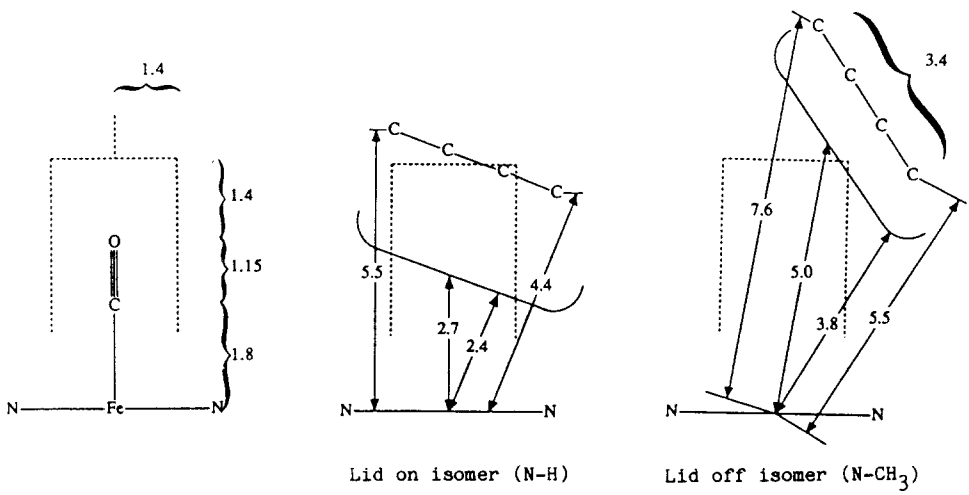

Fig. 8. Cavity sizes and the accommodation for CO in lacunar cyclidene complexes (distances reported in angstrom units).

Table I. Equilibrium constants for CO binding to iron(II) lacunar cyclidene complexes in acetonitrile at 0°C.

$[Cl^-]$, M	[Fe(Me,Me,*m*-xyl,cyclidene)]$^{2+}$	[Fe(Me,H,*m*-xyl,cyclidene)]$^{2+}$
0	4.27×10^{-1}	–
8×10^{-3}	2.5×10^{-2}	0.5
1.0×10^{-3}	1.2×10^{-3}	1.0×10^{-2}

The two *m*-xylylene bridged iron(II) complexes having the short, wide cavities (Figure 7) provide essentially identical space for small ligand binding; however, the relative rates of oxidation by O_2 of the two complexes differ by a factor of 10^4 [26, 28, 55]. The complex having only methyl groups flanking the cavity oxidizes with a half life of about an hour at -25°C while the species having the bulky phenyl and benzyl groups in the same positions oxidizes with an estimated half life of about 2 years under the same conditions. In fact, the latter species has a half life at ambient conditions of many hours. It has been proposed that the mechanism of autoxidation of these complexes involves a competition between O_2 binding and electron transfer when the O_2 molecule approaches the iron(II) complex [26, 55, 65]. In this context, the extra bulk appears to greatly impede the electron transfer process. This remarkable steric effect shows yet another way in which superstructure can help control the behavior of molecules.

2.2. SIMULTANEOUS METAL ION AND SUBSTRATE BINDING

The capability to simultaneously form inclusion complexes with organic molecules through the influence of hydrophobic interactions, was added to the superstructured cyclidene ligands by enlarging the permanent void (Figure 9) [66, 67]. The expected regiospecific mode of binding is shown in Figure 10.

The copper(II) complex was selected because it has a single unpaired electron, making it useful for the nuclear magnetic resonance technique used in these studies [68, 71]. The magnetic field due to the unpaired electron on the metal center affects the rates of relaxation of the protons of the organic *guest* molecule that invades the permanent cavity of the ligand. Because there are no bonds between the copper(II) atom and any part of the guest molecule, the rate of this relaxation process has a straightforward dependence on the distance between the metal atom and the protons in question [69, 70]. Thus, one can map the position of the guest, with respect to the metal atom, in these systems.

The data in Table II show that the α protons, and therefore the hydroxyl group, of an alcohol are furthest from the metal ion [71]. At the same time, the ω protons of the alcohol are nearest the metal center. Thus, the alcohols all enter into host/guest complexation regiospecifically. Also since the OH group is at about the same distance from the metal ion in all cases, it must remain in the solvent sheath of the host molecule. Thus, the binding arises from hydrophobic relationships involving the alkyl group of the alcohol and the hydrophobic interior of the cavity.

