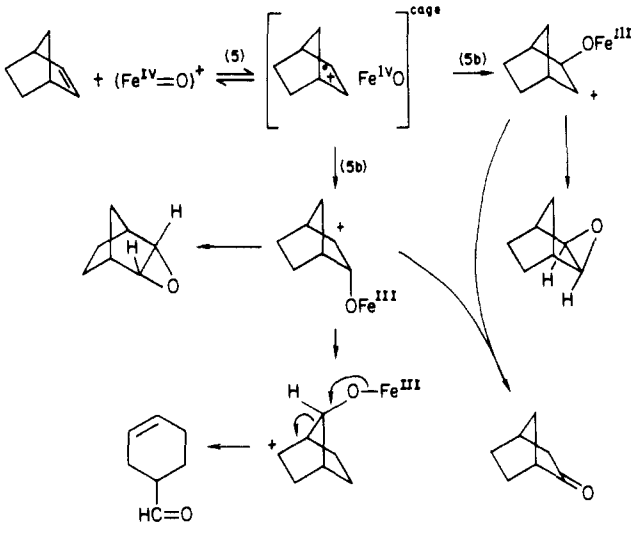

2,3-epoxynorbornane during hemin-catalyzed epoxidation is inconsistent with direct attack of the "oxohemin" on norbornene. The results are best explained as an electron transfer from the alkene followed by radical collapse to give a carbocation.

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Blue Copper Proteins. The Copper Site in Azurin from *Alcaligenes denitrificans*

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The blue copper proteins have been the focus of many spectroscopic and structural studies,[1] with much of the interest centering on the nature of the copper site. X-ray crystallographic results have previously been reported for two single-copper proteins, plastocyanin and azurin. The structure of the oxidized, Cu(II), form of poplar plastocyanin has been refined at high resolution,[2] but structural analysis of azurins (from two bacterial species, *Pseudomonas aeruginosa*[3,4] and *Alcaligenes denitrificans*[5]) have been reported only at medium resolution.

We have now refined the structure of *Alcaligenes denitrificans* azurin at high resolution (1.8 Å)[6] and we wish to report the details of the refined copper site. The refinement has clearly shown that its geometry is closer to a distorted trigonal-planar or trigonal-bipyramidal arrangement rather than the distorted tetrahedron usually quoted. This distinction may be crucial for a proper understanding of the spectroscopic and functional properties of azurin, in particular, and blue copper proteins in general.

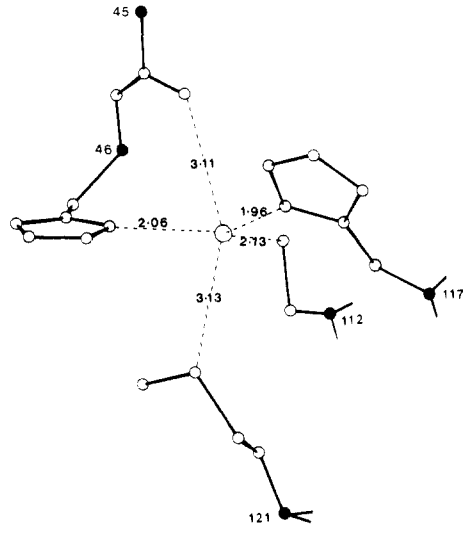

Figure 1. Copper site in azurin. Distances given are the mean of those found for the two independent molecules in the asymmetric unit.

