of regioselective metabolism of the R and S isomers of warfarin[17] and testosterone.[17,68] by eight different rat liver cytochromes P-450 and are using these results to initiate model building studies of the active sites.[68]

Concluding Remarks

What does the future hold? Our own efforts are directed toward elucidation of the general aspects of the

working hypothesis we have elaborated here. Further, if the general scheme is appropriate, many details remain to be filled in, such as the physical description of the iron–oxygen complexes involved and the kinetic and thermodynamic parameters of the individual steps. The modeling of the active sites has only begun. When more information becomes available, the catalytic activity of cytochromes P-450 may ultimately be understood in molecular terms and of course of reactions with new substrates may be predicted.

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Binuclear Oxygen Carriers: Hemerythrin

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With few exceptions, all living cells take up oxygen.[1] In simple, small animals, in which body surface is large compared to the mass of the organism, the oxygen essential for respiration reaches the cells by diffusion from the external medium. In larger animals, however, the ratio of surface to bulk drops markedly. Furthermore, internal tissues are too far from the exterior to have access to sufficient oxygen if it is supplied only by simple, slow diffusion from the outer environment. Thus in the course of evolution in animals from annelids upwards, nature has developed blood vessels to transport oxygen into proximity to deeper cells and has created several different molecular devices to provide for oxygen accumulation in blood.[1] The oxygen content of sea water, 0.5 mL of O_2/100 mL of solvent, has thereby been raised progressively to about 25 mL of O_2/100 mL of blood in warm-blooded mammals.[1]

There is surprising diversity in molecular structure, particularly at the functional site, of the different naturally occurring carriers of dioxygen.[2–5] All are constituted of protein frameworks, but the molecular weights, subunit constitution, and symmetry in supramolecular assembly vary enormously (Table I). Within the hemoglobins, which are the most familiar as well as the most ubiquitous O_2 carriers, the tetrameric oligomer of 64 000 molecular weight is the best known, but

monomeric myoglobin, of 16 000 molecular weight, and multisubunit extracellular hemoglobins, as high as 4 × 10^6 in molecular weight, have also been studied extensively. Hemoglobins are distributed throughout the animal kingdom (Figure 1) and even among some plants. On the other hand, the non-heme O_2 carriers hemerythrin and hemocyanin are found only among invertebrates, particularly annelids, molluscs, and arthropods. Most hemerythrins show molecular weights near 108 000 and are constituted of eight subunits.[2] Nevertheless, smaller oligomers, with two, three, or four subunits, are also known,[6–10] and even monomers have been found in some organisms. In contrast, in the hemocyanins, the natural form of the pigment is multisubunit in constitution, and functional monomers are unknown under physiological conditions.

Despite their many differences, hemerythrin, hemocyanin, and hemoglobin all function effectively as oxygen carriers. To a chemist, relationships between

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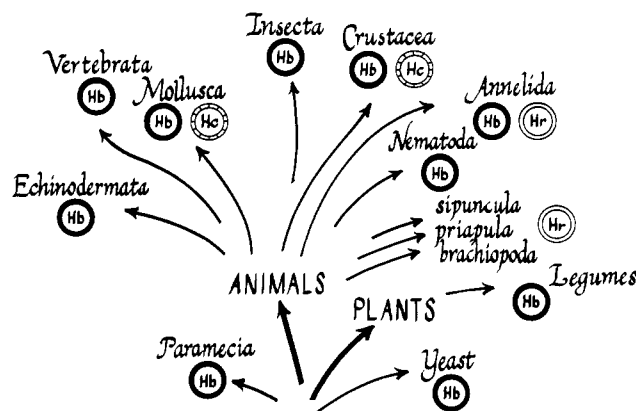(9) Manwell, C. *Comp. Biochem. Physiol.* 1977, *B58*, 331.
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Table I
Comparison of Some Properties of the Oxygen Binding Pigments

	hemoglobin	hemerythrin	hemocyanin
metal	Fe	Fe	Cu
metal:O_2	Fe:O_2	2Fe:O_2	2Cu:O_2
oxidation state of metal in the deoxy protein	Fe^{II}	Fe^{II}	Cu^{I}
coordination of metal	porphyrin	5 His, 1 Asp, 1 Glu residues of protein	protein side chains
no. of subunits	1, 4, up to 180	1, 2, 3, 4, 8	6, 12, 20, 24, 48
frequent symmetry in quaternary structure	C_2, D_6	D_4	D_3, D_5
frequent mol wt	64 000	108 000	400 000–9 000 000
color			
oxygenated	red	burgandy	blue
deoxygenated	red-purple	colorless	colorless

Figure 1. Distribution of oxygen-carrying proteins in nature. Hb, hemoglobin; Hc, hemocyanin; Hr, hemerythrin.

biological function and molecular structure pose intriguing structural questions. Since hemoglobin is the oxygen carrier in human blood, it has been a focus for chemical investigation in innumerable laboratories for more than a century, and its molecular structure has been unraveled in fine detail.[11] During the last three decades substantial progress has also been made in obtaining corresponding understanding of structure and function of the cognate protein hemerythrin. The attention of this Account will be focused on this latter oxygen carrier.

