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.

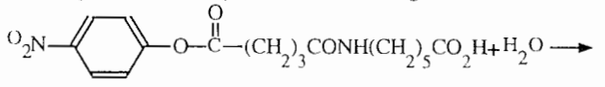
Unexpected features of the conformation of the bound FMP are (i) the ribose and base are in the anti conformation, rather than the syn conformation observed in the crystal structure of FMP, (ii) the furanose ring pucker is C1′-exo, different to that observed for the crystal structures of both FMP and 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.

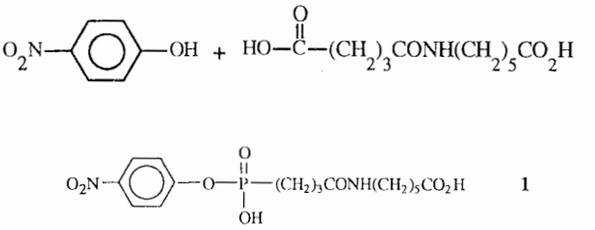
The hydrolysis of the N-glycoside bond by αMMC appears to proceed via a carboninum intermediate. The substrate adenosine is probably bound in a strained conformation (as observed in the bound FMP) thereby weakening the glycoside bond. Electrostatic and ring-ring interactions probably result in a force 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.e. 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].

