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The structure of β-lactamases

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The β-lactamases are widely distributed in both Gram-positive and Gram-negative bacteria. They all inactivate penicillins and cephalosporins by opening the β-lactam ring. Many varieties of the enzyme can be distinguished on the basis of their catalytic and molecular properties, but only amino acid sequence determination gives information upon which a molecular phylogeny can be based.

The present evidence suggests that the β-lactamases have a polyphyletic origin. All the β-lactamases of currently known amino acid sequence belong to one homology group, here called class A enzymes.† Class B consists of the mechanistically distinct *Bacillus cereus* β-lactamase II, which preliminary partial sequence analysis suggests to be structurally unrelated to the class A enzymes. It is predicted that sequence analysis will show that further classes will need to be created to account for particular β-lactamases of distinctive molecular and mechanistic properties.

Introduction

The β-lactamases have attracted much attention because of their clinical importance and their ecological and evolutionary interest. As many of the enzymes have favourable biochemical and molecular properties, the β-lactamases have become one of the best studied groups of bacterial enzymes (Hamilton-Miller & Smith 1979). Pollock (1971) has reviewed the evidence about the function of the β-lactamases and hypotheses about their evolution. The enzymes appear to occur only in bacteria, a finding that is consistent with their probable function in protecting the organisms against the action of β-lactam antibiotics, which are normally toxic only to bacteria. Such a distribution is also consistent with the hypothesis (Tipper & Strominger 1965) that the β-lactamases have evolved from one or other of the penicillin-sensitive enzymes involved in cell-wall synthesis (Spratt, this symposium).

Among the bacteria, β-lactamases are extremely widely distributed (Citri 1971), and because of the specificity and sensitivity of assays, they can be recognized even when present in very small amounts. The enzyme is produced at highest concentrations by certain Gram-positive organisms, and in particular by *Bacillus cereus* (Kogut *et al.* 1956; Kuwabara & Abraham 1967), *Bacillus licheniformis* (Pollock 1965) and *Staphylococcus aureus* (Richmond 1963, 1965). The enzyme is widely distributed in Gram-negative organisms, and many different types of enzyme can be recognized on the grounds of relative activity towards different β-lactams (Richmond & Sykes 1973) or isoelectric point (Matthew *et al.* 1975). The enzymes are synthesized at lower levels than in the Gram-positive organisms, even when fully induced, but are nevertheless very effective at protecting the organisms against the action of β-lactam antibiotics, possibly because they are precisely located at optimal positions in the periplasmic space.

† I apologize for proposing a new nomenclature. Unfortunately, Roman numerals have been used both for distinguishing between the two enzymes of *Bacillus cereus* (Kuwabara & Abraham 1967) and by Richmond & Sykes (1973) for the classification of Gram-negative β-lactamases.

The structures of various β-lactamases have been investigated at the primary (Ambler 1979), secondary (Robson & Pain 1976) and tertiary levels (DeLucia *et al.*, this symposium; Aschaffenburg *et al.* 1978; J. Moult & L. Sawyer, unpublished results). Structural information is necessary for the elucidation of the mechanism of action of the enzymes. It can also provide evidence about the origin of the β-lactamases, and form the basis of a reliable classification.

Diversity in β-lactamases

The β-lactamases vary widely in their enzymic and molecular properties. A systematic comparison between the known enzymes is difficult, as different workers concentrate their attentions on particular properties, and methods for the determination of even such simple properties as molecular mass vary so much in their reliability. This can be illustrated by the case of the R_{TEM} enzyme from *Escherichia coli*. The first molecular mass reported, from ultracentrifuge studies, was 16700 (Datta & Richmond 1966). Gel-filtration measurements indicated a molecular mass of 21000 (Dale & Smith 1971) or 25000 (Richmond & Sykes 1973), while sodium dodecyl sulphate gel electrophoresis suggested 27000 (Scott 1972). The molecular mass from the sequence is 28500 (Ambler & Scott 1978; Sutcliffe 1978).

The properties that have been used to differentiate and classify the β-lactamases are: (1) isoelectric point, (2) molecular mass, (3) relative activity towards different β-lactams ('substrate profile'), (4) interaction with inhibitors and inactivators, (5) the nature of the active site, (6) amino acid sequence, and (7) three-dimensional structure. These approaches differ in the amount of material and labour that they require, and in the information they can provide about the origin and relationships of β-lactamases.

