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Polarized Single-Crystal Spectroscopic Studies of Oxyhemerythrin

Sir:

We report the room and low temperature polarized single-crystal electronic spectra of oxyhemerythrin and methemerythrin fluoride and azide. A number of the absorption bands are found to be extremely polarized, and a comparison between the oxy and met forms allows the oxygen to iron charge-transfer transition to be determined to be dominantly polarized perpendicular to the Fe-Fe axis. Using a transition dipole vector coupling model, the observed polarizations require that the Fe-O axis make only a small projection on the Fe-Fe axis, thereby placing strong restrictions on the proposed modes of oxygen binding to the active site.

The data available on hemerythrin, the binuclear iron oxygen binding protein, is rapidly evolving to a point where a detailed picture of the active site can be obtained. In particular, resonance Raman studies¹ have shown that O₂ binds asymmetrically as peroxide to the binuclear iron site, which has further been shown to be antiferromagnetically coupled high-spin Fe(III)'s.² Recent x-ray crystal structure determi-

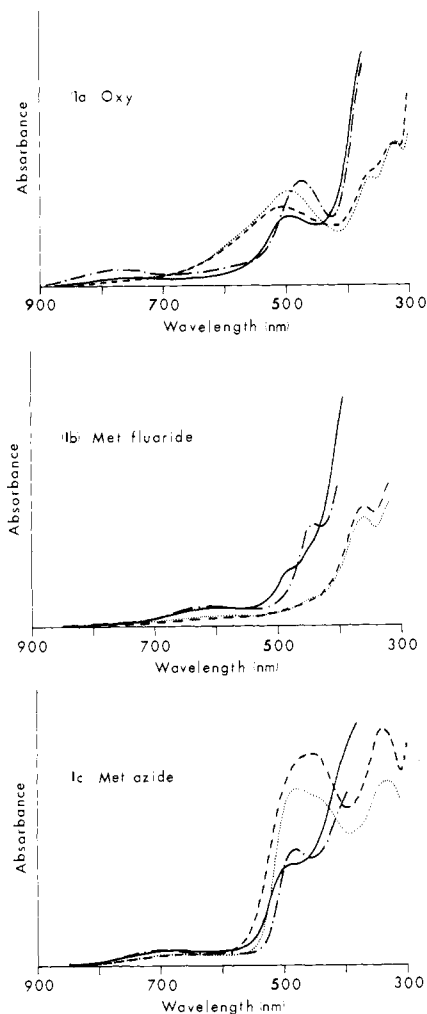


Figure 1. Polarized single-crystal absorption spectra of hemerythrin: (a) oxyhemerythrin; (b) methemerythrin fluoride; (c) methemerythrin azide. Polarizations and temperatures are indicated as follows: (—) 273 K, parallel; (---) 77 K, parallel; (····) 273 K, perpendicular; (— · —) 77 K, perpendicular. Polarizations refer to the orientation of the E vector relative to the needle axis.

nations³ have shown this site to be approximately a trigonal antiprism with the Fe-Fe axes for all eight of the subunits in the octamer approximately parallel.^{3a} This structure is ideal for polarized single-crystal spectroscopic studies which should provide some extremely detailed insight into this active site. Further, the dilute yet oriented nature of the active site of protein crystals makes them ideally suited for obtaining polarized spectra⁴ of charge-transfer and other intense absorption processes.

Hemerythrin crystals suitable for spectroscopic study were prepared by dialysis of the purified protein⁵ (from *Golfingia gouldii*) against 15% ethanol. The crystals obtained were of the approximate dimensions $2 \times 0.6 \times 0.1$ mm. These small crystals required modification of our McPherson RS-10 spectrometer for low temperature polarized studies, as described elsewhere.⁶ The orientation of the Fe-Fe axis in these crystals was determined by comparison with those forms used for x-ray analysis. The colors observed under polarized light for the crystal form of the met azide derivative used in the structural studies^{3b} require that the intense visible absorption⁷ be dominantly polarized perpendicular to the crystallographically defined Fe-Fe axis. As shown in Figure 1c, the intense 480-nm band in our met azide crystals is strongly polarized perpendicular to the needle axis, defining the Fe-Fe vector to be oriented along the needle axis.

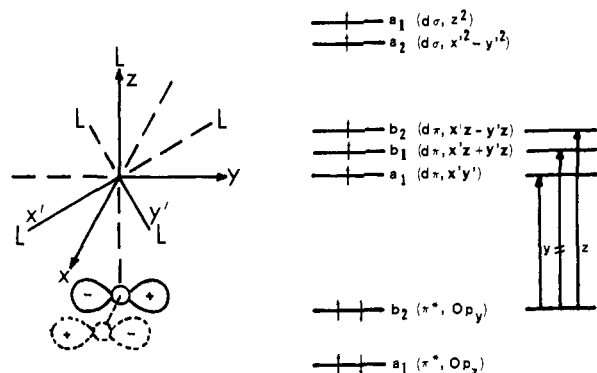


Figure 2. Monomer geometry and order of the energies of the valence orbitals of the iron-peroxide system in oxyhemerythrin. The predicted polarizations of the lowest energy transitions are shown at the right.

