Fe–Mo Complexes of Cysteinyl Peptides

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Information regarding the coordination environment of the molybdenum atom in the Fe–Mo protein component of nitrogenase systems, recently has become available [1]. The information obtained by X-ray absorption fine structure analyses (EXAFS), suggests that the molybdenum atom is coordinated to approximately four sulphur atoms at 2 Å and approximately to two irons atoms at 2.7 Å.

As a consequence of EXAFS studies on nitrogenase, a new impetus has been generated for the synthesis of active site analogue complexes containing Fe, Mo and S.

Our research efforts directed toward the same goal have resulted in the synthesis of various such complexes obtained by the coordination of the tetrathiomolybdate ligand (Mo_4^{2-}) to iron [2–4]. One of these complexes $[(C_6H_5S)_2FeS_2MoS_2]^{2-}$ (I) has a visible spectrum [2] similar to that of the Fe–Mo protein form *D. Gigas* [5]. This observation and a study that indicated the presence of MoS_4^{2-} in solutions of denatured nitrogenase [6] prompted us to explore the possibility that MoS_4^{2-} may serve as a ligand to iron in the presence of cysteine sulphur donors in appropriate peptides. In this communication we present evidence that complexes analogous to I form with cysteinyl peptides.

The reactions of the $Ac–Gly_2–[Cys–Gly_2]_n–Cys–Gly_2–NH_2$ peptides (n = 1, II; n = 2, III) [7] with Fe(II) and MoS_4^{2-} in the presence of base Et_3N were monitored using electronic spectroscopy. These experiments were performed in dimethyl sulphoxide under a rigorously oxygen free nitrogen atmosphere.

Addition of two equivalents of II and an equivalent quantity of Et_3N to one equivalent of $Fe(II)Cl_2$ or $Fe(II)(ClO_4)_2$ in dimethyl sulphoxide results in a pale yellow solution and a spectrum (Fig. 1, curve A) with a band at *ca.* 315 nm, very characteristic of reduced rubredoxin [8]. Treating this solution with one equivalent of MoS_4^{2-} results in a golden brown solution and a spectrum (Fig. 1, curve B) with features at 600 nm (sh), 535 (sh), 480 (ϵ-6320), 415 (7200), 325 (12630) and 308 (12125)$^{\neq}$. This spectrum is very similar to the spectrum of $[(S)_5FeS_2MoS_2]^{2-}$, [605 (nm) (ϵ - 1864) 545 (sh), 480 (7242), 410 (9924), 340 (sh), 305 (14894)] [3] and acid-base treated Fe–Mo protein of *Clostridium Pasteurianum* [6]. Furthermore this solution exhibits an intense band in the near IR region (930 nm, ϵ = 105). Similar bands have been observed in the near IR reflectance spectra of I and other Fe–Mo binuclear complexes and have been interpreted as associated with Fe–Mo transitions [9]. These observations and the electronic perturbations of the MoS_4^{2-} chromophore apparent in this spectrum (*cf.* Fig. 1, curve D) are evident of the MoS_4^{2-}–Fe interactions.

A similar spectrum is also obtained on treating the independently synthesized $[Cl_2FeS_2MoS_2]^{2-}$ complex [4] with II and Et_3N. These results suggest that the solution at this stage contain a complex with the 'FeS_2Mo' chromophore. In a reaffirmation of this suggestion, addition of excess of benzene thiol results in a red–brown solution and a spectrum (Fig. 1, Curve C) identical to that of I, [620 (nm) (sh), 550 (sh), 487 (ϵ = 8645), 425 (7773), 330 (14705) [2]. These experiments are summarized in the equation below.

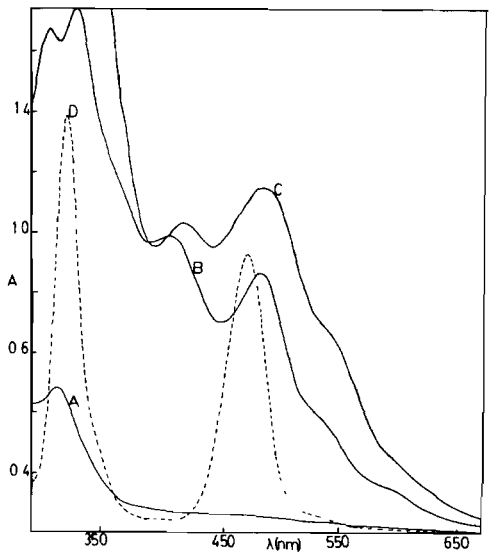

Fig. 1. A) Dimethyl sulphoxide (300 μl) + $FeCl_2$, 8μl (0.48 μmol) + II, 10μl (2.00 μmol) + Et_3N, 0.7 μl (5.04 μmol)**. B) A plus $(NH_4)_2MoS_4$, 8μl (0.48 μmol). C) B plus C_6H_5SH, 1 μl (9.74 μmol). D) $(NH_4)_2MoS_4$, 350 μl (0.30 μmol), (Included for comparison).

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$^{\neq}$Extinction coefficient is relative to the C_6H_5SH exchange product.

**Standard solutions of $FeCl_2$, $(NH_4)_2MoS_4$ and peptides were made in dimethyl sulphoxide.

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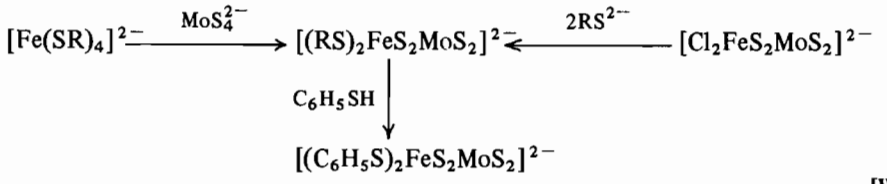

[Where 2RSH = II]

Similar reactions were explored in attempts to stabilize the trinuclear chromophore, 'FeS_2MoS_2Fe', in dimethyl sulphoxide using III. However treating the reduced rubredoxin analogue formed by III and Fe(II) with half an equivalent of MoS_4^{2-} or adding the $[Cl_2FeS_2MoS_2FeCl_2]^{2-}$ complex [4] to a solution of III and Et_3N resulted in a spectrum similar to that of the Fe–Mo complex stabilized by II. This is evident in the formation of the 'FeS_2Mo' chromophore and not 'FeS_2MoS_2Fe'. The formation of 'FeS_2Mo' was confirmed by extrusion of this core with benzene thiol. It should be noted that due to the solubility properties of the peptides, experiments could not be performed in nonpolar solvents such as CH_2Cl_2, in which $[Cl_2FeS_2MoS_2FeCl_2]^{2-}$ has been shown to be stable [4].

On the basis of these experiments we conclude that both II and III in solution stabilize complexes containing the binuclear chromophore 'FeS_2Mo' as shown diagrammatically in Fig. 2. **A** and B respectively*.

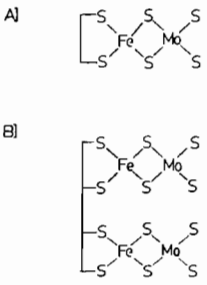

Fig. 2. The binuclear cromophore 'FeS_2Mo'.

*Attempts to crystallize these complexes were unsuccessful Hence in the absence in X-ray structural results other polymeric structures cannot be ruled out

It seems possible, therefore, that MoS_4^{2-} may serve as an exogenous ligand for cysteinyl bound iron in certain one iron, non-heme iron proteins.

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