ESRF	**Experiment title:** Time-resolved crystallographic studies on the catalytic mechanism of elastase.	**Experiment number:** LS-505
Beamline: BM14	**Date of experiment:** from: 18-Oct-96 7:00 to: 20-Oct-96 7:00	**Date of report:** 27 August 1997
Shifts: 6	**Local contact(s):** Thompson, A.	*Received at ESRF:* 28 AOUT 1997

Names and affiliations of applicants (* indicates experimentalists):

Dr I. J. Clifton*, Mr R. C. Wilmouth*, Miss P. A. Wright*, Dr. C. J. Schofield

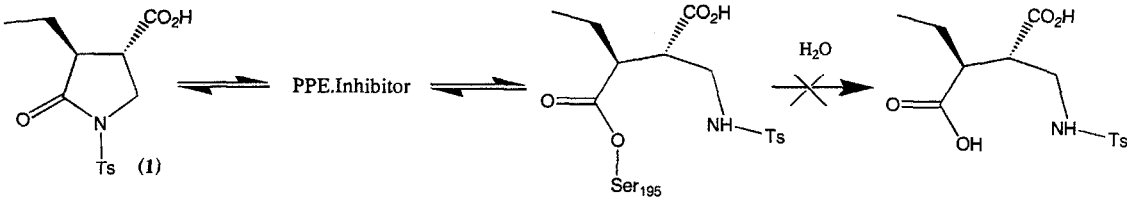
University of Oxford, Dyson Perrins Laboratory, South Parks Road, Oxford, OX1 3QY, UK.

Report:

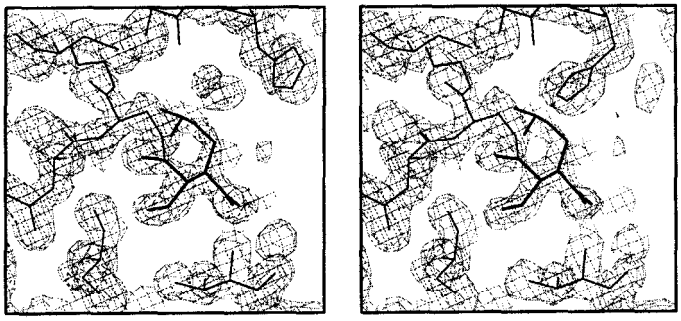
Following screening by mass spectrometry, β-casomorphin-7 (BCM7), YPFVEPI, was found to form a stable O-acyl (ester) enzyme complex with porcine pancreatic elastase (PPE). BCM7 was co-crystallized with PPE and the structure solved (to 1.9 Å) at pH 5 where PPE is inactive (R.C. Wilmouth et *al).* The structure led to the assignment of a specific water molecule (the 'hydrolytic' water, Wat-317) as being in an ideal position to effect hydrolysis of the ester. Subsequently, a second, high resolution, data set has been collected and the structure refined to 1.05 Å resolution (unpublished results, data collected at BM14). PPE has a pH optimum of 8.8, thus, by subjecting the 'ester' crystals to pH jumps and flash-freezing at different time points, we hope to capture intermediates in the reaction pathway thereby constructing a time-resolved structural picture of PPE during catalysis. Whilst inconclusive, the preliminary results are tantalising and merit further investigation.

pH jumps were performed at a series of time periods; pH 9, 1 minute: A small amount of electron density for BCM7 was visible in the active site, but 'extra' density was observed protruding from the 0, atom of Ser-195. The electron density around the position of the carbon atom of the ester carbonyl appeared tetrahedral in nature but the rest of the isoleucine residue was poorly defined. Significantly there was no density in the location of the hydrolytic water. Thus it appears that Wat-317 had reacted and it is possible that the density in the region of the ester linkage represents the putative tetrahedral intermediate. pH 10, 1 minute: The BCM7 peptide had even less electron density associated with it than the pH 9 structure. Slight

density for the isoleucine residue was still observable and some faint 'ghost' electron density was visible in the position of the oxygen atoms of the putative 'tetrahedral intermediate' in the pH 9 structure. pH 10, 2 minutes: No electron density for any part of BCM7 was visible in the active site i.e. the substrate had left. The side chain of Ser-195 had no extended density and closely resembled the resting state serine residue, but Wat-317 had reappeared. Analysis of other electron density in the time resolved studies has led to proposal of a short 2 water molecule channel leading to the hydrolytic position.



After extensive synthesis and kinetic screening, a γ-lactam (1) was shown to form a stable O-acyl enzyme complex with PPE in an analogous manner to BCM7. (1) was soaked into PPE crystals and the structure solved at pH 5. The structure showed the ester carbonyl located in the oxyanion hole in a similar conformation to that observed in the PPE:BCM7 structure, with the ethyl group located in the S_1 subsite. The γ-lactam closely follows a similar line in the active site as BCM7 up to the point of the C_2 methylene group. At this carbon atom, the γ-lactam turns sharply towards His-57 which appears to have been displaced 90° away from the standard native elastase conformation. The position of the γ-lactam nitrogen atom precisely displaced Wat-317. Thus cleavage of the ester linkage is only possible via attack of the tosyl nitrogen atom, leading to ring closure and reformation of the original γ-lactam structure (in accord with unpublished solution studies). Using the pH jump methodology a structure obtained after 1 minute at pH 9 showed a 90° rotation of His-57 to the position seen in native elastase structures. The His-57 $N_{\varepsilon 2}$ was situated 2.5Å from the nitrogen atom of the γ-lactam ring, ***i.e.*** in position to deprotonate the sulphonamide nitrogen. This is an exciting result since it is clearly the best quality time-resolved study on elastase catalysis. Further efforts will be directed towards obtaining further snapshots of the reacting PPE:γ-lactam complex and overcoming the technical difficulties encountered with the PPE:BCM7 complex.



Electron density maps (2FoFc, contoured at σ) for the PPE:γ-lactam structures at pH 5 (left) and pH 9, 1 minute (right). The γ-lactam (1) is shown in bold lines forming a covalent ester link with Ser-195.