The details of the molecular structure of hemerythrin have been elucidated within the last two to three decades.[2,12] Some molecular information, such as iron content, Fe/O_2 ratio, and molecular weight, had been reported in earlier literature, but the values given were markedly in error. (The first was off by 25%, the second by 50%, and the third by 100%.) Accurate elemental analyses in 1957 established[13,14] that two Fe atoms are associated with each bound O_2 (rather than the three Fe previously claimed). Studies of molecular weights under different ambient conditions revealed that the basic building block of the oligomer is a monomer of 13 500 molecular weight,[15] which corresponds to one 2Fe:O_2 unit, and that an octameric aggregate is the common natural arrangement of subunits.

Oxidation State of Fe in Hemerythrin

At this stage one might ask, what oxidation state should be assigned to the iron atoms in the cluster pair? Answers depend on the state of the hemerythrin and are summarized in Figure 2. In the deoxy form the iron pair is in the (II,II) state, in the oxy and met forms (III,III), and in the semimet (II,III) (Figure 2).

The oxidation state in the deoxy form was established readily[12] by a conventional chemical procedure: in the absence of O_2 the Fe was displaced from the protein by addition of H$^+$ ions and its state established by colorimetric tests. The results showed unequivocally that (II,II) was the proper oxidation number to be assigned to the iron pair. In the met state, obtained by oxidation of either deoxy- or oxyhemerythrin with ferricyanide, displacement with acid (in the absence of O_2) is still a convenient route for confirming the oxidation number as (III,III). Although immersion of oxyhemerythrin in acid releases iron that gives color tests corresponding to a III oxidation state, one cannot exclude reactions with oxygen in this case, and hence this test with released metal is not a reliable indicator of the state of the iron in the functional site. Definitive assignment of (III,III) for the iron pair in oxyhemerythrin was provided by Mössbauer spectroscopy,[16-18] which also established the binuclear, μ-oxo-bridge nature of the functional site, as will be described below. Finally the oxidation number of the semimethemerythrin, (II,III), was revealed in conventional chemical titrations[19-21] and confirmed in EPR and Mössbauer experiments.[22-24]

Oxo-Bridged, Binuclear Functional Site

The establishment of a stoichiometry of 2Fe:O_2 (Table I) suggested that the iron atoms are in a pairwise steric arrangement at the functional site of hemerythrin. It was also noted early[25] that the electronic absorption spectra of oxyhemerythrin and of a variety of met-

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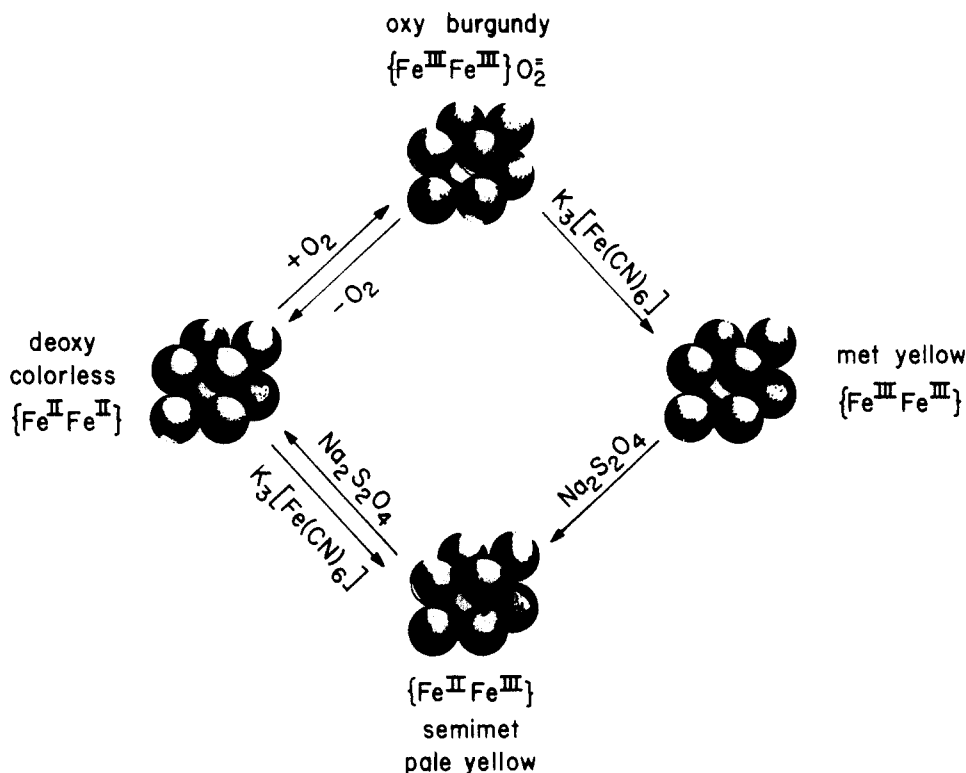(25) Keresztes-Nagy, S.; Klotz, I. M. *Biochemistry* **1965**, *4*, 919.

