of approximately 100°. These structural changes are expected for the substitution of the oxo bridge with sulfide.

NMR studies on the semimet and deoxy forms reveal the N-H signals to be further downfield, indicating that the extent of antiferromagnetic coupling between the iron atoms is significantly decreased. For the semimet azido complex, the N-H resonances are found at 73 and 54 ppm downfield with relative intensities of 2:3; these have been assigned to the histidine N-H protons coordinated to the ferric and ferrous sites, respectively. Thus the azide is coordinated to the ferric site, in agreement with resonance Raman data showing the persistence of the azide-to-Fe(III) charge transfer transition in this complex [6]. The temperature dependence of these shifts is best fitted with a J value of -10 cm^{-1}.

The NMR spectrum of the semimet sulfido complex is somewhat more complex. Five N-H resonances are observed in the region of 23-54 ppm downfield with relative intensities of 3:2:2:2:1. The temperature dependences of these peaks show both Curie and anti-Curie behavior, similar to that observed for the β-CH_2 resonances of the cysteines in reduced Fe_2S_2-ferredoxins [7]. The data may be interpreted in terms of both possible mixed-valent complexes with the unpaired electron localized on one of the iron atoms, *i.e.* electron transfer is slow on the NMR time scale.

Deoxyhemerythrin exhibits N-H resonances at 44, 46, and 62 ppm downfield. These signals are perturbed upon addition of azide with new resonances observed at 47, 66, and 77 ppm downfield. These spectral comparisons show that azide can coordinate to the iron sites in deoxyhemerythrin.

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PS1.8 — TU

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RESONANCE RAMAN STUDY OF THE HYDROXIDE ADDUCT OF HEMERYTHRIN

Hemerythrin is the non-heme iron-containing respiratory protein of several marine invertebrates. When O_2 binds to the binuclear active site, the two iron atoms are oxidized to the ferric state and dioxygen is reduced to peroxide [1]. Methemerythrin, the unligated form of the protein in which both irons are oxidized to the Fe(III) state, no longer binds O_2, but readily binds small anions such as N_3^-, SCN^-, OCN^-, and CN^-. At high pH, it binds hydroxide to form hydroxomethemerythrin. X-ray crystallographic studies show that the binuclear iron site of met- and oxyhemerythrin contains a μ-oxo bridge [2]. This Fe-O-Fe moiety gives rise to intense near-UV charge transfer transitions [3]. Excitation within these charge transfer bands gives strong, selective enhancement of a Raman peak near 500 cm^{-1} which has been assigned as the symmetric Fe-O-Fe vibration, ν_s(Fe-O-Fe) [4,5]. Whereas most methemerythrins have only a single Raman peak in the ν_s(Fe-O-Fe) region, hydroxomethemerythrin has a strong peak at 492 cm^{-1}, and a smaller peak at 565 cm^{-1} (Fig. 1a). Substitution of ^{18}O into the oxo bridge position causes the 492 cm^{-1} peak to shift to 478 cm^{-1} (Fig. 1c), prompting assignment of this peak as ν_s(Fe-O-Fe). Preparation of hydroxomethemerythrin in $H_2{}^{18}O$, under conditions where the bridge does not exchange, results in a shift of the 565 cm^{-1} feature to 538 cm^{-1} (Fig. 1b). This shift agrees with the calculated value (540 cm^{-1}) for the replacement of ^{16}O by ^{18}O in the iron-oxygen

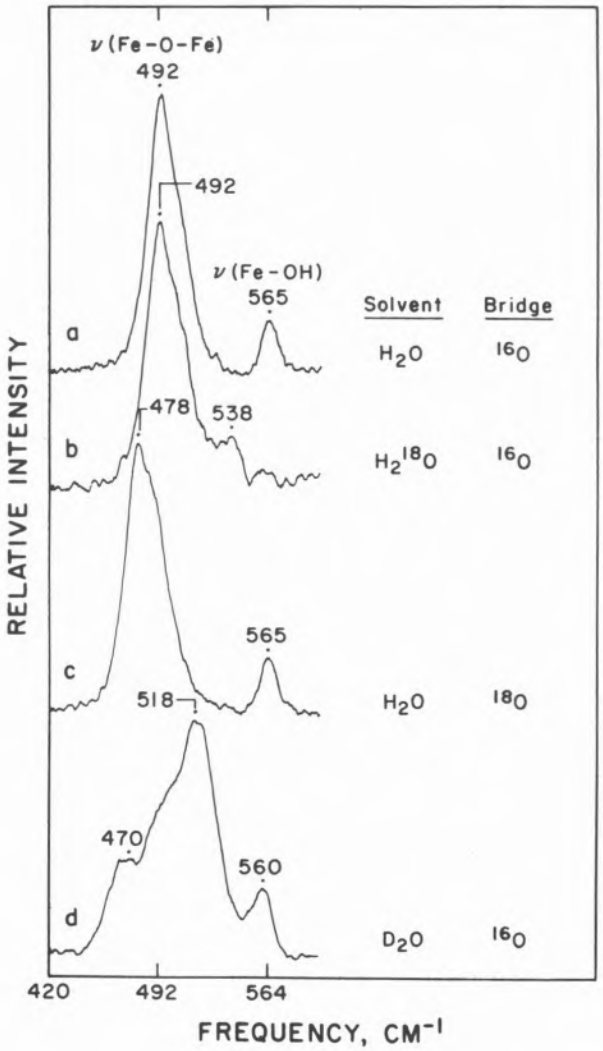

Fig. 1

stretch of a bound hydroxide, and we now assign it as such.

