02.1-12 CRYSTALLOGRAPHIC STUDIES OF Mb:Cu(II) COMPLEX. By E.E. Castellano, O.R. Nascimento and Y.P. Mascarenhas. Instituto de Física e Química de São Carlos, 13560 São Carlos, S.P., Brazil.

Three-dimensional diffractometric data of sperm-whale Mb and Mb:Cu(II) were collected to 2.0 Å resolution using Cu Kα radiation. Difference synthesis with Fourier coefficients $F_{oDop}-F_{oNat}$ using phases calculated from Takano's 2.0 Å resolution model (J.Mol.Biol. (1977). 110, 537), were calculated aiming the localization of the Cu(II) ions in the complex. Although a similar two-dimensional determination was already done by Banaszak, Watson and Kendrew (J.Mol.Biol. (1965). 12, 130-137), a recent single-crystal EPR study (Nascimento, Ribeiro and Bemski (Biophys.J. (1977). 19, 95-101) was indicative of conformational modifications of the quaternary structure in the vicinity of the Cu(II) ion and so a more detailed three-dimensional information of the structure was needed to interpret their results. The doped crystal diffraction data were obtained from two different samples prepared following the procedures described by Banaszak et al. and Nascimento et al. The difference electron density maps did not show any significant peaks indicating that in both procedures the site occupation number of the Cu(II) ion must be fairly small. A new EPR study was then performed on several crystals doped following the procedure by Nascimento et al, plotting the intensity of the EPR, signal against volume of the sample. A non-linear behaviour of the plot was indicative that rather than volumetric, the occupation of the Cu(II) ion was essentially at the surface of the crystals. The same study could not be performed on crystals doped following Banaszak et al. because in this case an isotropic component completely masks the crystalline Cu(II) EPR signal.

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02.1-13 THE REACTION OF FERRIC SPERM WHALE MYOGLOBIN WITH IMIDAZOLE: X-RAY AND BINDING STUDY

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Sperm whale met-Mb,whose structure is refined at 2.0 Å resolution,is an ideal and reliable system for the investigation of protein-ligand(s) interactions in crystals. We report here a study on the reactivity of crystalline Sperm whale met-Mb towards imidazole and on the structural features of the same complex at 2.0 Å resolution. The absorption spectra of Sperm whale met-Mb and its imidazole adduct were found to be identical in solution and in the crystals at several pH values. The equilibrium constants measured between pH 5 and 11 are also identical in the two states. It is noticeable that even for the crystalline state titration with the ligand conforms to expectations for a simple monomeric system,indicating that the active site is not influenced by the restraints imposed by lattice interactions. Crystals of the imidazole adduct of Sperm whale Mb have unit cell edges: a = 64.72, b = 30.74, c = 34.82 Å, = 105.72°;the space group is $P2_1$. These parameters compare favourably with those characteristic of the unligated protein. Difference Fourier analyses at 2.0 Å indicate that imidazole binds at the sixth coordination position of the iron in an orientation roughly perpendicular to the heme plane,and displacing the iron-bound water molecule. Binding of such a bulky ligand to the distal site induces conformational rearrangements of a number of side chains. Between them His E7 is characterized by the largest shift;other residues affected include Val E11,Arg 45 and Asp 60. Minor modifications are observed also in the stereochemistry of the porphyrin system and in the position of the iron atom.

02.1-14 X-RAY CRYSTAL STRUCTURE ANALYSES OF MYOGLOBINS RECONSTITUTED WITH SYNTHETIC HEMES.

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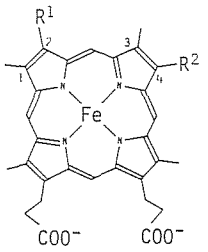
Three-dimensional structures of myoglobins reconstituted with synthetic hemes have been determined by means of X-ray diffraction in order to elucidate the functions of heme-proteins. The following four synthetic hemes were used.



(a) R^1= -CH=CH_2, R^2= H
isopemptoheme
(2-vinyldeuteroheme)
(b) R^1= H, R^2= -CH=CH_2
pemptoheme
(4-vinyldeuteroheme)
(c) R^1= -CH_2CH_3, R^2= H
2-ethyldeuteroheme
(d) R^1= H, R^2= -CH_2CH_3
4-ethyldeuteroheme
cf. R^1=R^2= -CH=CH_2 *native*

Crystals of four reconstituted myoglobins, all of which belong to the monoclinic system (space group $P2_1$), are isomorphous with the native metmyoglobin. Intensity data of (a), (b) and (d) to 2.2 Å resolution(*ca.* 7000 independent reflections) were collected on a Rigaku four-circle diffractometer. Data collection of (c) is in progress. In Fourier syntheses, phase angles used were calculated from atomic coordinates of metMb except for the porphyrin ring(Takano, *J. Mol. Biol.* (1977) 110, 537).

Electron density map on the heme plane of (a) and (d) are presented in the Figure. In isopemptoheme-Mb(a), the modified heme is inserted into apo-Mb in the same position and orientation as that in the native Mb. On the other hand, the heme group is recombined inversely with apo-Mb in 4-ethyldeuteroheme-Mb (d). In pemptoheme-Mb(b), most of hemes in crystal locate similarly as those in the native Mb and the rest inversely.

The difference Fourier syntheses ($|F(\text{modified})|-|F(\text{native})|$) do not give any significant peaks except for those of hemes, which show no large change in the main chains and residues in these reconstituted myoglobins.

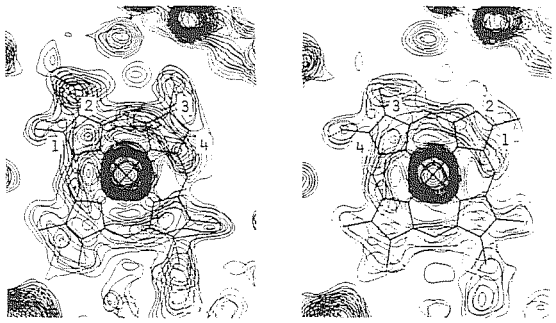


Figure. left: (a), right: (d)