ENZYMATIC ACYLATION OF MONOBACTAMS

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Monobactams are a new class of monocyclic β-lactams isolated from bacteria,[1,2] they are *N*-acyl derivatives of 3-aminomonobactamic acid. As with penicillins and cephalosporins, the nature of the *N*-acyl group affects their antibiotic activity.[3]

N-Acylation of penicillins and cephalosporins can be achieved enzymatically[4] as well as chemically and here we report on the use of a penicillin acylase from *Escherichia coli* for generating various *N*-acyl monobactams.

Benzylpenicillin acylase was purified from *E. coli* and was chemically modified and copolymerized in a polyacrylamide matrix by Boehringer-Mannheim.[5] One g of the immobilized enzyme could hydrolyze 166 μmol benzylpenicillin per minute.

Enzyme reactions were carried out in 50-ml Erlenmeyer flasks and shaken at *ca.* 60 rpm to keep the immobilized enzyme dispersed. The enzyme reaction was monitored by bioautography using *Bacillus licheniformis* (SC 9262) or *E. coli* (SC 12,155) and by densitometry after separation of products from substrates by TLC or high voltage electrophoresis.

When 2-formamidothiazol-5-yl acetic acid (SQ 28,113) was incubated with 3-amino-4α-methylmonobactamic acid (SQ 26,771) in the presence of the immobilized acylase at an acid pH, SQ 28,114 was generated. (Fig. 1). The SQ 28,114 was identified by comparison with an authentic sample. When a Silica Gel 60 F_{254} plate was developed in acetonitrile - water (10: 1) SQ 28,114 had a Rf of 0.7. When developed in acetonitrile - ethyl acetate - acetic acid - water (4: 4: 1: 1) the Rf was 0.5. By high voltage electrophoresis (70 V/cm, pH 4.5, 30 minutes), the mobility of SQ 28,114 was 0.7 with respect to sodium *p*-nitrobenzenesulfonate.

The enzyme reaction product was recovered by elution from a TLC plate and subjected to fast atom bombardment mass spectrometry. The molecular ion $(M+H)^+$ 371, the monosodium ion $(M+Na)^+$ 393, and the disodium ion $(M+2Na-H)^+$ 415 were identified. The fragmentation pattern was identical to authentic standard.

The reaction in Fig. 1 was tested over the pH range 4 to 9. pH was adjusted by addition of NaOH or HCl. Deacylation was assayed using SQ 28,114 (1 mg/ml) as substrate and 50 mg/ml enzyme resin. Activity was monitored by densitometry measurements (E 275 nm) of SQ 28,113 on a TLC plate. It was found (Fig. 2) that acylase activity was optimal at pH 4.5 whereas deacylase activity was optimal at pH 7.1.

The optimum concentration of SQ 26,771 in the acylase reaction was 3 mM whereas a two-fold higher concentration of side chain (SQ 28,113) was optimal. The optimum temperature for acylation was 45°C and the equilibrium constant for the reaction was 5×10^{-5} M^{-1}.

The specificity of the acylase was studied by incubating 3-amino-4α-methylmonobactamic acid together with various side chains in the presence of immobilized acylase (pH 4.5, 37°C). Table 1 shows that aminothiazole acetic acid and phenoxyacetic acid were active as substrates in the reaction. Also, 3-aminomonobactamic acid could be used as effectively as the 3-amino-4α-

Fig. 1. Acylation of SQ 26,771 using benzylpenicillin acylase.
The reaction was carried out at 37°C in a 50-ml Erlenmeyer flask rotated at 60 rpm. The 2 ml reaction mixture contained 100 mg enzyme resin, 6 μmol SQ 26,771 and 12 μmol SQ 28,113 at pH 4.5.

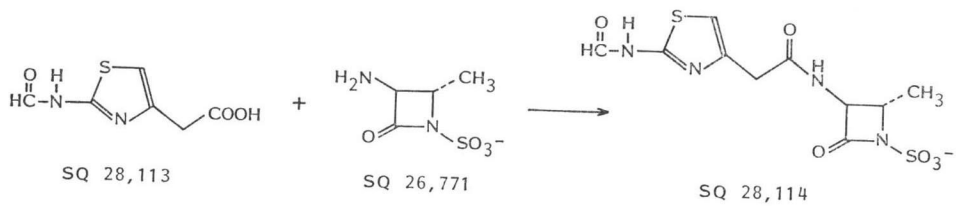
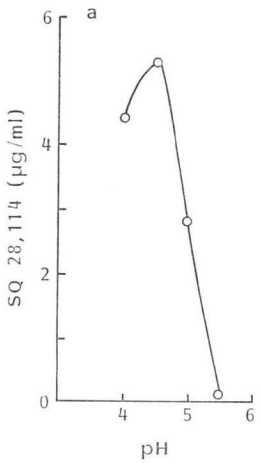


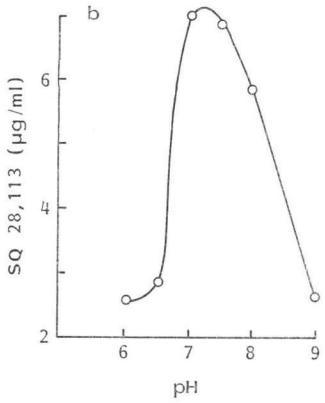
Fig. 2. The pH optimum for acylation and deacylation of monobactams.

Product was measured in both cases after separation of the reaction mixture by TLC.

(a) Acylation was monitored by appearance of SQ 28,114 after 16-hour incubation under the conditions described in Fig. 1.

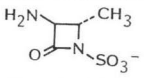
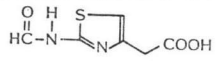
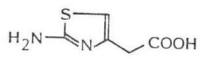
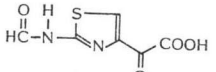
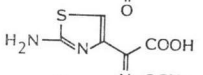
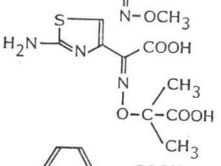
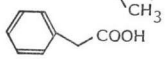
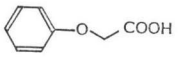
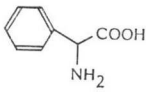


(b) Deacylation was monitored by appearance of SQ 28,113 after 2-hour incubation; the reaction mixture contained 50 mg/ml enzyme resin and 1 mg/ml SQ 28,114.



methylmonobactamic acid in the acylation reaction. When substitutions were made to the α carbon of the aminothiazole acetic acid (SQ 27,710, SQ 28,756, No. 776) and of that of phenylacetic acid (phenylglycine), no acylation occurred. When monobactams with these substituted side chains were tested as substrates in a deacylase reaction (pH 7.5) no evidence of deacylation was seen, whereas those compounds which were produced by acylation could be readily deacylated. That the acyl residue is more important for the substrate specificity of the enzyme than the nucleus, is consistent with earlier reports using various phenylacylated penicillins[6,7] and amino acids.[7]

Table 1. Specificity of monobactam acylase.[a]

	Structure	
		
SQ 28,113		+[b]
SQ 272		+
SQ 27,710		—[c]
SQ 28,756		—
No. 776		—
Phenylacetic acid		+
Phenoxyacetic acid		+
Phenylglycine		—

[a] Assays were carried out as in Fig. 1. Activity was determined by bioassay.

[b] + Acylation.

[c] — No acylation.

Further, when the natural monobactams[1,8,9] were tested as substrates for the deacylase reaction, no activity was detectable. This is consistent with biosynthetic studies[10] where the addition of possible side chain precursors failed to affect the monobactam produced.

Acknowledgment

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