Comparison of β -Lactamase II from *Bacillus cereus* 569/H/9 with a β -Lactamase from *Bacillus cereus* 5/B/6

By R. B. DAVIES and E. P. ABRAHAM

Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, U.K.
and

JOAN FLEMING and M. R. POLLOCK

Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, U.K.

(Received 22 October 1974)

A mutant of *Bacillus cereus* 5/B, strain 5/B/6, produces a β -lactamase II-like enzyme but no β -lactamase I. β -Lactamases II and II 5/B/6 appear to show a high degree of homology, but there are significant differences in their enzymic properties.

Two types of exo- β -lactamase are produced by strains of *Bacillus cereus*. The first, β -lactamase I, is mainly a penicillinase. The second, β -lactamase II, is a zinc-requiring enzyme which catalyses the hydrolysis of both penicillins and cephalosporins at comparable rates. The inducible β -lactamase I produced by strain 569 is apparently identical with the corresponding constitutive enzyme produced by strain 569/H, a spontaneous mutant of strain 569 (Kogut *et al.*, 1956). On the other hand, the constitutive β -lactamase I produced by *B. cereus* 5/B, a spontaneous mutant (Sneath, 1955) of the micro-constitutive wild-type, strain 5 (Pollock, 1957), is distinguishable from the enzyme of strain 569/H in its physico-chemical and immunological properties (Kogut *et al.*, 1956). The β -lactamase II from *B. cereus* 569 and 569/H is similar in enzymic properties to the corresponding enzyme from *B. cereus* 5/B (Crompton *et al.*, 1962), but no detailed comparison of these β -lactamases has been reported.

A mutant of *B. cereus* 5/B produces a β -lactamase II-like enzyme (named here II 5/B/6), but no β -lactamase I. In the present communication some of the properties of this enzyme are compared with those of β -lactamase II from *B. cereus* 569/H/9 (Davies *et al.*, 1974). Strain 569/H/9 is a 'hyper' magno-constitutive mutant obtained by Dr. J. Imsande from strain 569.

Materials and methods

Organisms. *B. cereus* 569/H/9 is that used by Davies *et al.* (1974). *B. cereus* 5/B/6 is a spontaneous mutant of *B. cereus* 5/B isolated after repeated subculture in liquid medium and selected on Andrade agar (Kogut *et al.*, 1956) for its relatively low β -lactamase activity.

β -Lactamase production. *B. cereus* 5/B/6 was grown on a laboratory scale (six 1.5-litre cultures) from a spore suspension as described by Davies *et al.* (1974) for β -lactamase production from *B. cereus* 569/H/9

and cultures were harvested 9 h after inoculation. The purification procedure was that used by Davies *et al.* (1974) for the β -lactamases from strain 569/H/9.

General methods. Chromatography and electrophoresis on paper, amino acid analysis, polyacrylamide-gel electrophoresis, the preparation of tryptic-digest 'maps', the buffers used, determinations of the rates of hydrolysis and Michaelis constants with different substrates and assays of β -lactamase activities were carried out as described by Davies *et al.* (1974). Protein was determined by the method of Lowry *et al.* (1951). The unit of activity is the amount of enzyme required to hydrolyse 1 μ mol of substrate/min at pH 7.0 at 30°C.

Results

The supernatants from cultures of *B. cereus* 5/B/6 contained 3.0–3.5 units of β -lactamase II-like activity/ml but no detectable amount of β -lactamase I. In its adsorption on Celite and subsequent elution the enzyme behaved like the β -lactamase II from *B. cereus* 569/H/9 (Davies *et al.*, 1974). It also behaved like β -lactamase II when chromatographed on Sephadex CM-50, being eluted in the same position as the latter enzyme as a single peak, but the elution curve contained no protein peak in a position corresponding to that found by Davies *et al.* (1974) for β -lactamase I. The apparent overall yield of purified enzyme was 100%. In view of its resemblance to β -lactamase II it was named β -lactamase II 5/B/6.

The preparation β -lactamase II 5/B/6 obtained by this procedure required Zn^{2+} as a cofactor and was inactive in the presence of 5 mM-EDTA. Dr. P. H. Lloyd kindly determined its molecular weight by the sedimentation-equilibrium procedure of Lloyd & Peacocke (1970) and obtained a value of 17600. The value obtained by sodium dodecyl sulphate-polyacrylamide-gel electrophoresis was 22500, close to that (22000) for β -lactamase II (Davies *et al.*, 1974).

Table 1. *Enzymic properties of β -lactamase II and β -lactamase II 5/B/6*

Except where otherwise indicated rates of hydrolysis were measured at pH 7.0 and at 30°C with substrate concentrations of 1.5 mg/ml. For substrates whose Michaelis constants were determined, the rates were near to V_{max} .

Compound	β -Lactamase	...	Rate of hydrolysis (relative)		K_m values (mM)	
			II	II 5/B/6	II	II 5/B/6
Benzylpenicillin			100	100		
Carbenicillin			81	68	—	—
Ampicillin			44	30		
Methicillin			129	95		
Phenoxymethylpenicillin			96	88		
6- α -Methylphenoxymethylpenicillin*			<0.1	<0.1		
6-Aminopenicillanic acid†			1.5	0.2		
Cephalosporin C			62	30	0.22	0.31
Cephalothin			48	21	0.11	0.16
Cephaloridine			30	17	0.40	0.40
Cephazolin			4	2		
Cephalexin			1.3	0.5		
Deacetylcephalosporin C lactone			<0.001	<0.002		
7-Aminocephalosporanic acid			7.6	2.2	0.17	0.17
7-Methylcephalosporin‡			<0.1	<0.1		

* Substrate concentrations 0.77 mg/ml.

