02.1-15 ON THE DIFFUSION OF SMALL MOLECULES IN MYOGLOBIN AND HEMOGLOBIN CRYSTALS. O. Baffa Filho, J. Ruggiero Neto, M. Tabak, O.R. Nascimento, Mol. Biophys.Lab. Institute of Physics and Chemistry of São Carlos, USP, 13560 São Carlos, SP, Brazil.

The process of soaking has been extensively used in protein crystallography. One important example is the soaking of heavy metals used to solve the phase determination problem. The use of X-ray methods together with EPR crystallography can be very useful in the study of single crystal proteins. In the present work we have studied the diffusion of a small paramagnetic molecule, the 2,2,6,6-tetramethylpiperidine-1-oxyl(I), in myoglobin and hemoglobin single crystals. The aim of this study was an attempt to obtain information on the hydrophobic interactions in these proteins. The crystals of sperm whale myoglobin (Mb) and human hemoglobin (Hb) were grown according to the procedures of Kendrew and Perutz, respectively. The process of diffusion was made as reported previously for Cu^{2+} ions (Biophys.J.19,95-101) and consisted of soaking of the crystals in a solution of mother liquor containing the molecule I. As in the case of Cu^{2+} ions it has been noticed that the diffusion of I is somehow facilitated if the incubation temperature is 40°C instead of room temperature. This is especially true for the hemoglobin crystals. The analysis of the EPR spectra obtained showed that in the case of Mb there is one slightly anisotropic site and for Hb there are at least two anisotropic sites, one of them showing a strong anisotropy of the EPR parameters. On the other hand attempts to diffuse Cu^{2+} ions in the Hb crystals were not successful. These results seem to have a strong correlation with the structure and packing of both Mb and Hb crystals: the Mb crystal has the monoclinic structure with two molecules in the unit cell and one molecule in the asymmetric unit which is magnetic unequivalent. On the other hand the Hb crystal has the tetragonal structure with four Hb molecules in the unit cell, two of which are magnetic inequivalent. So EPR results show that label I has one slightly anisotropic site in the Mb molecule and one strongly anisotropic site in the Hb molecule. The orientation of this site relative to crystal structure is being studied. The results with the diffusion of Cu^{2+} ions show that the process of diffusion strongly depends of the packing of the molecules in the crystal, being facilitated in the Mb crystal in respect to the Hb. Preliminary studies have been done in order to find if the molecule of I is superficially bound or in the crystal volume, showing that the first is true.

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02.1-16 STRUCTURAL STUDIES ON HAEMOGLOBIN FROM THE LANCEOLATE FLUKE *DICROCOELIUM DENDRITICUM*. J.D.G. Smit, Laboratorium für Biochemie I, ETH, 8092 Zürich, Switzerland.

The monomeric haemoglobin of the liver fluke *Dicrocoelium dendriticum* is the most primitive animal haemoglobin characterized to date (Tuchschmid, P.E., Kunz, P.A. & Wilson, K.J., Eur.J.Biochem. (1978) 88, 387). Its structure will shed more light on the globin fold and haem pocket evolution. The haemoglobin has several remarkable properties: a very high oxygen affinity (P_{50} =0.085 mm Hg), a high partition coefficient M(=pO_2.[Hb.CO]) / (pCO[Hb.O_2])) between CO and O_2, an oxyhaemoglobin absorption spectrum with an $A_{540\ nm}$ to $A_{570\ nm}$ ratio greater than unity, the replacement of the distal histidine E7 with a glycine, and a low (∿15%) sequence homology with other globins. Crystals of the CN-Met form of this haemoglobin have been grown from polyethylene glycol 2000 solutions, containing 0.1 M potassium acetate and 5 mM KCN at pH 4 to 5. Two crystal forms have been obtained: a) hexagonal: $P6_2$ or $P6_4$, a=b=91.5 Å, c=28.3 Å, α=β=90°, γ= 120°, Z=6; b) triclinic: P1, a=37.1Å b=39.9 Å, c=49.0 Å, α=88.8°, β=76.8°, γ=64.6°, Z=2. The triclinic crystals have a density of 1.24 g ml^{-1} and contain 35% (v/v) crystal solvent. This form has been selected for crystallographic studies because its diffraction pattern extends well beyond 2.2 Å and irradiation damage is low. Spectroscopic evidence for the triclinic crystals shows that in the presence of cyanide the molecules are still in the CN-MetHb form at pH 4.5. Data to 3.3 Å, collected on a 4-circle diffractometer, have been used to determine the shortest distance (about 28.5 Å) between iron atoms. Three potential heavy atom derivatives have been prepared with $Pb(NO_3)_2$, K_2HgI_4 and $NaAuCl_4$. Derivative data collection aiming to a first electron density map is in progress.

02.1-17 THREE-DIMENSIONAL STRUCTURES OF PHYCOBILIPROTEINS. By J.D. Conway, F.C. Tsui, N.E. Woods, R.G. Fisher, H.E. Fuchs, and R.M. Sweet. U. Cal. Los Angeles

The phycobiliproteins perform important light-harvesting functions in the photosynthetic red algae and cyanobacteria. There are several different color classes of these homologous proteins and together they improve an organism's ability to grow under poor light conditions. The ability of the proteins to capture light energy efficiently depends upon their organization within the light-harvesting organelle, the phycobilisome. As part of a program to understand the structure and functioning of the phycobilisome, we are engaged in crystal structure analysis of several of these colored proteins.

Phycocyanin from Anabaena variabilis consists of six α and six β subunits. A 3-D x-ray analysis of this protein at 5Å resolution shows that the molecule is 110Å in diameter, 40Å thick. It can be divided into three separate protomers related by the crystallographic 3 and contains long, columnar regions of density, presumed to represent α helix. B-Phycoerythrin from Porphyridium cruentum is composed of 6α, 6β, and a γ subunit. A 5.25Å resolution study indicates that the molecule is 107Å in diameter and 60Å thick, has 32 symmetry, and can be divided into 6 regions. The γ chain lies on a crystallographic 3 and undergoes symmetric disordering. A region of low and unstructured density in the center of the molecule is presumably occupied by this γ chain. Both of these structures are being extended to atomic resolution and structures of other phycobiliproteins are being explored.