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PS–03.07.06 STRUCTURE COMPARISON BETWEEN TRICHOSANTHIN AND MOMORCHARIN. By Gao Ben*, Wang Yaoping, Chen Shizhi, Wu Shen, Ma Xingqi and Dong Yicheng, Institute of Biophysics, Academia Sinica, Beijing, 100101, PRC.

The similarities and differences between the two RIPs (ribosome inactivating proteins) molecular structures were determined and analysed on the basis of the refined structure models of Trichosanthin at 1.73Å resolution (Gao Ben, et al., Scientia Sinica B, 1993, 3, in press) and α-Momorcharin at 2Å resolution provided by Ren Jingshan and Wang Yaoping (Private communication). The superposition of the two models was performed using the main-chain atoms and the RMS deviation for all the main-chain atoms of the 246 residues is 0.70Å. Dividing the two models into many pairs of different polypeptide fragments, the superpositions of the pairs of all polypeptide fragments have done in turn and the RMS deviations for the main-chain atoms of four fifth polypeptide fragments are smaller than 0.30Å. The RMS deviation for the side-chain atoms of more than half of all residues are smaller than 0.50Å and the great majority of these residues form six hydrophobic cores in the interior of the two proteins. These results indicates that the backbones of the two proteins have very similar three-dimensional arrange. There are three domains of the greatest deviation for the main-chain conformations of the two proteins, 38-45, 172-182, and 216-222, which are the flexible loops on the surfaces of the two proteins and corresponding to the sequence fragments with the greatest residue differences. Therefore, this result indicates that the residue differences have evidently brought about the three-dimensional conformational differences.

There are the residue differences corresponding to primary amino acid sequences for one third residues of the two models. As the result of these residue differences, the differences were found to exist not only in the main-chain conformations but also evidently in the secondary structures and in distributions of the other hydrogen bonds relative to the main-chain atoms and bound waters which form hydrogen bonds to the main-chain atoms. The patterns of thirteen percent of the hydrogen bonds for the α helics of the two models, that of seventeen percent of the hydrogen bonds for the β sheets, and that of thirty eight percent of the hydrogen bonds for the turns are different from each other, respectively. The patterns of thirty percent of the other hydrogen bonds relative to the main-chain atoms and thirty seven of the waters bound to the main-chain atoms are different, respectively.

Ten highly conserved residues among primary amino acid sequences of 12 RIPs (Funatsu, G., et al., Biochimie, 1991, 73, 1157-1161. Gao Ben, et al., Scientia Sinica B, 1993, 3, in press) were analysed and those corresponding to Trichosanthin are 14Tyr, 22Arg, 70Tyr, 111Tyr, 122Arg, 132Leu, 160Glu, 161Ala, 163Arg, and 192Trp. Superposition of ten residues of Momorcharin on those corresponding to Trichosanthin was done together using all the 40 atoms of the main-chains. The RMS deviation for the main-chain atoms is 0.30Å and that for the side-chain atoms is 0.18Å. The differences in distributions of hydrogen bonds and bound waters relative to the corresponding ten residues in the two models are a little. A summary of the analysis statistics by superimposing, in turn, ten pairs of residues of the two models indicates that the RMS deviations for the main-chain atoms are all smaller than 0.10Å and that for the side-chain atoms of those residues except 122Arg are smaller than 0.15Å. Therefore, the three-dimentional structures of nine residues which are invariant among the known sequences of those RIPs are highly conserved. These results have an important significance for researching of the RIPs structure-function relationship.

PS–03.07.07 THREE-DIMENSIONAL STRUCTURE OF THE COMPLEX OF TRICHOSANTHIN WITH NADPH AT 1.7Å RESOLUTION. By J.-P. Xiong*, L. Zhang, Z.-X. Xia, and Y. Wang. Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China.

