INORGANIC SYNTHETIC OXYGEN CARRIERS TO TEST THE ENTATIC STATE HYPOTHESIS

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CONTENTS

A C K N O W L E D G E M E N T

It is my pleasure to extend my deep gratitude to Professor D.J. Sardella, of the Chemistry Department, Boston College, for his willingness to serve as my mentor and for his copious donation of time and advice.

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I. INTRODUCTION

In 1938 Tsumaki noted that tetradentate Schiff-Base chelates of Cobalt darkened in air. His discovery was followed up by much research on the nature of the metal-oxygen binding, especially after Basolo showed that the bond was formed reversibly, thus demonstrating the biological significance.

In 1967, Vallee and Williams proposed a mechanism which very resonably helped to elucidate the enzymatic mechanism of rate enhancement. Their theory, known as the Entatic State Hypothesis, pertains to the chemistry of enzymes on the level of electronic structure. Standard methods of studying proteins, such as optical rotary dispersion, ultracentrifugation, and X-ray crystallography, are not sensitive to this level.

This thesis couples these two lines of research. In the THEORY section, findings from both lines are discussed in detail, with some relevant chemical background, and it is shown that inorganic synthetic oxygen carriers can be used as models to test the Entatic State Hypothesis.

The object of this research was to fabricate a new series of model complexes and use these to gather information to support or refute the hypothesis. The unexpected complexity of the reactions and the need to carefully monitor reaction conditions did not allow us to complete all of our intended experiments.

II. THEORY

A. Enzymes and Rate Enhancement

An understanding of enzyme reaction mechanisms is one of the most fruitful lines of research in biological chemistry. Nearly all cellular syntheses and degradations are accomplished in a large number of small steps, each catalyzed by a protein molecule known as an enzyme. Enzymes can accomplish rapidly in the mild conditions of the cell, and with high yield, reactions which would otherwise take hours, even at conditions much harsher than physiological.

Moreover, enzymes can be rigorously specific with regard to a substrate. The specificity of enzymes affords the cell an opportunity to control the rates of reactions by alternately inhibiting or activating enzyme activity. (Several models which explain this control have been expostulated.) Of particular interest to biochemists is the possibility of exploiting these properties of enzymes to artificially control cellular reactions, and thus aid in the treatment of many illnesses.

We know that an enzyme has two relatively small sites of importance. One is the binding site, to which a particular substrate can cling. The other is the so called catalytic site, which achieves a rate enhancement of up to 10^{20} for the reaction to be catalyzed. The central problem of enzyme theory is to account for this remarkably intense rate enhancement.

Four major factors contribute to these rate accelerations. The first is that the active site of an enzyme provides <u>orbital steering</u>, lining up the bonding orbitals of substrate and enzyme in a specific relationship which aids bond making and bond breaking.

Another is the formation of an enzyme - substrate intermediate which has a high probability of entering the transition state and thus lowers the energy barrier to reaction. A third is by providing functional groups at the active site which are capable of acting as general acid-base catalysts. These factors have been studied in detail by such workers as Jencks. Jencks was able to assign a specific amount of rate enhancement to be expected from each of these factors. However, he was only able to account for a rate enhancement of 10^8. Apparently, these organic chemical considerations alone cannot explain enzyme action.

The remaining rate enhancement has been attributed to the fourth factor, that of the contribution from the large portion of the enzyme which is not part of the active site. It can be hypothesized that the protein chains of an enzyme induce strain in both the susceptible bond and in the enzyme, allowing them to move into the transition state more readily.

The first theory to take into account parts of the molecule other than the active site was Koshland's Induced Fit model. Previously, enzymes were considered to have rigid active sites into which a rigid substrate would precisely fit much as the way a key fits a lock. Because of contradictions which arose from assuming the active site to be rigid, Koshland proposed that the chains of the enzyme shifted around to fabricate the active site when the substrate was binding.

Vallee and Williams have also proposed a theory which accounts for the function of the rest of the large enzyme and throws light on rate enhancement not explained by standard organic chemistry. Their theory is not incompatible with that of Koshland. The theory is based on the observations that selective chemical

modifications of specific amino acid side chains of proteins have indicated that particular amino acid residues are exceptionally reactive toward certain reagents, when compared with analogous model complexes or, even more significantly, with catalytically inert regions of the protein. Can this unusual reactivity be attributed to structural or electronic abnormalities of the side chains?

Metalloenzymes (as distinct from enzymes which do not contain a stoichiometrically bound metal at the active site) offer an opportunity to probe the physicochemical properties of enzymes and to determine if these atypical sites exist. The electron environments of metal ions can be elucidated through a systematic use of absorption and EPR spectra, magnetic susceptibility studies, and redox potentials. Unusual electronic structures were indeed found.

In order to proceed further it will be necessary to define some terms more precisely and to review what is known about the electronic structure of transition metal ions.

B. Transition Metal Electronic Structure

a. Lift of Degeneracy. Two classes of metals are known to be important biologically. The main group or free ions, such as Na^{+}, K^{+}, Ca^{+2}, and Mg^{+2}, and those of the first transition series, which are found bound to proteins. A metalloenzyme is such a protein. These enzymes depend on a metal ion, called a cofactor, for their activity. Their protein molecules are termed ligands, and the ligand-metal combination is referred to as a chelate, or complex. The electronic structure of the metal, upon which our understanding of the chemistry is based, depends upon the spatial arrangement of the ligands surrounding the metal.

