01.6-3 A NEUTRON & X-RAY STUDY OF HAEMOGLOBIN

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Using the High Flux Reactor at the ILL a neutron data set has been collected to 3.5 Å d-spacing from a large partially deuterated crystal of a T-state haemoglobin (space group $P2_12_12$; a=95.8, b=97.8, c=65.5 Å). Data were collected using the recently commissioned instrument D19 with its 2-d position sensitive detector (M. Thomas et al. in Position Sensitive Detection of Thermal Neutrons, P. Convert & J.B. Forsyth, eds., Academic Press, London, 1983).
The data have been processed using the programs developed for the instrument by R.F.D. Stansfield based on a 3-d minimum $\sigma(I)/I$ method of integration (C. Wilkinson & H.W. Khamis in Convert & Forsyth, eds., loc. cit.).
Starting from a carefully refined 1.5 Å X-ray model, joint refinement with the neutron data is in progress. The results will be discussed in terms of the H/D exchange of amide hydrogen atoms. The non-crystallographic symmetry in these crystals will allow a comparison between the chemically identical but conformationally distinct dimers of the haemoglobin tetramer.

01.7-1 THE "MISSING CONE" PROBLEM IN ELECTRON CRYSTALLOGRAPHY: INTEPRETABILITY OF HIGH RESOLUTION DENSITY MAPS.

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When electron microscopy is used for a three-dimensional structure analysis, practical limitations often prevent the collection of data within a solid cone (typical half-angle of 30 degrees) centered at the origin of reciprocal space. The cone of missing data in turn results in an anisotropic, three-dimensional point spread function in real space. Experience with 3-D reconstruction at modest resolution (down to ~ 15 Å - 20 Å) has shown that this point spread function can lead to the creation of artifactual positive densities, as well as producing the expected elongation or "streaking" of densities. Substantial effort has been devoted to computational and experimental methods by which the "missing cone problem" might be overcome. It does not seem unreasonable to think, however, that the missing cone of data might have only minor effects (i.e., ~ a 40 percent elongation of densities) on density maps that approach atomic resolution, where density features become highly localized and well separated from one another. We have tested this conjecture by computing 3-D density maps at 3.5 Å resolution for monellin, a protein of ~ 10,000 MW which has representative secondary structure features of α-helix, β-sheet and random coil. High resolution density maps can be as easily interpreted, in terms of tracing the peptide chain, when a 30° cone of data is deleted as when the full data set is used. The "missing cone" density map is no more sensitive to simulated experimental error than is the map for complete data. Thus, while the missing cone problem can be a serious one at lower resolution, it appears that it should not limit the applications of electron crystallography at resolutions which are high enough to permit chain tracing and the initial placement of side chain residues.

01.7-2 ELECTRON MICROSCOPY STUDIES OF PROTEIN CRYSTALS FOUND in vivo.

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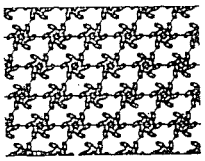
Protein crystals are known to be formed in vivo in many cases. These range from one-dimensional fibrous crystals as for example in actin, over two-dimensional as in gap junctions and bacterial surface layers (S-layers) to three-dimensional crystals. The 3-D crystals may be storage proteins or the result of a metabolic disease. Whereas 3-D crystals in general are not functional in the crystalline form, the 2-D and 1-D crystals are.

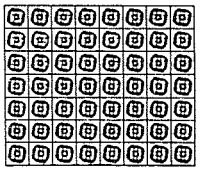
We have studied 2-D crystals of bacterial S-layers and 3-D crystals found in mitochondria from diseased human skeletal muscle fibers.

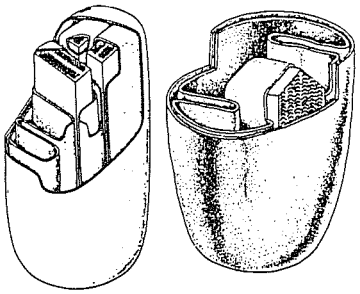
S-layers are crystalline networks of protein or glycoprotein molecules covering the entire surface of the bacterium. S-layers have been found for hundreds of different bacterial species. The S-layers do not contain lipids, so the structures are porous and will allow a free passage of molecules up to the size of small proteins through the S-layer network. The functions of the S-layers are still largely unknown, but they are known to be important for adhesion to host cells in at least one case.

We have determined the 3-D structures of several S-layers by electron microscopy of negatively stained specimens. At resolutions of 2 to 3 nm, the shape and assembly of the proteins were seen. In Bacteroides buccae (Sjögren et al. J. Bacteriology 164 (1985)1278) the S-layer was hexagonal p6 with a=b=21.5 nm and c=7.7 nm. In a strain of Eubacterium the lattice was also p6, with a=b=15.7 nm and a thickness of 16 nm. Hexagonal symmetries are the most common among S-layers, but in many cases tetragonal symmetries are also found. An example of this is a strain of Bacillus with p4 symmetry, a=b=10.7 nm.



Bacterial S-layers





The two types of crystalline inclusions in mitochondria.

In muscle biopsies from humans suffering from severe muscle diseases, especially ophtalmoplegia, large numbers of crystalline inclusions in mitochondria may be found. These three-dimensional crystals were studied by electron microscopy of thin sections of embedded skeletal muscle tissue. A goniometer stage was used which enabled us to obtain all three axial projections of the crystals. The images were scanned in a microdensitometer and the Fourier transforms calculated. By this crystallographic approach we could conclude that the there were two different crystal types, and not one as previously suggested. The large unit cell sizes clearly indicated that the crystals were composed of macromolecules, presumably proteins. Ref. Farrants, Hovmöller & Stadhouders Muscle & Nerve (1987) In press.