

Fig. 1. (a) Shows the $g = 2.09$ feature of the MV- α_3 -NO EPR spectrum from 12 to 77 K. (b) Shows the changes in the MV- α_3 -NO EPR spectrum near $g = 2.00$ from 20 to 77 K.

to 40 K, occurs at NO-liganded cytochrome α_3 . Or, a dipolar spin-spin interaction occurs between the NO-liganded α_3 center and a paramagnetic metal center in cytochrome α ; this spin-spin interaction is modulated by temperature-dependent spin-lattice relaxation (which we have independently measured) of the center in cytochrome α . If the latter explanation is correct, the distance between α_3 -NO and the interacting center in cytochrome α can be estimated at 15 Å, and the more likely interacting center in cytochrome α predicted to be heme a .

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Crystallographic Studies on Horse Isoferritins

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Ferritin is an iron-storage protein which is found in eucariotes as well as in procarotes [1]. In mammals ferritin is present in almost every tissue, at higher concentrations in iron-rich organs such as spleen and liver. Ferritins extracted from a single tissue contain families of different isoferritins which can be distinguished according to their surface charges and/or immunological properties [2]. The prototype ferritin molecule is an oligomer of 24 subunits, arranged in 432 symmetry, which form an inner cavity of approximately 75 Å diameter. This hollow oligomeric molecule readily accommodates an inorganic matrix of hydrated ferric oxide phosphate (up to 4500 iron atoms stored) which possesses crystalline order. Ferritin heterogeneity can be related at a molecular level to the different associations of two subunit types in the protein shell. In liver ferritins the proteic shell is composed mainly of the so called L subunits (18,500 mol. weight), while H-type subunits (21,000 mol. weight) are predominant in heart ferritins [3].

Most of what is presently known of the three-dimensional structure of ferritins comes from the crystallographic investigations on spleen ferritin [4], which readily crystallizes from cadmium sulfate solutions (Space Group F432; $a = b = c = 184$ Å, one subunit per asymmetric unit). Much less is known about the structure of the H subunit-rich isoferritins, dealt with in this communication.

In the course of the last year we have achieved the crystallization of horse heart isoferritin (both holo and apo forms) under different physico-chemical conditions. In particular we were able to grow crystals from protein solutions containing MPD (2-methyl-2,4-pentane diol) and in the presence of polyethylene glycol (av. mol. weight 4.000 ÷ 6.000), but not from CdSO_4 (Fig. 1 a,b). Thus heart ferritins cannot be crystallized under the same physico-chemical conditions which are used for the crystallization of spleen ferritins. It is interesting to note that in the presence of MPD the reverse is also true.

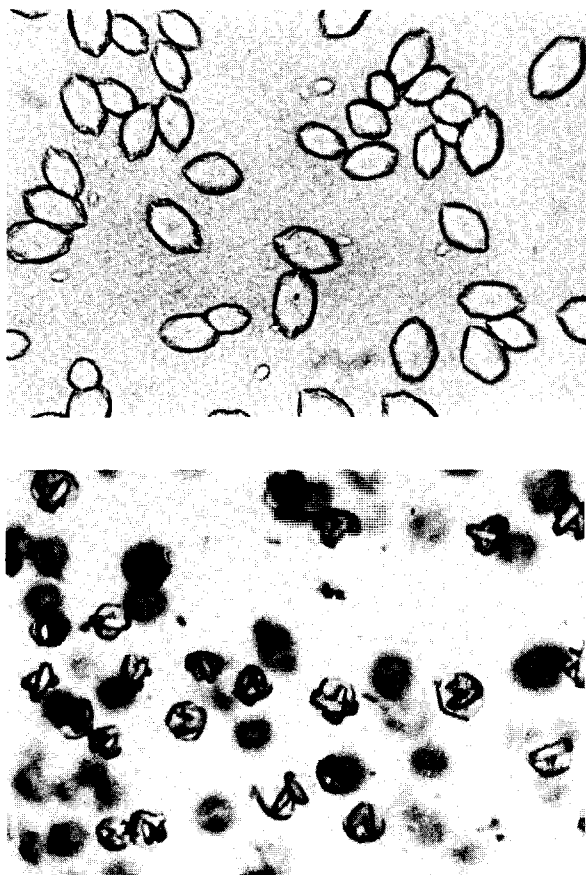


Fig. 1. Crystals of horse heart ferritin grown from MPD (a), and from polyethylene glycol (b). In both cases the longest dimensions of the crystals are approximately 0.15 μ m.

The dimensions of the heart ferritin crystals obtained so far are too slight to allow any crystallographic investigation. All the crystals obtained are isotropic under the polarizing microscope, and thus belong to a cubic space group. This observation is in keeping with the molecular symmetry of the ferritin oligomer, which, in the case of the spleen protein, is coincident with crystallographic symmetry. Details of the crystalline forms isolated as well as of their growth solutions will be presented.

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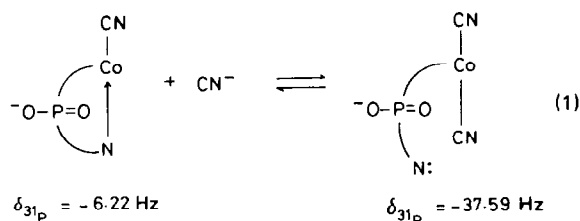
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C-13 and P-31 NMR Studies of ^{13}C -Cyanocobalamin in Sulfuric Acid–Water Mixtures

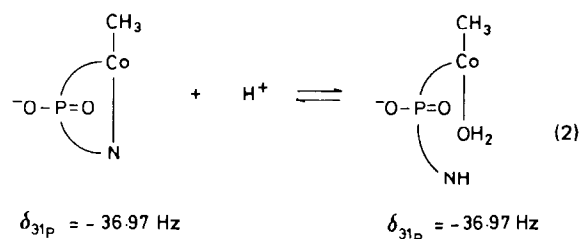
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In a previous study [1] we observed, in agreement with Satterlee [2, 3] an upfield shift of 31.4 Hz of the chemical shift of the phosphorus atom of the nucleotide loop of cyanocobalamin (CNCbl) upon displacement of the axial benzimidazole ligand by excess cyanide (Eqn. 1). However, we observed no change in chemical shift for methylcobalamin (CH_3 -



Cbl) upon displacement of axial benzimidazole by protonation (Eqn. 2, $\text{pK}_a = 2.89$) although further



lowering the pH causes an upfield shift of the phosphorus resonance due to protonation of the phosphodiester [1]. We consequently have attempted to determine if a change in phosphorus chemical shift occurs upon displacement of the axial base of CNCbl by protonation (Eqn. 3, $\text{pK}_a = 0.1$ [4]).

