02.1-42 THE STRUCTURE OF HUMAN LACTOFERRIN AT 3.2Å RESOLUTION

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Lactoferrin, a member of the transferrin family of proteins is an iron binding glycoprotein (M_r ~80 000 daltons), present in mammalian milks, mucous secretions and white blood cells. Because of its ability to bind iron tightly, (K ~10^{20}) lactoferrin exerts a bacteriostatic effect in vitro by depriving micro-organisms of essential iron (Bullen, J.J., Rogers, H.J. & Leigh, L., Brit. Med. J., 1972, 3, 69-75). As well as a role in non-immunological host defence, there are reports which suggest it may modulate immune and inflammatory processes (Birgens, H.S., Scand. J. Haematol., 1984, 33, 225-230) and prevent the catalytic formation of potentially harmful oxygen radicals (Baldwin, D.A., Jenny, E.R. & Aisen, P., J. Biol. Chem., 1984, 259, 13391-13394).
The three dimensional structure of lactoferrin determined at 3.2Å resolution will be presented. The 703 amino acid residues are organised into two homologous lobes that are connected by a short α helix. Each of these lobes carries one iron binding site, one carbohydrate chain, and is divided into two domains of supersecondary structure. The iron is found at the domain interface where it is bonded to four protein ligands, 2 tyrosine, 1 histidine and 1 aspartate. The fifth and sixth coordination sites correspond with a region of positive electron density adjoining the iron and provide a possible location for the associated anion (CO_3^{2-} or HCO_3^-). This region is adjacent to an arginine sidechain and a helix N-terminus. Some remarkable structural similarities between lactoferrin and certain other binding proteins are also apparent.

02.1-43 THE CRYSTAL STRUCTURE OF 4-ZN RHOMBOHEDRAL DES-B30 CROSS-LINKED HUMAN INSULIN. By U. Derewenda, Z. Derewenda, G. G. Dodson, Dept. of Chem., Univ. of York, U.K. & J. Markusson, NOVO Research Institute, Denmark.

Human des-B30 insulin in which there is a peptide bond formed between Lys B29 and Gly A1 by the synthetic action of trypsin is a byproduct of the industrial conversion of pig to human insulin. This modification leaves the hormone without detectable activity. Rhombohedral crystals of this insulin have been grown in conditions that produce 4 Zn insulin with native hormone. The crystals' cell dimensions and diffraction pattern showed they were similar to native rhombohedral 4Zn insulin. Refinement of the structure proceeded smoothly from the 4Zn insulin coordinates and converged with the agreement factor R =.175 . The main structural change in this cross-linked insulin is the movement of B27 - B29 into an approximate helix which is continuous with the helix at A1 - A9. There are two zinc ions on the 3-fold axis coordinated by the B10 His, in contrast to the off-axial coordination seen in native 4Zn insulin. The overall structure of the des-B30 insulin molecule is, apart from the cross-bridge, essentially the same as the native molecule. This structure of des-B30 cross-linked insulin is therefore similar to the beef diamino suberic acid B29 - A1 cross-linked insulin. The latter modification leaves some flexibility in the molecule but is associated with a large drop in potency (to ca. 15%). We conclude that the explanation for the absence of activity in the des-B30 is the more complete loss of flexibility at the B chain C terminus.

02.1-44 A NEW CRYSTAL FORM OF INSULIN. Z.H. Rao and N.W. Isaacs. St. Vincent's Institute of Medical Research, Victoria Parade, Melbourne, Victoria 3065, Australia.

A new crystal form of pig insulin has been obtained. The crystals are orthorhombic, with space group $C222_1$, and cell dimensions a = 60.2A, b = 228A, c = 222A.

Matthews (J. Mol. Biol., (1968) 33, 491) has found that for protein crystals the value of the crystal volume per unit mass, Vm, lies within the range 1.8 to 3.6 with a median value of 2.4. For a Vm of 1.8 the asymmetric unit of these crystals would contain 36 insulin molecules and for a Vm of 3.6 there would be 18 molecules. Assuming the insulin exists as a hexamer, for a Vm of 2.7, which is close to the median of observed values, there would be 24 molecules or 4 hexamers in the asymmetric unit.

A model for the structure, based on the packing of nearly spherical hexamers subject to the constraints of the space group symmetry, has been obtained.

The crystals diffract to a resolution of about 3.0A on precession photographs. The same crystals can be obtained from bovine insulin.