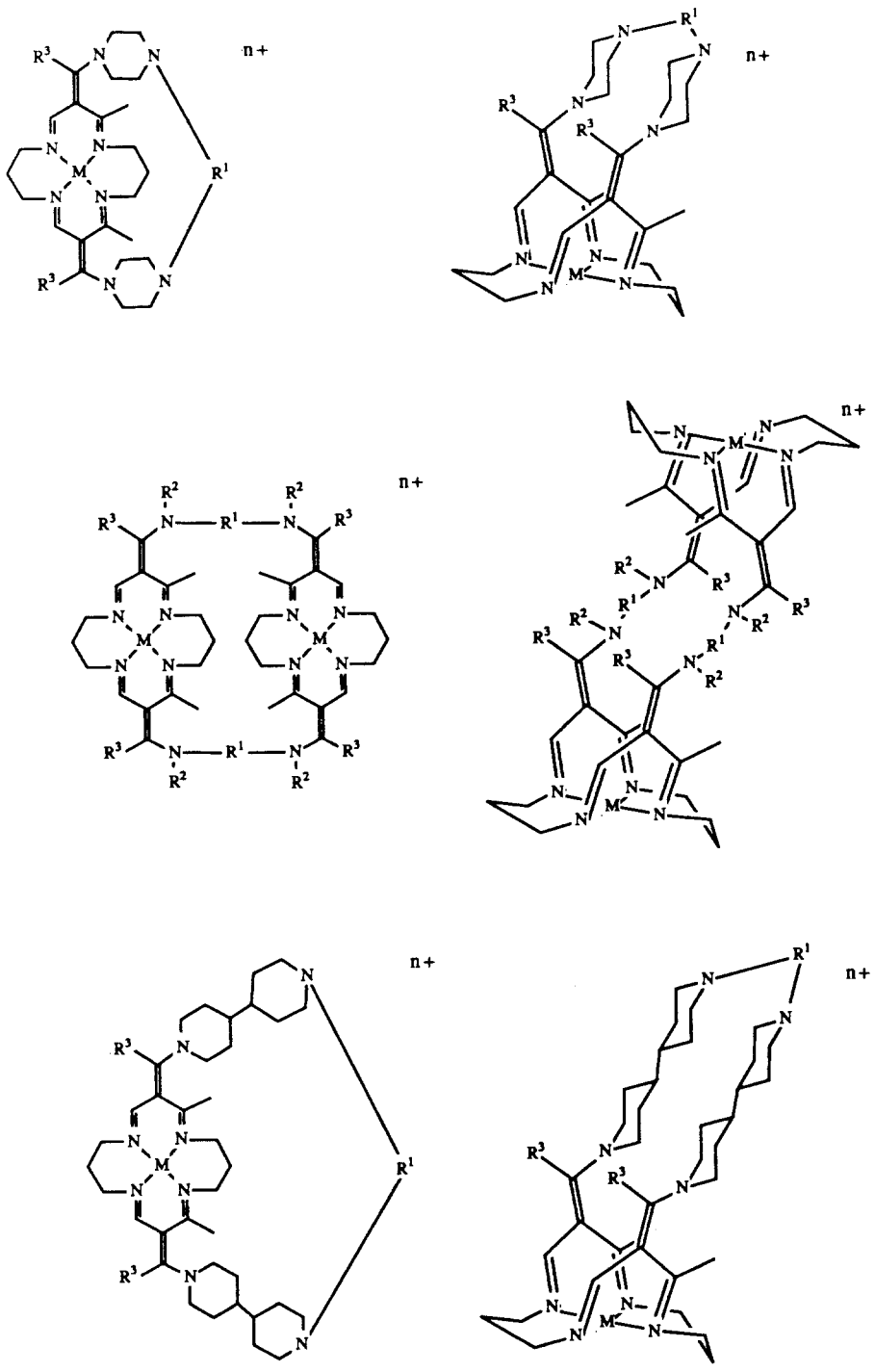

Fig. 9. Cyclidene complexes containing large cavities for guest binding.

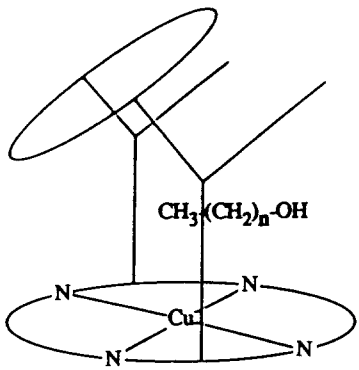

Fig. 10. Schematic representation of guest binding to a copper(II) lacunar cyclidene complex.

