

(pH ~ 7.0) containing PIH and  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ . It analyzed as a  $[\text{Fe}(\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}_3)_2]_2\text{SO}_4$  crystalline compound of space group  $\text{C}2/c$ ,  $z=8$ ,  $a=14.487$ ,  $b=18.586$ ,  $c=27.508 \text{ \AA}$ , and  $v=7224 \text{ \AA}^3$ .

PIH is shown to function as a neutral tridentate ligand, forming a non-planar tricyclic system which comprises pyridoxal, an hexatomic and a pentatomic chelate rings with dihedral angles of  $13.01^\circ$  and  $8.45^\circ$ , respectively, between them. The coordination plane deviates from coplanarity, showing significant departure from the ideal octahedron. The hydrazidic central donor atoms are *trans* related to each other and the two phenolate and enolate oxygens mutually *cis*. The coordinated ligands retain the neutrality characteristic of its free form by a transfer of protons from the phenolic oxygens and the hydrazidic nitrogens to the pyridine nitrogens. The sulphate ion is a counterion [2]. The transition of  $\text{Fe(II)}$  to  $\text{Fe(III)}$  occurs even in presence of a strong reducing agent, suggesting that PIH lends itself to reversible two-stage redox reaction. This was fully corroborated by polarographic measurements, allowing its likening to «viologenes» of potential biological importance.

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## MOBILIZATION OF FERRITIN-BOUND IRON BY REDUCED D,L-LIPOATE AND REDUCED D,L-LIPOAMIDE

The mobilization of ferritin-bound iron, an issue of great physiological and clinical relevance [1], was investigated by studying several iron chelators [2], either in the absence or in the presence of iron-reducing agents [3] and of activators of the removal process [4].

In the course of other studies, we noticed the unusual ease of formation and stability of a complex between iron(III) and D,L-dihydrolipoate (DHL-COOH). This complex was characterized to some extent and the tentative formula  $[\text{Fe}_2(\text{DHL-COO}^-)_4]^{-6}$  was attributed to it [5,6]. In view of the unusually high stability of this complex, of considerations about the amphiphilic nature of the ligand and about the molecular architecture of ferritin, we tested the ability of both DHL-COOH and D,L-dihydrolipoamide (DHL-NH<sub>2</sub>) in the mobilization of ferritin-bound iron.

Fig. 1 shows the electronic spectra obtained upon incubation of horse-spleen ferritin (HSF) with DHL-NH<sub>2</sub>, DHL-COOH and with dithiothreitol as a control dithiol. Progressive appearance of the spectral features of  $[\text{Fe}_2(\text{DHL-R})_4]^{(-6,-4)}$  is evident, whatever the amidation state of the ligand. DHL-NH<sub>2</sub> appears to react faster than DHL-COOH. Nevertheless, after 20 hours incubation, an identical absorbance at 620 nm was attained in both reactions. By assuming  $\epsilon_{620} = 4,000 \text{ (g atom iron)}^{-1}\text{cm}^{-1}$  for  $[\text{Fe}_2(\text{DHL-R})_4]^{(-6,-4)}$ , the amount of iron released

