RESONANCE RAMAN SPECTROSCOPIC EVIDENCE FOR STRUCTURAL VARIATION AMONG BACTERIAL FERREDOXIN, HiPIP, and $Fe_4S_4(SCH_2Ph)_4^{2-}$*

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Summary. Resonance Raman spectra have been obtained for C. pasteurianum ferredoxin, Chromatium HiPIP and $Fe_4S_4(SCH_2Ph)_4^{2-}$, all of which contain a cubane-like Fe-S cluster. They show bands assignable to Fe-S stretching vibrations of both bridging and terminal sulfur atoms. The frequencies shift significantly among the three molecules, and an extra polarized band gives evidence of symmetry lowering in the proteins.

The iron-sulfur electron transfer proteins continue to generate much biochemical interest (1), and considerable progress has been made in elucidating their structural chemistry. Particularly striking are the relationships which have emerged among the bacterial ferredoxins, the high-potential iron protein (HiPIP) of Chromatium and the synthetic clusters $[Fe_4S_4(SR)_4]^{2-}$ (2-6). All three contain cubane-type iron-sulfur clusters in which the iron and sulfur atoms occupy alternating corners of an approximate cube, and each iron atom is additionally bound to a sulfur atom of a cysteine residue. The bacterial ferredoxins generally contain two such clusters which in the P. aerogenes protein are ~12Å apart (5). Some four-iron ferredoxins are also known (7). Within the current resolution of the protein structure determinations no significant

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Abbreviations used: Ph- phenyl; HiPIP - High-potential iron protein.

differences are observable between the clusters found in HiPIP, ferredoxin from P. aerogenes, and $[Fe_4S_4(SCH_2Ph)_4]^{2-}$. Yet the redox potential of HiPIP, 0.35V, is ~0.8V higher than that of ferredoxin (-0.4 to -0.5V) (1). Moreover the reduced form of ferredoxin, but the oxidized form of HiPIP, contains an odd number of electrons (per cluster).

Magnetic and spectroscopic evidence for $[Fe_4S_4(SR)_4]^{2-}$ demonstrates that these dianions, in which the formal oxidation state of the iron is half-way between (II) and (III), possess the same total oxidation level as reduced HiPIP and oxidized ferredoxin proteins (3,4). From the highest occupied molecular orbital of the cluster one electron can be removed from HiPIP and one electron (per cluster) can be added to ferredoxin (1). The dianions $[Fe_4S_4(SR_4]^{2-}$ can be both oxidized and reduced, and the difference between the two potentials is about 1.0 V (4). Recently Cammack (8) has lent further support to this picture by showing that reduced HiPIP can be further reduced by dithionite if the solution contains in excess of 70% dimethylsulfoxide, which apparently denatures the protein in a reversible manner. The inference is that the protein conformation plays a crucial role in determining the redox behavior of the iron-sulfur proteins. This behavior must also be reflected in the structure of the iron-sulfur clusters, but at a level of detail which may escape the present resolution of protein x-ray crystallography. Fine structural differences may be revealed in changes in iron-sulfur vibrational frequencies, however. These are obtainable through the technique of resonance Raman spectroscopy, in which laser excitation within an electronic absorption band produces selective enhancement of Raman lines which arise from vibrations of the chromophoric unit (9). The visible spectra of the iron-sulfur proteins and analogs are dominated by intense S → Fe charge transfer absorptions (4), and enhancement of Fe-S vibrations can be expected. Such modes have been reported for rubredoxin (10) and adrenodoxin (11). Here we report preliminary resonance Raman spectra for HiPIP, C. pasteurianum ferredoxin and $Fe_4S_4(SCH_2Ph)_4^{2-}$.

RESULTS AND DISCUSSION

Figure 1 compares Raman spectra for $Fe_4S_4(SCH_2Ph)_4^{2-}$, reduced HiPIP, and oxidized ferredoxin, obtained with 4880 Å excitation. Their quality is lower than is optimal, because the materials present special problems with respect to air and photo-sensitivity, and the fluorescent backgrounds are high. The data must therefore be considered preliminary. The peaks which are labelled in the figures have been reproduced several times however, and are considered reliable. All of them are polarized, and arise from totally symmetric vibrations.

