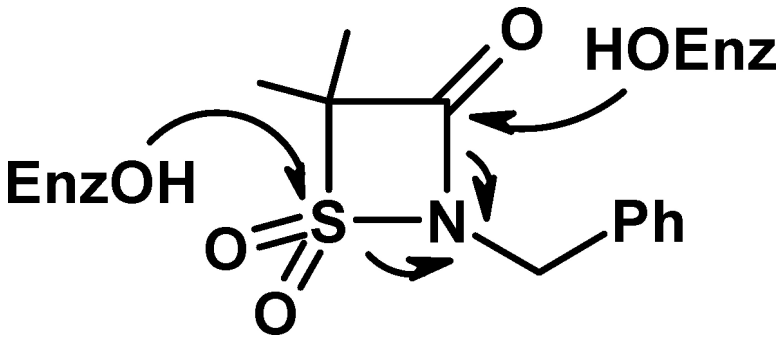
Communication

Acylation versus Sulfonylation in the Inhibition of Elastase by 3-Oxo-β-Sultams

Wing Y. Tsang, Naveed Ahmed, Lindsay P. Harding, Karl Hemming, Andrew P. Laws, and Michael I. Page

J. Am. Chem. Soc., **2005**, 127 (25), 8946-8947• DOI: 10.1021/ja050787z • Publication Date (Web): 01 June 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 9 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 06/01/2005

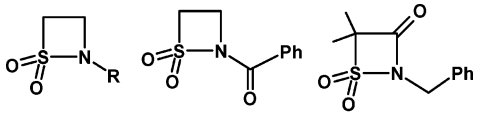
Acylation versus Sulfonylation in the Inhibition of Elastase by 3-Oxo-β-Sultams

Wing Y. Tsang, Naveed Ahmed, Lindsay P. Harding, Karl Hemming, Andrew P. Laws, and Michael I. Page*

Department of Chemical and Biological Sciences, The University of Huddersfield, Huddersfield, HD1 3DH, U.K.

Received February 7, 2005; E-mail: m.i.page@hud.ac.uk

We have previously shown that β-sultams **1**, the sulfonyl analogues of β-lactams, are 10^2- to 10^3-fold more reactive than β-lactams in their rates of hydrolysis, compared with the 10^4-fold *slower* rate of hydrolysis of acyclic sulfonamides compared with analogous amides.[1] β-Sultams are, therefore, unusual in undergoing sulfonyl transfer faster than the corresponding acylation reaction. We have also shown, by X-ray crystallography, that *N*-benzoyl β-sultam **2** inhibits the enzyme elastase by sulfonylation of the active site serine to give a sulfonate ester (Scheme 1).[2] We report here that the 3-oxo-β-sultam **3** is unusual in that it inhibits elastase by acylation resulting from substitution at the carbonyl center, C−N fission, and expulsion of the sulfonamide (Scheme 2).

