



	Experiment title: Structural analysis of a chimeric bacterial α -amylase.	Experiment number: LS1532
Beamline:	Date of experiment: from: 10/12/99 to: 12/12/99	Date of report: 28/08/00
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Report:

α -Amylases (1,4- α -glucan 4-glucanohydrolase; EC 3.2.1.1) are ubiquitous enzymes which catalyse the breakdown of amylose and amylopectin through the hydrolysis of internal α -1,4-glycosidic linkages with net retention of anomeric configuration. Together with many other enzymes that work on α -linked glucopyranose-derived substrates, such as α -glucosidases, cyclodextrin glycosyltransferases (CGTases) and pullulanases, they are found in family 13 of the glycoside hydrolase sequence-classification, often termed the " α -amylase" superfamily [1,2].

A feature common to all reported α -amylase structures is their $(\beta\alpha)_8$ catalytic core-domain, termed domain A. An excursion between barrel strand β -3 and helix α -3 forms the B domain, whilst the C domain, frequently an eight-stranded β -sheet, lies at the C-terminal extremity of the barrel. All family 13 members possess a constellation of three acidic residues, located at the C-terminal face of the $(\beta\alpha)_8$ -barrel, which are implicated in catalysis. α -amylases perform catalysis with net retention of anomeric configuration in a double-displacement mechanism. The mechanism involves the formation, and subsequent breakdown, of a covalent glycosyl-enzyme intermediate *via* oxocarbenium-ion like transition state (for a review see [3]). One of the catalytic acidic residues functions as the catalytic nucleophile whilst another functions as the catalytic acid/base.

α -amylase family members find widespread use in a diverse array of industrial processes ranging from the breakdown of starch to glucose-syrups through to the production of cyclodextrins for the pharmaceutical industry. Many strategies have been used to generate new, more effective, amylases for these processes. One such construct of a chimeric α -amylase, termed BA2, was formed by the fusion of two bacterial α -amylase genes encoding for residues 1-300 from the *B. amyloliquefaciens* α -amylase and 301 to 483 from the *B.*

licheniformis enzyme. The three-dimensional structure of BA2 was solved by multiple isomorphous replacement using data collected at home and at the EMBL outstation in Hamburg. Subsequently, data were collected at the European Synchrotron Radiation Facility (ESRF) beamline ID14-4 at a wavelength of 0.9836 Å using an ADSC QUAD-4 CCD detector. Crystals were mounted in Rayon fibre loops and immediately placed in a stream of boiling N₂ gas at 100 K. Data were collected to a resolution of 1.7 Å with an oscillation range of 0.3° / image. The structure was further refined to this resolution with the addition of hydrogen scattering from “riding” hydrogen atoms as a fixed contribution as justified by a drop in R and R_{free} of 2.1 / 2.0%. The BA2 native model, at 1.7 Å, has a crystallographic R value of 0.14, with a corresponding R_{free} of 0.19 using all observed data between 20 and 1.7Å. This model shows deviations from stereochemical target values of 0.010Å for 1-2 bonds and 0.028Å for the 1-3 bonding distance. There is a single outlier on the Ramachandran plot, Tyr 148, as was also seen in the Ca²⁺-depleted *B. licheniformis* α-amylase. This residue lies in well-defined electron density in a type II β-turn as residue i+2, a position more usually associated with a glycine. The model also contains a single *cis*- peptide in a type IV β-turn between Trp184 and Glu185.

The structure of BA2 amylase contains 483 amino acids with a molecular weight of 55kDa. The structure is made up of three globular domains ordered A, B and C with respect to sequence, which lie in an approximately linear arrangement in the order B,A,C. Residues 1-103 and 206-395 make up domain A, an excursion between barrel-strands β-3 and helix α -3 (residues 104-205) forms domain B, and residues 396-483 complete domain C. This gives rise to an elongated molecule, the longest axis being some 85 Å. The chimeric nature of this enzyme reveals the potential for gene shuffling to generate novel enzymes for industrial applications.

In order to investigate the effect of pH on the unusually close interaction of two of the catalytic acid residues, crystals were grown at various pH values. A number of data sets have been collected, one of which on station ID14-4 to 1.8 Å resolution. The completed series of structures will provide us with an illustration of a pH titration of the active site. Further work on this is in progress.

References

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2. Henrissat, B., & Bairoch, A. (1996) *Biochem. J.* 316, 695 - 696.
3. Davies, G., Sinnott, M. L., & Withers, S. G. (1997) in *Comprehensive Biological Catalysis* (Sinnott, M. L., Ed.) pp 119-209, Academic Press, London.