Figure 2. Oxidation numbers in iron pairs in various states of hemerythrin.

hemerythrin–ligand complexes show a band near 330 nm, very close to that, 335 nm, found in an [Fe(III)]$_2$ dimer of ferric perchlorate whose magnetic properties[26,27] correspond to those of an antiferromagnetic, spin-coupled system. Thus it was suggested[25] that the functional site in oxy- and methemerythrins is binuclear, with the iron atoms linked by a μ-oxo bridge: Fe–O–Fe. A more detailed analysis[28] of the absorption bands, including a comparison of their wavelengths and intensities with those of model iron chelates, showed remarkable similarities in the spectra of model oxo-bridged complexes, L$_n$Fe^{III}–O–Fe^{III}L$_n$, and the hemerythrins. This strengthened the presumption of a μ-oxo-bridged iron pair in the protein. Similar conclusions were reached from an examination of circular dichroic spectra.[28]

Definitive evidence of a μ-oxo bridge was provided by Mössbauer spectroscopy.[16-18] Deoxyhemerythrin shows a single quadrupole doublet, with a large quadrupole splitting and high isomer shift (1.15 mm/s) characteristic of high-spin Fe(II). In contrast, for oxyhemerythrin and the various ligand complexes of methemerythrin, the isomer shifts are in a range (0.45–0.55 mm/s relative to natural iron) characteristic of high-spin Fe(III). (Model inorganic binuclear bridged Fe(III) complexes have isomer shifts of 0.4–0.6 mm/s.[2] The quadrupole splittings, however, are large compared to those of simple high-spin Fe(III) salts, but they are in the range found for binuclear high-spin iron(III) bridged complexes. Thus the Mössbauer parameters are consistent with antiferromagnetically coupled high-spin Fe(III). This coupling is also manifested by a decreased susceptibility to a magnetic field compared to isolated high-spin Fe(III) and by a drop in suscep-

tibility with decreasing temperature. Methemerythrin and oxyhemerythrin both become diamagnetic near absolute zero[29,30] due to an antiferromagnetic exchange coupling between the two Fe(III) atoms. The exchange coupling constants, J, of -134 cm^{-1} for aquomethemerythrin and of -77 cm for oxyhemerythrin are comparable to the values of -90 to -131 cm^{-1} for model μ-oxo-bridged compounds.[31] For semimethemerythrin, with S as ligand, two doublets are seen, at 0.57 and 1.16 mm/s, respectively, providing direct evidence for the Fe(II)–Fe(III) state.

The presence of a μ-oxo bridge has also been evident in the resonance Raman spectra of methemerythrins.[3,32] Resonance Raman spectroscopy selects out vibrational modes coupled to the Fe transition. A peak near 510 cm^{-1} is found for a variety of ligand complexes of methemerythrin in H$_2$^{16}O. This frequency is in the correct range for the symmetric stretch of a bent μ-oxo bridge.[33,34] Furthermore, if H$_2$^{18}O is used for solvent water, the peak is shifted to about 490 cm^{-1} [3,32,35] for a number of methemerythrin complexes.