Table I. Bond Lengths and Angles for the Copper Site in Azurin from *Alcaligenes denitrificans*

bond lengths, Å	1	2	bond angles, deg	1	2
Cu-O(45)	3.14	3.08	O(45)-Cu-$N_{\delta 1}$(46)	72	75
Cu-$N_{\delta 1}$(46)[a]	2.08	2.04	O(45)-Cu-S_γ(112)	103	104
Cu-S_γ(112)[a]	2.10	2.16	O(45)-Cu-$N_{\delta 1}$(117)	78	82
Cu-$N_{\delta 1}$(117)[a]	1.98	1.94	O(45)-Cu-S_δ(121)	146	148
Cu-S_δ(121)	3.13	3.13	$N_{\delta 1}$(46)-Cu-S_γ(112)[a]	137	136
			$N_{\delta 1}$(46)-Cu-$N_{\delta 1}$(117)[a]	100	106
other distances			$N_{\delta 1}$(46)-Cu-S_δ(121)	77	74
S_γ(112)···N(47)	3.54	3.44	S_γ(112)-Cu-$N_{\delta 1}$(117)[a]	121	117
$N_{\epsilon 2}$(46)···O(10)	2.68	2.63	S_γ(112)-Cu-S_δ(121)	110	105
			$N_{\delta 1}$(117)-Cu-S_δ(121)	93	97

[a] Bond lengths and angles within the trigonal plane.

Our azurin was purified from *Alcaligenes denitrificans* NCTC 8582[7] and crystallized in its oxidized, Cu(II), form at pH 6.0. The unit cell data, and the results of the medium-resolution (2.5 Å) analysis have been published previously.[8,5] One notable feature is that the crystallographic asymmetric unit contains two molecules of azurin, thus giving two copies of the same structure and a valuable internal check on the reliability of structural observations.

Refinement of the structure was based on diffractometer data to 1.8-Å resolution (2θ = 50.7°). Because of the relatively low ratio of observations to parameters for an asymmetric unit containing over 2000 atoms, all X-ray data between 10 and 1.8 Å were used, with no σ cutoff, and only negative intensities and misset reflections excluded.[9] For the same reason, restrained least-squares procedures[10] were used for the refinement, with protein bond lengths and angles being restrained close to standard values.[11] No restraints were, however, imposed on any of the distances or angles involving the copper atom, and the two molecules in the asymmetric unit were allowed to refine quite independently.

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(7) The strain number, NCTC 8582, is important as this organism has recently been reclassified as belonging to the genus *Alcaligenes faecalis* and should not be confused with the type strain *Alcaligenes faecalis* NCIB 8156, on whose azurin numerous other studies have been performed.

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(9) From an estimated total of 25 200 unique reflections to 1.8-Å resolution, 15 330 (i.e., 61%) were measured with $I > 2\sigma_I$ and 18 680 (74%) with $I > \sigma_I$. The inclusion of all nonnegative intensities increased the number of observed data to 21 980 (87% of total).

(10) Programs used were PROLSQ, the restrained least-squares program of Hendrickson and Konnert (See: Hendrickson, W. A.; Konnert, J. H. *Biomolecular Structure, Function, Conformation and Evolution*; Srinivasan, R., Ed.; Pergamon: Oxford, 1980; Vol 1, pp 43-57), and TNT, a restrained least-squares procedure using fast Fourier methods: D. Tronrud and L. Ten Eyck, Institute of Molecular Biology, University of Oregon.

(11) In the final protein model, root mean square (rms) deviations from standard values were 0.017 Å for bond lengths and 3.1° for bond angles.

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The final refined model of 1956 protein atoms and 218 water molecules[12] gives a crystallographic R value of 0.164 for the 21 779 reflections between 10- and 1.8-Å resolution. The *upper limit* for the average error in atomic positions, from the variation of R with resolution, is 0.15 Å, but for well-ordered parts of the structure, such as the copper site, the error is much less. Our estimate of the standard deviations in the copper site geometry, based on the level of agreement between the two molecules,[13] is ~0.05 Å for Cu–ligand bond distances and ~3.0° for ligand–Cu–ligand angles.

