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β-Sultams—A novel class of serine protease inhibitors

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_N_-Benzoyl β-sultam is an irreversible inactivator of elastase by sulfonation of the active site serine.

Human neutrophil elastase (HNE) is a serine enzyme which is one of the most destructive proteolytic enzymes, being able to catalyse the hydrolysis of the components of connective tissue. It has been implicated in the development of diseases such as emphysema, cystic fibrosis and rheumatoid arthritis and there have been numerous studies attempting to find small molecule inhibitors of HNE.[1] The structure of HNE has been determined by X-ray crystallography,[2] but most structural and inhibition studies have been conducted with the related, but more readily available, porcine pancreatic elastase (PPE).[3]

The majority of elastase inhibitors are based on a similarity to other serine protease inhibitors, such as peptidyl fluoroketones[4] or ketones attached to a strongly electron withdrawing group.[5] An alternative strategy has been to use acylating agents as inhibitors that generate an acyl enzyme which does not turnover. For example, bicyclic [3.3.0] systems containing a γ-lactone or γ-lactam which is *trans*-fused to the other 5-membered ring inhibit HNE by acylating the nucleophilic hydroxy group of serine-195 in the active site of the enzyme.[6]

Interestingly, the classical β-lactams, traditionally used as anti-bacterial agents by inhibiting serine transpeptidases,[7] have also been shown to be mechanism based inhibitors of elastase when used as neutral derivatives.[8] ESI-MS and NMR studies have shown that the first step is an acylation process in which the four membered β-lactam ring is opened.[9] The requirements for inhibition of elastase by monocyclic lactams have been recently described.[10]

In principle, sulfonation of serine proteases offers an interesting but largely unexplored strategy for inhibition as an alternative to the traditional mechanism-based acylation process. In addition to their normal acyl substrates, serine proteases are known to react with other electrophilic centres such as phosphonyl derivatives.[11] The main reason why sulfonation of serine enzymes is not a well studied process is because sulfonyl derivatives are much less reactive than their acyl counterparts.[12] For example, sulfonamides are extremely resisitant to alkaline and acidic hydrolysis and, in general, sulfonyl transfer reactions are 10^2 to 10^4 fold slower than the corresponding acyl transfer process. However, we have recently shown that the rates of alkaline and acid hydrolysis of *N*-alkyl and *N*-aryl β-sultams are 10^2 to 10^3 fold greater than those for the corresponding β-lactams.[13] β-Sultams show extraordinary rate enhancements of 10^9 and 10^7, respectively, compared with the acid and base catalysed hydrolysis of the corresponding acyclic sulfonamides.[14] Therefore β-sultams are excellent candidates to explore the mechanism of sulfonation and possible inhibition of serine protease enzymes. Ring opening of the β-sultam would give the sulfonate ester, by analogy to the acyl enzyme intermediate formed during the hydrolysis of normal substrates. Either formation of the sulfonated enzyme could itself lead to inactivation because of its resistance to hydrolysis or the sulfonate ester could undergo a further reaction giving rise to a loss of enzyme activity.

1

The unsubstituted β-sultam, 1,2-thiazetidine 1,2-dioxide,[13] was reacted with benzoyl chloride in the presence of triethylamine with a catalytic amount of DMAP to give *N*-benzoyl β-sultam, **1**, which was found to be a time dependent inactivator of PPE. The activity of PPE was measured by the rate of the enzyme catalysed hydrolysis of the chromophoric substrate *N*-succinyl-L-$(Ala)_3$-*p*-nitroanilide (6.0×10^{-5} M) at 390 nm at 30 °C in 0.1 M TAPS buffer, pH 8.5 and I = 1.0 M (KCl). The substrate stock solution was dissolved in MeOH but because it was found that MeOH decreased enzyme activity (although not as much as the commonly used solvent DMSO) all assays were conducted at a constant MeOH concentration of 1.5% v/v. The enzyme (8.0×10^{-5} M) and the β-sultam, at a variable concentration of up to 5×10^{-3} M, were incubated together at 30 °C, 0.04 M buffer 20% v/v acetonitrile. Aliquots of this solution were assayed for PPE activity at various time intervals by injection into pH 8.5 TAPS buffer solution with final assay conditions of 6.0×10^{-5} M substrate, 1.5×10^{-6} M PPE, 6% v/v acetonitrile and 1.5% MeOH v/v. In all cases, control incubations were performed at the same time and under identical conditions, except for the omission of the β-sultam. Initial rates were determined, using $\Delta\varepsilon$ = 12 400 M^{-1} cm^{-1}, to give a measure of enzyme activity and taking the control as 100% activity. The results show exponential rates of inactivation which give first order rate constants, $k_{obs,}$ dependent on the β-sultam concentration. The corresponding second order rate constants for inactivation, k_i, were obtained from the slopes of plots of k_{obs} against β-sultam concentration. At pH 7.0, this was 4 M^{-1} s^{-1} but the rate constant was found to vary with pH. Where necessary the inactivator concentration was normalised due to the competing hydrolysis of the β-sultam. A plot of the dependence of k_i on pH indicates a similar behaviour to that for the hydrolysis of the substrate (Fig. 1). It appears that the inactivation of PPE by the β-sultam uses the same catalytic machinery as hydrolysis at the active site.