The distinguishing characteristic of the first transition series is that its members contain partially filled 3d orbitals. The five d-orbitals are degenerate if the metal is isolated. However, ligand atoms act to remove the degeneracy. This can be understood if we imagine a transition metal cation to be surrounded by six anions (assumed for simplicity to be point charges in an octahedral arrangement.) Remembering the spatial arrangement of the five d-orbitals, it is clear that a single electron would prefer to enter an orbital that points in between these point charges, rather than to enter an orbital whose main axis lies in the direction of the negative point charges. Thus the d_{z2} and d_{x^2-y2} orbitals (e_g set) are higher in energy than the d_{xy}, d_{yz}, and d_{xz} orbitals (the t_{2g} set), based on the ease with which an electron will enter them.

A set of four negative point charges arranged in a tetrahedron will also lift the degeneracy of the five d-orbitals. In this case the t_{2g} set will have higher energy than the equivalent e_g set.

If we remember that the rise in energy of one set of d-orbital must be matched by the decrease in energy of the other set, and using the symbols $\triangle_o$ and $\triangle_t$ to represent the energy difference between the t_{2g} and e_g sets for the octahedral and tetrahedral cases, respectively, we can draw the following well-known diagram:

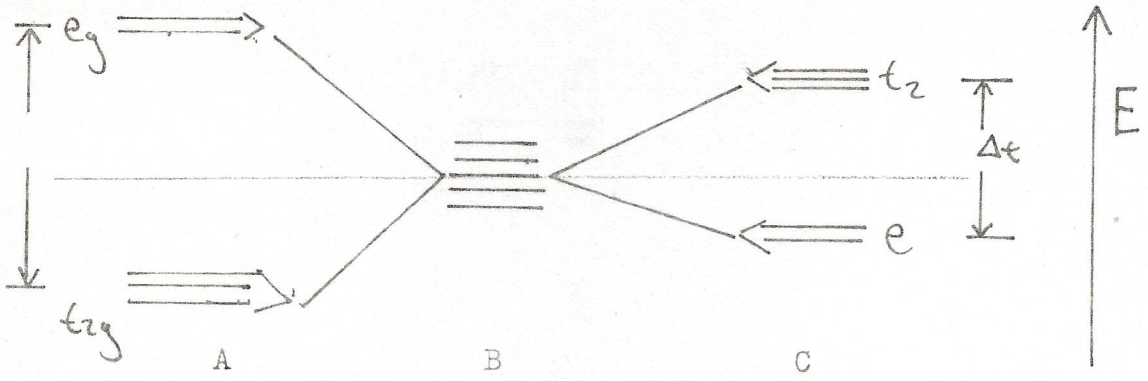

Fig. 1. Lifting of degeneracy in a crystal field is depicted. A. Octahedral field. B. Five degenerate 3d orbitals. C. Tetrahedral field.

Tetragonally distorted octahedral complexes and square planar complexes can also be treated in transition metal electronic structure theories. A tetragonally distorted octahedral complex is one in which the two z-axis ligands are drawn away, with the limiting case of a planar complex being reached when the z-axis ligands have dissociated from the metal. The effect of these distortions is to lift the degeneracy of the e and t orbitals, as indicated in the diagram below.

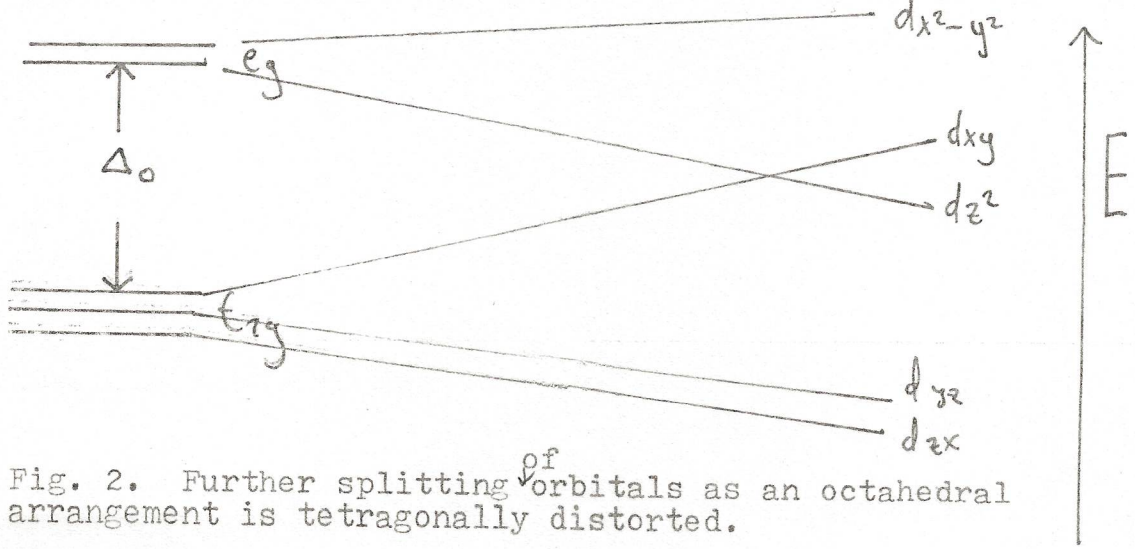

Fig. 2. Further splitting of orbitals as an octahedral arrangement is tetragonally distorted.

b. Absorption and Magnetism. This discussion is a simple, yet highly useful formalism known as Crystal Field Theory. Two consequences of the departure from the five-fold d-orbital degeneracy are important to this discussion: 1) magnetic moments and spin states,,and 2) absorption spectra.

The importance of absorption spectra can be exemplified using the tetrahedral case. In this instance, electrons will tend to occupy the e_g set of orbitals, for they are of lower energy (Fig. 1).. It is possible for an electron in the lower set of orbitals to capture a quantum of energy and be excited to a higher-energy t_{2g} orbital.