Structural classes of β-lactamases

There is already enough structural information available to indicate that there are at least two distinct classes of β-lactamases, and that the enzymes therefore have a polyphyletic origin. In addition, there are suggestions that there are further classes that may have had separate origins.

Class A

The amino acid sequences of four different β-lactamases are substantially known (figure 1). The four proteins match well throughout their sequences, and very few gaps need to be postulated to obtain the optimal matching. The amount of similarity is so great that divergence from a single ancestral gene is the most reasonable explanation, and is such that similar tertiary structures can be confidently predicted. I propose that enzymes of this sequence type be called class A β-lactamases (see footnote to abstract).

Although the similarity extends throughout the enzymically active parts of the molecules (as exemplified by the sequence of the extracellular β-lactamase of *S. aureus* shown in figure 1), no similarity can be seen between the leader sequence of the *E. coli* R_{TEM} enzyme (deduced from the DNA sequence; Sutcliffe 1978) and the various *N*-terminal extensions of membrane-bound precursor forms of the *B. licheniformis* enzyme (Lampen *et al.*, this symposium). Nothing is yet known about *N*-terminal extensions of the *S. aureus* or *B. cereus* I proteins, although there is evidence for the existence of precursors. The DNA studies show that there is no *C*-terminal

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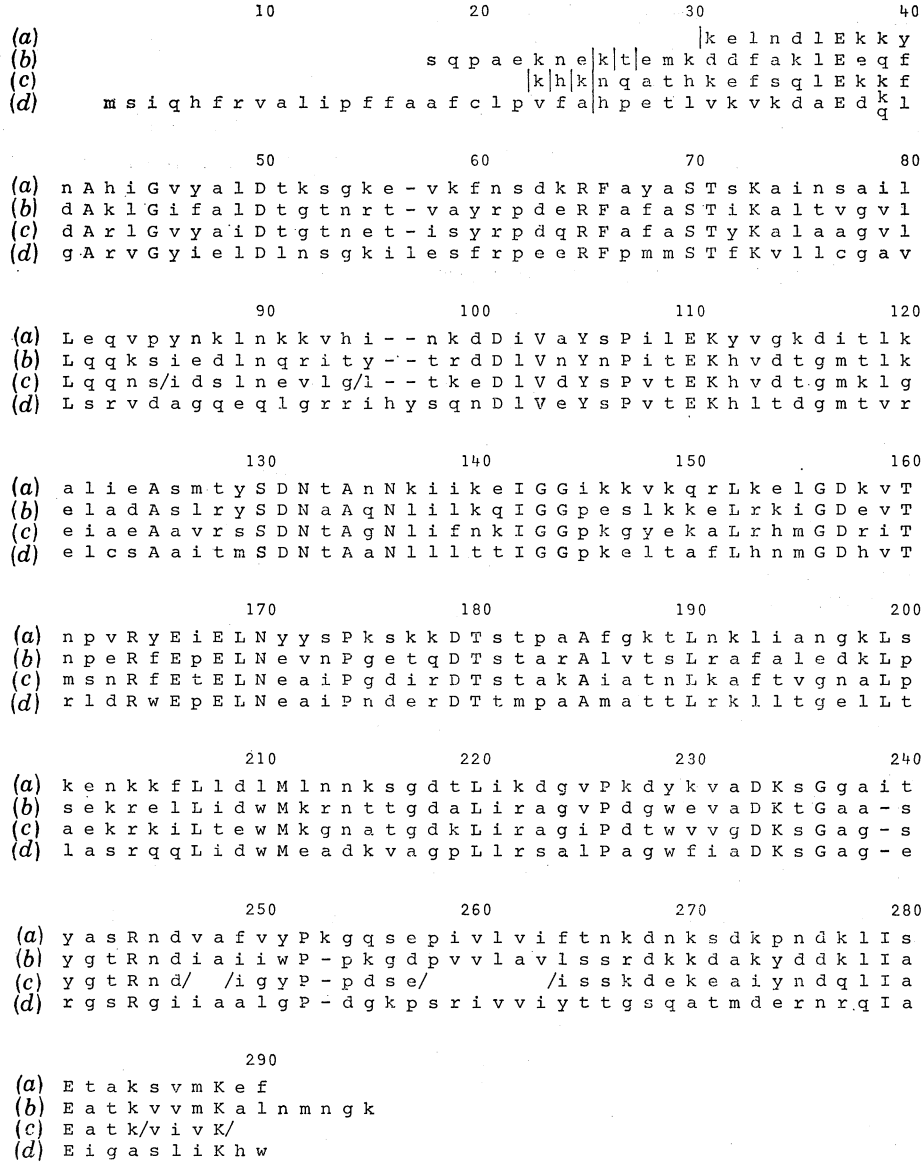

