COMPARATIVE AMINO ACID CONTENTS OF PURIFIED β-LACTAMASES FROM ENTERIC BACTERIA

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1. Introduction

It is already well established that a variety of different types of β-lactamases can be detected among enteric bacteria if the relative rate of hydrolysis of various substrates and the effect of various inhibitors on the enzymes are used for comparative purposes [1,2]. Although eight distinct types of enzyme may be detected by these tests, all fall into the following four main classes, [see 3]:

Class I : Enzymes predominantly active against cephalosporins (two types: Ia and Ib)
Class II : Enzymes predominantly active against penicillins (two types: IIa and IIb)
Class III : Enzymes with approximately equal activity against penicillins and cephalosporins and which are inhibited by cloxacillin but resistant to *p*-chloromercuribenzoate and other SH inhibitors (one type: III)
Class IV : Enzymes with similar substrate profile to Class III but which are resistant to cloxacillin and sensitive to *p*-chloromercuribenzoate (three types: IVa, IVb and IVc).

In view of this wide range of properties among enzymes that have the common property of hydrolysing the β-lactam bond, it was interesting to try to discover whether any underlying molecular similarity could be detected among this group of enzymes. The considerable difference in electrophoretic mobility and in substrate profile, together with the absence of immunological cross-reaction between enzymes from the different classes suggested that the enzymes might differ widely: yet Ambler and Meadway [4] have shown a considerable homology in the amino acid sequence of the β-lactamases from *Bacillus licheniformis*, strain 749/C, and *Staphylococcus aureus*, strain PC1, despite wide differences in substrate profile and physico-chemical properties.

Measurement of the amino acid composition of pure preparations of enzymes from three of the four classes of β-lactamases from enteric bacteria (types Ia, III and IVa) and comparison of these data with the analysis published of a type Ib enzyme from *Escherichia coli* K12, strain D31, [5] shows that all four types of enzymes have a very similar amino acid composition despite their markedly different enzymic characteristics. This suggests that the wide range of properties encountered among β-lactamases from enteric bacteria may reflect relatively minor variations in structure of a basic molecule with β-lactamase activity and this, in turn, implies a common evolutionary ancestry.

2. Methods

2.1. *Strains and enzyme characteristics*

Table 1 summarises some of the enzymic and physico-chemical properties of the β-lactamases in these experiments together with the characteristics of the bacterial strains that produce them. The three enzymes comprise one from class I (type Ia), one from class III (type III) and one from class IV (type IVa) - see table 1. Table 1 also includes characteristics of the enzyme synthesised by *E.coli* K12, strain D31, described in detail by Lindström, Boman and Steele [5] (see also [2], strain no. 16). The type Ia enzyme (from *Enterobacter cloacae*, strain 214) was purified as described by Hennessey and Richmond [6], the type III enzyme from *E. coli* (R^{+}_{TEM}) by the method of Datta and Richmond [7], and type IV enzyme (from *Aerobacter*

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Table 1

Summary of substrate profiles and physico-chemical properties of β-lactamases involved in these experiments

Enzyme Type	References	Bacterial source	Electrophoretic mobility at pH 8.5	Substrate profile*					Inhibited by	
				Pen G	Amp	Cephine	Cephx	Carb	pCMB	Clox
Ia	6, 11	*E. cloacae* 214	+ ve	100	0	8000	620	0	R	S
Ib	2, 5	*E. coli* D31	+ ve	100	0	350	80	0	R	S
III	7, 12	*E. coli* (R^{+}_{TEM})	- ve	100	180	140	2	10	R	S
IVa	13	*A. cloacae* 53	+ ve	100	120	150	0	13	S	R

* Substrate profiles are quoted as V_{max} values relative to an arbitrary value of 100 for Pen G hydrolysis.

Abbreviations: Pen G = benzylpenicillin; Amp = ampicillin; Cephine = cephaloridine; Cephx = cephalexin; Carb = carbenicillin; pCMB = *p*-chloromercuribenzoate; Clox = cloxacillin; R = resistant to inhibition; S = sensitive to inhibition.

Table 2

Amino acid composition of four distinct β-lactamases from enteric bacteria

Enzyme type *	Amino acid composition (%, by wt.)			
	Ia	III	IVa	Ib
Amino acid				
Lys	9.1	8.5	9.4	8.2
His	2.1	1.0	1.0	1.9
Arg	4.4	5.6	5.7	5.5
Cys	0	0	0.8	0
Asp	13.8	13.6	11.8	9.8
Thr	7.2	7.4	7.4	6.5
Ser	4.3	4.4	6.4	4.9
Glu	12.7	15.2	12.2	14.6
Pro	5.5	5.7	3.5	5.5
Gly	3.2	3.4	4.6	4.5
Ala	5.0	6.3	6.2	6.6
Val	4.9	5.8	5.8	5.6
Met	1.9	1.9	1.9	1.9
Ile	4.0	4.2	4.1	5.6
Leu	8.8	10.0	9.1	8.8
Tyr	5.8	2.4	4.8	5.8
Phe	8.4	4.4	5.6	4.3
Try	?	?	?	?

* See [3].

cloacae, strain 53) by an as yet unpublished method. All enzyme preparations were more than 90% pure as judged by specific enzyme activity and homogeneity on starch-gel electrophoresis. Purity of the β-lactamase prepared from *E. coli* K12, strain D31, was checked by polyacrylamide-gel electrophoresis, ultracentrifugation analysis and immunodiffusion analysis and found to be homogeneous [5].

2.2. *Enzyme hydrolysis and amino acid analysis*

Enzyme samples (about 1 mg) were hydrolyzed following performic acid oxidation [8] and the resulting amino acids prepared for analysis are described by Hennessey and Richmond [6].

3. Results and discussion

Table 2 shows the relative abundance of each amino acid in the three enzymes that have been purified in this laboratory. The information is given in the form of weight percentage content of each amino acid since this method of presentation avoids the difficulties arising from doubts as to the precise molecular weights of the enzymes concerned. Table 2 also contains the analysis of the type Ib enzyme synthesised by *E. coli* K12, strain D31 [5], recalculated so that the values are directly comparable with the others in table 2.

Comparison of the amino acid composition of the four enzymes reveals a very high degree of similarity despite the wide range in substrate profile and physico-chemical properties of the enzymes (cf. table 1). Apart from the presence of cysteine in the type IVa enzyme (the probable cause of the *p*-chloromercuribenzoate sensitivity of this enzyme) the main differences in analysis concern the content of phenylalanine and tyrosine. Even this relatively wide variation, however, may be more apparent than real since analysis of these two amino acids in hydrolyzed protein preparations is less reliable than is the case for other amino acids in the hydrolyzate.

There is, of course, no reason for concluding that two proteins of unknown function but similar amino

acid composition share any common peptide sequences. However, enzymes with related activities do often have considerable stretches of primary sequence in common [9] and the β-lactamases – at least on the evidence obtained with enzymes from Gram-positive species [4] – are no exception. Under these circumstances the extremely similar amino acid content of the four enzymes compared here strongly suggests a high degree of similarity in their primary sequences despite the variation observed in their detailed enzymic properties.

Comparison of the amino acid analysis of the β-lactamases from Gram-positive species [4,10] with the analyses shown in table 2 reveals much less similarity, and, as yet, it is impossible to say whether there are any similarities in the primary sequences of the β-lactamases from these two classes of organism.

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