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CHELATION EFFECT OF A Cys-X-Y-Cys TETRAPEPTIDE SEQUENCE FOR THE 4Fe-4S CLUSTER

Physical and chemical properties of the 4Fe-4S cluster in simple alkane- or arylthiolato model complexes have been established by HOLM's group [1,2]. The differences between native ferredoxin and the model complexes have been discussed in terms of redox potential, electron transfer rate, and redox stability. These differences are caused by a peptide chain of native ferredoxin. For example, invariant amino acid residues in the protein sequence of *P. aerogenes* ferredoxin play a crucial role in the construction of an unusual 4Fe-4S core environment. Previously we reported the importance of a Cys-Gly-Ala fragment of $[\text{Fe}_4\text{S}_4(\text{Z-cys-Gly-Ala-OMe})_4]^{2-}$ (Z = benzyloxy-carbonyl) with NH---S hydrogen bonding which is supported in a nonpolar solvent [3]. This paper presents a study on the chelation effect of an invariant Cys-X-Y-Cys sequence to a 4Fe-4S cluster and the effect of two amino acid residues placed between two Cys residues. A simple 4Fe-4S model complex with Cys-Gly-Gly-Cys ligands has already been synthesized by QUE *et al.* [4].

$[\text{Fe}_4\text{S}_4(\text{Z-cys-Gly-Ala-cys-OMe})_2]^{2-}$, **1**, and $[\text{Fe}_4\text{S}_4(\text{Z-cys-Ile-Ala-cys-OMe})_2]^{2-}$, **2**, having a conservative sequence of *P. aerogenes* ferredoxin were synthesized from the ligand exchange reaction of $[\text{Fe}_4\text{S}_4(\text{S-}t\text{-Bu})_4]^{2-}$ and the corresponding tetrapeptides. The $^1\text{H-NMR}$ spectrum of **1** in $\text{Me}_2\text{SO-d}_6$ exhibits two $\beta\text{-CH}$ signals of Cys resi-

dues at 11.0 and 12.3 ppm which are observed separately, with different contact shifts from the 4Fe-4S core to the $\beta\text{-CH}$ groups of two Cys thiolato ligands. The redox potential of **1** was -0.95 V (SCE) with a positive shift (0.04 V) from that (-1.00 V , SCE) of $[\text{Fe}_4\text{S}_4(\text{Z-cys-Gly-Ala-OMe})_4]^{2-}$ in *N,N*-dimethylformamide (DMF) and -0.91 V (SCE) in dichloromethane. These values may be compared with that (-0.98 V , SCE) of $[\text{Fe}_4\text{S}_4(\text{Z-cys-Gly-Ala-OMe})_4]^{2-}$ at room temperature. This positive shift in DMF is ascribed to a chelation effect by Cys-X-Y-Cys to the 4Fe-4S cluster. A decrease in the temperature for **1** in dichloromethane results in a positive shift of the redox potential; it attains -0.85 V (SCE) at 243 K, which is similar to the redox potential of $[\text{Fe}_4\text{S}_4(\text{Z-cys-Gly-Ala-OMe})_4]^{2-}$ at 233 K, indicating a preferable conformation for the NH---S hydrogen bonding, frozen at low temperature (233 K). In the case of **2**, the Cys-Ile-Ala-Cys sequence was found to chelate to the 4Fe-4S core in spite of the disadvantageous hairpin turn structure of the Cys-Ile-Ala sequence [5].

Redox behaviors of **1** and **2** in aqueous micellar solutions will be discussed as a model for a metalloprotein which acts as an electron transfer mediator.