Trichosanthin is a toxic protein (Mr 27,000) used as a traditional Chinese drug for inducing abortion and recently found to be an anti-human immunodeficiency virus agent. Trichosanthin is a type-I ribosome-inactivating protein (RIP) with the acitivity of RNA N-glycosidase, and it was reported that ricin, a type-II RIP, catalyzes the cleavage of the N-glycosidic bond of a specific adenine within 28s rRNA, resulting in the inhibition of protein synthesis (Endo et al., J. Biol. Chem., 1987, **262**, 8128-8130). We have determined at 3Å resolution the three-dimensional structure of trichosanthin crystallizing in monoclinic space group C_2 (Xia et al., chinese J. Chem., 1991, **9**, 563-564) and it has been refined at 2.7Å resolution (Xia et al., Abstracts of 6th FAOB Congress, 1992, **16-21**, 99). The molecule shows a cleft near the interface of the two domains and the cleft is likely to be the active site region in which several absolutely conserved residues are located.

The complex of trichosanthin with nicotinamide adenine dinucleotide phosphate (NADPH), a substrate analogue, was prepared and crystallized in space group $P2_12_12_1$ with unit cell dimensions a=38.39Å, b=76.81Å and c=79.93Å, similar to orthorhombic native crystals. The diffraction data up to 1.7Å resolution were collected on an X-200B area detector. The three-dimensional structure of the complex has been solved by molecular replacement method (program MERLOT) using one molecule of the monoclinic trichosanthin structure as the search molecule. The complex structure was refined at 1.7Å resolution, using program PROFFT, in which 170 bonded water molecules were included but NADPH was absent in the model, giving an R-factor of 18.9% in the resolution range 5.0-2.0Å with the rms deviation of 0.025Å from ideal bond lengths. The resulting (2Fo-Fc) map shows excellent electron density for the protein and an additional piece of continuous electron density. The NADPH has been fitted into it with the adenine ring in the strong and flat electron density which is located between the aromatic rings of Try70 and Try111. The adenine interacts with Arg163 which is absolutely conserved and located in the deep center of the cleft, Ser159 which is conserved in some of the RIPs', and the main chain of the protein. The phosphate at the position O_2' of the ribose of the adenosine interacts with serveral conserved residues in the cleft and is important for stabillizing the complex, as shown by the fluorescence spectra. The further refinement with NADPH present in the model is in progress.

PS–03.07.08 X-RAY STUDIES ON THE TRYPSIN INHIBITOR I-2 FROM WHEAT GERM AND ITS COMPLEX WITH TRYPSIN. By A. Suzuki[1], T. Kurasawa[1], C. Tashiro[1], T. Yamane[1]*, T. Ashida[1], and S. Odani[2], [1]Department of Biotechnology, School of Engineering, Nagoya University, Nagoya 464-01, Japan, [2]Department of Biology, Faculty of Science, Niigata University, Niigata 951, Japan.

03-Crystallography of Biological Macromolecules

The double-headed Bowman-Birk type trypsin inhibitor(BBI), I (Mr=14,000, 123 amino acid residues) was isolated from wheat germs. To compare the tertiary structures of BBI's in cereal grains with those in leguminous plants (for example, Suzuki et al, J. Biochem.(Tokyo), 1987, **101**, 267-274), the structural study of I-2, the major component of the group I, have been carried out.
I-2 and its 1:2 complex with bovine β-trypsin have been crystallized. For I-2 two morphologically different crystal forms were obtained. Crystal form (1) is tetragonal, P4122 or P4322, a=55.45(2), c=129.1(2)A, Vm=3.55A^3/dalton, Do=1.30gcm^{-3}, Z=8 and solvent content(S)=65%. Crystal form (2) is monoclinic, space group C2. The cell parameters show significant variation even for crystals in a same batch. The median parameters are: a=83.9, b=41.5, c=45.7A, β=95.9° , Vm=2.83A^3/dalton, Do=1.33gcm^{-3}, Z=4, and S=44%. The crystals of trypsin-I-2 (2:1) complex are orthorhombic, P212121, a=73.49(2), b=120.56(3), c=70.04(2)A, Vm=2.58A^3/dalton, Do=1.28gcm^{-3}, Z=4 and S=50%. The X-ray intensity data of trypsin-I-2 complex were collected using a Weissenberg camera (Sakabe, J. Appl. Cryst, 1983, **16**, 542-547) at the BL6A2 station, Photon Factory, National Laboratory for High Energy Physics. A total of 26,565 independent reflections between 25 and 2.3A resolution were measured, giving the merging R of 0.130.
The structure of the trypsin-I-2 complex was determined by the molecular replacement method using 6~3.5A data and the structure of bovine trypsin. The structure of I-2 was traced on a 3A difference-Fourier map, and the model building was carried out using TURBO-FRODO (Cambillau, 1992). Currently 18 residues were constructed. The R-factor was 0.324 for 10,674 reflections (Fobs>5σ(F)) between 6 and 3A resolution. The partial structure of the two reactive site regions of I-2 is essentially similar to that of the 1:1 complex between trypsin and adzuki inhibitor AB-I(Tsunogae et al,. J. Biochem.(Tokyo), 1986, **100**, 1637-1646).