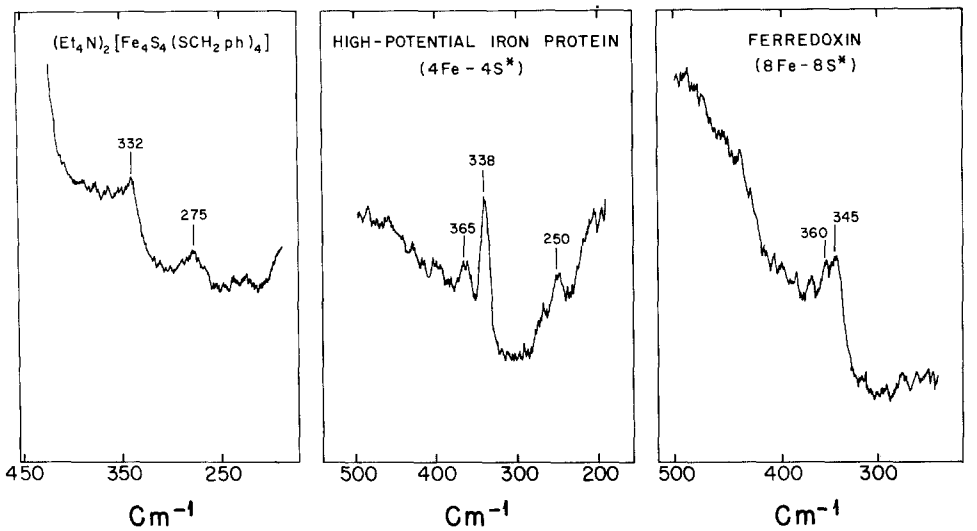

Figure 1: Resonance Raman spectra of dilute solutions of the indicated species, held in 1mm glass capillaries, obtained by transverse excitation with a 4880 Å Ar^+ laser line (20-50 mW). The spectrometer is described elsewhere (16). Solutions (preparation procedures given in the cited references), and instrument conditions:

$(Et_4N)_2$ $[Fe_4S_4(SCH_2Ph)_4]$ (17), 10^{-3} M in acetonitrile; Chromatium HiPIP (18), 10^{-4} M in 0.05M aqueous phosphate buffer, pH7; C. pasteurianum ferredoxin (19), 2.5 x 10^{-3} M in 0.05M tris buffer, pH 8.0.

Instrument conditions: slit width, 10 cm^{-1}; scan rate 20 cm^{-1}/sec., period 1 sec.

As in the cases of rubredoxin (10) and adrenodoxin (11), these peaks occur in the frequency region, 250-400 cm^{-1}, appropriate for Fe-S stretching. For tetrahedral $Fe_4S_4(SCH_2Ph)_4^{2-}$ two totally symmetric Fe-S stretching modes are expected, corresponding to breathing modes of the terminal (mercaptide) and bridging sulfur atoms, respectively. Two bands are observed in the Raman spectrum. (The sharp rise in Raman intensity beyond 400 cm^{-1}, shown in Figure 1, is due to a band of the acetonitrile solvent). We tentatively assign the higher of these, 332 cm^{-1}, to the terminal mode; the corresponding Fe-S(cysteine) mode in adrenodoxin is at 350 cm^{-1} (11). The lower frequency, 275 cm^{-1}, is then assignable to the bridging sulfur mode.

The Raman spectrum of HiPIP shows two similar bands, shifted apart somewhat to 250 and 338 cm^{-1}, and an additional polarized band at 365 cm^{-1}. Since only two totally symmetric Fe-S modes are expected, the appearance of an extra polarized band in the region strongly implies a lowering of the cluster symmetry from tetrahedral, and induction of totally symmetric character into what would otherwise be a non-totally symmetric mode (and probably not subject to resonance Raman enhancement (9)). From the present data we cannot determine the character or extent of the symmetry lowering. Vibrational calculations, currently in progress, may throw some light on this question.

It should be noted that the X-ray crystal structure shows the Fe_4S_4 core of $Fe_4S_4(SCH_2Ph)_4^{2-}$ to be slightly distorted from T_d toward D_{2d} symmetry, via compression along a two-fold axis (3). Four of the bridging Fe-S distances are 2.24 Å and the remaining eight are 2.31 Å in length. It is unclear whether this distortion is too small to affect the Raman spectrum, at its present level of resolution, or whether the distortion is a crystal packing effect and disappears in solution. The extra Raman band in HiPIP implies a greater distortion than the solution distortion, if any, of $Fe_4S_4(SCH_2Ph)_4^{2-}$.

Like HiPIP, ferredoxin also shows two bands in the 330-370 cm^{-1} region, indicative of a similar lowering of tetrahedral symmetry.

Their frequencies, 345 and 360 cm^{-1}, are closer together than those of HiPIP. In the case of ferredoxin, the lower frequency, bridging sulfur mode near 250 cm^{-1} has not yet been resolved from the high fluorescent background.

The cubane-like iron-sulfur clusters should possess an additional breathing mode, which can be described either in terms of deformation of interior cluster angles, or in terms of Fe-Fe stretching (12). In either description the frequency is expected to be near or below 200 cm^{-1} (13,14). This region is at present obscured in our spectra by high backgrounds. The intensity of the mode is expected to depend on the degree of direct Fe-Fe bonding (13). That such bonding may be significant is suggested by the relatively short Fe-Fe distances, which average to 2.75Å, in $Fe_4S_4(SCH_2Ph)_4^{2-}$ (3) and the evidence for extensive electron delocalization in a framework of equivalent iron centers (15).

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