In contrast to the straightforward results from the oxygen isotope experiments, preparation of hydroxomethemerythrin in D_2O causes several anomalous shifts in the resonance Raman spectrum (Fig. 1d) [6]. The major peak at 492 cm^{-1} in H_2O (ν_s(Fe-O-Fe)) shifts to 517 cm^{-1} in D_2O. This shift cannot be due to deuterium-induced conformation changes of the protein since analogous peaks of deuterium-substituted met-, azidomet-, and perchloromethemerythrin do not shift. In addition, the iron-oxygen stretch of the bound hydroxide at 565 cm^{-1} shifts only 5 cm^{-1} in D_2O, which is less than half the shift expected for the substitution of OH^- by OD^- (calc. shift to 553 cm^{-1}). This indicates that the hydroxide ligand is involved in the anomalous D_2O effect on ν_s(Fe-O-Fe).

Although the spectral shifts of hydroxomethemerythrin in D_2O are unusual, similar results have been obtained for oxyhemerythrin [5]. Both hydroxomet- and oxyhemerythrin show anomalous deuterium isotope effects on ligand as well as Fe-O-Fe vibrations. In addition, these forms of the protein have ν_s(Fe-O-Fe) frequencies (492 and 486 cm^{-1} for hydroxomet- and oxyhemerythrin, respectively) which are considerably lower than those of the other ferric hemerythrins (507-516 cm^{-1}) [4,5]. We have proposed that these effects in oxyhemerythrin are the result of a hydrogen bond between the proton of the hydroperoxide ligand and the oxygen of the oxo-bridge. Because hydroxide is the only other methemerythrin ligand that is capable of forming a hydrogen bond analogous to that proposed for oxyhemerythrin, it is not unreasonable to propose a similar model for hydroxomethemerythrin. In this case, we suggest that the proton of the bound hydroxide is hydrogen bonded to the oxo-bridge. Though this appears to be a strained structure, the strain may be responsible for the large deuterium effect on ν_s(Fe-O-Fe). We are currently studying model compounds which contain elements of this proposed hydroxomethemerythrin structure.

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PS1.9 — TH

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PRELIMINARY STRUCTURAL STUDIES ON BACTERIOFERRITIN

Bacterioferritin is a novel multimeric cytochrome which appears to function both as an electron transport protein and as a means of storing iron. A cytochrome, named b_1, was isolated from *Escherichia coli* by DEEB and HAGER [1] who suggested it was a key component of membrane electron transport systems in this organism. A similar cytochrome (named cytochrome $b_{557.5}$), but which also contained non-haem iron was purified from *Azotobacter vinelandii* by BULEN and co-workers [2] and subsequently characterised more fully by STIEFEL and WATT [3]. These authors showed that the protein resembled mammalian ferritin in several respects and identified it as a bacterioferritin. A protein from *E. coli* could be similarly described [4], since it contained an «iron-core» surrounded by a shell of protein, although its diameter, measured from electron micrographs, was somewhat smaller than that of ferritin and the magnetic properties of its inorganic complex differed from those of ferritin's iron-core.

We report here preliminary X-ray crystallographic data for three crystalline forms of bacterioferritin isolated from *E. coli* grown anaerobically on nitrate. These crystals are monoclinic, tetragonal and cubic in form. The monoclinic crystals grown from aqueous solutions containing $MnCl_2$ have cell dimensions a = 122.2 Å, b = 209.6 Å, c = 118.6 Å, β = 118.3° and space group $P2_1$. Tetragonal crystals grown from 2 M $(NH_4)_2SO_4$ solution, also containing $MnCl_2$, have a = b = 210.6 Å, C = 145.0 Å and space group $P4_22_12$ but show marked pseudo-cubic symmetry. Soaking the tetragonal crystals in tetrakisacetoxymercurimethane solution causes conversion to a cubic form with a = 146.9 Å, space group I432. We interpret these data in terms of a unit cell containing two molecules each having 24 protein subunits arranged in 432 molecular point symmetry to give a packing diameter of 127 Å. This now clearly shows that bacterioferritin does indeed closely resemble horse spleen ferritin which has a similar diameter and the same number of subunits and symmetry, although it contains no haem. Crystals of all three forms of *E. coli* bacterioferritin diffract to high resolution (at least 1.6 Å) and are all suitable for three dimensional structure determination. This is now being undertaken.

Structural relationships between the three crystalline forms of *E. coli* bacterioferritin, between the *E. coli* and *A. vinelandii* proteins and between ferritin and bacterioferritin will be outlined and the functions of these exciting proteins discussed.

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