PS-03.07.09 STRUCTURE OF OLIGO-1,6-GLUCOSIDASE REFINED AT 2.0 Å RESOLUTION. By Y. Hata[1]*, H. Kizaki[2], K. Watanabe[2], Y. Suzuki[2] and Y. Katsube[3], [1]Institute for Chemical Research, Kyoto University, [2]Department of Agricaltural Chemistry, Kyoto Prefectural University, and [3]Institute for Protein Research, Osaka University, Japan.

Bacillus oligo-1,6-glucosidases exhibit a strong correlation between their proline content and thermostability. In order to elucidate the contribution of proline to their thermostability as well as the enzyme function, we have analyzed the structure of oligo-1,6-glucosidase (Mr=66010, 558 residues) from a mesophile *B. cereus* by X-ray method. Crystals of the enzyme grew in hanging drops (Watanabe, K., Kitamura, K., Hata, Y., Katsube, Y., & Suzuki, Y. (1991). *FEBS Lett.* 290, 221-223). The structure was solved by MIR method with three derivatives prepared in $HgCl_2$, $UO_2(NO_3)_2$ and $Sm(NO_3)_3$ solutions. The intensity data were collected using synchrotron radiation (λ=1.04 Å) at BL6A$_2$ station in KEK-PF, Tsukuba. The data were recorded on Fuji image-plates using a Weissenberg camera. The structure model was built with the FRODO on PS390 graphics by interpreting a 3.0 Å resolution electron density map ($\langle m \rangle$=0.76). The structure refinement was started at this resolution with the program XPLOR, and the resolution has been extended to 2.0 Å. The *R*-factor for the current model with 221 waters is 0.196 for 43328 reflections (92%) between 8.0 and 2.0 Å resolution. The structure of *B. cereus* oligo-1,6-glucosidase is similar to those of α-amylases and can be subdivided into three domains. The N-terminal domain has a $(\beta/\alpha)_8$-barrel with four additional α-helices. The subdomain between the third β-strand and α-helix of the N-domain is a long loop containing a small sheet of three β-strands. The C-terminal domain forms an irregular β-barrel of eight strands. The sequence homology and consequent structural similarity between the oligo-1,6-glucosidases of a thermophile and a mesophile suggest that most of the prolines responsible for increase of the thermostability should be positioned on second sites of β-turns.

PS-03.07.10 CRYSTAL STRUCTURE OF ALKALINE SERINE PROTEASE OF BACTERIAL ORIGIN. By T. Yamane[1], T. Hatanaka[1], T. Kani[1], T. Narusc[1], A. Suzuki[1], T. Ashida[1]*, T. Kobayashi[2] and S. Ito[2], [1]Department of Biotechnology, School of Engineering, Nagoya University, Nagoya 464-01, Japan, [2]Tochigi Research Laboratories, Kao Corporation, Tochigi 321-34, Japan.

