# 03-Crystallography of Biological Macromolecules



conserved across the RIP family. Two charged residues, Glu160 and Arg163, are on the left-hand side of the box, making a salt bridge with each other. The side chain of Tyr70, sitting at the front, undergoes significant conformational changes upon substrate binding. The right-hand side of the box consists of the main chains of Gly109, Asn110 and Tyr111. The side chain of Tyr111 projects across the cleft to form a H-bond with OE1 of Glu160.

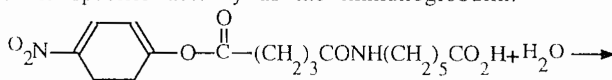
Unexpected features of the conformation of the bound FMP are (i) the ribose and base are in the anti conformation, rather than the syn conformation observed in the crystal structure of FMP, (ii) the furanose ring pucker is C1′-exo, different to that observed for the crystal structures of both FMP and AMP. This suggests that FMP may be bound in a strained high energy conformation. In the product bound structures, the adenine rings are in essentially the same position. However, this position is rotated by 15° (around an axis approximately along C6-N1) in comparison to the orientation in the FMP-bound structure, so that N9 moves 0.8Å toward the side chain of Tyr111. Two active site water molecules are conserved in all the five structures, both are H-bonded to FMP. OH2(247) forms a tight H-bond (2.67Å ) to O3′; OH0(247) makes four H-bonds to OP2, O5′, O4′ and N8 of the FMP molecule. Thus OH0(247) could possibly be ionized by the negatively charged phosphate. The latter may therefore be the most likely candidate for nucleophilic attack on the C1′ atom of the ribose.

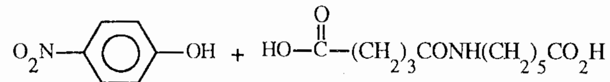
The hydrolysis of the N-glycoside bond by αMMC appears to proceed via a carboninum intermediate. The substrate adenosine is probably bound in a strained conformation (as observed in the bound FMP) thereby weakening the glycoside bond. Electrostatic and ring-ring interactions probably result in a force on the adenine ring which drives atom N9 of the substrate towards Tyr111. The strain is then secured by tight contacts, locking the ribose in position. Protonation at N3 by Arg163 or partial protonation at N3 and N1 by the H-bonds from Arg163 and Ile71 would then break the N-glycoside bond and produce the oxycarbonium ion on the ribose. Rotation of the freed adenine ring would then allow nucleophilic attack at C1′ and protonation at N9 of the adenine.

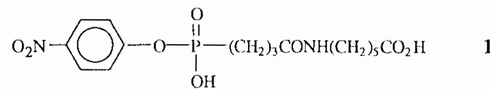
The formation of a covalent intermediate is prohibited simply by the long distance from Glu160 to the carbon atom C1′ of the ribose (3.96Å , adjusting the side chain alone can not produce a sufficiently close approach to the C1′ atom).

**DS-03.07.04** CRYSTAL STRUCTURE OF AN ABZYME HAVING AN ESTERASE ACTIVITY. By B. Golinelli[1], B. Gigant[1], T. Bizebard[1], J. Navaza[2], P. Saludjian[2], R. Zemel[3], Z. Eshhar[3], B.S. Green[3] and M. Knossow[1]* (1) Laboratoire de Biologie Structurale Bat 433 Université Pais Sud 91405 Orsay Cedex France (2) Laboratoire de Physique, Centre Pharmaceutique, 92296 Chatenay Malabry Cedex France (3) Department of Chemical Immunology, Weizmann Institute, Rehovot Israël.

Antibodies catalysing the following paranitrophenol ester hydrolysis have been obtained by immunising mice with the phosphonate transition state analog 1 conjugated to KLH ; Fab fragments of these were prepared and purified ; they were shown to have the same specific activity as the immunoglobulin.




Crystals of one of these Fab fragments have been obtained ; they belong to space group P1 with : a= 99.35 Å, b= 68.06 Å, c= 83.66 Å,α= 71.9°,β= 112.1°,γ= 119.6°, Z=4. Diffraction has been measured to 3 Å resolution, the $R_{merge}$ value being 0.07 for a 93% complete data set. This abzyme has a kcat/kuncat value of 1600 and exhibits turnover. The crystal structure has been solved by molecular replacement and refined ; the R factor value is 0.21 , with a standard stereochemistry.

In the antibody combining site several tyrosine residues are oriented in such a way to enable them to make hydrogen bonds to the nitro and phophonate groups of 1; they are therefore most likely to be involved in the transition state stabilisation ; their importance is confirmed by labelling experiments, which also indicate the presence of an argininine and an histidine. These crystallographic and biochemical results will be described and their implications for the mechanism of catalysis discussed.

**DS-03.07.05** CONVERSION OF THE 3-D STRUCTURE OF ACETYLCHOLINESTERASE TO BUTYRYLCHOLINE-STERASE: MODELING AND MUTAGENESIS by M. Harel[a]*, I. Silman[b] & J. L.Sussman, [a]Dept. of Structural Biology [b]Dept. of Neurobiology Weizmann Institute of Science, Rehovot, Israel

In vertebrates, two enzymes efficiently catalyze acetylcholine (ACh) hydrolysis: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The principle role of AChE is the termination of impulse transmission at cholinergic synapses. BChE derives its name from the fact that it hydrolyses butyrylcholine (BCh) at rates similar to or faster than ACh, whereas AChE hydrolyses BCh over 100-fold more slowly. AChE and BChE are further distinguished by their differential susceptibility to various inhibitors. There exists a striking sequence homology between AChE and BChE with 53% identity and no deletions or additions. This marked structural similarity encouraged us to use the three-dimensional structure of AChE[1] to model BChE, in order to gain an understanding of how the structural differences between the two enzymes might account for the known differences in specificity between them.
The catalytic triad in AChE is located close to the bottom of area 20A deep marrow cavity, which we named the aromatic gorge, since about 40% of its surface area is lined with the rings of 14 aromatic amino acids. Six aromatic residues in the gorge of AChE are substituted by a non-aromatic residue in BChE. We suggested a plausible model for the docking of ACh, in an all-trans configuration, within the active site of AChE. This model was confirmed by structures of complexes of AChE with several competitive inhibitors[2]. When we model a bound BCh it is clear that the bulkier butyryl moiety of BCh cannot fit into the 'esteratic' locus. In the BChE model, however, the bottom of the gorge is enlarged by the substitution of two aromatic amino acids by smaller residues, i.c. F288L and F290V, permitting the butyryl group to fit into the larger 'esteratic' pocket. Following the predictions, it was possible to convert AChE to an efficient BCh-hydrolysing enzyme by site-directed mutagenesis of only two residues[3].

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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.