02.1-45 CRYSTAL STRUCTURES OF BOWMAN-BIRK PROTEASE INHIBITOR AND ITS COMPLEX WITH TRYPSIN. by Y. Tsunogae, A. Suzuki, I. Tanaka, T. Yamane and T. Ashida, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya 464, Japan

Bowman-Birk inhibitors found in the seeds (beans) of the leguminous plants are small proteins which inhibit the serine proteases by making stable enzyme-inhibitor complexes. The inhibitors usually consist of 60-80 amino acid residues including 7 disulfide linkages, all are evolutionarily conserved. They are double-headed inhibitors consisting of two tandem homologous domains each with a binding site. Each domain consists of three peptide loops made by disulfide linkages, and each loop is made of 8-11 residues. Crystals have been obtained of AB-I and IIa from azuki beans, A-I, II and B-III from peanuts, and their complexes with trypsin or chymotrypsin.

A-II, 3 A study: The molecule has an elongated shape with an approximate dimension of 45x15x15 A, consisting of two distinct domains which are connected by two rather flexible chains (Fig. 1.). The structures of the domains are very similar to each other and are related by an intramolecular pseudo two-fold symmetry. The binding sites are in the outermost loops, which protrude from the core of the molecule to the opposite direction. The electron densities for both binding sites are very low, indicating a considerable flexibility or a disorder in the conformations.

AB-I+trypsin complex, 2.3 A study: The SIR method with the MR method solved the structure. Of the inhibitor only the structure of the trypsin-binding domain could be determined. The electron density for the chymotrypsin-binding domain, however, is so low that any model could not be built. The structure was refined to R=0.21 including trypsin, the trypsin-binding domain of 29

residues (Cys18-Arg38 and Asn65-Cys72) and 111 water molecules. The overall conformation of the trypsin binding domain is very similar to those of two domains of A-II. The mode of interactions between trypsin and the inhibitor (Fig. 2) is very similar to those of e.g. trypsin-BPTI and trypsinogen-PSTI complexes. The 'front side', Cys24-Lys26-Met28, contacts the trypsin active center with several hydrogen bonds and van der Waals contacts, the distance between Ser195 OY-Lys26 C' being 2.3 A. The structure of the trypsin-binding loop in the complex is stabilized by several intra-loop hydrogen bonds and van der Waals contacts, thus the conformation change of the inhibitor necessary for the proteolysis seems to be inhibited.

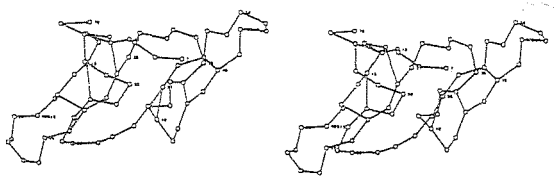

Fig. 1. A-II

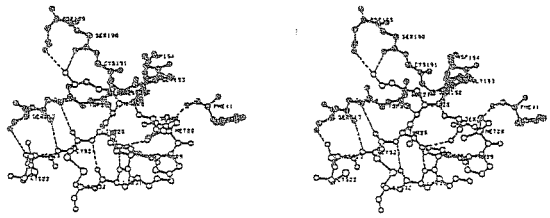

Fig. 2. AB-I(white)+trypsin(black) binding site

02.1-46 THE CRYSTAL STRUCTURES OF $(D\text{-}Trp)^{A1}$-INSULIN AND $(L\text{-}Trp)^{A1}$-INSULIN. By D.C. Liang and Z.L. Wan Institute of Biophysics, Academia Sinica, Beijing, China