Finally the μ-oxo bridge has been confirmed in X-ray diffraction studies[36,37] and by X-ray absorption spectroscopy.[38,39] Crystal structures of azidometheme-

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rythrins have been refined to a resolution of 2.2–2.5 Å. Originally there were significant disagreements between different crystallographical groups concerning the presence of an oxo bridge and the proper value to be assigned to the Fe–Fe distance. Values first reported for the distance ranged from 3.05 to 3.44 Å. More recent studies have narrowed this range very much, to 3.30–3.34 Å, and there is now general agreement that a μ-oxo bridge is present. X-ray absorption spectroscopy also shows the oxo bridge and gives independent values for the distances and angles at the binuclear site. Reasonable current choices for the structural parameters at the oxo center (in the met form) are

$$\text{3.40 Å} \qquad 165° \quad \overset{\text{Fe}}{\underset{\text{Fe}}{\diagdown}}\!\!\text{O} \quad \text{1.75 Å}$$
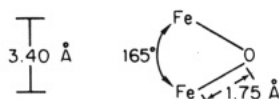
The Fe–Fe distance of 3.40 Å is close to that inferred from one of the early X-ray diffraction studies.[36] Extended X-ray absorption fine structure (EXAFS) analysis permits one to establish the oxidation state of the absorbing atom since more energy is needed to pluck out a core electron from Fe if it carries a larger positive charge; the analysis confirms the Fe(III) assignment. EXAFS also gives a value of about 1.75 Å for the Fe–O distance and of 165° for the Fe–O–Fe bridging angle. This bond distance is consistent with those of an –O– bridge, rather than an –O(H)– in model complexes.[31,42] It and the bridging angle are also reasonable for an antiferromagnetic exchange coupling constant $-J \sim 100$ cm^{-1} as found for oxy- and methemerythrin.

Location of Iron in Protein

Since there is no heme or other prosthetic group in hemerythrin, the pair of iron atoms must be held at the active site by direct coordination to side chains from amino acid residues of protein. The primary structure of hemerythrin has been known for some time.[2] Of the 113 residues in each subunit (from *Phascolosoma gouldii*), 44 are potential ligands to the iron pair: 1 Gly, 1 Ile, 1 Met, 1 Cys, 11 Lys, 7 His, 5 Tyr, 11 Asp, 6 Glu. Nucleophilic side chains bound to the iron atoms should be relatively unreactive toward externally added group-specific reagents. Potential ligands selected by such chemical means, combined with comparisons of sequences among genetic variants, were as follows:[3] His-25, His-54, His-73, His-77, His-101, Tyr-8, Tyr-67, Tyr-109.

Before jumping to conclusions, one must recognize that side chains submerged within the protein matrix may also be chemically unreactive, and that some liganded moieties may fluctuate enough in orientation to be accessible to an external reagent. It is of interest, therefore, to compare the ligand candidates suggested by chemical means with those ultimately identified by X-ray diffraction.[36,37,40] Although the ligands selected by crystallography changed as resolution improved, there is general agreement now that the five histidines

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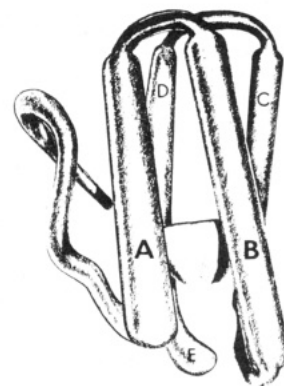
Figure 3. Molecular model of monomeric myohemerythrin at low resolution. The four approximately parallel helical segments are labeled A–D. The C-terminal stub is designated E. The electron density spanning the four helices, slightly below the center, is a manifestation of the iron pair. Subsequently it has been found that this 4-α-helical-fold is a recurrent structural motif among proteins.[56,57]
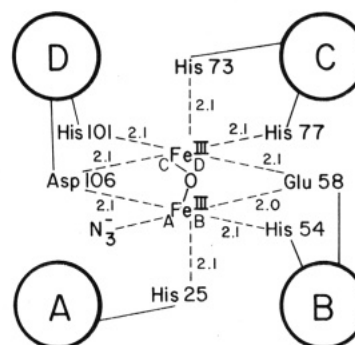
Figure 4. Bonding of iron atoms at functional site to each other and to their respective helices.

identified chemically are indeed coordinated to the Fe$_2$ pair. However, no tyrosine is involved, although Tyr-109 seems to be near the active site. Furthermore, two carboxylates, Glu-58 and Asp-106 provide binuclear bridging groups, but these were overlooked by chemical probes.

Even at 5-Å resolution,[2] crystallography clearly placed the Fe$_2$ pair at a definite locus between the four helices of the subunit (Figure 3). Taking into account the results of diffraction analysis near 2-Å resolution,[36,37,40,41] we can draw a schematic representation (Figure 4) of the specific attachments of each iron (designated by $_A$Fe$_B$ and $_C$Fe$_D$) to the helical columns of the protein framework and to each other. It is evident that there are actually three bridges between the binuclear irons and that each Fe is hexacoordinate in metazidohemerythrin. It is likely that O$_2^{2-}$ in oxyhemerythrin and most other ligands in methemerythrin occupy the site shown for N$_3^-$ in Figure 4. However, in metaquohemerythrin, the $_A$Fe$_B^{III}$ seems to be pentacoordinate, the site for (non-oxo) external ligand being unoccupied.