Since both the t_{2g} and e_g sets are d-orbitals, this electron motion is known as a d-d transition. An absorption band will result for each such electronic transition. By analyzing the position, breadth, and intensity of the absorption, it is possible in principle to deduce the initial and final orbitals of the electron. Information concerning the electronic structure can be inferred from these transition assignments. Both tetrahedral and octahedral Co(II) complexes, for example, have three "spin allowed"* electronic transitions which are qualitatively identical. However, because the two cases differ quantitatively, the intensity of absorption in the visible and near-infrared regions can be assigned to their unique d-d transitions. Thus, the tetrahedral state can be distinguished

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* a discussion of this term is beyond the scope of this paper.

from the octahedral state.

Magnetism is also of outstanding importance in transition metal chemistry. For our purposes, it will be sufficient to review the two most important types; paramagnetism (presence of unpaired electrons) and diamagnetism (the absence of unpaired electrons) and relate these to the so called high and low spin states. Most magnetic behavior is traceable to the number and distribution of electrons within the nondegenerate d-orbitals just discussed.

Specifically, we wish to determine how many unpaired electrons are present. A primary consideration is that electron pairing requires energy and is thus unfavored. We should note that two electrons sharing the same orbital will repel each other. Thus, electrons will tend to spread themselves out in the available orbitals. This tendency is known as Hunds' Rule. Of course, because the five d-orbitals in a crystal field are not degenerate, electrons must also choose between settling in a lower energy orbital or higher energy one. The actual position of the electron will depend upon whether the pairing energy is greater or less than the energy difference between the two sets of orbitals.

The configuration in which a maximum number of unpaired electrons is of lowest energy is known as high spin; that in which the minimum number is most stable is low spin.

For ions containing 1,2,3,8,9, or 10 electrons in an octahedral environment, the number of unpaired electrons is fixed. For those containing 4,5,6, or 7 electrons, both high- and low-spin states are possible. Which of these will actually

occur depends on the magnitude of $\triangle_o$, which in turn depends on the nature of the ligands. In a tetrahedral environment, $\triangle_t$ is so small that the pairing energy is the dominant force, and all such complexes are high spin.

Since Co(II) and Co(III) have 7 and 6 electrons in the third orbital, respectively, they can, in an octahedral environment, be either high or low spin. The biological significance of the spin state can be illustrated for the case of Fe in Hemoglobin, which will be discussed shortly.

C. Entatic State Hypothesis

We now return to the anomalies observed by Vallee and Williams, who found unusual electronic spectra for a number of metalloenzymes. The absorption spectra of the "Copper blue enzymes", for example, differ strikingly in intensity and fine structure from the spectra of any known copper protein complexes (not enzymes) and copper complex ions. Cobalt substituted for zinc* in the hydrolytic enzyme Carboxypeptidase A, shows activity and spectral bands which differ significantly from those of model cobalt complexes. These exceptional spectra signify an atypical electronic structure for metal ions at the catalytic sites of enzymes.

Correspondingly, EPR spectra of metalloenzymes such as Pseudomonas aeuruginosa blue and Laccase have indicated an unusual

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* zinc, with 10 d-electrons, has filled e_g and t_{2g} orbitals so no electronic transitions are possible. Substitution by Co^{2+} retains enzyme activity while permitting spectroscopic observations.

electronic distortion about the cupric and ferric ions. Also, redox potentials of "copper blue enzymes" are much higher than those of all simple copper complexes, except distorted complexes. Redox data for the redox proteins cytochromes indicate that the protein environment destabilizes the Fe(III) with respect to Fe(II) to a degree unknown in inorganic complexes.

In addition, findings from other studies (optical rotatory dispersion, magnetic suseptibilities, ligand binding constants) also support the implication of an unusual electronic structure around the metal in metalloenzymes. By analogy this unusual situation can be extended to the active site of nonmetalloenzymes, for which one cannot obtain data of this type.

Vallee and Williams constructed a system by which this unusual electronic structure could explain rate enhancement. They recalled that the electronic structure of simple metal complexes is in a state of lowest free energy. The metalloenzymes, however, have large protein chains acting as the multidentate ligands. The disparity of the spectral data between simple complexes and proteins can be understood by realizing that protein chains, which surround the active site, are subject to precise secondary and tertiary conformations which give the molecule as a whole lowest energy. The conformational demands of the large protein chains may induce geometric and electronic strain around the active site. In other words, the local region immediately surrounding a metal ion in the active site of a metalloprotein is not in a state of minimum free energy.

Vallee and Williams call this a "state of entasis: the existence in the enzyme of an area with energy, closer to that of

a unimolecular transition state, than to that of a conventional, stable molecule, thereby constituting an energetically poised domain." The superb catalytic ability of enzymes which Jencks could not account for may be attributable to this entatic nature of the active site. An example will illustrate. The functioning of cytochromes involves interconversion of Fe(II) and Fe(III). As noted earlier, although both oxidation states involve octahedral coordination, Fe(III) is considerably destabilized. The instability of the Fe(III) state in cytochromes may be explained by postulating a longer axial Fe to protein bond than that expected for inorganic model complexes. The intermediate stages of this change must involve some compromised geometry between these two states. If the Fe is in the entatic state with an elongated bond, it is held in a geometry closer to that of the transition state than it would be otherwise. It's ground state energy is increased and thus, the activation energy is lowered, and in this way a major contribution is made toward rate enhancement. The cytochrome will be able to pick up an electron more easily.

D. Application of the Entatic State Hypothesis to Hemoglobin

The merits of the Entatic State Hypothesis have yet to be put to experimental test. Several problems stand in the way. For example, the energy enhancement of the active site can be inaugurated by distortions too small to be noticed by X-ray crystallography, or (for nonmetalloenzymes) spectroscopic methods. This problem can be overcome by restricting attention to metalloenzymes, as we have done so far. As noted above, absorption spectra, magnetic susceptibility studies, and redox potentials

can be utilized to detail the electronic structures of the metals in metalloenzymes.