FIGURE 1. Amino acid sequence alignment of class A β-lactamases. The sequences are: (*a*) *Staphylococcus aureus* PC1 (Ambler 1975); (*b*) *Bacillus licheniformis* 749/C (Meadway 1969; further details in Ambler 1979; *N*-terminal eight residues, Lampen *et al.*, this symposium); (*c*) *Bacillus cereus* 569/H β-lactamase I (Thatcher 1975; also pers. comm.); (*d*) *Escherichia coli* pBR322 (Sutcliffe 1978) and R_{TEM} (Ambler & Scott 1978). In sequence (*d*) the only supposed difference between the plasmids is at residue 39. The residues are numbered on the system proposed by Ambler (1979). Numbering starts from the *N*-terminus of the longest form of the *B. licheniformis* enzyme that has been isolated (Lampen *et al.*, this symposium), and takes account of the gaps postulated in the currently known sequences to obtain optimal matching. The vertical bars (|) show the *N*-termini of naturally released enzymically active molecules. Residues that are identical in all four sequences are shown in capitals, other residues in lower-case letters. The one-letter notation used is that recommended by the IUPAC–IUB Commission on Biochemical Nomenclature [*Biochem. J.* (1969) **113**, 1–4]: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; X, unknown or other amino acid; Y, tyrosine.

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postsynthetic modification to the R_{TEM} enzyme, but there is evidence for *C*-terminal modification of the *B. licheniformis* enzyme (Kelly & Brammar 1973).

The tertiary structure of three class A β-lactamases is under investigation by X-ray crystallography. The progress of the investigations is summarized in table 1.

As is discussed in the next section, three different class A β-lactamases have been shown to react with mechanism-based inhibitors in similar ways, suggesting that they all have similar mechanisms of action.

TABLE 1. CRYSTALLOGRAPHIC DATA FOR CLASS A β-LACTAMASES

	Staphylococcus aureus PC1†	*Escherichia coli* R_{TEM}‡	*Bacillus cereus* I569§
crystal size/mm	5 × 0.5 × 0.2	1 × 0.5 × 0.5	1 × 0.2 × 0.2
space group	I222	P2 or $P2_1$, Z = 4	C2
unit cell size/Å¶	54.5 × 93.9 × 138.7	47.5 × 75.5 × 73.5 β = 97.0°	143.0 × 35.8 × 52.7 β = 98.6°
heavy atom derivatives	$Pt(CN_4)^{2-}$ $Au(CN)_2^-$	$SmCl_3$ $K_3UO_2F_5$	
resolution of electron density map	5 Å	3.3 Å	
molecular dimensions/Å	35 × 45 × 40 (ellipsoidal); molecules stack as dimers	40 overall diameter	

† J. Moult, L. Sawyer, L. Jones, O. Herzberg, M. Harding & D. Green, unpublished results.
‡ DeLucia *et al.* (1980).
§ Aschaffenburg *et al.* (1978).
¶ 1 Å = 10^{-10} m = 10^{-1} nm.

The known class A β-lactamases differ widely in their physical and enzymic properties. Although the soluble forms of all of them have molecular masses of about 29000, their isoelectric points vary from pH 5.7 to above pH 9, and they have great differences in substrate profiles and other kinetic parameters. Several other β-lactamases are reported to have molecular masses of about 29000 (Richmond & Sykes 1973), and dimeric enzymes in which the subunits are of this size have been recognized (Dale & Smith 1976). However, there is so much variation in the published molecular masses of β-lactamases that it would be unwise to include even tentatively in class A other 29000 molecular mass enzymes on the criterion of size alone.

