Conversion of *Clostridium pasteurianum* Rubredoxin into a Four-iron Ferredoxin

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Summary Denatured *C. pasteurianum* rubredoxin in 90% aqueous dimethyl sulphoxide is converted, by addition of sodium sulphide, iron(II) chloride, and iron(III) chloride, into a four-iron ferredoxin, which is partially reconverted into the rubredoxin on renaturation of the protein by dilution with water.

WE have recently shown[1] that the formation of analogues of four-iron ferredoxins from their components proceeds through the rubredoxin analogues. It seemed possible, therefore, that a natural rubredoxin (**1**) could be converted into a four-iron ferredoxin (**2**) by treatment with sodium sulphide, iron(II) chloride, and iron(III) chloride as shown in equation (1), where $(SR)_4$ = the peptide chain of the rubredoxin. We now report such a conversion starting from *C. pasteurianum* rubredoxin; this contains four cysteine

$$\underset{(\mathbf{1})}{[Fe^{III}(SR)_4]^-} + 2Fe^{2+} + Fe^{3+} + 4S^{2-} \longrightarrow \underset{(\mathbf{2})}{[Fe_2^{II}Fe_2^{III}S_4(SR)_4]^{2-}} \quad (1)$$

residues with the spacing[2] –Cys·X_2·Cys·X_{29}·Cys·X_2·Cys– (X = an amino-acid residue other than cysteine) which should not in any way hinder the formation of a cubic cluster.

Owing to the precipitation of iron sulphide, the reaction could not be satisfactorily studied spectroscopically in wholly aqueous solution. This difficulty did not arise in 90% aqueous dimethyl sulphoxide and the reaction was accordingly studied spectroscopically in this solvent, buffered at pH 8·3 with tris-hydrochloride, under strictly anaerobic conditions; the spectra were measured against a blank containing all the reagents except the protein. The results are shown in the Figure. Curve A shows the

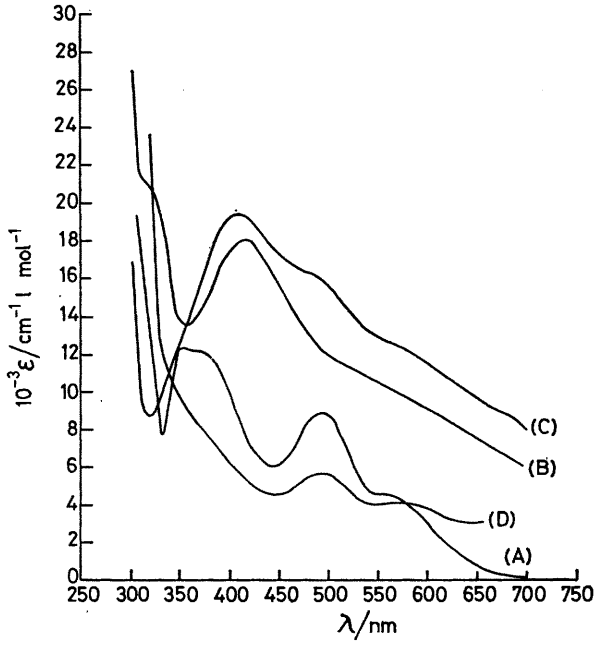

FIGURE. (A), Oxidized rubredoxin (0·094 μmol) in Me_2SO–H_2O (9:1 v/v, aqueous portion 50 mmol in tris-hydrochloride, pH 8·3) (3·0 ml); (B), as (A) plus 0·4 M aqueous Na_2S (2 μl), 0·2 M aqueous $FeCl_2$ (2 μl), 0·1 M aqueous $FeCl_3$ (2 μl), 2 h after mixing; (C), as (B) after diluting with two volumes of 50 mmol tris-hydrochloride, pH 8·3, buffer; (D), as (C) plus $HOCH_2CH_2SH$ (2 μl) and a limited amount of air, 24 h after mixing.

spectrum of the oxidised form of the rubredoxin (**1**); it is very similar to that observed in aqueous solution[3] and in unbuffered 80% aqueous dimethyl sulphoxide.[4] The spectrum changes dramatically on addition of sodium sulphide (8 equiv.), iron(II) chloride (4 equiv.), and iron(III) chloride (2 equiv.) (this excess of reagents is necessary for complete conversion) and after 2 h is as shown in curve B; the peak at 414 nm is typical of a four-iron ferredoxin and its intensity (ϵ = 18,000) is comparable to that observed for a naturally occurring eight-iron ferredoxin (per four-iron cluster) in a similar solvent.[5]

Dimethyl sulphoxide is a well-known reversible denaturing agent for proteins in general and iron–sulphur proteins in particular.[6] The addition of aqueous buffer to reverse the denaturation by reducing the dimethyl sulphoxide concentration to 30% caused some precipitation of iron sulphide, giving rise to considerable background absorption only partially compensated for by the blank; for this reason the absorption spectra for the diluted solutions (curves C and D) have only qualitative significance. The spectrum of the diluted solution (curve C) showed the expected blue shift of the main absorption band[5] from 414 to 408 nm and also shoulders at 480—500 and 560—570 nm, indicating some reformation of the rubredoxin. Since control experiments had shown that the sodium sulphide caused partial reduction of the oxidized form of rubredoxin, a little oxygen was admitted to convert the rubredoxin into the oxidized form, after the addition of a little 2-mercaptoethanol to prevent disulphide formation in the protein. After 24 h the final spectrum was as shown in curve D; although the high energy region is obscured by background absorption, the bands at 490 and 550—590 nm clearly indicate partial re-formation of the original oxidized rubredoxin; a rough estimate of the additional background absorption indicates that about a third of the original rubredoxin has been reformed in the complete cycle of operations.

This appears to be the first time that experimental evidence has been obtained for the direct conversion of a natural rubredoxin into a ferredoxin,[7] with replacement of the central single tetrahedrally co-ordinated iron atom in the former by the larger central Fe_4S_4 cube in the latter. The ready formation of a ferredoxin from the 'random' peptide chain of the denatured protein is not unexpected in the light of work with simple ligands, including cysteine peptides, which indicates that the four-iron type of structure is the most stable of the possible iron-containing complexes.[1,8] There seems no reason why such 'ferredoxins' should not be similarly obtainable from other denatured proteins containing four or more, suitably spaced, cysteine residues. The re-formation of part of the original rubredoxin on re-naturation of the protein by adding water is clearly due to the 'squeezing out' of the four 'inorganic' sulphur atoms and the three extra iron atoms in (**2**) as the peptide chain of the protein takes up its ordered conformation, with the four cysteine sulphur atoms sufficiently close together to co-ordinate to a central iron atom and *ipso facto* too close together to accommodate a Fe_4S_4 cube; the intrinsic stability of the native conformation of the peptide chain outweighs that of the Fe_4S_4 cubic cluster.

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