The N-terminus of the insulin A-chain is one of important positions for maintaining the biological activity of insulin. It is known that the removal of A-chain N-terminal residue A1-Gly from the molecule causes the insulin molecule to lose almost the whole biological activity(Brandenburg,D. et al.,Hoppe-Seyler's Z. Physiol. Chem., 356(1975) 916) and modification of A1-Gly would more or less reduce the activity as well (Geiger, R., Chemiker-Zeitg., 100(1976) 111). In our case, the modified insulin molecule with replacement of A1-Gly by L-configurational tryptophane had only 14 % of natural insulin activity (fat cell assay), nevertheless the $(D\text{-}Trp)^{A1}$-insulin molecule still retained 82 % biological activity of insulin. It indicated that the spacial arrangement of differently configurational amino acid residues in A1 had significant effect on the molecular conformation on the whole and on the activity of the insulin molecule in particular. The single crystals of this pair of insulin analogue suitable for X-ray diffraction were grown in citrate buffer system by still-setting method. They both belong to the trigonal system with space group R3. The parameters of the unit cell of $(L\text{-}Trp)^{A1}$-insulin are a=80.31Å, c=37.45Å and those of $(D\text{-}Trp)^{A1}$-insulin a=78.65Å, c=50.01Å. There are two molecules in an asymmetric unit. The crystal structures of this pair of insulin analogue have been solved by the methods of isomorphous replacement and molecular replacement at 2.1Å and 2.0Å resolution respectively. The studies on three-dimensional structure and function relationship of insulin in our lab pointed out that the binding of the insulin receptor probably occurs on a surface of the insulin molecule and this surface should be possessed of two parts. One of them is a hydrophobic surface with an area of about 150Å^2. Another is the charged and polar groups dispersing around the hydrophobic surface. The α-amino group of A1-Gly with positive charge rightly located at the edge of the hydrophobic surface is one of very important charged groups for the interaction of insulin molecule with its receptor. Recently the refined structures of $(L\text{-}Trp)^{A1}$-insulin and $(D\text{-}Trp)^{A1}$-insulin show that the L-configurational side chain of indole ring at A1 in the $(L\text{-}Trp)^{A1}$-insulin molecule has a conformation towards up from the amphipathic binding surface of the molecule and thus the interaction of the α-amino group with the insulin receptor is greatly screened. On the other hand, the side chain of the D-configurational amino acid at A1 in the $(D\text{-}Trp)^{A1}$-insulin structure goes to the other part of the molecular surface outside the amphipathic surface and does not obstruct the α-amino group to contact with the receptor. These refined crystal structures of $(L\text{-}Trp)^{A1}$-insulin and $(D\text{-}Trp)^{A1}$-insulin confirmed our proposal concerning the amphipathic binding surface of insulin molecule and gave us a better understanding of the interaction mechanism on the amphipathic surface of insulin molecule with its receptor. The structural comparison of this pair of insulin analogue with the structures of 2Zn pig insulin and despentapeptide (B26-B30) insulin is now in progress.

02.2-1 STRUCTURE OF A LOW-POTENTIAL [4FE-4S] FERREDOXIN DETERMINED BY ANOMALOUS SCATTERING OF NATIVE IRON ATOMS. By K. Fukuyama*,††, Y. Nagahara*, T. Tsukihara*, Y. Katsube†, T. Hase†† and H. Matsubara††, *Faculty of Engineering, Tottori Univ., Tottori 680; †Institute for Protein Research, Osaka Univ., Osaka 565; ††Faculty of Science, Osaka Univ., Osaka 560, JAPAN.

The structure of a low-potential [4Fe-4S] ferredoxin (Fd) from *Bacillus thermoproteolyticus* has been solved by the anomalous scattering information of iron atoms in the diffraction data of native crystal. This Fd consists of one [4Fe-4S] cluster as a prothetic group and 81 amino acid residues. The four iron sites were derived from the Patterson map computed with the coefficient of $(\Delta F)^2$ at 2.56 Å resolution and refined by the least-squares method to R=0.296 against 20% largest $|\Delta F|$'s. The model was built based on the best Fourier map calculated from the anomalous scattering and the partial structure informations. The structure was refined by alternate cycles of Hendrickson-Konnert restrained least-squares and model revision. The current R factor is 0.33 for 6.0-2.3 Å resolution reflections with $F>3\sigma_F$.

The folding of the present Fd is closely similar to that of *Peptococcus aerogenes* Fd, although both Fd's are distinct in the numbers of the clusters and amino acid residues. (*P. aerogenes* Fd consists of two [4Fe-4S] clusters and 54 residues.) The present Fd has three turns of α-helix before the fourth ligand as well as insertions of peptide segments relative to *P. aerogenes* Fd. The helical region in *B. thermoproteolyticus* Fd corresponds to the second cluster binding region in *P. aerogenes* Fd. Structural correspondence strongly supports that both Fd's evolved from a common ancestor. The significance of the α-helix in the [4Fe-4S] Fd's and the evolutionary relationship among bacterial Fd's will be discussed based on the known primary and tertiary structures.