There are still some discrepancies between X-ray and EXAFS in regard to bond angles and distances, but their resolution will not change appreciably the geometric disposition of the irons at the functional site.

## Oxidation State and Disposition of Bound Dioxygen and of Other Ligands

Resonance Raman spectroscopy has provided revealing insights into the state of bound oxygen. That substantial electron transfer from metal (in its lowest

oxidation state) to bound oxygen occurs in the oxygen carriers was suggested for hemerythrin and hemocyanin in 1955,[12] and a corresponding model was proposed for hemoglobin in 1964.[43] Resonance Raman spectra show vibrational bands that clearly support these suggestions.[44]

Isotope shifts and normal coordinate analyses indicate that the frequency associated with the O–O stretch is not highly mixed with other interatomic motions. Thus the oxygen stretching frequency provides a guide to the O–O bond order. Simple ionic salts and gaseous dioxygen provide reference points for the correlation of bond orders with frequencies: O_2^+ (bond order $2^1/_2$), 1865 cm^{-1}; O_2 (bond order 2), 1560 cm^{-1}; O_2^- (bond order $1^1/_2$), 1100 cm^{-1}; O_2^{2-} (bond order 1), 850 cm^{-1}. In addition to leading to the assignment of general bond orders, the O–O ligand stretching frequency also implies the oxidation state of the ligand. Vibrational spectra of well-defined model metal–dioxygen complexes indicate that a superoxide ligand should display an O–O stretch in the range of 1075–1165 cm^{-1} and a peroxide ligand in the 738–880-cm^{-1} region.

Optical absorption bands of hemerythrin provide a footing for a vibrational probe to assign an oxidation state to bound dioxygen. Charge-transfer transitions of this protein overlap several blue and green laser lines. For oxyhemerythrin the vibrations that are coupled to the $O_2(-II) \rightarrow Fe(III)$ charge-transfer transition at 500 nm are localized at the oxygen-binding site. Peaks in the resonance Raman spectrum have been found at 844 and 504 cm^{-1} and have been assigned[45,46] to symmetric O–O and Fe–O stretching frequencies, respectively, in oxyhemerythrin. These assignments were confirmed by an examination of the mass dependence of peak frequencies when $^{16}O_2$ was replaced by $^{18}O_2$. The observed displacement in O–O stretching frequency from 844 to 798 cm^{-1} agrees almost exactly with the shift to 796 cm^{-1} calculated for a diatomic oscillator. Thus the O–O stretching frequency in oxyhemerythrin clearly indicates a peroxide-type oxidation state for bound dioxygen.

Turning to the steric disposition of bound O–O, we can visualize several idealized structural representations at the functional site:
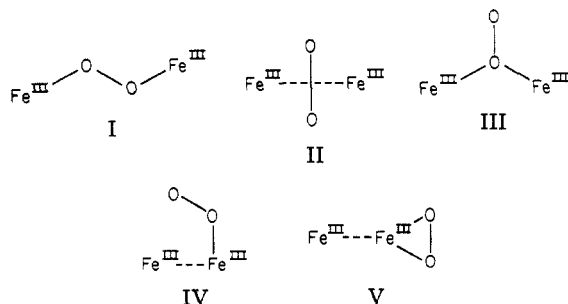
In view of the mass dependence of the O–O and Fe–O stretching frequencies, it became apparent[47] that un-
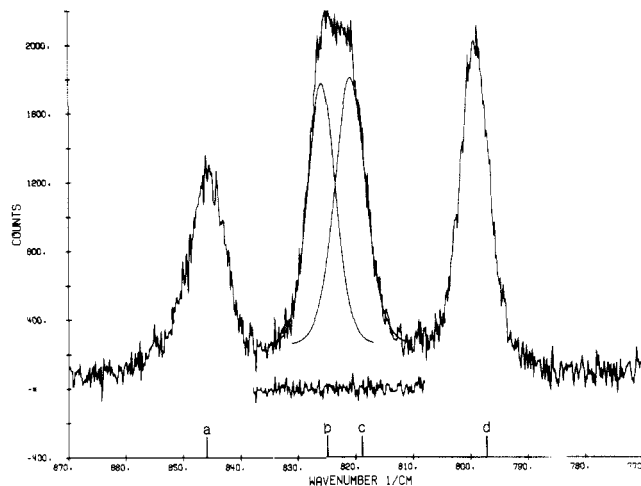
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Figure 5. Resonance Raman spectrum in the ν_{O-O} region of oxyhemerythrin equilibrated with a mixture of the isotopic species $^{16}O_2$, $^{16}O^{18}O$, $^{18}O_2$. The smooth curves fitted into the central peak ($^{16}O^{18}O$) represent the deconvolution of the 822-cm^{-1} feature into two components. The difference between observed and fitted curves is shown below the spectrum in the 822-cm^{-1} region. The vertical lines a–d show the calculated peak positions for models III and IV of Fe–$^{16}O_2$ (846 cm^{-1}), Fe–^{16}O–^{18}O (825 cm^{-1}), Fe–^{18}O–^{16}O (819 cm^{-1}), and Fe$^{18}O_2$ (798 cm^{-1}), respectively.