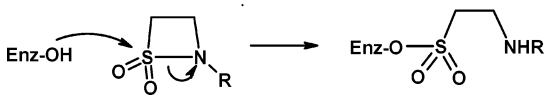


1 2 3

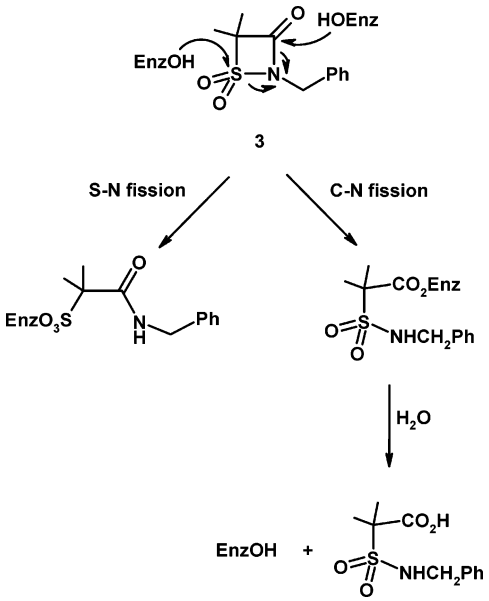
The inhibition of proteolytic enzymes is a common goal for designing new therapeutic agents. This is often achieved by using mechanism-based or active site directed inhibitors[3] and generally involves the covalent modification of an active site residue which may be slowly regenerated. Human neutrophil elastase (HNE) is a serine enzyme which is one of the most destructive proteolytic enzymes, being able to catalyze 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 reports of inhibitors of HNE.[4] Many structural and inhibition studies have been conducted with the related, but more readily available, porcine pancreatic elastase (PPE).[5] The majority of elastase inhibitors are based on other serine protease inhibitors and often act by acylating 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] Sulfonylation of serine proteases offers an interesting but largely unexplored strategy for inhibition as an alternative to the mechanism-based acylation process. The main reason why sulfonylation is not a well studied process is because sulfonyl derivatives are normally much less reactive than their acyl counterparts.[9,10] Although *N*-acylsulfonamides are often used to inactivate serine enzymes,[11] their mechanism of inactivation involves acylation and C−N bond fission, with the serine hydroxyl group attacking the amide to displace the sulfonamide as the leaving group.[12]

The incorporation of an acyl group next to the nitrogen in β-sultams may be exo- or endocyclic, giving *N*-acyl β-sultams **2** or 3-oxo-β-sultams **3**, respectively. Nucleophilic attack on *N*-acyl β-sultams **2** involves ring-opening, arising from sulfonylation.[1,10] The mechanism of inactivation of elastase by *N*-acyl β-sultams **2** also occurs by sulfonylation of the active site serine (Scheme 1).[2] Nucleophilic attack on 3-oxo-β-sultams **3** could involve either acylation or sulfonylation resulting from substitution at the carbonyl center and expulsion of the sulfonamide or from substitution at the sulfonyl center and expulsion of the amide, respectively (Scheme 2). Hydrolysis of 3-oxo-β-sultams occurs by S−N fission,[13] but herein, we report that the inhibition of elastase occurs by C−N fission and acylation of the active site serine.

Scheme 1



Scheme 2



The activity of porcine pancreatic elastase (PPE) was monitored by measuring the rate of the hydrolysis of *N*-succinyl-(L-ala)$_3$-*p*-nitroanilide as substrate at 390 nm using a molar extinction coefficient change, $\Delta\epsilon_{390}$, of 1.24×10^4 M^{-1} cm^{-1}. The kinetic analysis was undertaken with the anilide substrate (0.08 mM) in 0.1 M pH 8.5 TAPS buffer at 30 °C in 1.5% methanol v/v and I = 1.0 M (KCl) in the assay cells. Inhibition was studied by incubating the *N*-benzyl-4,4-dimethyl-3-oxo-β-sultam **3** with PPE together in a buffered solution (the incubation cell). Aliquots of the incubation solution containing enzyme and potential inhibitor were then removed from the incubation cell after an incubation time, t_i, and assayed for PPE activity against the anilide substrate in separate assay cells. These assays showed a time-dependent decrease in enzyme activity, given by the slope of absorbance at 390 nm (A_{390}) against time plots, and tended toward zero with increasing t_i. If the

10.1021/ja050787z CCC: $30.25

enzyme was incubated alone under exactly the same conditions but in the absence of β-sultam, the observed assay slope remained constant throughout the experimental time frame. By taking the slope of these control experiments as being equal to 100% activity, the percentage of the activity of PPE remaining in the inhibitor experiments could be calculated and plotted against t_i. This gave apparent first-order rate constants for inactivation, which were first order with respect to inhibitor concentration. The concentration of the enzyme is much lower than that of the inhibitor at time zero, and therefore, the rate of inactivation is effectively pseudo-first order. Second-order rate constants for inactivation, k_i, were obtained graphically from slopes of the plots of the observed rate of inactivation against several inhibitor concentrations.

