



	<b>Experiment title:</b> Visualising the hydrolysis of the acyl-enzyme complex formed between $\beta$ -casomorphin-7 and porcine pancreatic elastase	<b>Experiment number:</b> <b>LS-1629</b>
<b>Beamline:</b> ID14, EH3	<b>Date of Experiment:</b> from: 14 June 2000 to: 17 June 2000	<b>Date of Report:</b> 27 February 2001
<b>Shifts:</b> 9	<b>Local contact(s):</b> Dr. Wim Burmeister	<i>Received at ESRF:</i>

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**Report:**

Elastase is a serine protease of the chymotrypsin sub-family. A natural heptapeptide, human  $\beta$ -casomorphin-7, forms a stable acyl-enzyme intermediate with porcine pancreatic elastase (PPE) at approximately pH 5.0. At this pH this acyl-enzyme complex readily crystallises and its X-ray structure [1] revealed the heptapeptide bound in the active site cleft and covalently bound via an ester linkage to Ser-195 of the catalytic triad. Additionally a well-defined water molecule is visible which forms a hydrogen bond with N<sub>ε</sub> of His-57. At this pH hydrolysis does not occur (or is very slow) due to His-57 being protonated. Raising the pH of the enzyme within crystals deprotonates His-57 and initiates the hydrolysis of the ester bond.

This experiment sought to study the process of deacylation during serine protease catalysis. This represented the conclusion of a series of experiments which first characterised (at medium resolution) the X-ray structure of the acyl-intermediate [1]. The results from this experiment completed the structural elucidation of the tetrahedral intermediate which forms during deacylation, and also revealed the structure of the acyl-enzyme complex at 0.94 Å resolution. A manuscript describing the X-ray structure of the tetrahedral intermediate is now at an advanced stage of review [2], and a manuscript describing the atomic resolution study of the acyl-intermediate is in preparation.

The methodology pursued was rather straight-forward. Increasing the pH within the crystal (through immersion into a buffer) allows deacylation to be initiated, since the

catalytic histidine becomes deprotonated at high pH. Following a specific time-delay (typically a few tens of seconds) the crystal was then plunged into liquid nitrogen. Monochromatic X-ray diffraction data was then collected at cryogenic temperature. Due to the high quality of the diffraction data recorded at ID14 subtle changes in electron density at the active site could readily be observed.

The mechanistic highlights of the study relate to a mechanism by which the hydrolysis of the ester linkage couples to the release of the cleaved peptide from its binding pocket. In particular, during the formation of a tetrahedral intermediate the oxyanion hole remains rigid, and hence the position of the peptide must adjust to compensate for the changed bond-lengths and angles at the active site. Interestingly the peptide was observed to disorder during the formation of the tetrahedral intermediate. These results are described in detail in [2].

The mechanistic highlights which arise from the 0.94 Å resolution study of the acyl-intermediate relate to the specific geometry of the ester-linkage. Indeed the somewhat more planar than expected geometry is of interest from a calculational chemistry perspective, as is the visualisation of H-bond donors of the peptide backbone nitrogens which form the oxy-anion hole.

[1] Wilmouth, R. C., Clifton, I. J., Robinson, C. V., Roach, P. L., Aplin, R. T., Westwood, N. J., Hajdu, J., Schofield, C. J., *Structure of a specific acyl-enzyme complex formed between beta-casomorphin-7 and porcine pancreatic elastase*, Nature Structural Biology, 4, 456-462 (1997).

[2] Wilmouth, R. C., Edman, K., Neutze, R., Wright, P. A., Clifton, I. J., Schneider, T. R., Schofield, C. J. & Hajdu, J. *X-ray snapshots of serine protease catalysis reveal a tetrahedral intermediate*, manuscript under review.