symmetrically labeled dioxygen, $^{16}O^{18}O$, would be ideally suited to discriminate among alternative geometric arrangements. With $^{16}O^{18}O$ a single symmetric O–O stretch, ν_{O-O}, would be expected for structures I, II, and V, whereas ν_{O-O} should be a doublet if dioxygen is bound as in III or IV because of the two possible modes of attachment of the unsymmetrical ligand.

Because a sample of pure $^{16}O^{18}O$ was not experimentally accessible at the time, the ν_{O-O} frequencies were obtained with a mixture of the isotopic species $^{18}O_2$, $^{16}O^{18}O$, and $^{18}O_2$. In the gaseous form, this mixture showed resonance Raman peak heights and areas on the ratios 1/2.6/1.4 for the ν_{O-O} values of the respective isotopic species. The same ratios should be seen in oxyhemerythrin prepared with this gaseous isotopic mixture if structure I, II, or V is the correct representation. Instead the resonance Raman spectrum of oxyhemerythrin with this isotopic mixture (Figure 5) showed a central peak of much reduced height, although the relative areas, 1/2.7/1.5, were essentially unchanged. Furthermore, this central peak, a manifestation of ν_{O-O} for $^{16}O^{18}O$, is significantly broader (10.0 cm^{-1} at half-height) than that for $^{16}O_2$ (7.1 cm^{-1}) or $^{18}O_2$ (5.4 cm^{-1}), and the top is flattened. These observations indicate two closely spaced features contribute to the 822-cm^{-1} band. In fact, a good least-squares fit was achieved by the insertion of two components of 5.5-cm^{-1} width (at half-height) separated by 5 cm^{-1} (Figure 5). One can conclude, therefore, that structure III or IV is the correct representation of the steric disposition at the functional site.

This conclusion has been confirmed in a subsequent examination[48] of the ν_{Fe-O} stretch in oxyhemerythrin. For these spectra, an 89 mol % sample of $^{16}O^{18}O$ was available. With an almost pure sample of $^{16}O^{18}O$, one should see a single Fe–O vibration if one of the symmetric models I, II, or V is correct. From simple valence force field considerations, one can calculate that this

(48) Weddell, L. L. D. Ph.D. Dissertation, Northwestern University, Evanston, IL, 1981; 75.

frequency should be near 494 cm^{-1}. (The observed values for Fe–^{16}O$_2$ and Fe–^{18}O$_2$ are 504 and 484 cm^{-1}, respectively.) Actually what one sees[48] for oxyhemerythrin with unsymmetrically labeled O$_2$ is two peaks, one at 503 cm^{-1} and the other at 485 cm^{-1}, which can be assigned to Fe–^{16}O–^{18}O and Fe–^{18}O–^{16}O, respectively. Thus one of the unsymmetrical representations, III or IV, must be the proper one.

Corresponding experiments were performed with another unsymmetrically isotopic ligand ^{15}N^{14}N^{14}N$^-$. Examination of the resonance Raman spectrum of azidomethemerythrin prepared with K^{15}N^{14}N^{14}N of 99% isotopic purity reveals two clearly resolved peaks in $\nu_{N\equiv N}$ region, one at 2044 cm^{-1} and one at 2032 cm^{-1}, both clearly distinct from that for azidomethemerythrin with ^{14}N$_3^-$ (2050 cm^{-1}) or with ^{15}N$_3^-$ (1983 cm^{-1}). These observations are consistent with structures analogous to III or IV with N$_3^-$ in place of O$_2^{2-}$, that is, the atoms of the ligand are in nonequivalent positions.