Hemoglobin is a good biomolecule to focus upon. Although not an enzyme, it exhibits two properties characteristic of enzymes: hemoglobin is composed of subunits, and displays allosterism. enhancement of the ability of one subunit to act in response to a physical displacement of another subunit. The actual physical dislocations of chains in hemoglobin have been measured by X-ray crystallography. Furthermore, the uptake and transport of oxygen in biological systems is of obvious importance, and a study of hemoglobin may help elucidate the still turgid nature of oxygen binding, as well as the nature of allosterism in general. Finally, a theoretical treatment of the molecule shows that it may exhibit entasis, as will now be shown.

Hemoglobin and myoglobin have a Fe(II) porphyrin at the active site, coordinated to a histidine, referred to as proximal, and distal. The Fe(II) has an unpaired electron. This unpaired electron is in the d_{xz} orbital, is in a high spin assignment, and therefore has the effect of increasing the radius of Fe. Coupled with the pull from the proximal histidine, the larger radius forces the Fe out of the plane of the porphyrin group. On addition of O_2 the Fe becomes low spin and moves back into the plane. The position of the Fe ion relative to the heme has been postulated to be of paramount importance to the heme-heme interactions by Perutz in his "Trigger Mechanism".

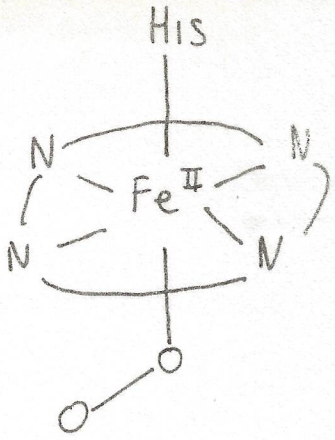

Fig. 3. Heme group of hemoglobin, where the porphyrin ring is greatly simplified. The position of the proximal histidine and dioxygen are shown.

Studies have shown how these changes in spin state are dependent on the stereochemistry and basicity of the ligands surrounding the Fe. The chosen biological ligand, porphyrin, and the natural axial ligand, histidine, place the iron very close to the balance point of the spin state equilibria.

It is known that Fe in the high spin state cannot be bound to O_2. Low spin Fe will bind but will only release O_2 slowly. Of course, hemoglobin takes up and releases O_2 rapidly. Since steric factors can control spin state, and spin state change is accompanied by a change in stereochemistry, then an Entatic strain on the Fe-N bond would facilitate a stereochemical change and thus a spin state change. The final effect would be to increase the speed of O_2 uptake and release.

What is the evidence that an Entatic state may exist? In a random coil of myoglobin, we would expect two basic amino acids to become axial ligands of Fe, thus making the Fe low spin. The protein is actually in a rigid conformation demanded by the thermodynamics of the protein chain as a whole. The Fe is held in an open Fe high spin octahedral structure, slightly distorted because the Fe-histidine bond is lengthened. The effect of this

distortion is that the Fe is held in an energy state that is 5 to 10 Kcal higher than it would be in a random coil. This high energy, strained system fits the description for an Entatic state. In fact, this entatic condition matches a conventional S_{N1} intermediate of an octahedral substitution reaction.

Upon oxygen binding, a new and different strain is induced in the metalloprotein system, which is used to abet oxygen release. This strain accounts for the unusually rapid release of O_2 from a low spin Fe. Entrance into the transition state, and subsequent release of the substrate of any enzyme (including non-metalloenzymes) could be explained in an analogous manner.

Thus, a dynamic picture of the hemoglobin molecule emerges which makes it a likely candidate for study of the Entatic state theory. The stereochemistry of the protein chains in hemoglobin as a whole are such that the z-axis ligand, histidine, is held at a longer bond distance than would be expected. This places a strain upon the electronic structure of the Fe ion which holds it close to the crossover of high to low spin state. Changes in spin state can also cause large changes in the stereochemistry of the protein. Thus here is a dynamic flow of energy and information, where the metal influences the conformation of the protein, and the protein influences the electronic structure of the metal, all centering around the entatic nature of the active site.

E. Use of Synthetic Oxygen Carriers to Test the Entatic State Hypothesis

For purposes of laboratory control, work with actual enzymes is not advantageous. Since we will concentrate on the geometry of the active site alone, analogs of the smaller active site will suffice for study and have the advantage of being easier to maintain control over. Model systems are also required because the

metals of prime biological interest, Fe and Cu, have rather intractable chemistry. For instance, Fe is diamagnetic when complexed to oxygen, and thus electron paramagnetic resonance (epr), which gives insights into the electronic and geometric structure, cannot be used. Cobalt, on the other hand, is more easily studied using a variety of methods; such as optical and epr spectra, redox potentials and chemical reactions. In addition, a vast amount of literature on cobalt chemistry is available.

Crumbliss and Basolo, on the basis of previous reports by Tsumaki, Calvin, Caldazarro, and others, synthesized such a model using Co(II) as the metal and a Schiff Base as the ligand.

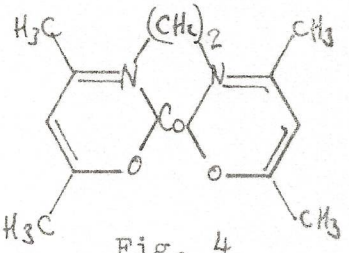

Fig. 4
N,N'-ethylenebis(acetylacetoniminato)cobalt(II)
Co(acacen)

They found that molecular oxygen will react with N,N'-ethylene bis(acetylacetoniminato)cobalt(II) in nonaqueous solvents containing added base to form a monomeric oxygen adduct (Co (SB)BO_2). This adduct is similar to oxyhemoglobin in several respects.