The copper site is shown diagrammatically in Figure 1, and relevant bond distances and angles in Table I. The copper atom makes three strong bonds, with the thiolate sulfur of Cys-112 (mean Cu–S 2.13 Å) and the imidazole nitrogens of His-46 and His-117 (mean Cu–N 2.06 and 1.96 Å, respectively). These distances, including the unusually short Cu–S bond, agree very closely with those deduced from X-ray absorption spectroscopy on azurin[14] and X-ray crystallographic and X-ray absorption spectroscopy[15] measurements on plastocyanin. The three ligands form a distorted trigonal-planar arrangement about the copper atom. Much longer approaches are made to the thioether–sulfur of Met-121 on one side of this plane (Cu–S 3.13 Å) and the peptide carbonyl oxygen of Gly-45 on the other side (Cu–O 3.11 Å), making overall a distorted axially elongated trigonal-bipyramidal arrangement.

Whether the coordination geometry is best described as trigonal planar, trigonal pyramidal, or trigonal bipyramidal depends on whether the Cu–S(Met) and Cu–O approaches are regarded as weak bonds or not. The Cu–S(Met) interaction almost certainly is, while the Cu–O distance of 3.1 Å is probably right at the limit for a weak Cu–O bond.[16] We note, however, that this carbonyl oxygen is buried in a nonpolar environment, with no possibility of interaction with any other polar or polarizable atom, and that this copper–oxygen (carbonyl) interaction has been detected by NMR spectroscopy.[17] The relative positions of the copper atom and these more distant axial groups probably has a role in tuning the redox potential in different proteins.

The copper atom lies very close to the N_2S plane formed by the three strong ligands, its deviation (toward Met-121) being 0.13 Å in molecule **1** and 0.08 Å in molecule **2**. During the refinement, and again at the end, we tried artificially moving the copper about 0.3 Å toward Met-121 (decreasing the Cu–S distance and increasing Cu-O). In each case, refinement brought it right back to its "in-plane" position. The bond angles within the trigonal plane are quite irregular (mean values 103°, 119°, and 136°) emphasizing the low symmetry of this site. This low symmetry must clearly be taken into account in any theoretical analyses of (for example) ESR spectra. The long approaches to S_δ(121) and O(45) are both bent away from a precise axial disposition. The copper site groups are tightly constrained by the surrounding protein structure, through hydrogen bonds (to His-46 $N_{\epsilon 1}$ and Cys-112 S_γ) and van der Waals contacts.

Finally, the structure described above is entirely consistent with that determined earlier for *Pseudomonas aeruginosa* azurin[18] (although the latter has not yet been refined) and this, together with the high level of consistency between the two independent molecules in our crystal asymmetric unit, further validates the above results.

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(12) Coordinates and thermal parameters have been deposited with the Brookhaven Protein Data Bank, Brookhaven National Laboratory, Upton, NY 11973.

(13) Superposition of the two copper sites and their immediate surrounds (112 atoms) gives an rms difference in atomic positions of 0.14 Å. A better guide to the likely error in copper site geometry, however, is given by comparison of distances and angles that were not restrained during refinement. For copper bond lengths and angles the rms *differences* between the two molecules are 0.05 Å and 3.6° and for hydrogen bond lengths and angles 0.10 Å and 6.2° (for 55 pairs of hydrogen bonds). Standard deviations should be less than these values.

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Reversible Thermochromism in Photopolymerized Phosphatidylcholine Vesicles

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There is substantial interest in modified phospholipids that can be polymerized. Adding a polymer backbone to noncovalent lipid assemblies such as liposomes and Langmuir/Blodgett films should make them more rugged and useful in biotechnology.[1-4] Perhaps the best known have been phosphatidylcholines incorporating diacetylenic moieties within their acyl side chains, which can be polymerized by ultraviolet light or γ radiation.[5,6] The solid-state polymerization is accompanied by the development of a characteristic blue color, which can be converted to red upon heating or treatment with solvents;[7] in some cases, cooling reverses the change. We report here that an aqueous suspension of polydiacetylenic phospholipid vesicles can exhibit reversible thermochromism and examine the implications of this phenomenon for models of polydiacetylene thermochromism.[6,8,30]

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