On the Chemical Binding of 6β-Bromopenicillanic Acid to β-Lactamase I

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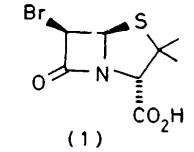
Summary 6β-Bromopenicillanic acid (**1**) binds to serine-44 of the β-lactamase I from *B. cereus* as a dihydrothiazine derivative, which undergoes further chemical changes during cleavage of the enzyme.

WE have recently reported on the rapid, irreversible inhibition of β-lactamase I from *B. cereus* by interaction with 6β-bromopenicillanic acid.[1] Subsequent tryptic and acidic digests of the bound enzyme have shown that the penicillanic acid interacts with serine-44 of the enzyme.[2] Herein we report on the chemical transformations that appear to occur during the inhibition of the enzyme and on subsequent chemical changes which are observed during the degradation of the peptide chain.

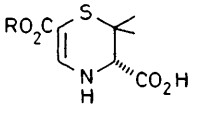
6β-Bromopenicillanic acid (**1**) is a relatively reactive substance and may be prepared either as a mixture with the (enzymically) inactive 6α-bromo-isomer, by equilibration of the latter at pH 9·2 for 3—4 days or by selective reduction of 6,6-dibromopenicillanic acid with tributyltin hydride.[1,2]

On reaction between the enzyme and the 6β-bromopenicillanic acid a new absorbance forms at λ_{max} 326 nm. This λ_{max} of the bound enzyme shifts (reversibly) to 314 nm when the protein is treated with 3M guanidinium chloride, a change consistent with the unfolding of the native enzyme under these conditions. After tryptic digestion the bound fragment corresponds to the T7 peptide unit.[3] The first step in the purification of the T7 fragment was paper chromatography with the solvent, butan-1-ol–acetic acid–

water–pyridine (15:3:12:10). On subsequent electrophoretic purification at pH 4 and 2 the peptide behaved as a monoacidic base, whereas on electrophoretic separation immediately after the tryptic digestion it behaved as a neutral component (zero net charge at pH 6·5), an observation indicating a structural change during the former isolation procedure. The parent tryptic peptide T7 was basic. It appears, therefore, that the initial, bound T7 peptide has gained a carboxy group and that, subsequently this is lost (or that a new basic centre is formed). The chemical change in the bound T7 fragment was also effected by leaving it in solution at pH 6 for several hours. The structure of the modified T7 peptide (Phe-Ala-Phe-Ala-Ser′-Thr-Tyr-Lys) suggests the latter explanation if one assumes that the bound penicillanic acid residue on the serine unit (Ser′) also initially donates one extra carboxylic group to this fragment and that this extra carboxy group is lost during the chromatography step.

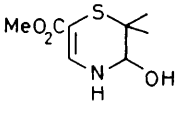


(1)

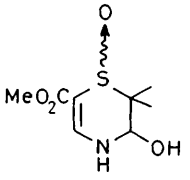


(2) R = Me

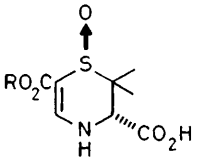
(6) R = enzyme



(3)



(4)



(5) R = Me

(7) R = enzyme

(8) R = H

That this explanation is correct is corroborated by circumstantial evidence gained by examination of the behaviour of 6β-bromopenicillanic acid with methanol. The reaction of the acid (**1**) with 2 equiv. of sodium methoxide in methanol affords the known dihydrothiazine ester (**2**),[4] λ_{max} 314 nm, identical to that observed for the bound β-lactamase under denatured conditions. Whilst this species is stable at pH 7, at lower pH, as in 1% aq. AcOH, a new product forms, identified as the alcohol (**3**),† λ_{max} 303 nm. This change appears to be the result of an autoxidation reaction since it sometimes showed an induction period and it did not occur under strictly anaerobic, peroxide-free conditions. Moreover, paper chromatography in the solvent given converted the acid (**2**) into the neutral species (**3**), λ_{max} 303 nm. Thus the treatment that altered the charge in the substituted peptide T7 affected the model compound similarly. Further oxidation of the alcohol (**3**), to the isomeric sulphoxides (**4**), λ_{max} 275 nm, can also be observed by prolonged autoxidation at pH 4.

Conversion of the acid (**2**) into the sulphoxides,[5] the isomer (**5**) predominating, λ_{max} 275 nm, could also be effected by use of sodium periodate. Compared to the sulphide (**2**) the sulphoxide (**5**) is relatively stable to dilute acid. Oxidation of the intact bound enzyme with sodium periodate also shifts the absorbance at 314 nm to a new position at λ_{max} 275 nm (denatured conditions). The agreement in absorption characteristics of the bound enzyme fragments with those of the model series strongly supports assignment of the former as the dihydrothiazine derivatives (**6**) and (**7**), respectively.

Further evidence for this assignment was obtained by hydrolysis of the dihydrothiazine ester sulphoxide (**5**) with 1N aqueous sodium hydroxide at 40 °C for 4 h to give the dicarboxylic acid (**8**), λ_{max} 277 nm. Storage of the bound enzyme (**7**) under neutral or mildly alkaline conditions also afforded the identical acid.

After reaction with 6β-bromopenicillanic acid the enzyme must therefore be inactivated by the presence of the dihydrothiazine residue on serine-44, as in (**6**). The reasons why the dihydrothiazine ester of the β-lactamase is stable to release, compared to the esters derived from normal substrates of the 6β-acylamidopenicillanic type, are under investigation. It should be noted that the dihydrothiazine unit can be compared with the vinylogous urethane system assigned to inactivated complexes arising from clavulanic acid and the *E. coli* RTEM β-lactamase.[6] Since vinylogous urethanes are relatively resistant to base-catalysed hydrolysis compared to simple esters the stability of the bound enzyme can be partly explained in terms of electronic effects on the serine–ester bond as well as steric factors.

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† Satisfactory microanalytical and/or mass spectral information has been obtained for all new compounds other than the enzyme derivatives.

[1] V. Knott-Hunziker, S. G. Waley, B. S. Orlek, and P. G. Sammes, *Biochem. J.*, 1979, **177**, 365.
[2] V. Knott-Hunziker, S. G. Waley, B. S. Orlek, and P. G. Sammes, *F.E.B.S. Letters*, 1979, **99**, 59.
[3] D. R. Thatcher, *Biochem. J.*, 1975, **147**, 313.
[4] R. J. Stoodley, *J. Chem. Soc.* (*C*), 1968, 2891.
[5] R. J. Stoodley and R. B. Wilkins, *J.C.S. Perkin I*, 1974, 1572.
[6] R. L. Charnas, J. Fisher, and J. R. Knowles, *Biochemistry*, 1978, **17**, 2185.