At pH 6.0, the second-order rate constant, k_i, for the inactivation of PPE by *N*-benzyl-4,4-dimethyl-3-oxo-β-sultam **3** is 768 M^{-1} s^{-1}. For comparison, *N*-benzoyl β-sultam **2**, which is also a time-dependent inhibitor of the enzyme, shows a second-order rate constant for inactivation of 0.85 M^{-1} s^{-1} at pH 6.0.[2] The rate difference of nearly 10^3 is too great to be attributed to favorable binding interactions in the S_1 pocket of elastase. For example, the second-order rate constant, k_i, for the inactivation of PPE by sulfonylation with *N*-benzoyl-4,4-dimethyl-β-sultam is similar to that for *N*-benzoyl β-sultam **2**.

Electrospray ionization-mass spectrometry (ESI-MS) of solutions of PPE incubated with *N*-benzyl-4,4-dimethyl-3-oxo-β-sultam **3** in a 1:1 ratio shows the enzyme bound to 1 equiv of β-sultam (M_{EI} = 26 133 $\pm$ 1 Da) (98%). The native enzyme has a mass of 25 892, and therefore, a mass difference of +241 is consistent with the formation of a covalent bond between the enzyme and β-sultam **3**. The enzyme-bound hydrolysis product would give a mass of 26 148. Inactivation of PPE by **3** could be due either to attack of the active site serine residue, Ser-64, on the sulfonyl group, leading to S−N fission and sulfonylation to form an inactive sulfonyl enzyme, or to attack on the β-lactam, giving C−N fission and acylation to form an acyl enzyme (Scheme 2). Enzyme inactivation with *N*-benzoyl β-sultam **2** is irreversible with no return of enzyme activity over 4 weeks.[2] By contrast, the enzyme inactivated by *N*-benzyl-4,4-dimethyl-3-oxo-β-sultam **3** slowly regains full activity over a period of days, which at pH 8.5, occurs with a rate constant of 7.35×10^{-6} s^{-1}. Furthermore, the rate of recovery of enzyme activity shows the same dependence on pH as that for the rate of hydrolysis (k_{cat}/K_m) of the anilide substrate.[2] Both reactions are critically dependent on the ionization of a group with a pK_a of 6.9. The dependence of k_{cat}/K_m for hydrolysis of a substrate on pH reflects the acylation process, whereas the rate constant for recovery of activity is deacylation. The much faster rate of enzyme inactivation by the 3-oxo-β-sultam **3** compared with that of *N*-benzoyl β-sultam **2** and the recovery of enzyme activity suggests different mechanisms for the two processes. It thus appears that inactivation of elastase by the 3-oxo-β-sultam **3** is occurring by serine attack on the β-lactam center to form an acyl enzyme of intermediate stability, which is then slowly hydrolyzed in the conventional manner (Scheme 2).

Interestingly, the alkaline hydrolysis of *N*-benzyl-4,4-dimethyl-3-oxo-β-sultam **3** occurs exclusively by S−N fission as a result of attack on sulfur and displacement of the carboxamide with a second-order rate constant, k_{OH}, of 1.83×10^5 M^{-1} s^{-1}, which is, surprisingly, only 10-fold greater than that of the *N*-acyl β-sultam **2** with an exocyclic group.[13] Attack at either electrophilic center of **3** involves opening the four-membered ring. Preferential attack by hydroxide-ion on the sulfonyl group of 3 is consistent with the 10^3-fold greater reactivity of β-sultams compared with that of β-lactams toward alkaline hydrolysis.[10] Attack at the acyl center expels a better leaving group (the sulfonamide anion) than attack at the sulfonyl center to expel the amide anion. However, based on the similar reactivities of imides and *N*-acylsulfonamides toward alkaline hydrolysis, the nature of the leaving group does not have a large effect.[14] With elastase, there may be some molecular recognition of the β-lactam to facilitate attack at the carbonyl center. It is known that the α-chymotrypsin family of enzymes has a well-established binding pocket adjacent to the active serine residue in the S_1 position. In the elastase enzymes, this binding pocket is relatively small and has a preference for small hydrophobic substituents. The inhibition of PPE by monocyclic β-lactams has also been improved by the introduction of alkyl substituents at the 3-position, which are thought to bind strongly in the S_1 subsite of elastase.[15,16]