Of the two possible structures III and IV, a preference for the latter was expressed[3] after examination of the resonance Raman spectra of thiocyanatohemerythrin. The vibrational frequencies observed corresponded to those for an N-bonded ligand rather than S-bonded. Since no compounds are known in which SCN$^-$ bridges two metal atoms through N, it was suggested that SCN$^-$ is bonded to only one Fe in thiocyanatomethemerythrin. A similar choice was made after examination of linearly polarized charge-transfer spectra of single crystals of oxyhemerythrin,[49] using light polarized either parallel or perpendicular to the Fe–Fe axis. The observed polarizations, analyzed in terms of a transition dipole–vector coupling model, strongly favored the geometry of representation IV.

A valence orbital energy level diagram for this geometry is the following:[49]
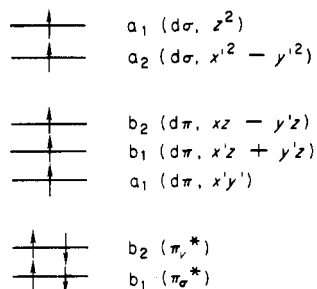
From comparisons with other systems, one can estimate that the splitting of the π* level of peroxide upon coordination with a metal should be of the order of 5000 cm^{-1}, whereas the splitting of the dπ orbitals should be no greater than a few hundred wavenumbers.[49,50] We assume also a formal Fe(III)·O$_2$(–II) ground state and effective C_{2v} symmetry, since only one oxygen is directly involved in the iron–peroxide bonding. Under these circumstances, the two lower energy O$_2^{2-}$ → Fe(III), charge-transfer transitions predicted to be electric-dipole allowed in various polarizations are π$_v$*(O$_{p_y}$) → dπ and π$_\sigma$*(O$_{p_z}$) → dπ. The former, lower energy transition has been assigned to the 508-nm band seen in polarized single spectra of oxyhemerythrin.[49] The higher energy transition has not yet been assigned. The spectroscopic conclusions regarding geometry have now been confirmed by high-resolution X-ray crystallography[36,37,40,41] (Figure 4).

Structural Changes in Transformation of Oxidation States

As has been mentioned, deoxyhemerythrin contains iron in the Fe(II) state. Furthermore its magnetic and Mössbauer properties[2,16,18,51] correspond to those of high-spin ferrous ions, not coupled antiferromagnetically. EXAFS[38,39] also indicates the absence of a μ-oxo bridge in deoxyhemerythrin but the continued coordination of the Fe atoms to the same protein ligands. Furthermore, repeated oxygenation and deoxygenation of hemerythrin in the presence of H$_2$^{18}O does not introduce isotopic oxygen into the functional site, at least as manifested by the constancy of the ν_{Fe-O} frequency.[3,32] Also no pH change, i.e., no proton release, has been observed when dioxygen is bound by deoxyhemerythrin,[51,52] and, complementarily, there is no Bohr effect, i.e., oxygen binding is not a function of pH.[53,54] With this information a reasonable structure for the functional site in deoxyhemerythrin is
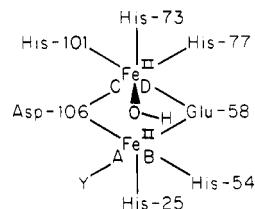
EXAFS studies[38,39] indicate that the iron–iron distance is shorter and that the metal atoms are subject to much more thermal motion than in oxy- or methemerythrin. The $_C$Fe$_D^{II}$–OH bond contains the O atom from the μ-oxo bridge and is presumably strong enough or inaccessible enough to be kinetically stable toward exchange with solvent. The ligand Y could be an OH$^-$ from the solvent; but it is intriguing to recall that Tyr-109 is near the $_A$Fe$_B^{II}$ and it can provide a phenolate ligand to the metal at the same time as it transfers a proton to the μ-oxygen.

An entering O$_2$ could thus approach the pentacoordinate $_A$Fe$_B^{II}$, displacing the Y ligand, which in turn by accepting the proton from the $_C$Fe$_D$–O–H would facilitate formation of the binuclear μ-oxo bridge. The molecular architecture of the functional site would become that shown in Figure 4. Electron density could then be transferred to the antibonding orbitals of the dioxygen[12,43] to generate the oxidation state that is evidently the stable form. This stability is a thermodynamic one with respect to O$_2$ uptake, for this process is reversible. On the other hand, there is also a slow

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conversion of oxyhemerythrin to methemerythrin (and peroxide); to this reaction the barrier must be the kinetic activation energy.