Class B

After the four class A enzymes shown in figure 1, the best studied is the *B. cereus* β-lactamase II (Kuwabara & Abraham 1967). It differs from the class A enzymes in so many features that class B is created to comprise it. It has not yet been found in any organisms except *B. cereus* and very similar bacilli, and in natural isolates occurs together with a class A enzyme. A mutant of *B. cereus* has been isolated that only produces the class B enzyme (Davies *et al.* 1975).

The *B. cereus* β-lactamase II is slightly but significantly smaller than the class A enzymes, the present estimate of the molecular mass, from sodium dodecyl sulphate gel electrophoresis, being about 23000. It is the only known β-lactamase that requires a metal cofactor, which is normally Zn II, although some other ions can substitute (Davies & Abraham 1974). The metal binding involves histidines and the solitary cysteine residue, and metal reactivation is inhibited

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by thiol reagents. The particular histidine residues that act as zinc ligands have been recognized (Baldwin *et al.* 1978, 1979).

A start has been made with the determination of the amino acid sequence of the protein. In figure 2 are shown the sequences around the histidine and cysteine residues (J. M. Hermoso & R. P. Ambler, unpublished observations). The longer fragments have been compared with the sequences of the class A enzymes shown in figure 1, by using similar procedures to those described in table 3. None of the fragments matched at above 'background' level with any part of any of the A enzyme sequences. The best of the 'high background' matches were inspected for (1) matching with residues identical in all the four known class A enzymes, and (2) matching of structurally similar amino acids ('conservative substitutions': leucine–isoleucine–valine, tyrosine–phenylalanine, aspartic acid–glutamic acid, etc.). No sequence similarity was detected.

In view of the differences in mechanism and size between the class A and the class B enzymes, and the apparent lack of sequence similarity, it is suggested that the two classes had independent origins.

```
. I K A H S T A L T A E L A K .  (1)

. A H A N .         . L H T .  (2 ?)

. N I N A V V P G H G E V K G G .  (3)

. F G N M K V E T F Y P G K G H .  (4)

. I L V G G C L V K S T S A K D .
```

FIGURE 2. Cysteine and histidine containing sequences from the class B enzyme *Bacillus cereus* 569/H β-lactamase II. The sequences were deduced from the study of tryptic and thermolysin digests of performic acid oxidized protein. The histidine residues are numbered to correspond with the peptides isolated by Baldwin *et al.* (1979). The one-letter notation used is described in the legend to figure 1.

Other possible classes of β-*lactamases*

Some β-lactamases are known that have been reported to have molecular masses that are either too large or too small for them to be closely similar in structure to either the class A or the class B enzymes. The β-lactamases encoded by the *Pseudomonas aeruginosa* plasmids R151 (Matthew 1978) and Rms149 are believed to have molecular masses of about 12000. There is some evidence that the *S. aureus* class A enzyme is a multi-domain protein (Mitchinson *et al.*, this symposium), so it is possible that the small β-lactamases could be similar in structure to just one of the domains. The β-lactamase from *Enterobacter cloacae* P99 (Ross & Boulton 1973; Ross 1975) and the inducible enzyme from *Ps. aeruginosa* (Sabath *et al.* 1965; McPhail & Furth 1973) have been purified and are larger than the class A enzymes, with molecular masses of about 40000 by sodium dodecyl sulphate gel electrophoresis. Both enzymes are much more active against cephalosporins than against penicillins, and together may be representatives of a further class of β-lactamases.

Some of the β-lactamases of Gram-negative organisms are inhibited by thiol reagents such as *p*-chloromercuribenzoate (Smith 1963; Jack 1971; Richmond & Sykes 1973). These *p*-chloromercuribenzoate-sensitive enzymes may form a mechanistically distinct class. Little is yet known about the structure of any of them.

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Structure and activity of β-lactamases

The most successful approach towards relating the activity of β-lactamases to their structure has been through the use of mechanism-based reagents that form a relatively stable covalent derivative of the enzyme. This can be degraded and the modified peptide or amino acid residue recognized (Knott-Hunziker *et al.* 1979; Cartwright & Coulson 1979 and this symposium; Fisher *et al.*, this symposium; Hill *et al.*, this symposium). Similar results were found with three different reagents and three of the class A enzymes shown in figure 1. In each case the evidence is consistent with the modified residue's being serine-70 (figure 1), although the possibility that the modification is to another of the adjoining hydroxyl amino acids has not been excluded in each case.