Combining the results of the solution NMR studies on the guest/host complexes with the X-ray determined coordinates for the atoms comprising the host molecule, it is possible to use modern computer graphics and molecular mechanics to produce reasonable images of these species [71]. Figure 11 shows the results which indicate that the guest molecule resides near the top of the cavity, leaving ample space for the binding of O_2 in case the metal ion were appropriate for that reaction, as well.

Corresponding measurements with phenols in place of the alcohols but using the same host molecules revealed that the cavity in this cyclidene complex is too small to accommodate a benzene ring (Figure 12) [66]. Replacing the piperazine riser in the host molecule by a bipiperidine moiety produces a much larger cavity that accommodates the phenols very easily [72].

Table II. Calculated distances for various protons in several alcohols for 'vaulted' cyclidene hosts (^{1}H NMR $\nu = 300$ MHz; [Host] $= 6.48 \times 10^{-5}$ M; [guest] $= 10^{-2}$ M).

	distance, Å			
alcohols	α	β	γ	δ
CH_3OH	8.6			
CH_3CH_2OH	8.4	7.5		
$CH_3CH_2CH_2OH$	9.0	8.2	7.5	
$CH_3CH_2CH_2CH_2OH$	9.0	7.7	7.4	6.6
average	8.8 ± 3	7.8 ± 3	7.5	6.6
t-$(CH_3)_3COH$		7.4		
i-$(CH_3)_2CHOH$	9.2	8.2		
$CH_2{=}C(CH_3)CH_2OH$	9.0		7.6	
		(7.2)		
overall average	8.9 ± 2	7.8 ± 3	7.4 ± 1	(6.6)

3.1 Multisite Hosts Containing Transition Metal Ions

The concept that a single ligand, of admittedly somewhat complicated design, might organize several molecular entities within a single guest/host/coordination entity is particularly intriguing. Models that exist in nature are enzyme/cofactor/substrate

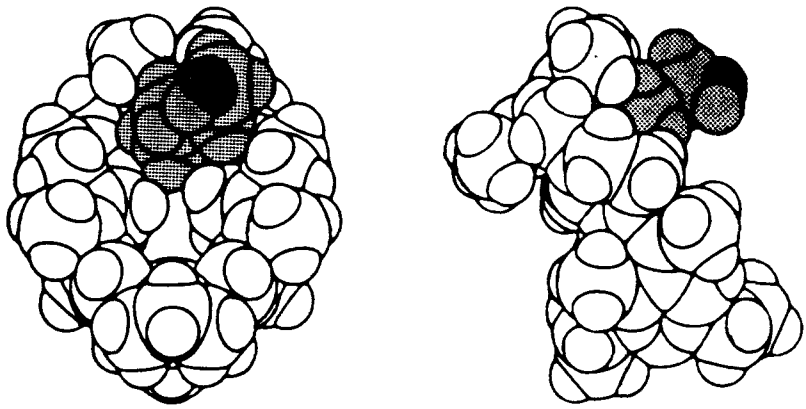

Fig. 11. Binding of *n*-BuOH to a 'vaulted' cyclidene complex.

complexes, or the complicated clusters of proteins that are associated with some functions, e.g., cytochrome c oxidase. Relatively unimaginative examples of the various classes of species that might be involved are given in Figure 13. In fact, if chemists are to gain control over intricate molecular processes, then some control must be gained over the organization of molecules during the course of the crucial events. The coordination template effect [73] constituted the first example of such control, but it was concerned with only a single kind of organizational process. The range of possibilities for organization of molecular events within multisite guest/host chemistry appears limitless. Again, one thinks of biomimicry, catalysis, and molecular machines, including molecular switches.

Here we consider a rare example of a quaternary complex in which four separate, distinctly different molecular species reside simultaneously within a single complex [74]. The general concept, shown in Figure 14, is a model for the so-called *ternary complex* of cytochromes P450 [75]. This is a misnomer since the complex is actually quaternary; i.e., it involves the enzyme protein, the heme prosthetic group, the substrate, and the O_2 cofactor. Our complex involves the *vaulted* cyclidene ligand, the cobalt ion, the O_2 molecule and a guest molecule [74].