The enzyme was completely deactivated by **1** and, after inactivation, the enzyme showed no sign of recovery after 4 d at 30 °C. ESI-MS analysis indicated that the β-sultam (theoretical M_r = 211.24 Da) reacted with PPE (25904, 25943 (potassium adduct) Da) to give both mono- (26115, 26153 Da) and di- (26326, 26364 Da) sulfonated adducts. Furthermore, sulfonation was confirmed by X-ray crystallography of crystals obtained by soaking native PPE crystals for 24 h at pH 5 in a saturated solution of the β-sultam inhibitor (**1**). The soaked crystals were isomorphous to native PPE and there was no significant change in unit cell parameters. The resultant 1.67 Å resolution structure (Fig. 2) shows the β-sultam ring has been opened and there is well-defined electron density for a sulfonate ester between the inhibitor and Ser-195. Although the atoms originating from the β-sultam ring are clearly defined, no density is visible for the benzoyl group, probably due to

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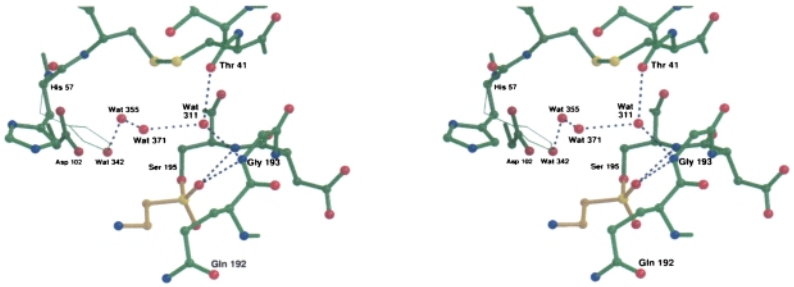

Fig. 2 Stereo views of the active site of PPE (in green) showing the *N*-benzoyl β-sultam (**1**) (in beige) covalently linked *via* a sulfonate ester to Ser-195 and the 'native' position of His-57 in thin lines.

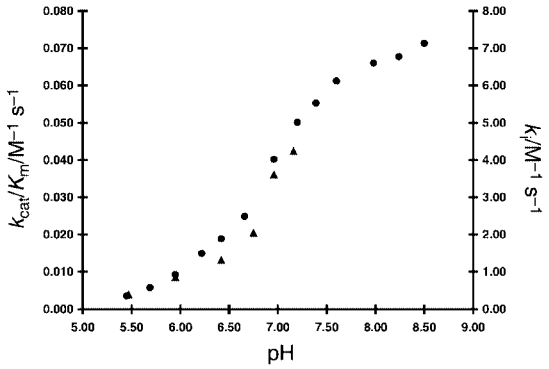

Fig. 1 The pH dependence of the rate constants k_{cat}/K_m, for the hydrolysis of *N*-succinyl-L-(ala)$_3$-*p*-nitroanilide by PPE (● Left Hand scale), and k_i, for the inactivation of PPE by *N*-benzoyl β-sultam (▲ Right Hand scale).

disorder. An alternative possibility is that the amide bond has been hydrolysed either before or after ring-opening by Ser-195, but there was no evidence for this in the ESI-MS analysis.

One oxygen atom of the sulfonate is located in the oxyanion hole within hydrogen bonding distance of the amido-nitrogen atoms of Ser-195 (3.02 Å) and Gly-193 (3.23 Å). The other sulfonate oxygen atom is located in the upper part of the S_1 pocket. It is worth noting that the native enzyme crystallises with a sulfate ion in the oxyanion hole. The other atoms of the ring-opened β-sultam are located in the P_1/P_2 region. The side chain of His-57 has been displaced by approximately 90° from its normal location and to a position similar to that observed in the structure of a γ-lactam inhibitor bound to PPE.[15] The electron density map is consistent with the presence of two water molecules (Wat-342 and Wat-355) occupying the 'native' position of His-57.[16] Another water molecule in the vicinity (Wat-371) is located in a similar position to the hydrolytic water observed in the structure of an acyl-enzyme intermediate between PPE and a peptide inhibitor.[17]

The electron density map indicates that the side chain of His-57 clearly has extra density extending from its $N_{\varepsilon 2}$ atom. This may reflect the sulfonation of His-57 by another molecule of the β-sultam inhibitor consistent with the ESI-MS results. However the 'extra' electron density is not sufficiently well-defined for any atoms to be built in. We are improving the selectivity of inactivation by synthesising β-sultams with suitably placed substituents.

