Lipopolysaccharides (LPS) are characteristic components found in the outer leaflet of the so-called outer membrane of all gram-negative bacteria. As an exposed component of the cell surface LPS play an important role in the interaction of the bacteria with the host during infection and is responsible for a variety of immunologic and toxic effects (Kieschnel, Galanos, Lederits, Westphal, in "Immunopharmacology", D. Webb, ed., (1982), pp. 183-229).

Furthermore, LPS are mainly responsible for the permeation-barrier properties of the outer membrane, thus providing the very reason for the resistance of gram-negative bacteria against many antibiotics. In order to learn about the possible relationships between these important properties and the conformational features of this unique molecule, a X-ray diffraction study on isolated LPS from wild type bacteria and on LPS samples differing in the length of the polysaccharides portion connected to its lipid A part as well as on its lipid A portion itself was undertaken.

The results showed, that LPS and lipid A can form bilayered structures in the dry state as well as in solution. The fatty acid chains of the lipid A portion were oriented perpendicular to the membrane surface and were packed remarkably well ordered in a two dimensional hexagonal lattice. The phase transition behaviour of dried multilayers as well as aqueous solutions of lipid A and LPS-samples has been studied using Fourier-transform-infrared spectroscopic techniques.

Using the experimental data so far obtained, a model of the three dimensional architecture of the LPS will be presented. For the lipid A portion, a molecular model stemming from conformational energy calculations will be shown to be compatible with the X-ray diffraction data and seems to be capable of explaining the well-known barrier function properties of the LPS even for lipophilic molecules.

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02. STRUCTURAL MOLECULAR BIOLOGY

02.10-3 DISCRETE DISORDER IN PROTEIN CRYSTALS. By Janet L. Smith, Wayne A. Hendrickson, Richard B. Hozakto and Steven Sheffer, Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375 USA.

Proteins in solution are widely recognized to be flexible molecules. We have recently observed the manifestations of such flexibility in the crystal structures of four proteins and have modeled several discretely ordered side chains in each. The four structures are: Crambin with R = 0.178, 0.045A resolution (W. N. Teeter, Nature 1981) 290, 107); Erabutoxin B with R = 0.182 to 1.4A resolution (JLS with W. Wash; B. M. Haw and P. E. Bourne; Kimball et al., Biochim. Biophys. Res. Comm. 1979 88, 950); Myohemerythrin with R = 0.159 to 1.7/A resolution (SS with W. AN and JLS; Hendrickson, Kippenstein and Ward, Proc. Natl. Acad. Sci. USA 1975 72, 2160); and Lamprey hemoglobin with R = 0.142 to 2.0A resolution (RBH with W. Wash; Hendrickson, Love and Karle, J. Mol. Biol. 1973 75, 331).

Models from restrained least-squares refinement have indicated thermal parameters (anisotropic for crambin) and individual positional disorder for six side chains in cRAMBIN, seven in erabutoxin, seven in myohemerythrin and ten in lamprey hemoglobin. Most disorder mates are related by rotation of side chain torsional angles and all make sensible nonbonded or hydrogen bonded contacts. Two of the disordered side chains in crambin and one in lamprey hemoglobin are cases of heterogeneity in the amino acid sequence, and both of those in crambin exhibit further positional disorder. In most cases conformational heterogeneity is modeled only in side chains, although its effects are likely felt in the protein backbone as well. Many of the disordered side chains are associated with disorder in the solvent structure. There are also numerous pairs of water-of-water or water-ion sites which reproductively refine too close to one another to be simultaneously occupied. The crambin and erabutoxin crystals both exhibit mutually exclusive networks of water molecules. We expect that further discrete disorder in solvent regions may be masked by the relatively high thermal parameters typically associated with solvent sites.

The effect of resolution is seen dramatically in the extent of disorder observable in these four structures. In lamprey hemoglobin (2.0A) only widely separated alternate side-chain conformers can be assigned with certainty; four of ten disordered side chains are not well resolved but were built into persistent Fo-Fc electron density. By contrast, in crambin (0.945A) features with very low occupancy can be reliably refined and some three-way disorder is observed. There is no evidence in crambin or in crambin electron-density maps of continuous large-scale motion of any part of the protein. Rather we see alternate, partially occupied conformers. As we compare this to electron densities at lower resolution from which such large-scale motion could be postulated, we conclude that lack of resolution obscures much discrete disorder. The crambin results combined with the fact that discrete disorder can be stably refined at 1.4 to 2.0A resolution support the hypothesis that large atomic displacements in protein molecules generally result in discrete conformers separated by energy barriers high enough that flexible groups spend little time between stable states.

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