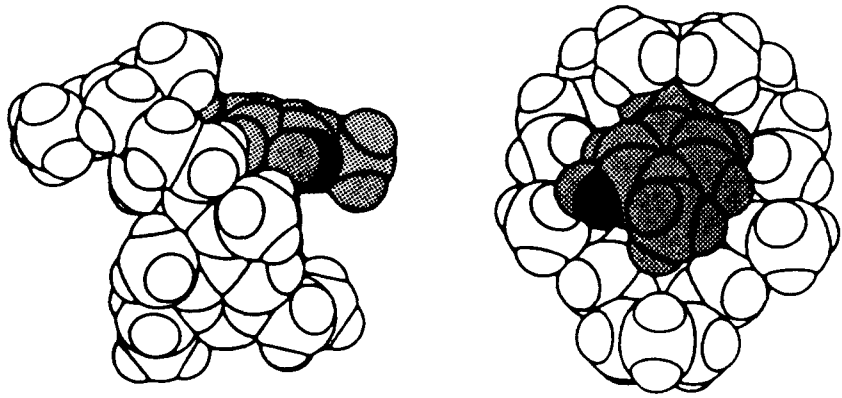

Fig. 12. Binding of 2,5-dimethylphenol to a 'vaulted' cyclidene complex.

Probable Guest Species—
- Transition metal ion
- Substrate molecule
- Cofactor or cofactors

Examples of Metal Ions—
- Redox enzyme models: Fe, Co, Mn, Cu
- Solvolytic enzyme models: Zn, Co, Mg, Mn

Examples of Substrates—
- Select linear hydrocarbons for oxidation
- Select ester or amide groups for hydrolysis

Examples of Cofactors—
- O_2 binding for monooxygenase models
- Nucleophile binding for esterase model

Fig. 13. Multisite hosts containing transition metal ions.

Most of the properties favorable to reversible O_2 binding have been preserved or redesigned into the ligand molecule used in this work. The critical experiments involve the same kinds of measurements used in studying guest/host complexes as described above [68, 71, 74]. However, they differ in that the dioxygen complex of the cobalt/cyclidene complex is used to define the regiospecific binding of the substrate molecule.

The cobalt(II) complexes of the cyclidene ligands are low spin and excellent O_2 carriers [25, 53]. Their O_2 adducts are typical for such cobalt derivatives and have a single unpaired electron, that is localized mainly on the O_2 moiety. Thus, the unpaired electron of the bound O_2 can be used to probe the protons of the

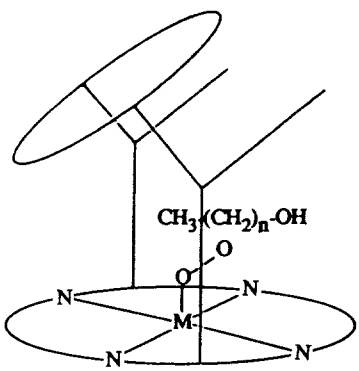

Fig. 14. Model for the ternary complex of cytochromes P450.

guest/host molecule. A difficulty is associated with the fact that the delocalization of the electron deviates from the simple model used previously to calculate distances on the basis of magnetically accelerated relaxation rates (as described above) [69, 70]. However, the very fact that this system gives the same effect on guest proton relaxation rates is proof of the presence of the guest within the cavity of the host molecule [74].

Additional problems that had to be confronted derived from the stability of the O_2 adduct. The great stability of the cobalt/cyclidene/O_2 adducts depends on the presence of a reasonably small lacuna in the structure [25, 53]. The vaulted complexes have very large cavities and the resulting cobalt/O_2 adducts autoxidize relatively rapidly. Consequently, it was necessary to study the quaternary complex at the lowest possible temperature; 1°C for the solvent D_2O. This assured the saturation of the O_2 forming equilibrium so that only a single NMR relaxing agent was present in the solvent. At the same time, the lower temperature reduced the quality of the measurement to some extent.

Within the limits of the measurements, the results show unequivocally that the cobalt(II)/O_2 complex is serving as host for the organic guest molecule. The calculated distances between the center of electron spin density and the protons are shorter than in the cases where the electron is confined to a metal ion. This is expected since the O_2 moiety resides between the metal ion site and the guest site. The limitations described advise against quantitative interpretation of the difference [53].

Acknowledgement

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