It is generally accepted that nucleophilic substitution at acyl centres proceeds through the formation of an unstable tetrahedral intermediate (TI). Furthermore, it is assumed that there is some preferential direction of nucleophilic attack such that the incoming nucleophile approaches at approximately the tetrahedral angle to the carbonyl group. By contrast, the mechanism for sulfonyl group transfer often involves a pentacoordinate intermediate or transition state with trigonal bipyramidal geometry.[13] It is often assumed, but with little actual supporting evidence, that enzymes catalyse reactions by an exquisite positioning of the catalytic groups. If this were the case then it is doubtful if an enzyme with a primary function, say, as a catalyst for acyl transfer could be an effective catalyst for sulfonyl transfer because of these geometrical differences. However, we have shown, for example, that β-lactamase is almost as efficient at catalysing P–N fission in phosphonamidates as it is at increasing the rate of C–N cleavage in β-lactams.[18] It appears also that similar catalytic machinery is used by elastase for both hydrolysis of peptides and for its inactivation by β-sultams.

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Notes and references

1 P. R. Bernstein, P. E. Edwards and J. C. Williams, *Prog. Med. Chem.*, 1994, **31**, 59.
2 W. Bode, A. Z. Wei, R. Huber, E. Meyer, J. Travis and S. Neuman, *EMBO J*, 1986, **5**, 2453; M. A. Navia, B. M. McKeever, J. P. Springer, T. Y. Lin, H. R. Williams, E. M. Fluder, C. P. Dorn and K. Hoogsteen, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 7.
3 E. Meyer, G. Cole, R. Radhakrishnan and O. Epp, *Acta Crystallogr., Sect. B*, 1988, **44**, 26.
4 L. H. Takahashi, R. Radhakrishan, R. E. Rosenfeld, Jr., E. F. Meyer, Jr. and D. A. Trainer, *J. Am. Chem. Soc.*, 1989, **111**, 3368; A. Renaud, P. Lestienne, D. L. Hughes, J. G. Bieth and J.-L. Dimicoli, *J. Biol. Chem.*, 1983, **258**, 8312.
5 M. J. Costanzo, B. E. Maryanoff, L. R. Hecker, M. R. Schott, S. C. Yabut, H.-C. Zhang, P. Andrade-Gordon, J. A. Kauffman, J. M. Lewis, R. Krishnan and A. Tulinsky, *J. Med. Chem.*, 1996, **39**, 3039; P. D. Edwards, D. W. Andisik, A. M. Strimpler, B. Gomes and P. A. Tuthill, *J. Med. Chem.*, 1996, **39**, 1112.
6 S. J. F. McDonald, G. D. E. Clarke, M. D. Dowle, L. A. Harrison, S. T. Hodgson, G. G. A. Inglis, M. R. Johnson, P. Shah, R. J. Upton and S. B. Walls, *J. Org. Chem.*, 1999, **64**, 5166.
7 M. I. Page, ed. *The Chemistry of β-Lactam Antibiotics*, Blackie, Glasgow, 1992.
8 R. Chabin, B. G. Green, P. Gale, A. L. Maycock, H. Weston, C. P. Dorn, P. E. Finke, W. K. Hagmann, J. J. Hale, M. MacCross, S. K. Shah, D. J. Underwood, J. B. Doherty and W. B. Knight, *Biochemistry*, 1993, **32**, 8970.
9 D. J. Underwood, B. G. Green, R. Chabin, S. Mills, J. B. Doherty, P. E. Finke, M. MacCoss, S. K. Shah, C. S. Burgey, T. A. Dickinson, P. R. Griffin, T. E. Lee, K. M. Swiderek, T. Covey, W. M. Westler and W. B. Knight, *Biochemistry*, 1995, **34**, 143 344.
10 R. C. Wilmouth, S. Kassamally, N. J. Westwood, R. J. Sheppard, T. D. W. Claridge, R. T. Aplin, P. A. Wright, G. J. Pritchard and C. J. Schofield, *Biochemistry*, 1999, **38**, 7989.
11 M. I. Page, in *Comprehensive Medicinal Chemistry*, vol. 2, ed. P G Sammes, Pergamon, Oxford, 1990, pp 61–87.
12 J. F. King, R. Rathore, J. Y. L. Lam, L. E. R. Gao and D. F. Klassen, *J. Am. Chem. Soc.*, 1992, **114**, 3028.
13 N. J. Baxter, A. P. Laws, L. J. M. Rigoreau and M. I. Page, *J. Am. Chem. Soc.*, 2000, **112**, 3375.
14 J. Baxter, A. P. Laws, L. J. M. Rigoreau and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 1996, 2245.
15 R. C. Wilmouth, S. Kassamally, N. J. Westwood, R. J. Sheppard, T. D. W. Claridge, R. T. Aplin, P. A. Wright, G. J. Pritchard and C. J. Schofield, *Biochemistry*, 1999, **38**, 7989.
16 E. Meyer, G. Cole, R. Radhakrishnan and O. Epp, *Acta Cryst.*, 1988, **B44**, 26.
17 R. C. Wilmouth, I. J. Clifton, C. V. Robinson, P. L. Roach, R. T. Aplin, N. J. Westwood, J. Hajdu and C. J. Schofield, *Nat. Struct. Biol.*, 1997, **4**, 456.
18 M. I. Page and A. P. Laws, *Chem. Commun.*, 1998, 1609; M. I. Page, *Curr. Pharm. Des.*, 1999, **5**, 895; M. J. Slater, A. P. Laws and M. I. Page, *Bioorg. Chem.*, in press.