Direct Formation of Peptide Analogues of Rubredoxins and Four-iron Ferredoxins from their Components

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Summary Cysteine peptides react with iron(III) chloride in dimethyl sulphoxide in the presence of base to form rubredoxin analogues (**2**), which are converted by addition of aqueous sodium sulphide into four-iron ferredoxin analogues (**4**).

ALTHOUGH peptide analogues of four-iron ferredoxins have been prepared by ligand exchange of cysteine peptides with the cluster compound $[Fe_4S_4(SBu^t)_4]^{2-}$, their direct synthesis from their components has not previously been achieved.[1] We now report their formation, in solution, from their components; the corresponding rubredoxin analogues are intermediates in the process, thus establishing a direct experimental link between these two major types of iron–sulphur protein.

The reactions between a number of cysteine peptides, iron(III) chloride, and sodium sulphide, in the presence of base, in dimethyl sulphoxide under strictly anaerobic conditions was studied spectroscopically. The results for the tetracysteine peptide (**1**; $n = 3$)[2] are shown in the Figure. Addition of triethylamine to a solution of the

$$Ac.Gly_2.Cys.(Gly_2.Cys)_n.Gly_2.NH_2$$
(**1**)

peptide and iron(III) chloride results in the immediate appearance of a red-violet colour; at this stage the spectrum (curve B) is very similar to those of the oxidised rubredoxins[3] and of (**2**; 4SR = 2 $SCH_2.C_6H_4.CH_2S$)[4] and it seems clear that the solution contains the oxidised rubredoxin analogue [**2**; 4SR = (**1**; $n = 3$)].[5] The colour fades

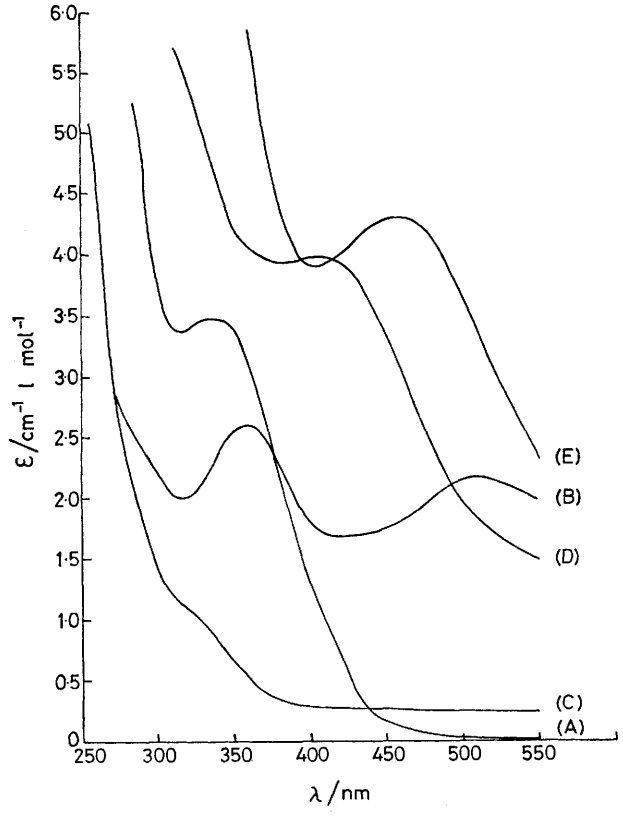
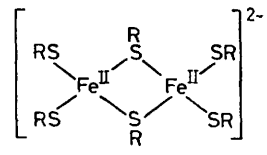


FIGURE. (A), $FeCl_3$ (1·2 μmol) and (**1**; $n = 3$) (1·5 μmol) in Me_2SO; (B), as (A) plus NEt_3 (6·0 μmol) immediately after mixing; (C), as (B) after 5 min; (D), as (C) plus Na_2S (1·96 μmol) and H_2O (2·4%); (E), as (D) plus PhSH (20 μmol). The values of ε are based on the molar concentration of Fe, however combined.

$[Fe^{III}(SR)_4]^-$ (**2**)



(**3**)

$[Fe^{II}_2Fe^{III}_2S_4(SR)_4]^{2-}$ (**4**)

rapidly and the final spectrum (curve C), observed after 5 min, is consistent with the major component at this stage being the dimeric bridged compound [**3**; 4SR = (**1**; $n = 3$)], the *o*-xylylene analogue of which was prepared by Holm *et al.*;[4] the shoulder at *ca.* 315 nm is ascribed to the presence of some reduced (Fe^{II}) rubredoxin analogue, resulting from the reduction of (**2**) by excess of peptide, since its intensity depends on the amount of excess of peptide present in the reaction mixture. Addition of sodium sulphide results in the rapid development of a stable golden-brown colour and a spectrum (curve D) identical with that of the four-iron ferredoxin analogue [**4**; 4SR = (**1**; $n = 3$)], prepared by ligand exchange between the peptide (**1**; $n = 3$) and the cluster compound (**4**; R = Bu^t). The presence of the ferredoxin analogue is supported by the e.s.r. spectrum and confirmed by the addition of an excess of benzenethiol, which causes the rapid development of a spectrum (curve E), identical with that of the independently synthesised phenyl cluster compound (**4**; R = Ph);[1] the intensity of absorption at 458 nm shows that 98% of the original iron(III) chloride has been converted into (**4**; R = Ph). Similar results have been obtained with several other cysteine peptides, *e.g.* (**1**; $n = 0$, 1, and 2).

This is the first time that peptide analogues of four-iron ferredoxins have been prepared directly from their components. The method is rapid and especially useful for the

preparation of such analogues in solution for studies of physical properties (*e.g.*, e.s.r. spectra) for which the isolated compounds are not needed.

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[1] *Cf.* L. Que, J. R. Anglin, M. A. Bobrik, A. Davison, and R. H. Holm, *J. Amer. Chem. Soc.*, 1974, **96**, 6042; B. V. De Pamphilis, B. A. Averill, T. Herskovitz, L. Que, and R. H. Holm, *ibid.*, p. 4159.
[2] R. J. Burt, B. Ridge, and H. N. Rydon, unpublished work; R. J. Burt, Ph.D. Thesis, University of Exeter, 1975.
[3] W. Lovenberg and B. E. Sobel, *Proc. Nat. Acad. Sci. U.S.A.*, 1965, **54**, 193.
[4] R. W. Lane, J. A. Ibers, R. B. Frankel, and R. H. Holm, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2868.
[5] The formation of a reduced rubredoxin analogue from Boc.(Gly.Cys.Gly)$_4$.NH_2 and iron(II) chloride in Me_2SO solution has been reported by J. R. Anglin and A. Davison, *Inorg. Chem.*, 1975, **14**, 234.