ESRF	**Experiment title:** Time-resolved crystallographic studies on the catalytic mechanism of elastase.	**Experiment number:** LS-969
Beamline: BM14	**Date of experiment:** from: 09-July-98 7:00 to: 11 -July-98 7:00	**Date of report:** 1 October 1998
Shifts: 6	**Local contact(s):** Thompson, A.	*Received at ESRF':* 05 OCT. 1998

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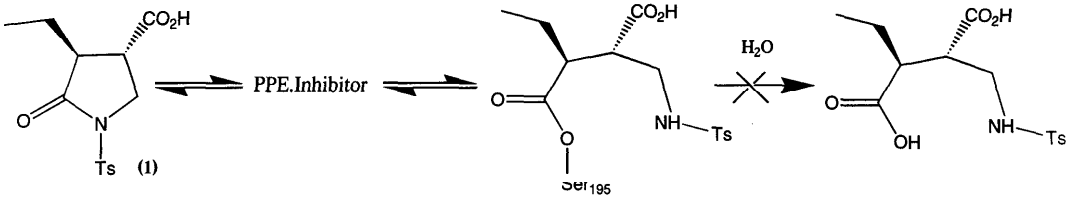
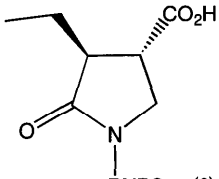
Report:

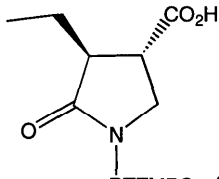


After extensive synthesis and kinetic screening, a γ-lactam (1) was shown to form a stable O-acyl enzyme complex with porcine pancreatic elastase (PPE). (1) was soaked into PPE crystals and the structure solved at pH 5. The structure showed the ester carbonyl located in the oxyanion hole in a manner analogous to the previously solved structure of PPE:BCM7[Wilmouth et al *Nature Structure Biology* 1997, 4, 456-462], with the ethyl group located in the S_1 subsite. The γ-lactam occupied a very similar position in the active site as that of BCM7 in the PPE:BCM7 complex up to the point of the C_2 methylene group. At this carbon atom, the γ-lactam 'turned sharply' towards His-57 which appeared to have been displaced 90" away from the standard native elastase conformation. The position of the γ-lactam nitrogen atom precisely displaced the putative hydrolytic water identified in the PPE:BCM7 structure. Thus cleavage of the ester linkage was 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of the tosyl nitrogen atom, leading to ring closure and reformation of the original γ-lactam structure (in accord with unpublished solution studies). 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 hoped to capture intermediates in the reaction pathway thereby constructing a time-resolved structural picture of PPE during catalysis.

pH jumps were performed at a series of time periods; pH 9, 1 minute: Weak electron density for His-57 was present in both the 90" displaced and native elastase position suggesting movement between the 2 positions. The His-57 $N_{\varepsilon 2}$ lying in the native elastase position was situated 2.5Å from the nitrogen atom of the γ-lactam ring, *i.e.* in position to deprotonate the sulphonamide nitrogen. pH 9, 2 minutes: Electron density for His-57 was seen only in the native elastase position, confirming the initial movement seen at pH 9, 1 minute. The distance between the His-57 $N_{\varepsilon 2}$ and the nitrogen atom of the γ-lactam was again 2.5Å, suggesting the presence of a low barrier hydrogen bond. pH 10: The crystals were unable to survive these pH jumps possibly due to major conformational changes at this pH.



PNBS (2)



PTFMBS (3)

During subsequent screens, two new γ-lactams, (2) and (3), were identified as being inhibitors of PPE. Crystallisation of these compounds was undertaken to confirm first the results for (1) and second to try to identify the position of the tosyl ring. At pH 5 (2) was shown to form a stable O-acyl enzyme complex with PPE in an analogous manner to (1). Excitingly the tosyl ring was visible, lying between His-57 and Arg-61. pH jumps on (2) were attempted at pH 9, 1 and 2 minutes. The structures confirmed the movement of His-57 observed with (l), however, the tosyl ring appeared to become more disordered as the pH was raised. The crystals were unable to survive at pH 10. (3) was also seen to form a stable O-acyl enzyme complex with PPE in an analogous manner to (1). A structure of (3) at pH 9, 3 minutes showed the His-57 displaced 90° from the native elastase position. (3) was a stronger inhibitor of PPE than (1) and it is possible that longer time periods are required to initiate cleavage of the acyl-enzyme intermediate. A pH 10 structure would be of considerable interest in an attempt to visualise the intermediates involved in the mechanism of serine proteases.