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Pressure-Induced Spectral Shifts in Hemoproteins

We have observed the pressure-induced spectral shift in the spectra of hemoglobin and its derivatives. The compounds investigated were methemoglobin (pH 6.9), methemoglobin fluoride, methemoglobin cyanide, oxyhemoglobin, deoxyhemoglobin, hematin, and hematoporphyrin IX. Figure 1 shows the results for the Soret band absorption. Within experimental error ($\pm 5 \text{ \AA}$) we saw no change of the position of the α and β band of oxyhemoglobin and hematin or in the 560-m μ band of deoxyhemoglobin up to the pressure of 2000 atm.

The effects observed are an order of magnitude greater than one would expect by analogy to other aromatic systems (1).

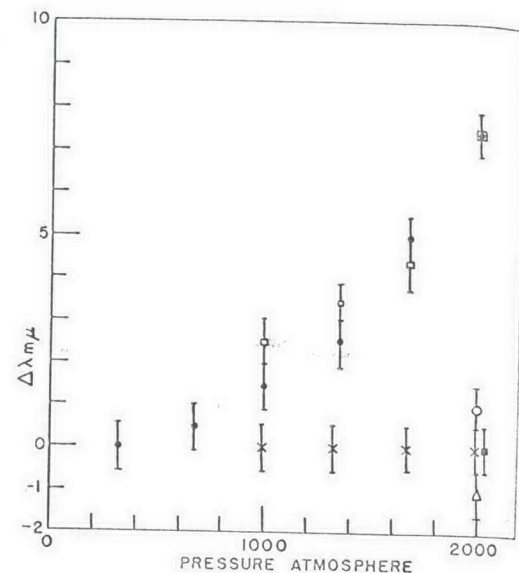


FIG. 1. Shift in the position of the Soret band at 25°C for different hemoproteins. Hemoglobin was prepared from the freshly drawn blood of an adult male by washing the red blood cells with 0.9% saline and centrifuging of the stroma after hemolysis. Concentrations were about 10^{-4} . Hemoglobin was oxidized with ferricyanide. Experiments were carried out at pH 7. Ligand concentrations were 10^{-3} ; phosphate buffer concentration, 10^{-4} . Sigma Hematin was dissolved in 10^{-2} N NaOH. Hematoporphyrin IX 2 HCl (California Corp. for Biochemical Research) was dissolved in distilled water. Δ , Hematin; O , oxyhemoglobin; X , hematoporphyrin; \bullet , methemoglobin; \square , methemoglobin fluoride; \blacksquare , methemoglobin cyanide.

It is well demonstrated that the position of the Soret band is proportional to the paramagnetic susceptibility of the methemoglobin compound (2). The advantages of investigating the high spin-low spin transition by pressure, rather than by temperature variation, are twofold: (1) proteins are stable under pressures up to 3000 atm, but they denature under relatively low temperature variations; (2) the Soret band positions in Fig. 1 cover the whole range from high to low spin forms, a range not available by temperature variations.

All the absorption bands investigated are porphyrin $\pi-\pi^*$ transition. According to previous observations (1), an approximately linear relationship can be obtained between wavelength shift under pressure and dielectric constant of the solvent. Since in the limited pressure range of our experiment, the dielectric constant of the solvent

changes linearly with pressure, one should obtain a linear relationship between applied pressure and wavelength shift of the Soret band. The lack of this linear relation indicates that the wavelength shift is not due to the effect of the solvent but that it is caused by a change in the protein structure. Since the heme proteins contain void volumes (the "heme pocket"), one would expect that the change in wavelength is caused by the approach of the E or F helix to the iron atom of the porphyrin ring.

The fact that neither the hematin nor the porphyrin spectra show any variation within experimental errors further strengthens the argument that the large effect is a particular property of the globin-heme complex; in particular it suggests the easy compressibility of the heme pocket.

The distance of the F helix to the heme is fairly well fixed, since the imidazole of the F8 histidine is in the fifth coordination position of the heme iron. Previous work on the pressure-induced frequency shift of the absorption bands of transition metal complexes indicates that much larger pressures are needed to compress this bond. The low-spin methemoglobin cyanide does not show any pressure-induced shift. The Soret band of methemoglobin and methemoglobin fluoride shifts toward the Soret band of methemoglobin cyanide. This suggests that what we observe is the displacement in the high spin-low spin equilibrium of the methemoglobin compounds.

This may be due to either the replacement of the low field ligand with the E7 imidazole or by the compression of the bond between the low field ligand and the heme iron.

Recently, Gouterman (3) suggested that both

the α and β bands and the Soret band have their origin in the heavily mixed, nearly degenerate $a_{2u}(\pi) \rightarrow e_g^*(\pi)$ and $a_{1u}(\pi) \rightarrow e_g^*(\pi)$ transitions. If the effect of the pressure is the shortening of the bond length between the ligand in the sixth coordination position and the iron atom, it is interesting that one observes such a large effect in the Soret band position but not in the position of the α and β bands.

In summary, the high pressure optical technique seems to be a promising approach to elucidate the nature of the high spin-low spin transition in hemoproteins. We are extending our measurements to cover all of the ultraviolet and visible spectral range.

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Erratum

Vol. 122, No. 2 (1967), in the article entitled, "Wurster's Blue Mediated Oxidation of NADH and Phosphorylation in Mitochondria," by M. G. Mustafa and Tsou E. King, pp. 501-508:

Page 503, Fig. 1, ordinate should read: " $\mu\text{M O}_2/\text{sec.}$ "

Page 507, Fig. 7, coupling site I should be located at reaction step 2.