02.9–2 ON THE MOLECULAR CONFORMATION OF THE LIPOPOLYSACCHARIDES OF THE OUTER MEMBRANE OF GRAM-NEGATIVE BACTERIA AS PROBED BY X-RAY DIFFRACTION AND MOLECULAR MODELING. By

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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 (Rietschel, Galanos, Lüderitz, 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 lipophibic molecules.

02.10–1 CONFORMATIONAL FLEXIBILITY IN THE 4–ZINC HUMAN INSULIN HEXAMER AS DETERMINED BY X-RAY CRYSTALLOGRAPHY, G. D. Smith, D. C. Swenson, E. J. Dodson, G. G. Dodson and C. D. Reynolds, Medical Foundation of Buffalo, Inc., Buffalo, NY 14203 and University of York, Heslington, York, England YO1 5DD.

The interaction of insulin with its receptor most likely requires changes in the conformation of the insulin molecule. One way of obtaining information concerning the flexibility of the insulin molecule is by comparing the crystal structures of different crystalline forms of insulin from various species. The structure of 4–Zn human insulin has been refined using 1.85Å resolution data to a residual of 0.17. In the 2–Zn porcine insulin structure, pairs of monomers are related by a local two fold axis and each of the independent B–chains consists of two extended sections of peptide chain connected by a well defined α–helix, B9 to B19. The most significant change in conformation in the 4–Zn human insulin structure is observed in the B–chain of molecule 1 and involves a shift of over 30Å for B1 Phe. As a result of this change involving residues B1 through B8, the extended conformation observed in the 2–Zn structure is transformed into an α–helical conformation producing a continuous α–helix from B1 through B19. A change in coordination around one of the zinc ions is observed and an additional zinc ion in a general position is bound by the hexamer. Four additional hydrogen bonded interactions between monomers help to stabilize the dimer and the hexamer. In 2–Zn porcine insulin, the zinc ions are coordinated by water molecules which lie in a shallow depression on the surface of the hexamer. In 4–Zn human insulin one surface has been drastically altered as a result of the change in conformation so that a zinc ion is now buried in a cavity in the center of three parallel α–helices. The only access to this zinc ion is through a tunnel 8Å long. Research supported by the Kroc Foundation and the James H. Cummings Foundation.

02.10–2 CONFORMATIONAL FLEXIBILITY IN THE THIRD DOMAIN OF THE TURKEY OVOMUCOID INHIBITOR BOUND TO SGPB AND α–CHYMOTRYPSIN. Randy Read, Masao Fujinaga, Anita Sielecki, Wojciech Ardelt*, Michael Laskowski, Jr.* and Michael James, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada, and *Department of Chemistry, Purdue University, West Lafayette, Indiana, U.S.A.

Avian ovomucoid inhibitors consist of three homologous tandem domains of approximately 60 residues. The third domain from turkey, OMTKY3, has 56 amino acid residues and inhibits those serine proteinases with chymotryptic specificity. The reactive bond of OMTKY3 is between Leu18I and Glu19I. The equilibrium association constants, K_a, of OMTKY3 for SGPA, SGPB, α–chymotrypsin and elastase are 2.2×10^{11}, 5.6×10^{10}, 2.0×10^{11} and 5.7×10^{10} M^{-1}, respectively. The crystal structure of the complex between SGPB, a bacterial serine protease, and OMTKY3 has been solved and refined at 1.8 Å resolution (Fujinaga *et al.*, PNAS *79*, 4868 [1982]). The crystallographic R after 58 restrained parameter least squares cycles for the 16,245 data in the range 10.0 – 1.8 Å with $I \geq \sigma(I)/2$ is 0.145. The inhibitor binds in a fashion similar to that of a good substrate but the reactive bond remains intact in the observed complex (Read *et al.*, Biochemistry *22*, 4420 [1983]).

Crystals of the complex of OMTKY3 with α–chymotrypsin are monoclinic, space group $P2_1$ with unit cell dimensions of a=44.9, b=54.5, c=57.2 Å and β=103.9°. The structure was solved by molecular replacement and has been refined by restrained parameter least–squares using the data from 8.0 – 1.8 Å resolution (19,178 reflections with $I \geq \sigma(I)$. The final R factor is 0.174. The figure shows the OMTKY3 complexed to α–chymotrypsin.

The ovomucoid domains are disc–shaped with a central α–helix flanked by a small three–stranded antiparallel