Oxyhemoglobin has a Fe atom that is octahedrally coordinated. Five of the positions are occupied by electron donating nitrogens, with the sixth position occupied by the dioxygen.* It appears that the purpose of the base in the Co(acacen) adduct is analogous

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* coordinated O_2 is termed dioxygen

to that of the proximal histidine of hemoglobin in that electron donors stabilize the cobalt-oxygen bond by enabling octahedral coordination around the metal to be achieved upon oxygenation. Co(acacen) can bind oxygen monomerically and reversibly. Esr data indicate one unpaired electron heavily localized on the dioxygen, leaving a low spin Co(III). The Co-O-O bond is bent. This formulation agrees with that proposed for oxyhemoglobin.

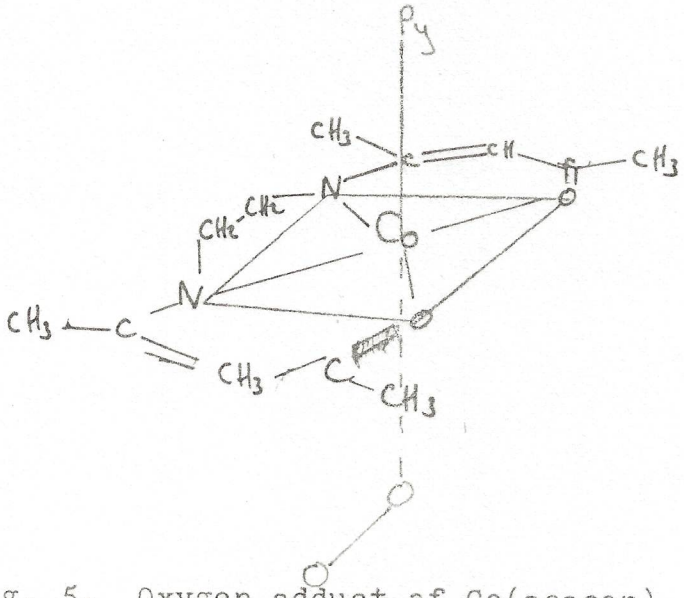
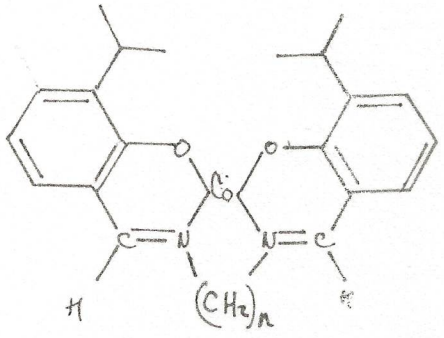


Fig. 5. Oxygen adduct of Co(acacen). Note similarity to heme depicted in Fig. 3.

A set of molecules, each similar to Co(acacen), was fabricated by Hariharan and Urbach, in which the number of methylene groups in the bridge was increased.



n=2-7

Fig. 6. N,N'-bis(isopropylsalicylidene)-polymethylenediaminecobalt(II)

These molecules lend themselves well to a study of the Entatic State Hypothesis, because Urbach and Hariharan discovered that the stereochemistry of these molecules was a function of the degree of distortion caused by the length of the polymethylene bridge.

The absorption spectra of the set of complexes with n = 4-7 were compared with the spectra of bis(N-phenylsalicylaldiminato)Co(II), a complex known to be high spin pseudotetrahedral by spectral and crystallographic studies. The spectra were found to be similar. Their findings were augmented by magnetic susceptibility studies which gave magnetic moments in the range characteristic of tetrahedral arrangements, (μ = 4.2- 4.7). Apparently, when the number of bridging methylene groups is 4-7, the ligands surrounding cobalt adopt a high spin pseudotetrahedral arrangement. Hariharan and Urbach theorized that the relatively large number of bridging groups allowed sufficient flexibility for the tetrahedral arrangement to occur.

Alternatively, the n=3 complex did not agree in either band position or general appearance with bis(N-phenylsalicylaldiminato)Co(II). The bands came at lower energy and were less intense, indicating that the energy difference for the d-d transition between the t_{2g} and e_g orbitals was not as large as the n = 4-7 cases. A highly flattened, or distorted, tetrahedron was invoked to account for the reduction in orbital splitting. The magnetic moment data were 4.48 and 4.83 for the solid complex and solution, once again in the range expected for a tetrahedral arrangement.

The spectra for the n=2 derivative showed a band in the near

IR which is characteristic of low spin Co(II) in a square planar environment. The magnetic moments of 2.35 and 2.38 (for solid and solution, respectively) are in the range (2.1- 2.9,) characteristic of a square planar configuration.

It is important to note that these results were obtained for the solid complexes or in non-donor solvents. In donor solvents such as pyridine, all complexes expand their coordination numbers and became high spin pseudo-octahedral. Recall Basolo found a similar trait in Co(acacen); that it would combine oxygen only if made six coordinate by a base. The electronic environment emerges as flexible, but subject to strict controls exerted by the number and flexibility of the ligands.

If axial ligands are unavailable (as in a non-donor solvent) the stereochemistry of the metal is determined by the steric control of the number of methylene groups in the bridge. This steric control is analogous to the restrictions imposed on the metal by protein ligands, that is, the steric control of the methylene bridge may be placing the molecule in a strained, or higher energy state. When placed in a donor solvent, the restriction is relieved, and the molecule moves toward the octahedral arrangement, which is apparently of lower energy and thus more stable.

