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. 1) is very similar to those of e.g., trypsin-BPTI and trypsinogen-P12A in complexes. The front side, Cys24-Lys26-His28, contacts the trypsin active center with several hydrogen bonds and van der Waals contacts, thus the conformational change of the inhibitor necessary for the proteolysis seems to be inhibited.

Fig. 1. A-II

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 conformational change of the inhibitor necessary for the proteolysis seems to be inhibited.

Fig. 2. A8-1(white)-trypsin(black) binding site

02.1-48 THE CRYSTAL STRUCTURES OF (D-Trp)A1-INSULIN AND (L-Trp)A1-INSULIN. By B.C. Jiang and Z.L. Han

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, J. 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., Dachez-Bréart, A., 120(1976) 111). In our case, the modified insulin molecule with replacement of A1-Gly by L-configurational tryptophane had only 1.4% of natural insulin activity (fat cell assay), nevertheless the (L-Trp)A1-insulin molecule still retained 82% biological activity of insulin. It indicated that the special arrangement of different configurational amino acid residues in A1 had significant effect on the biological conformation on the whole and on the activity of the insulin molecule in particular. The single crystals of this pair of insulin analogous 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-Trp)A1-insulin are a=69.51 Å, c=107.45 Å and those of (D-Trp)A1-insulin are a=78.65 Å, c=50.01 Å. 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 2.1 Å and 2.0 Å 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 hydrophilic surface and this surface should be possessed of two parts. One of them is a hydrophobic surface with an area of about 150 Å². Another is the charged and polar groups dispersing around the hydrophobic surface. The a-amino group of A1-Gly with positive charge sightly 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-Trp)A1-insulin and (D-Trp)A1-insulin show that the 1-configurational side chain of indole ring at A1 in the (L-Trp)A1-insulin molecule has a conformation towards up from the amphipathic binding surface of the molecule and thus the interaction of the a-amino group with the insulin receptor is greatly screened. On the other hand, the side chain of the 3-configurational amino acid at A1 in the (D-Trp)A1-insulin structure goes to the other part of the molecular surface outside the amphipathic surface and does not obstruct the a-amino group to contact with the receptor. These refined crystal structures of (L-Trp)A1-insulin and (D-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 analogous with the structures of zinc plus insulin and deepentepaside (325-350) 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. Pokucan*+*, Y. Nagaehara*, T. Tsukihara*, Y. Katsube*, T. Hase*++ and H. Matsubara*++*, *Faculty of Engineering, Tohoku Univ., Tottori 680; †Institute for Protein Research, Osaka Univ., Osaka 560; ‡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 atom in the diffraction data of native crystal. This Fd consists of one [4Fe-4S] cluster as a prosthetic group and 81 amino acid residues. The four iron sites were derived from the Patterson map computed with the coefficient of (DF) at 2.36 Å resolution and refined by the least-squares method to R=0.296 against 20% largest [DF]². 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-Kolmar restrained least-squares and model refinement. The current R factor is 0.33 for 6.0-2.3 Å resolution reflections with P<30°.

The folding of the present Fd is closely similar to that of Paracoccus 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 56 residues.) The present Fd has three turns of a-helix before the fourth ligand as well as insertions of peptide segments relative to P. aerogenes Fd. The helical region in S. 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 a-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.