The class A β-lactamases are sensitive to inactivation by iodine, but the molecular basis of the inactivation is not yet understood. The enzymes vary considerably in their sensitivity to iodine. The reaction of the class A enzymes with tetranitromethane has been studied. In all cases the invariant tyrosine-105 (figure 1) is modified rapidly, and there is some inactivation, but the amounts of modification and inactivation do not correlate. Evidence has been put forward that tyrosine-105 is not part of the active site of the enzyme, but that the loss of activity on treatment with tetranitromethane is due to intermolecular cross-linking (Bristow & Virden 1978).

Scott (1973) showed that the R_{TEM} enzyme was inactivated by photooxidation, with accompanying destruction of histidine, and put forward evidence that histidine-112 was the residue involved. The histidine residues are very variable among the class A β-lactamases. The only site that is occupied by histidine in more than one of the enzymes is position 112, which is histidine in all of the known sequences except that from *S. aureus*, where it is replaced by tyrosine.

Information about the mechanism of action of the enzymes can also be obtained from a study of the structure of artificial mutant forms in which the enzymic activity is altered. Pollock (1968) reported that simple mutations in the structural gene for β-lactamase in *B. licheniformis* caused the formation of proteins with profoundly altered enzymic properties. The mutants were selected as penicillinase-loss variants from magnoconstitutive strains. The activity changes detected were typically a considerable reduction in the specific activity towards benzylpenicillin, a change in substrate profile (such as a major increase in the relative activity towards cephalosporins) and decreased thermal stability. At about the same time, a chemical study of some of the proteins formed by the *S. aureus* penicillinase mutants characterized by Novick (1963) was undertaken (R. P. Ambler & M. H. Richmond, unpublished results). Mutants were chosen that had been shown to produce material that cross-reacted with antiserum to the wild-type β-lactamase, and proteins of much reduced specific activity were purified. In two cases the amino acid lesions were identified. In mutant P2, the replacement was Thr → Ile at position 71, the residue adjacent to the supposed active-site serine residue. In mutant P54, the replacement was Asp → Asn at position 179. These *S. aureus* mutants were then believed to have been lost, and independent attempts to isolate further similar mutants were unsuccessful (M. S. Davies, R. H. Pain & R. Virden, personal communication). Very recently, cultures of the original mutants have again been located, and further characterization of the mutant proteins is in progress. The latest experiments show that mutant P54 has less than 1 % of the activity of the wild-type enzyme, but that appreciable activation takes place when it is incubated at pH 9 and 25 °C for

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12 h, conditions that would favour deamidation of asparagine-170 (S. Yeo & A. F. W. Coulson, personal communication). Unfortunately, the isoelectric point of the protein is so high that separation of the mutant protein from the wild-type has not been achievable by isoelectric focusing.

Hall & Knowles (1976) isolated β-lactamases of altered specificity from *E. coli* R_{TEM} after mutation and selection for increased resistance to cephalosporin C, but they have not yet been able to locate the structural lesion or lesions.

The location of single amino acid substitutions in a protein of the size of the class A β-lactamases is still an arduous operation, but the results that have been achieved through the study of mutants demonstrate that this way of investigating the mechanism of action of the β-lactamases should be pursued more vigorously, particularly as high-resolution three-dimensional structures can be expected soon. The protein from *S. aureus* digests well to give good peptide maps, and is particularly easy to purify, and so is probably the best choice for further study, despite the genetic disadvantages of staphylococci.

TABLE 2. PERCENTAGE SIMILARITY MATRIX FOR CLASS A β-LACTAMASES

	(*a*)	(*b*)	(*c*)	(*d*)
(*a*) *Staphylococcus aureus* PC1	100	41	38	30
(*b*) *Bacillus licheniformis* 749/C		100	56	33
(*c*) *Bacillus cereus* 569/H I			100	34
(*d*) *Escherichia coli* R_{TEM}				100

The number of identities between sequences were scored when they were aligned as shown in figure 1. They are expressed as a percentage of 273, the maximum length of the alignment of the soluble forms of the enzymes.

