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.

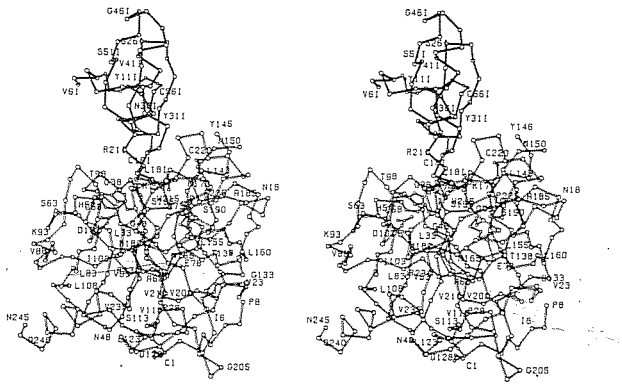
The interaction of insulin with its receptor most likely requires changes in the conformation of the insulin molecule. One way of obtaining information concerning the flexibility of the insulin molecule is by comparing the crystal structures of different crystalline forms of insulin from various species. 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



β-sheet. Along one edge of this central core runs the strand P_5-P_3' containing the reactive bond. Comparison of the structure of OMTKY3 bound to SGPB with that when bound to α-chymotrypsin shows that the strand P_5-P_3' rotates by 14.3° relative to the central core of the domain. This rotation can be modelled by a hinge-like motion about the rotation axis. This conformational flexibility of the ovomucoid domain allows the complementary segment of the inhibitor to bind to several serine proteinases in spite of differences in enzyme structure remote from S_6 to S_3'. The central α-helix and β-sheet seems to provide a relatively rigid scaffolding to support the conformationally labile reactive site. An additional conformational change in OMTKY3 or in elastase at Arg217A (or both) must occur in order for the complex between these proteins to form.

Research supported by the Medical Research Council of Canada.

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-water or water-ion sites which reproducibly refine too close to one another to be simultaneously occupied. The crambin and erabutoxin crystals both contain alternate, 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 hemoblogin (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 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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02.10–3 DISCRETE DISORDER IN PROTEIN CRYSTALS. By Janet L. Smith, Wayne A. Hendrickson, Richard B. Honzatko and Steven Sheriff, 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 disordered side chains in each. The four structures are:
Crambin with R=0.111 to 0.945A resolution (WAH with M. M. Teeter, Nature (1981) 290, 107);
Erabutoxin B with R=0.152 to 1.4A resolution (JLS with WAH, B. W. Low and P. E. Bourne; Kimball et al., Biochem. Biophys. Res. Comm. (1979) 88, 950);
Myohemerythrin with R=0.159 to 1.7/1.3A resolution (SS with WAH and JLS; Hendrickson, Klippenstein and Ward, Proc. Natl. Acad. Sci. USA (1975) 72, 2160); and
Lamprey hemoglobin with R=0.142 to 2.0A resolution (RBH with WAH; Hendrickson, Love and Karle, J. Mol. Biol. (1973) 74, 331).
Models from restrained least-squares refinement have individual thermal parameters (anisotropic for crambin) and very good stereochemistry (rms deviation from ideality in covalent bonds = 0.014-0.017A; mean magnitude of difference in B between bonded main chain atoms = 0.67-0.95A^2). We have observed in electron-density maps and included in refinement conformational heterogeneity for six side chains in crambin, ten 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

02.10–4 CONFORMATIONAL VARIABILITY OF THE COENZYME NAD^+ IN THE FREE AND BOUND STATES: NICOTINAMIDE SANDWICHED BETWEEN ADENINE AND WATER IN THE CRYSTAL STRUCTURE OF THE FREE ACID FORM OF NAD^+.[x] R. Parthasarathy and S.M. Fridey, Center for Crystallographic Research, Roswell Park Memorial Institute, Buffalo, NY 14263 USA.

The coenzyme NAD^+ plays a dominant role in the hydride transfer in biological redox processes. Its structure and conformation in the free state in solution as well as in the bound state complexed to enzymes have been investigated intensively over many years using a variety of spectroscopic techniques including ^{13}C and ^{31}P NMR and using theoretical calculations. X-ray crystallographic studies (at low resolution) of NAD^+ bound to several dehydrogenases demonstrated the extended form with the nucleotides exhibiting non-standard conformations (for a summary see Saenger, Reddy, Muhlegger and Weimann (1977) in Pyridine Nucleotide-Dependent Dehydrogenases, (Ed.) H. Sund, Walter de Gruyter, New York USA, pp. 222-236). A medium resolution single crystal study of Li^+-NAD^+ complex (Saenger, Reddy, Muhlegger and Weimann (1977) Nature 267, 225-229) shows an extended conformation of NAD^+, somewhat similar to that found in holoenzyme complexes. Here, we present a very accurate high resolution x-ray study of the free-acid form of NAD^+ in which the NAD^+ molecule adopts a totally different conformation. Crystals of NAD^+ tetrahydrate ($C_{21}H_{27}N_7O_{14}P_2\ 4H_2O$) are triclinic, a = 8.643(2), b = 8.857(1), c = 11.784(3)A, α = 109.74(2), β = 90.76(2), γ = 103.43(1)°, V = 779.9A^3, Z = 1, space group P1. Using full three dimensional data to the limit of the Cu-sphere for CuKα (3460 reflections, 2624 ≥3σ), the structure was solved using Patterson and difference-Fourier techniques and refined by full-matrix least-squares procedures to an R of 0.03. All the hydrogen atoms were located in difference electron-density maps and their parameters were refined by least-squares. The molecule exhibits