The structure of the semimet form of hemerythrin obtained by reduction of the full met form has been delineated from a variety of physicochemical studies. Mössbauer and EPR data[23] have established that in sulfidosemimethemerythrin the individual iron atoms are Fe(III), $S = 5/2$, and Fe(II), $S = 2$ in their oxidation and spin states, as originally suggested by Harrington et al.[19] Furthermore, they are antiferromagnetically coupled, to a resultant spin of $S = 1/2$. Chemical titrations[21] as well as EPR measurements[22-24] also indicate that almost all the iron pairs in octameric semimethemerythrin are in the hybrid oxidation state [$Fe^{II}Fe^{III}$]. These observations together with electronic and resonance Raman spectra[21] of azidosemimethemerythrin lead to a suggested structure closely analogous to that shown in Figure 4 for the full met form, with the difference that the Fe atoms have different oxidation states: $_CFe_D{}^{II}-_AFe_B{}^{III}$. The ligands to the protein framework are presumably unchanged, and the oxo bridge is retained. Semimethemerythrin can also be prepared by one-electron oxidation of deoxyhemerythrin. Since an intermediate with this oxidation level may exist on the pathway between oxy- and deoxyhemerythrin, the redox chemistry of semimethemerythrin may supply some relevant insights.

Superstructure of the Oxygen Carrier

The most common arrangement of the functional units of hemerythrin is as an octamer with D_4 symmetry. The supermolecule is composed of two layers, each a square of four subunits (Figure 6) in an end-to-side arrangement. In gross appearance each layer looks like a square doughnut. The subunits are each in an identical environment by virtue of the D_4 symmetry. The layers are related to one another by four twofold axes of two kinds (see ref 2 for detailed drawings). A side view of the two layers is also presented in Figure 6.

This arrangement creates a square channel (Figure 6, top) from the surface down the fourfold symmetry axis, each side being 20 Å wide. As one goes further down the symmetry axis, the cavity swells into a central chamber 30 Å in diameter and 15 Å high. In the side view of the two layers of subunits (Figure 6), one can also see in the center, down one of the twofold symmetry axes, a small portal into the central cavity of the octamer. Water presumably fills this chamber.

Space-filling models also show in each subunit a narrow channel between the four helices. Access to the outside is through the top, as shown in Figure 3. These openings are also visible in the model in Figure 6, bottom. In the subunit at the upper left and that at the lower right, the darkened sphere is an iron in the interior of the channel. Presumably it is through this narrow molecular passageway in hemerythrin that di-
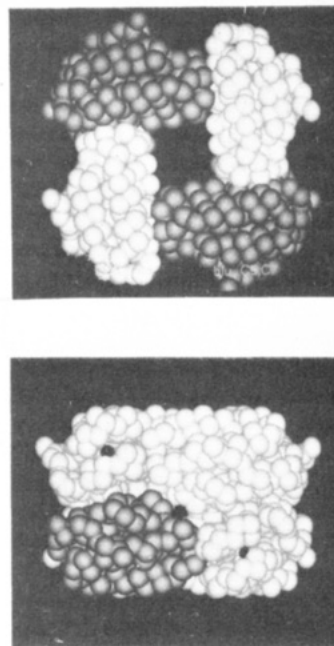
Figure 6. Computer-generated molecular graphics models of structure of hemerythrin (courtesy of Richard J. Feldmann, National Institutes of Health). Top photograph shows view down the fourfold symmetry axis. The subunits are identical, but differences in shading are used to delineate their dispositions in the quaternary structure. Bottom photograph shows side view down one of the twofold symmetry axes. The black hole in the center is a portal to the inner water chamber. The two shaded atoms, in the upper left and lower right subunits, respectively, draw attention to the iron atoms at the bottom of the channel between the four helices of each subunit.

oxygen and other ligands find access to the binuclear iron center.

Concluding Remarks

This binuclear oxygen carrier is another manifestation of the enormous versatility of the polyamino acid chain to provide a molecular framework for a host of biological functions. It has taken evolution about 10^9 years of trial and error to find a proper combination of a suitable polypeptide and an appropriate metal to generate an efficient oxygen carrier. In a period of a few times 10^1 years, chemists have unraveled the detailed molecular structure of this entity. The stage has thus been reached, therefore, where we can formulate a complementary goal. It should now be possible to synthesize novel entities in the laboratory, in principle, from coal, rocks, air, and water, that have molecular structures and properties analogous to those of the functional centers in biological oxygen carriers. Several laboratories have initiated substantial exploratory efforts in this direction. It will be of interest to see how many decades are required to synthesize an entity with features superior to those of hemerythrin, hemocyanin, or hemoglobin.