β-LACTAMASES AND MOLECULAR EVOLUTION

The class A β-lactamases are found in both Gram-positive and Gram-negative bacteria. The R_{TEM} protein, the only determined sequence from a Gram-negative organism, is the most divergent, and the two bacillary proteins are the most similar, but the differences in similarity are not great (table 2). The observations are consistent either with divergence from a precursor gene (coding for a β-lactamase or some functionally related protein) in a remote common ancestor of both Gram-positive and Gram-negative bacteria or with a more recent origin for the precursor gene, in one or other of the branches, followed by interspecific gene transfer to the other lines. The frequent occurrence of β-lactamase genes in readily transmissible plasmids, and the observed cases of chromosomal incorporation of such genes (Bobrowski *et al.* 1976) makes the latter hypothesis attractive, particularly as investigations of other systems (see, for example, Ambler *et al.* 1979) suggest that such reticulation is a common feature in bacterial evolution. There is no reason to expect the evolution of the β-lactamase leader sequences to conform with that of the enzyme-coding section of the gene, as species needs rather than the functional need of the enzyme class are likely to dominate selection in transmembrane processes.

Evolutionary stability of β-lactamases

Although β-lactamases are peripatetic, with the transposon mechanism enabling them to move readily from replicon to replicon (Richmond *et al.*, this symposium), there is evidence that the structural genes themselves are evolutionarily stable. Matthew & Hedges (1976) examined the β-lactamases specified by 100 naturally occurring R factors, and classed the

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enzymes on the basis of their behaviour on analytical isoelectric focusing. In 77 cases the β-lactamases were of the R_{TEM}-1 type, in 7 cases they were the very similar R_{TEM}-2 type, and the remainder were of other types. Although many neutral amino acid replacements would not be distinguished by analytical isoelectric focusing, the method is very precise, and the observations suggest a low level of polymorphism in this widely distributed gene. There is evidence for similar behaviour in the β-lactamase gene of *B. licheniformis*. J. Fleming & M. R. Pollock (personal communication) examined the β-lactamases produced by 12 isolates of *B. licheniformis*, and were able to class them into one or other of two distinct types on the basis of their substrate profiles. Few, if any, natural intermediate forms were found, but artificial hybrids could be constructed genetically (Dubnau & Pollock 1965). Sequence analysis has shown that there are very few amino acid differences (probably between three and six; R. J. Meadway, D. R. Thatcher & R. P. Ambler, unpublished observations) between the type strain enzymes of each class (Pollock 1965).

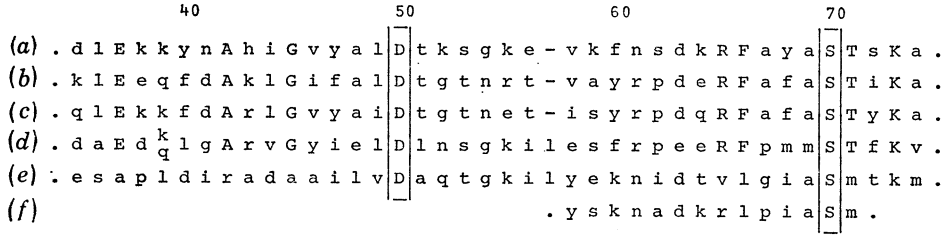

FIGURE 3. Amino acid sequence alignment of class A β-lactamases and two bacillary D-alanine carboxypeptidases (Yocum *et al.* 1979; Waxman *et al.*, this symposium). Sequences (*a*)–(*d*) are as described in figure 1. Sequence (*e*) is the carboxypeptidase from *Bacillus stearothermophilus*, sequence (*f*) that from *Bacillus subtilis*. The sequences are aligned so that the active site serine residue is at position 70. Residues common to all of the β-lactamases are shown in capitals, and residues common to all six sequences are boxed. The one-letter notation used is described in the caption to figure 1.

The origin of β-*lactamases*

An attractive hypothesis for the origin of β-lactamases is that they are derived from a penicillin-sensitive enzyme involved in cell-wall synthesis (Tipper & Strominger 1965). These enzymes have different functions and molecular weights (Spratt, this symposium), and some of them have been shown to act as very poor β-lactamases. The amino acid sequence around the penicillin binding site of the D-alanine carboxypeptidase of *Bacillus stearothermophilus* and *Bacillus subtilis* has been investigated (figure 3; Yocum *et al.* 1979; Waxman *et al.*, this symposium), and the results interpreted as showing homology with the sequence around the active site serine-70 of the class A β-lactamases (figures 1 and 3). The amount of sequence information is still small and statistical analysis of the similarities difficult (table 3). The sequence similarity seems to be confined to the immediate vicinity of the active site serine residue, but residues found in this region in all the β-lactamases are not found in the carboxypeptidases. I interpret the results as indicating slight structural similarity at the active site, but not giving any evidence at all for a common evolutionary origin of the two types of enzymes. Nevertheless, the results are very interesting, and progress in this area will be rapid and exciting. A crystallographic investigation of a similar cell wall synthesis carboxypeptidase has started (Knox *et al.* 1979).