In Figure 1a we present the 273 and 77 K polarized single-crystal spectrum of oxyhemerythrin. This spectrum has been obtained at temperatures down to 5 K but no significant change was observed on cooling from 77 K. As with the azide form, we find a number of the bands in oxyhemerythrin to be extremely polarized. To sort out the peroxide to iron charge-transfer transitions, we have included the polarized spectrum of the met fluoride derivative for comparison (Figure 1b). The fluoride to iron charge-transfer transition should be shifted to higher energy, out of the accessible spectral range, while charge-transfer transitions due to other ligands and the ligand field transitions of the metals should only be slightly affected. It is seen that the narrow, parallel polarized band at 494 nm (at 273 K) in the oxy spectrum also occurs in the met fluoride and met azide spectra; hence it cannot be associated with the charge-transfer transitions from the ligand being varied. Eliminating the contributions of this invariant band, Figure 1 then demonstrates that the peroxide to iron charge-transfer transition results in one broad absorption band centered at 508 nm (at 273 K) which is strongly polarized perpendicular to the Fe-Fe axis.

What now remains is to determine the requirements this perpendicular polarization imposes on the active site. We are therefore interested in determining the polarization characteristics of a dimer site in which each monomer remains high spin yet weakly coupled to the other. In our approach, we first determine the polarization of the $O_2^{2-} \rightarrow Fe^{3+}$ charge-transfer transition to be expected for the monomer, and then extend this to the dimer case using a transition dipole vector coupling model for various peroxide binding modes. The expected polarizations for the monomer are treated in Figure 2. The highest filled orbital in the peroxide ion is the π^* orbital. Upon coordination to a metal, bonding interactions will split the π^* level by $\sim 5000 \text{ cm}^{-1}$.⁸ The lowest energy unfilled metal orbitals are the half-filled $d\pi$ orbitals which may be split by, at most, several hundred wavenumbers owing to the low symmetry of the Fe^{3+} site. Using an effective C_{2v} monomer symmetry, two $\pi^*, Op_y \rightarrow d\pi$ transitions are group theoretically allowed (Figure 2) with overlap arguments requiring the z-polarized transition to be significantly more intense (where z refers to the Fe-O axis).

For each transition in the monomer, there are two transitions in the dimer, which are required by symmetry to be polarized either parallel or perpendicular to the dimer Fe-Fe axis. In Figure 3 we have calculated the ratio of parallel to perpendicular intensity for three modes of coordination of the peroxide ion for the three possible transition polarizations of the monomer. Eliminating the contribution of the band in parallel polarization found in all derivatives, an upper limit for the ratio of parallel to perpendicular intensity of $\sim 1/10$ is reasonable for the peroxide to iron charge-transfer transition (Figure 1a). As

Ratio of I_{\parallel}/I_{\perp} for a Monomer Transition in a Dimer

Dimer Geometry	Monomer Polarization		
	X	Y	Z
(3a)	0.44	0	9.1
(3b)	5.3	0	0.76
(3c)	$2 \tan^2 \theta$ (0.1 for $\theta = 13^\circ$)	0	$2 \cot^2 \theta$ (0.1 for $\theta = 77^\circ$)

Figure 3. Predicted polarization ratios for the active site dimer using a transition dipole vector coupling model. Numbers were obtained assuming the following bond lengths: Fe-Fe, 3.44 Å; Fe-O, 1.90 Å; O-O, 1.45 Å.

determined in Figure 2, an x-polarized monomer charge-transfer transition is forbidden by symmetry and thus cannot contribute to the dimer spectrum. For any of the geometries considered in Figure 3, the y-polarized monomer transition results in a completely perpendicularly polarized dimer transition, which would be consistent with our data. However, overlap requires the z-polarized transition to make the dominant contribution to the observed intensity. For a z-polarized monomer transition to result in a dimer band predominantly perpendicular in character, the projection of the Fe-O vector onto the Fe-Fe axis must be quite small. The estimated ratio of parallel to perpendicular intensity of $< 1/10$ requires an O-Fe-Fe angle of $> 77^\circ$ ($90 \pm 13^\circ$). This would preclude the bridging end-on geometries considered in Figure 3a and 3b. Resonance Raman studies^{1b} have also eliminated the bridging geometry shown in Figure 3b as well as other modes of ligand binding for which the oxygen atoms are equivalent. The observed polarization ratio is, however, consistent with the model in Figure 3c in which the peroxide is bound to only one iron with an O-Fe-Fe angle close to 90° .

These studies are now being extended to use the spectral effects of ligand variation to obtain further information on the active site.

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