These molecules therefore present themselves as moieties in which distortion of electronic structure (strain) is progressively increased as one goes from n=2 to n=7. A demonstration that these strained molecules exhibit enhanced reactivity would be evidence for the viability of the Entatic State Hypothesis.

F. Redox Potentials as a Method of Study

a. Application. The ease of oxygen uptake by metal complexes should depend on the ease of oxidation (redox potential) of the metal in a given complex. The redox potential could be the most important parameter in determining the oxygen affinity of the substrate. For example, easily oxidized metals, such as Fe(II), react with O_2 irreversibly; metals difficult to oxidize, such as Ni(II), do not react with O_2; and metals between the two extremes may react reversibly. However, since the redox potential is a function of the electron density on the cobalt, and the nature of the ligands surrounding the metal can affect electron density, the reversible oxygen uptake depends not only on the metal but also on the spatial deployment and nature of the ligands surrounding it. Since the entatic strain of a molecule may be defined as an energy requiring shift of electron density, measurement of redox potentials is important.

b. Background of Use on Co(acacen). Basolo et al determined the equilibrium constants, K_{O_2}, of this reaction:

$$Co(L)B + O_2 \underset{}{\overset{K_{O_2}}{\rightleftharpoons}} Co(L)(B)O_2$$

where L is a quadridentate ligand in the equatorial positions and B is a monodentate ligand in the axial position. The equilibrium constants were measured with the Hill equation:

$$\frac{Y}{(1-Y)} = \frac{(\ Co(L)B \cdot O_2\ )}{(\ Co(L)B\ )} = K_{O_2} P_{O_2}{}^{n}$$

Basolo endeavored to relate the ease of oxygen uptake to the redox potential of the Co in many ligand environments. Oxygen uptake was measured via spectral changes and redox potentials

were determined by cyclic voltammetry.

The effects of axial ligands, B, were noted by keeping the equatorial base constant. The Schiff base benacen was employed for this portion of the study (see structures on next page). The bases used as axial ligands had bonding properties ranging from pure sigma donation (in saturated amines) to the sigma and pi bonding of the imidazoles (like Histidine in hemoglobin).

The oxygen carrying ability of Co(benacen) was noted to increase with increasing base strength of the amines (with the concomitant increase of electron density on the Co). Steric effects were also found to be important. The four butylamines, t-$BuNH_2$, sec-$BuNH_2$, i-$BuNH_2$, and n-$BuNH_2$ have similar base strengths, and can only sigma bond, thus any change in the oxygen bursting of Co(benacen) must be due to steric hindrance. The oxygenation of Co(benacen) became slower as the bases were changed in the order: n-$BuNH_2$, i-$BuNH_2$, sec-$BuNH_2$, and t-$BuNH_2$.

Similarly, pi bonding was also proven to be a factor. The picture of a cobalt-oxygen adduct as basically Co(III)-O_2^- predicts that the strength of the cobalt-oxygen bond should reflect the extent of back-donation of electrons from the filled $3d_{xz}$ orbital of the cobalt atom into the half empty π^* orbital of the oxygen moiety. Bases coordinated <u>trans</u> to oxygen will be in direct competition with the oxygen moiety for the 3d π electrons of the cobalt atom. Thus, according to the theory that the ligands affect the density around the cobalt, a good pi donating base should force more electron density onto the cobalt, enhancing

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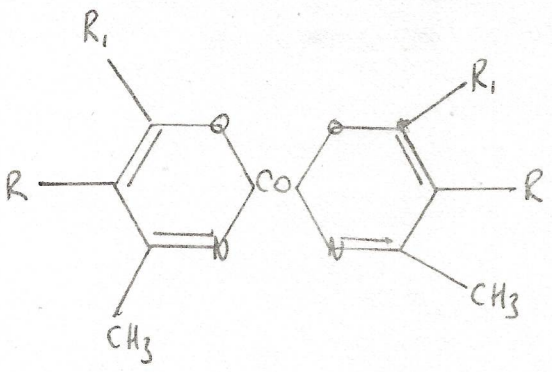
* a pi antibonding



Co (acacen)	R' = CH_3	R=H	
Co (phenacen)	R' = CH_3	R=C_6H_5	
Co (mecacen)	R' = CH_3	R=CH_3	
Co (benacen)	R' = C_6H_5	R=H	
Co (sacsacen)	R' = CH_3	R=H	O=S

Fig. 7. Equatorial Ligands used to Test Effect of Ligand Framework Modifications on Redox Potential

the pi binding electron flow from cobalt to oxygen. Furthermore, because of the symmetry of the orbitals involved, the axial ligand can effectively donate its electrons to the d_{xz} orbitals of cobalt which are the orbitals that π-bond to oxygen. (see Fig. 8).