A new alkaline serine protease(ALPT) was obtained from a Bacillus strain. It consists of 269 amino acids and has a molecular weight of 26,715. The sequence of ALPT is 60% homologous with subtilisin Carlsberg(SBC).
The enzyme inhibited with phenylmethylsulfonylfluoride was used for crystallization. Needle crystals (form 1) were grown in 0.05M phosphate buffer (pH 5.5), using 1.4M ammonium sulfate as precipitant. The space group is P212121 with a=57.79(2), b=75.82(2), c=54.19(1)A, Z=4 and Vm=2.15A^3/dalton. The cell parameters at first suggested that form 1 might be isomorphous to the crystal of SBC(Petsko & Tsernoglou, J. Mol. Biol., 1976, **106**, 453-456) if the a and b axes were exchanged. The other crystals (form 2) were obtained from 0.05M acetate buffer (pH 5) and belong to space group P212121 with a=47.3, b=62.5, c=75.6A, Z=4 and Vm=2.09A^3/dalton, which is isomorphous to the crystals of high-alkaline serine protease PB92 (van der Laan et al., Protein Engineering, 1992, **5**, 405-411).
The X-ray intensity data of form 1 were collected using a Weissenberg camera (Sakabe, J. Appl. Cryst, 1983, **16**, 542-547) at the BL6A2 station at the Photon Factory, National Laboratory for High Energy Physics. The needle (a*) axis was mounted parallel to the rotation axis of the camera. A total of 8,513 independent reflections up to 1.9A resolution were measured, giving the merging R of 0.119. The structure was solved by the molecular replacement method using 6~3.5A data and the structure of SBC (PDB entry number 1SBC). The crystallographic R-factor was 0.472 at this stage. The structure was refined by the X-PLOR system (Bruenger, 1990, X-PLOR Manual, Ver. 2.1, Yale Univ., New Haven, USA), with the amino acid sequence of SBC. The current R factor is 0.255 for 6404 reflections between 7 and 2.5A resolution. The structure of ALPT is essentially the same as that of SBC, though the orientation parameters from the rotation function revealed that it was not isomorphous to that of SBC.

PS-03.07.11 STRUCTURE ANALYSIS OF PHOSPHOLILASE A2 FROM VENOM OF *TRIMERESURUS FLAVOVIRIDIS*. By E. Matsueda[1], A. Suzuki[1]*, T. Yamane[1], T Ashida[1], H. Kihara[2], and M. Ohno[3], [1]Department of Biotechnology, School of Engineering, Nagoya University, Nagoya 464-01, Japan, [2]Department of Physiology, Faculty of Medicine, Kagoshima University, Kagoshima 890, Japan, [3]Department of Chemistry, Faculty of Science, Kyushu University, Higashi-ku, Fukuoka 812, Japan

Phospholipase A2 (PLA2) from the venom of *Trimeresurus flavoviridis* (habu snake) is a homo-dimeric enzyme composed of subunits with 122 residues. Crsytals belonge to space group P21 with a=44.1 Å, b=55.7 Å, c=48.8 Å, and β =92.4° . An asymmetric unit contains one dimer.
X-ray diffraction data up to 3.5Å resolution were collected on a four-circle diffractometer with CuKα radiation. High resolution data up to 2.0Å were recorded on imaging plates by using a Weissenberg camera installed at BL-6A2 station of Photon Factory. These two datasets were merged to give a total of 13822 unique reflections. The merging R was 0.054.
The structure analysis was proceeded with the molecular replacement method using the modified structure of PLA2 from the Western Diamondback rattlesnake as a search model. The search model was constructed by omitting two turn regions and side-chain atoms of residues different from the habu-snake PLA2 except C$_\beta$ atoms. Self- and cross-rotation search were performed with the Crowther fast rotation function using 6.0 to 3.5Å resolution data. Self-rotation search shows that two subunits in an asymmetric unit are related by a non-crystallographic 2-fold axis. Using this symmetry, the peaks on the cross-rotation function could be paired with one another. The pair containing the highest peak gave true orientations of two subunits in an asymmetric unit. Molecular translation along the **a** and **c** axes was determined for each subunit with the Crowther-Blow translation function using 6.0 to 3.5Å resolution data. Relative position along the **b** axis was determined by the R-search method using 6.0 to 4.0Å data.