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PS4.6 — TH

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EVIDENCES FOR THE FORMATION OF COMPLEXES OF D,L-DIHYDROTHIOCTIC ACID (REDUCED LIPOIC ACID) WITH Ni^{II} , Co^{II} AND Fe^{III} SALTS

We wish to report here very preliminar evidences about the complexing ability of D,L-dihydrothioctic acid (D,L-dihydrolipoate, DHL) towards some metallic ions. The occurrence of the Fe^{III} -DHL complex was first noticed in studies dealing with the enzymatic synthesis of iron-sulfur structures [1,3], and DHL was found able to remove ferritin-bound iron to an extent which compares more than favourably with the figures obtained by using other iron-chelators [4]. The possibility of using complexes of DHL with Ni^{II} and Co^{II} in the enzymic biosynthesis of hetero-metallic, sulfur-coordinated clusters, such as those known to occur in some bacterial hydrogenases [5] and nitrogenases [6], suggested the present preliminary investigation.

All experimental operations were performed anaerobically. DHL was prepared by NaBH_4 reduction of an aqueous solution of D,L-thioctic acid brought to pH 9.0 with NaOH. After acidification to destroy the excess reductant, DHL was extracted with chloroform, dried with sodium sulphate, and its concentration determined by sulfhydryl titration [7]. The same procedure was used for the synthesis of D,L-dihydrothioctamide, starting with an ethanolic solution of D,L-thioctamide.

Fig. 1 shows the electronic spectra of mixtures of DHL and of halides of the investigated metals.

All the spectra show two major charge-transfer absorption bands, and the Fe^{III} -DHL complex gave, on a metal-content basis, the most intense absorption. The iron complex can be reduced with dithionite to give an almost colorless solution. Careful air-oxidation of this dithionite-reduced

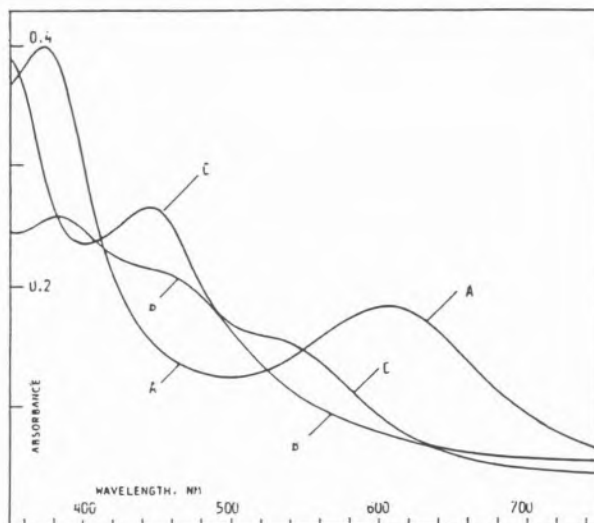


Fig. 1

Electronic spectra of buffered solutions of DHL in the presence of different metal ions. Spectra were recorded in 0.05 cm cuvettes and in 0.2 M Tris/sulfate pH 9.00. A — 20 mM DHL and 1 mM FeCl_3 ; B — 1 mM DHL and 2.2 mM CoCl_2 ; C — 1 mM DHL and 1.8 mM NiCl_2

sample allows recovery of the spectral features of the starting mixture (not shown). This and other evidences (3) suggest that iron is bound to DHL in the ferric form. When aqueous-detergent micellar solutions of D,L-dihydrothioctamide replaced DHL in the reaction with Fe^{III} , the same results were obtained, whereas D,L-thioctic acid and its amide did not display any chelating ability. These observations rule out the possible involvement of the carboxylate moiety of DHL as a ligand. Fig. 2 shows that, whatever the ion used, complexes are formed at an approximate 1/1 molar ratio between DHL and the metal. On the basis of the diamagnetic behaviour of the $\text{Fe}^{\text{II,III}}$ -DHL complexes, and of the strong resemblance of the electronic spectra of Fe^{III} -DHL to those of $\text{Fe}_2(\text{ethanedithiolate})_4^{2-}$, we are inclined to assign to the Fe-DHL complex the structure $\text{Fe}_2(\text{DHL})_4^{6-}$. The apparent 1/1 stoichiometry observed in the titration experiments could be explained by the reduction of some of the iron with concomitant oxidation of the thiol groups of DHL.