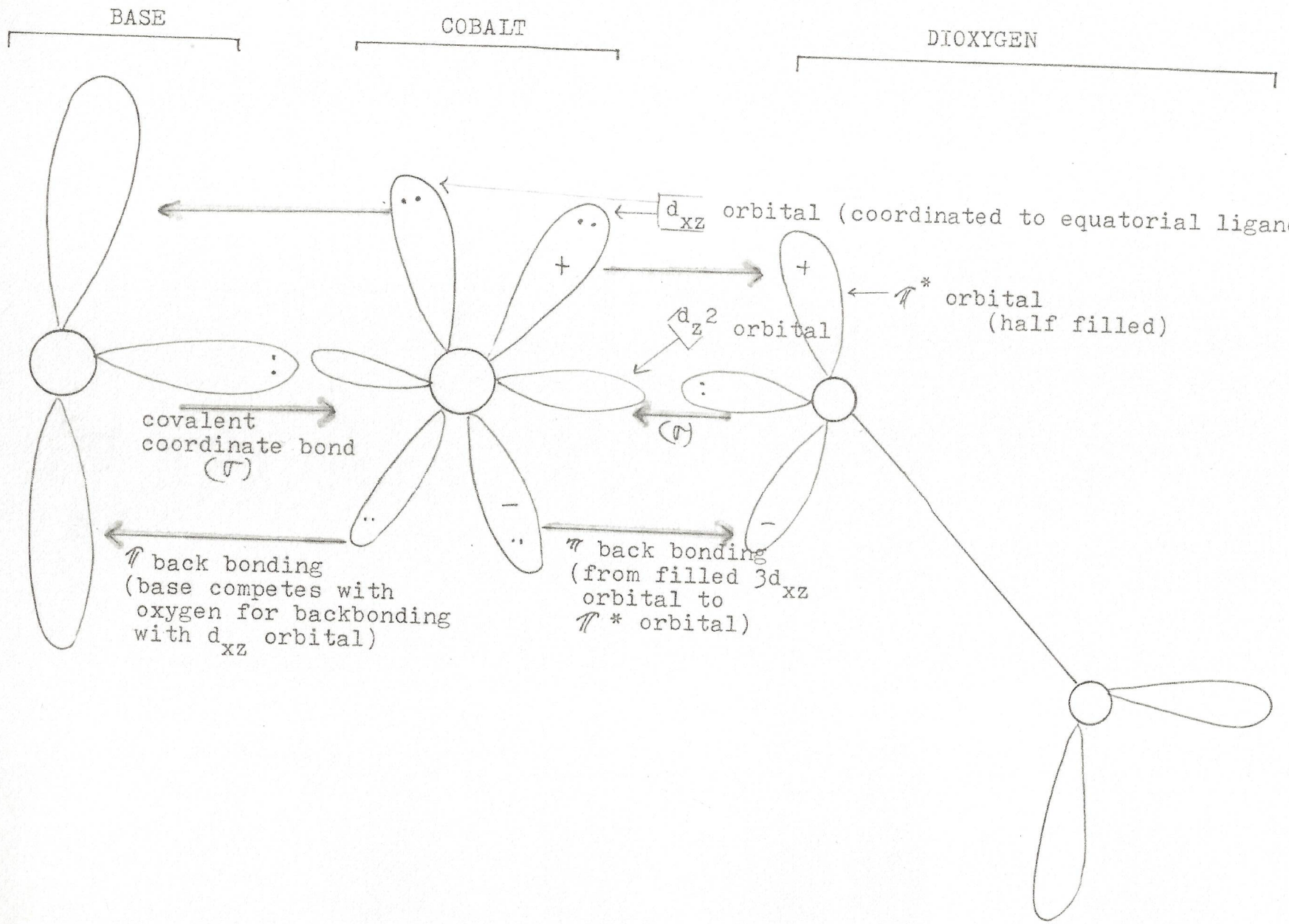
Thus, imidazoles, which may be better pi donors than pyridine, have the greater enhancement on the oxygen carrying ability of Co(benacen). A linear correlation was found between the redox potential and log K_{O_2}, mm^{-1}, no matter how the cobalt electron density was changed. From these studies, we can conclude that the principal function of the axial base is to "activate" the cobalt complex by making it a better reducing agent.

Equatorial ligands were studied by using pyridine as the axial base, and varying three parameters of the equatorial ligand: 1) the framework of acacen, 2) the electronegativity and pi withdrawal properties of the bonding atoms, and 3) the pi delocalization of the metal's electron density over the equatorial ligand.

In the first case, changes of the framework of acacen sometimes resulted in a greater and sometimes resulted in a lesser ability of the N and O coordinated directly to cobalt, to donate electrons, dependent upon substituted groups. (Fig. 7). Both the nature of the groups and their position on acacen proved important.

In the second case, a decrease in electronegativity of the ligand atoms should facilitate the oxygen carrying ability of the complex, because it will be accompanied by a concomitant decrease in pi electron removal. In a manner analogous to the effects of the axial base, this will increase the electron density around cobalt and make for better binding to oxygen.

Fig. 8 Effect of Axial Base on Oxygen Binding



Prior to evaluating the third factor important to the effect of the equatorial ligand, we will discuss work of Stynes and Ibers because of its biological significance.

After the discovery of the monomeric, reversibly oxygenating, cobalt-Schiff base complexes, it seemed prudent to study metallo-oporphyrins. It appeared that a model closely resembling the heme group of hemoglobin could be prepared with protoporphyrin IX dimethyl ester (PIXDME) as the equatorial ligand.

Fig. 9
Protoporphyrin IX dimethyl ester

PIXDME closely resembles the biological porphyrin. In addition, methylimidazole was used as the axial base in the fifth coordination site because this is analogous to the imidazole of the histidine found coordinated to heme in the same position. Also, it is well known that the dioxygen must travel through a hydrophobic pocket to reach the heme group; thus, toluene was used as the solvent to simulate the hydrophobicity. Moreover, in both the cobalt porphyrin and the hemoproteins, O_2 becomes spin paired upon coordination. Finally, the visible spectra of Co(PIXDME)B and Co(PIXDME)BO_2 are similar to those of deoxyhemoglobin and oxyhemoglobin, respectively.

The following reaction was carried out:

$$Co(PIXDME) + Base + O_2 \rightleftharpoons Co(PIXDME)BO_2$$

As a result of thermodynamic studies on this system, once again using the Hill equation to determine equilibrium constants, it was learned that the cobalt porphyrin bound dioxygen at higher temperatures than the Schiff base complexes did. Since more activation energy is required for the oxygenation, the porphyrin system is a poorer oxygen carrier than the Schiff base system.

