Peptide Cluster Analogue of Two Iron Ferredoxin

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The synthesis of simply alkyl and aryl thiolate analogues of 1-Fe, 2-Fe, and 4-Fe proteins has contributed greatly to the understanding of the structure and properties of iron–sulphur proteins [1]. These studies have been further augmented by the preparation and characterization of peptide cluster analogues of rubredoxin [2] and four iron ferredoxin [1–3].

Recently we reported that Fe–Mo–peptide complexes could be prepared by ligand exchange of cysteinyl peptides with preformed $[Cl_2FeS_2MoS_2]^{2-}$ [4]. This observation and the finding that arylthiols could displace chloride from $[Fe_2S_2(Cl)_4]^{2-}$ and $[Fe_4S_4(Cl)_4]^{2-}$ [5] prompted us to investigate the possibility of preparing peptide cluster analogues of oxidised two iron ferredoxin 2Fe-Fd_{ox} which has not been reported up-to-date**. In this communication we present evidence for the formation of a peptide cluster analogue of 2Fe-Fd_{ox} in solution.

The reaction of $[Fe_2S_2(Cl)_4]^{2-}$ [5] with Ac-Gly_2-Cys-Gly_2-Cys-Gly_2-NH_2 (I) [3] in the presence of base Et_3N were monitored using electronic spectroscopy. These experiments were performed in dimethylsulphoxide under a rigorously oxygen-free nitrogen atmosphere.

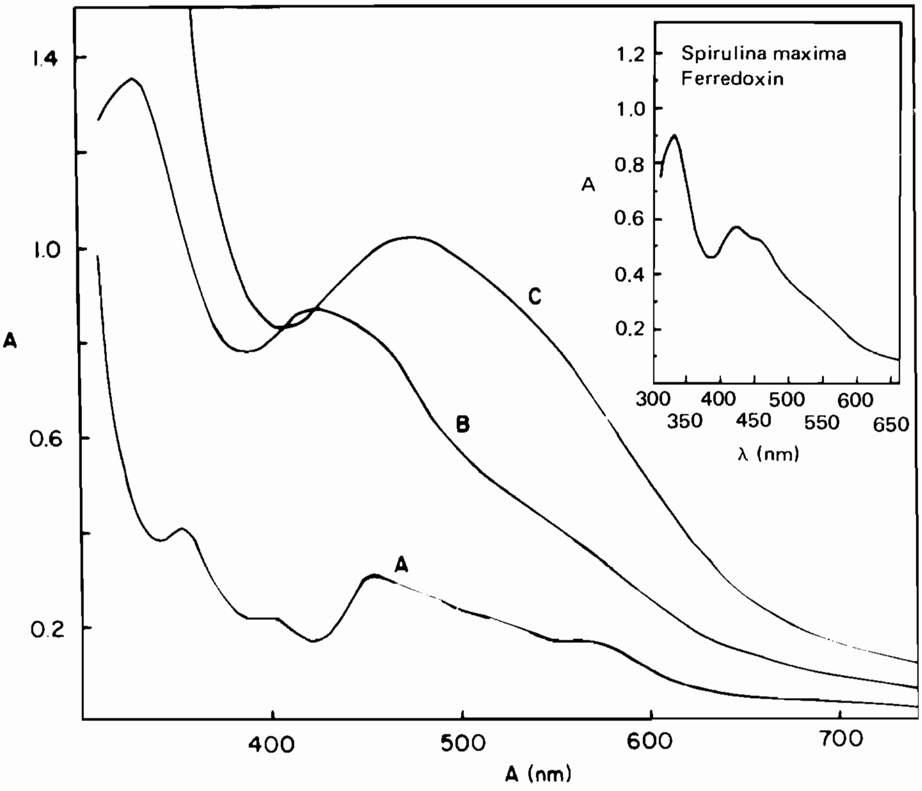

Fig. 1. A) Dimethyl sulphoxide (300 μl) + $[Fe_2S_2(Cl)_4]^{2-}$, 30 μl (0.32 μmol). B) A plus I, 10 μl (1.92 μmol) + Et_3N, 0.7 μl (5.04 μmol). Standard solutions of $[Fe_2S_2(Cl)_4]^{2-}$ and I were made in CH_3CN and $(CH_3)_2SO$ respectively. C) B plus C_6H_5H 2 μl (19.48 μmol). Insert: *Spirulina Maxima* Fd_{ox} (From Ref. 1).

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**Reduction of a complex formed by mixing $FeCl_3$, Li_2S, and Ac-Gly_2-Cys_2-Gly_2-NH_2 in 8% aqueous dimethyl sulphoxide resulted in an e.p.r. spectrum characteristic of 2Fe-Fd_{red} [6].

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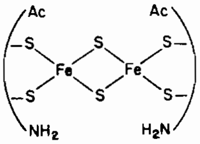

Fig. 2. Peptide cluster analogue of 2Fe-Fd_{ox}.

Addition of six equivalents of I and an equivalent quantity of Et_3N to a solution of $[Fe_2S_2(Cl)_4]^{2-}$ results in a light red-brown solution and a spectrum (Fig. 1, Curve B) with features at 333 (nm) (ϵ = 14.35), 423 (9.25), 458 (sh) (8.60)†. This spectrum is very similar to those of 2Fe-Fd_{ox} which have features at 325–333 (12–15), 410–425 (9.0), 455–470 (8.5–9.5), 720 (0.8) [1] (*e.g.*: Fig. 1 insert). Furthermore the absorbance ratio at 430 nm to that at 330 nm for this spectrum (0.64) compares well with those of 2Fe-Fd_{ox} (*e.g.*: A_{430}/A_{330} for *Spriulina Maxima* Fd is 0.69). These observations indicate that a peptide cluster analogue of 2Fe-Fd_{ox} has formed in solution.

Consistent with our observation, addition of excess of benzene thiol to the above solution resulted in a red solution and a spectrum (Fig. 1, Curve C), with a maximum at *ca.* 485 nm identical to that of $[Fe_2S_2(SPh)_4]^{2-}$ [1]. Furthermore the ratio of absorbance at 540 nm to that at 440 nm of this spectrum (0.95) compares well with that reported for pure $[Fe_2S_2(SPh)_2]^{2-}$ (1.01) [7]. This particular ratio of absorbance (A_{540}/A_{440}) of $[Fe_2S_2(SPh)_2]^{2-}$ and $[F_4S_4(SPh)_4]^{2-}$ (0.53) has been used as a sensitive criterion in determining the composition of proteins by core extrusion methods [7]. These experiments are summarized in the equation below.

$$[Fe_2S_2(Cl)_4]^{2-} \xrightarrow{4RS^-} [Fe_2S_2(SR)_4]^{2-} \xrightarrow{C_6H_5SH} [Fe_2S_2(SC_6H_5)]^{2-}$$

(where 2RSH = 1)

Based on these experiments it may be concluded that the ligand exchange of I with $[Fe_2S_2(Cl)_4]^{2-}$ results in the formation of a peptide cluster analogue of 2Fe-Fd_{ox}, as shown diagramatically in Fig. 2.#

Acknowledgement

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†Extinction coefficients are relative to C_6H_5SH exchange product.

#Attempts to crystallize this cluster were unsuccessful. Hence in the absence of X-ray crystallographic results, other polymeric structures cannot be excluded.