The penicillin-sensitive enzymes of cell wall synthesis are all larger molecules than the class A β-lactamases (Spratt, this symposium), and so it will not be possible for homology between their structures to be complete.

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The future of structural studies on β-lactamases

The study of protein structures has been revolutionized by the advent of rapid DNA sequencing methods, and direct amino acid sequencing will no longer be attempted for proteins of molecular mass larger than about 40 000 if the gene (or the mRNA) coding for it is accessible. Nevertheless, pure preparations of proteins will still be needed for mechanistic studies and for terminal amino acid sequence investigations. The DNA sequencing approach has already demonstrated its value for β-lactamase genes in the elucidation of the sequence of the *amp* gene coding for the R_{TEM} protein (Sutcliffe 1978); this has been particularly useful because it has enabled the whole sequence of the initial ribosomal product to be deduced. Genes for β-lactamases are very suitable for genetic manipulation, as selection of recombinants is likely to be easy. Brammar (1977) has transferred the β-lactamase structural gene of *B. licheniformis* into a λ-bacteriophage, and shown that the enzyme can be produced in *E. coli* infected with the recombinant bacteriophage. Such methods will allow both the isolation of pure DNA for sequencing and the production of large amounts of enzyme protein.

Table 3. Matching of amino acid sequences of β-lactamases and d-alanine carboxypeptidases

		sequence											
		(*a*)			(*b*)			(*c*)			(*d*)		
sequence	length of fragment	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)
(*e*)	40	8 =	8	(4)	6	7	(5)	6	9	(1)	8 =	8	(4)
(*f*)	14	6 >	4	(1)	4	5	(1)	5 >	4	(1)	4 =	4	(4)
(*e1*)	23	3	7	(1)	2	6	(1)	2	7	(1)	6†	7	(1)
(*e2*)	17	5 =	5	(2)	4	5	(1)	4	5	(2)	2	5	(1)
(*e*/*f*)	14	4 =	4	(2)	3 =	3	(7)	3 =	3	(3)	1	3	(3)

Sequences (*a*)–(*f*) are as in figures 1 and 2. Sequence (*e1*) is the first 23 residues of sequence (*e*), and (*e2*) the last 17 residues. (*e*/*f*) is a sequence consisting of the residues that are identical in sequences (*e*) and (*f*), i.e Y X K N X D X X L X I A S M. The scores are shown in columns (i)–(iii): (i) is the number of identities between two sequences when they are aligned as in figure 2, with the active site serine residues coinciding; (ii) is the largest number of identities found if the two sequences are compared in any other alignment; (iii) is the number of different alignment positions that match the same as the value in (ii). Values of (i) greater than or equal to (ii) support the hypothesis of sequence similarity between the active centres of the two sets of proteins. Such values are marked > or = in the table.

† This high value is because the tetrapeptide G K I L occurs in both *E. coli* R_{TEM} β-lactamase and *B. stearothermophilus* d-alanine carboxypeptidase. Residues 54–57 are not conserved among the different β-lactamases (figure 1).

Direct amino acid sequencing methods are still competitive for the study of small proteins that can easily be isolated in 100 mg quantities, as the techniques are cheap and reliable.

In the next few years I expect that the sequences of several more β-lactamases will be determined, both by the direct method and by DNA sequencing, and high-resolution three-dimensional structures will also have been determined. As knowledge of the most diverse β-lactamases increases, we shall come to know how many separate classes of these enzymes exist. Like proteases, β-lactamases hydrolyse peptide bonds, and it will be interesting to see how many mechanistic analogues of the proteases exist as β-lactamase classes. The class A β-lactamases have features resembling the serine proteases, and the class B enzyme has a zinc prosthetic group like thermolysin and the pancreatic carboxypeptidases. Will the *p*-chloromercuribenzoate-sensitive β-lactamases prove to resemble the thiol proteases like papain?

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