Thus, the nature of the equatorial ligand was shown to be essential in determining the oxygen carrying capacity of the complexes. Recall that pi electron withdrawal will weaken oxygen binding. The porphyrin ligand is thought to delocalize more electron density <u>from</u> cobalt into the ligand π system. In Co(PIXDME), the cobalt atom is coordinated to four nitrogen atoms which are part of a highly conjugated tetrapyrrole system, whereas in the cobalt-Schiff base complexes, the cobalt atom is coordinated to two oxygen and two nitrogen atoms which in turn are part of only a partially conjugated system. Thus, the dinegative charge of the ligand is less delocalized in the Schiff base complexes and hence there is <u>more</u> electron density on the metal in this case.

We now return to the work of Basolo. He concluded that regardless of the mechanism of electron withdrawal from cobalt, the redox potentials give a relative measure of the electron density on the cobalt, and the O_2 uptake correlates linearly with redox potential. (Fig. 10).

Although the point was not made by Basolo, some of the dynamics behind the Fe-O_2 binding become clear. The porphyrin ring around Fe in heme reduces the ability of Fe to bind to O_2, thus preventing the irreversible oxidation which would otherwise take place. Concurrently, the N of the proximal histidine

strengthenens the bonding, increasing the rate, and preventing premature loss of the O_2. Certainly any attempt at describing the Fe-O_2 interaction in detail will have to consider these forces.

Since Co(acacen) was demonstrated to be a reversible oxygen carrier, and the isopropylsalicyl molecules were shown to have electron structures which depend on the number of groups in the methylene bridge, we attempted to synthesize molecules which took advantage of both these attributes. The molecules selected for study were derivitives of Co(acacen) (Fig. 4), where the number of methylene groups would be varied from two to six.

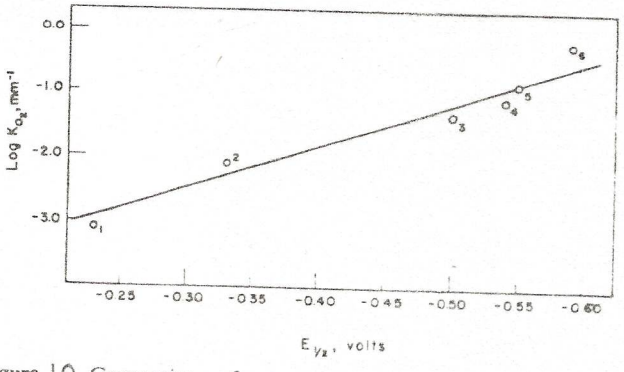
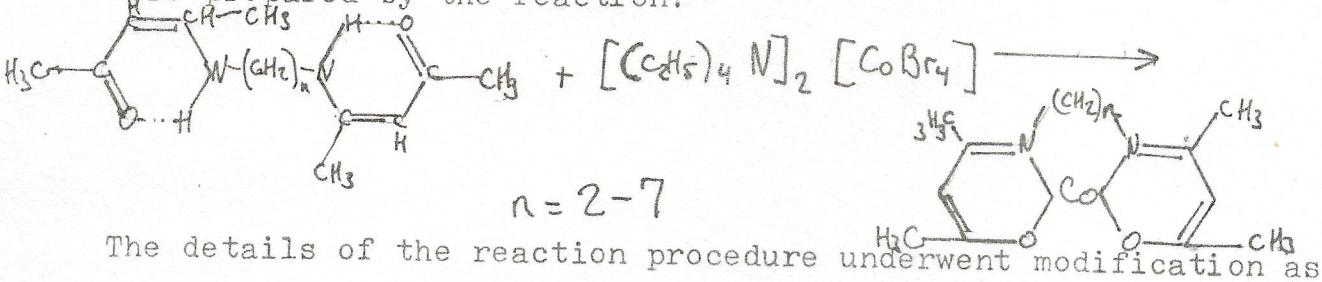


Figure 10. Comparison of oxygen uptake (log K_{O_2}) at −31° for Co(L)py to polarographic half-wave potentials ($E_{1/2}$) for Co^{II-III}-(L)py_2: 1, Co(*p*-MeOTPP); 2, Co(sacsacen); 3, Co(benacen); 4, Co(meacacen); 5, Co(Phacacen); 6, Co(acacen).

50 ml. of ethanol. They were combined quickly, and allowed to stir at room temperature for 15-20 minutes. The ethanol was removed on a rotary evaporator, giving a blue powder which was recrystallized from ethanol.

c. Synthesis of Cobalt Complexes. The cobalt complexes were prepared by the reaction:



The details of the reaction procedure underwent modification as proved necessary. A typical procedure follows. To 75 ml. of t-butyl alcohol (Fischer) was added 3.0 gm. of potassium t-butoxide (Aldrich). The solution was warmed to about 50°C., and 6gm. of the ligand, N,N-ethylenebis(acetylacetonamine) was added. The solution was allowed to cool to room temperature, with stirring, for about 15 minutes. Eight grams of tetraethylammonium tetrabromocobaltate, was placed in a curved tube (see figure 1). The apparatus in figure 1 was flushed with high purity dry grade N_2 (Linde) for about 15 minutes. The cobalt salt was added all at once, with stirring, and the reaction was allowed to proceed for 2.5 hours at room temperature.

The reaction mixture was removed from the flask via a septum and long needled syringe. It was filtered under N_2 by squirting into port A (fig. 1) and then placing a finger over port A to force the filtrate through under N_2 pressure. This first endeavor produced a green claylike mass which would not recrystallize from toluene or n-heptane. Since the literature reported that Co(acacen) was soluble in hot toluene or n-heptane and was bright orange, we concluded that we had failed to make the complex.