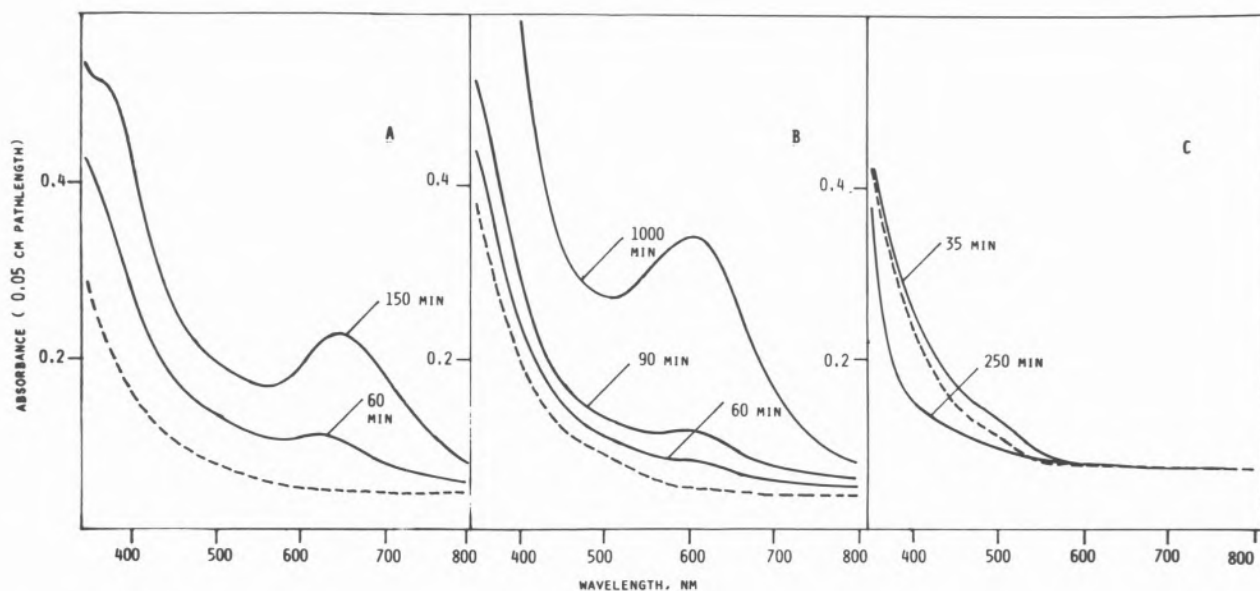


Fig. 1

Absorbance spectra of HSF incubated with different dithiols. HSF (Calbiochem, 2.2 mg/ml in 0.3 M Tris/HCl pH 9.0) was incubated at 20°C with: 4.55% (v/v) Triton X-100 and 32 mM dithiothreitol (C). Spectra were recorded before the addition of the dithiol (dashes) and at the times given

ranged between 49 and 52% of the original content of HSF (20% iron, by weight). Samples taken during the course of the incubation with DHL-COOH were chromatographed on an Ultrogel AcA54 column. This procedure separated a faintly-reddish ferritin band from the dark-green band of the iron-DHL complex, thus indicating that the complex actually leaves the ferritin molecule. Fig. 2 compares the time course of iron removal by different concentrations of either DHL-COOH or DHL-NH<sub>2</sub>. The addition of detergent, which is mandatory in order to solubilize DHL-NH<sub>2</sub> at the high concentrations used in the present study, does not affect the rate of iron release. The initial lag in the time course of the reaction can be ascribed to the process of penetration of the ligand into the protein shell, whereas the biphasicity of the reaction could be explained either by the presence of different forms of ferritin-bound iron [7] or by the attainment of a dynamic equilibrium among molecules of ligand entering the protein and molecules of the complex leaving it. DHL-NH<sub>2</sub> appears more effective than DHL-COOH, likely because it is more hydrophobic and diffuses more easily through the channels between the subunits of HSF.

Distinctive features of the present study are: *i* — a physiological, non-toxic, inexpensive com-

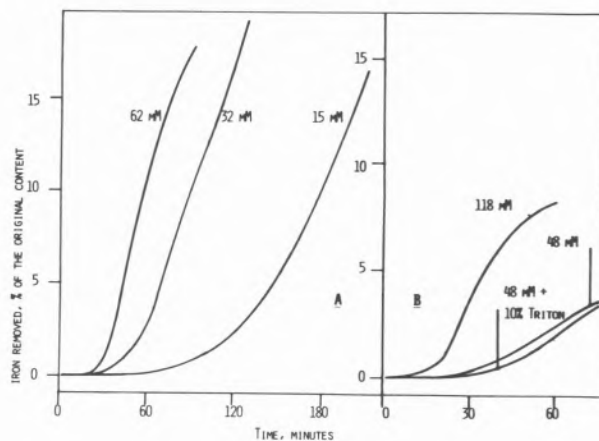


Fig. 2

Time-course of the removal of iron from HSF by DHL-NH<sub>2</sub> (A) or DHL-COOH (B). HSF was incubated as detailed in the legend to Fig. 1 with the given concentrations of dithiol. The absorbance increase at 620 nm was monitored continuously, and the percentage of iron-removal calculated as given in the text

pound is used as the iron-removing agent; *ii* — iron is released by either DHL-COOH or DHL-NH<sub>2</sub> in the ferric form; *iii* — after 5-hours incubation, the iron removal with DHL-COOH is 16.8%, thus higher than the figures at 5 hours reported for other iron-removing agents: 2.6% for

desferoxiamine B+ascorbate, 5-7% for catecoylamides+ascorbate and 2.7% for EDTA [2,3].