Interestingly, *N*-benzyl-4,4-dimethyl-3-oxo-β-sultam **3** also inactivates the serine enzyme β-lactamase from *Enterobacter cloacae* but at a rate that is 10-fold *less* than that with *N*-benzoyl β-sultam **2**. This is in contrast to the greater rate difference of nearly 10^3 seen with elastase. Furthermore, there is no recovery of enzyme activity with β-lactamase, and the indications are that inactivation of β-lactamase by the 3-oxo-β-sultam **3** is due to sulfonylation through S−N fission.

In summary, β-sultams are a novel class of inactivators of elastase which can act by either sulfonylation or acylation of the active site serine residue.

Acknowledgment. We thank the University of Huddersfield for support (N.A., W.Y.T., and L.P.H.).

Supporting Information Available: Complete refs 6 and 11. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Baxter, N. J.; Rigoreau, L. J. M.; Laws, A. P.; Page, M. I. *J. Am. Chem. Soc.* **2000**, *112*, 3375−3385; *J. Chem. Soc., Perkin Trans. 2* **1996**, 2245−2246.
(2) Hinchliffe, P. S.; Wood, J. M.; Davis, A. M.; Austin, R. P.; Beckett, R. P.; Page, M. I. *Org. Biomol. Chem.* **2003**, *1*, 67−80.
(3) Page, M. I. Enzyme Inhibition. In *Comprehensive Medicinal Chemistry*; Sammes, P. G., Ed.; Pergamon: Oxford, 1990; Vol. 2, pp 61−87.
(4) Metz, W. A.; Peet, N. P. *Prog. Inflammation Res.* **1999**, *9*, 853−868.
(5) Bode, W.; Meyer, E., Jr.; Powers, J. C. *Biochemistry* **1989**, *28*, 1951−1963.
(6) Macdonald, S. J. F. et al. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 895−898.
(7) Page M. I., Ed. *The Chemistry of β-Lactams*; Blackie: Glasgow, 1992.
(8) (a) Chabin, R.; Green, B. G.; Gale, P.; Maycock, A. L.; Weston, H.; Dorn, C. P.; Finke, P. E.; Hagmann, W. K.; Hale, J. J.; MacCoss, M.; Shah, S. K.; Underwood, D.; Doherty, J. B.; Knight, W. B. *Biochemistry* **1993**, *32*, 8970−8980. (b) Beauve, C.; Bouchet, M.; Touillaux, R.; Fastrez, J.; Marchand-Brynaert, J. *Tetrahedron* **1999**, *55*, 13301−13320.
(9) Wood, J. M.; Page, M. I. *Trends Heterocycl. Chem.* **2002**, *8*, 19−34.
(10) Page, M. I. *Acc. Chem. Res.* **2004**, *37*, 297−303.
(11) Macdonald, S. J. F. et al. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 243−246.
(12) Sykes, N. O.; Macdonald, S. J. F.; Page, M. I. *J. Med. Chem.* **2002**, *45*, 2850−2856.
(13) Ahmed, N.; Tsang, W. Y.; Page, M. I. *Org. Lett.* **2004**, *6*, 201−203.
(14) (a) Eriksson, S. O.; Jakobsson M. *Acta Pharm. Suec.* **1973**, *10*, 63−74. (b) Matsui, S.; Aida H. *J. Chem. Soc., Perkin Trans. 2* **1978**, 1277−1280.
(15) Wilmouth, R. C.; Westwood, N. J.; Anderson, K.; Brownlee, W.; Claridge, T. D. W.; Clifton, I. J.; Pritchard, G. J.; Aplin, R. T.; Schofield, C. J. *Biochemistry* **1998**, *37*, 17506−17513.
(16) Firestone, R. A.; Barker, P. L.; Pisano, J. M.; Ashe, B. M.; Dahlgren, M. E. *Tetrahedron* **1990**, *46*, 2255−2262.

JA050787Z