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.

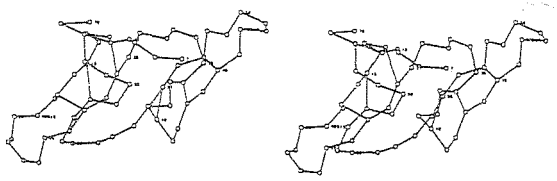

Fig. 1. A-II

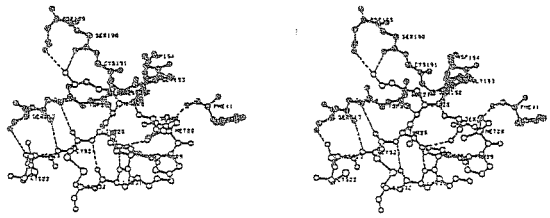

Fig. 2. AB-I(white)+trypsin(black) binding site

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.