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## ELECTROCHEMICAL PROPERTIES OF $[\text{Fe}_4\text{S}_4(\text{SC}_6\text{H}_5)_4]^{-2}$ , ANALOGUE OF THE ACTIVE SITE OF IRON-SULFUR PROTEINS, IN AQUEOUS MICELLAR SOLUTIONS

In order to elucidate the mechanism by which the protein region of iron-sulfur proteins modulates the redox potentials of otherwise identical 4Fe-4S clusters, we investigated the properties of the title cluster ( $t^{-2}$ ) in water, the physiological solvent for proteins. The title cluster is water-insoluble, but becomes soluble in the presence of detergents [1], and by using detergents differing in structure and

in charge we attempted to simulate different protein-cluster interactions.

Synthesis of  $[(\text{Et})_4\text{N}]_2[\text{Fe}_4\text{S}_4(\text{SC}_6\text{H}_5)_4]$  was performed in aqueous solution as described by KURTZ *et al.* [2,3]. A stock solution of  $t^{-2}$  was anaerobically prepared in DMF, and  $t^{-2}$  concentration determined using  $\epsilon_{457} = 17,700 \text{ M}^{-1} \text{ cm}^{-1}$ . For electrochemical measurements the stock solution of  $t^{-2}$  in DMF was anaerobically diluted in 5% (v/v) detergent in 0.2 M Tris/sulfate buffer pH 9.00, to a final concentration of 1.34 mM  $t^{-2}$  and 5% (v/v) DMF. A three-electrodes configuration was used. All the potentials quoted here refer to saturated calomel electrode (SCE). Fig. 1 shows the cyclic voltammograms of  $t^{-2}$  dissolved in buffer/detergent mixtures of different composition and in the presence of excess thiophenol.

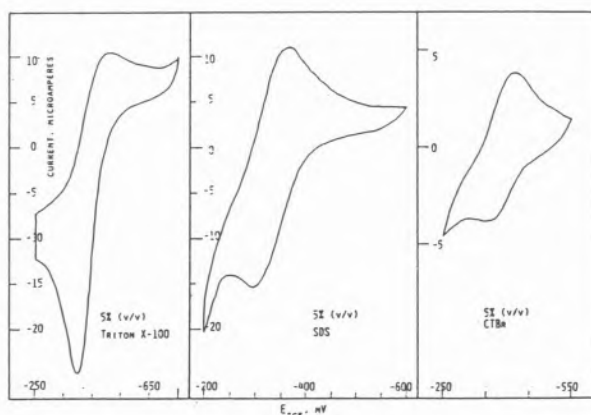


Fig. 1

Cyclic voltammograms of aqueous solutions of  $t^{-2}$  in the presence of different detergents. Cyclic voltammograms of 1.34 mM  $t^{-2}$  (in 0.2 M Tris/sulfate pH 9.0, 5% (v/v) DMF, 100 mM thiophenol containing also detergents as given in the figure) were recorded at a scan rate of 50 mV/sec

In the absence of this latter no oxidation (anodic) peak can be detected, likely because of the hydrolytic decomposition of the  $t^{-3}$  species generated in the reduction process. Excess thiophenol prevents the hydrolytic process, thus allowing the monoelectronic, quasi-reversible redox process involving the  $t^{-2}/t^{-3}$  couple to be observed. Both anodic and cathodic currents ( $i_a$  and  $i_c$ ) were found to be directly proportional to the concentration of  $t^{-2}$ . In aqueous detergents, the measured values of  $E_0$  are by far higher than those reported for the same compound dissolved in DMF