† Hydrolysis followed potentiometrically at pH 8.5.

‡ 7- α -Methyl-7-phenoxyacetamidodeacetycephalosporanic acid.

The preparation behaved as a homogeneous protein in the ultracentrifuge, but it was resolved by isoelectric focusing into two components with pI values (at 0.4°C) of 8.42 and 9.08 respectively. These components were present in approximately equal amounts and showed almost the same specific activity expressed as units/ml per E_{280} unit. They were not separated from each other for the determination of the properties described here.

The amino acid composition of β -lactamase II 5/B/6 was very similar to that of β -lactamase II (Davies *et al.*, 1974). With both enzymes 1 mol of cysteic acid/mol was obtained on oxidation and hydrolysis. 'Maps' of the products of tryptic digestion of the two enzymes were similar, though not identical. Thus 29 peptides were observed in the digest of β -lactamase II 5/B/6 and 31 in that of β -lactamase II, and 38 peptides were observed in a mixture of the digests. The thermostability of β -lactamase II 5/B/6 was similar to, though significantly less than, that of β -lactamase II, the activity remaining after 30 min at 60°C in 'Tris-zinc' buffer, pH 7, being 84 and 98% respectively.

The catalytic-centre activity of β -lactamase II 5/B/6 at 30°C and pH 7.0 with cephalosporin C as substrate (mol of substrate hydrolysed/min per mol of enzyme) was 0.24×10^5 , about 47% of that of β -lactamase II. The pH-activity curves of the two enzymes with benzylpenicillin as substrate were very similar, both showing a maximum at pH 6.0. The relative rates of hydrolysis of different substrates in similar

concentrations by the two enzymes are shown in Table 1, together with Michaelis constants for several substrates determined from Lineweaver-Burk plots. Both β -lactamases readily hydrolysed a number of cephalosporins, unlike β -lactamase I (Abraham & Newton, 1956; Davies *et al.*, 1974), but cephazolin and cephalexin were hydrolysed relatively slowly. A 6- α -methylpenicillin, a 7- α -methylcephalosporin and deacetylcephalosporin C lactone were hydrolysed very slowly, if at all, and may have low affinities for the enzymes (Davies *et al.*, 1974). Despite the similarities in the substrate profiles of the two enzymes, however, there were significant differences between them. Thus with β -lactamase II 5/B/6 the rates of hydrolysis of several cephalosporins, relative to that of benzylpenicillin, were less than half the rates with β -lactamase II.

Discussion

The properties of *B. cereus* 5/B/6 are consistent with the mutation of strain 5/B being in a structural gene for β -lactamase I. However, there is little precise genetic information about *B. cereus* and nothing is known about the map location of the β -lactamase genes. Although co-ordinate production of β -lactamases I and II in strain 569 on induction and in the magno-constitutive strain 569/H and different loss mutants of the latter (M. R. Pollock & J. Fleming, unpublished work) suggests that the corresponding genes may be part of a single operon and share com-

mon control loci, this cannot be definitely concluded. There was no detectable precipitation between the culture supernatant of strain 5/B/6 and antiserum to β -lactamase I from strain 569/H (as is apparent with culture supernatant from strain 5/B) when tests were made on an Ouchterlony plate (see Pollock, 1964). Further, no protein similar to that of β -lactamase I was detected during the chromatographic purification of β -lactamase II 5/B/6. Hence, no evidence was obtained in these experiments for the presence of a missense mutation in the β -lactamase I gene of strain 5/B/6.

β -Lactamase II 5/B/6 is clearly similar to, but not identical with, β -lactamase II from *B. cereus* 569/H/9. It is likely that there is a high degree of homology between these two enzymes, although differences exist in their amino acid sequences which are responsible for detectable differences in the properties of their active sites. The relationship of β -lactamase II to enzyme II 5/B/6 appears to be analogous to that of the β -lactamase I from *B. cereus* 569/H and 5/B respectively. It is likely, though not

yet certain, that the β -lactamases II 5/B/6 and II 5/B are identical.

We are grateful to Dr. J. E. Dolfini for samples of 6-methylpenicillins and 7-methylcephalosporins and are indebted to the Medical Research Council and the National Research Development Corporation for financial support.

- Abraham, E. P. & Newton, G. G. F. (1965) *Biochem. J.* **63**, 628–634
Crompton, B., Jago, M., Crawford, K., Newton, G. G. F. & Abraham, E. P. (1962) *Biochem. J.* **83**, 52–63
Davies, R. B., Abraham, E. P. & Melling, J. (1974) *Biochem. J.* **143**, 115–127
Kogut, M., Pollock, M. R. & Tridgell, E. J. (1956) *Biochem. J.* **62**, 391–401
Lloyd, P. H. & Peacocke, A. R. (1970) *Biochem. J.* **118**, 467–474
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Pollock, M. R. (1957) *Ciba Found. Symp. Drug Resist. Micro-Organisms*, pp. 78–96
Pollock, M. R. (1964) *Immunology* **7**, 707–723
Sneath, P. H. A. (1955) *J. Gen. Microbiol.* **13**, 561–568