

The active-site-serine penicillin-recognizing enzymes as members of the *Streptomyces* R61 DD-peptidase family

Bernard JORIS,* Jean-Marie GHUYSEN,*|| Georges DIVE,* André RENARD,† Otto DIDEBERG,‡
Paulette CHARLIER,‡ Jean-Marie FRÈRE,* Judith A. KELLY,§ Jeffrey C. BOYINGTON,§ Paul C. MOEWS§
and James R. KNOX§

*Service de Microbiologie, Université de Liège, Institut de Chimie, B6, B-4000 Sart Tilman (Liège 1), Belgium,

†Eurogentec S.A., Campus du Sart Tilman, B6 Bldg, B-4000 Liège, Belgium, ‡Service de Cristallographie, Université de Liège, Institut de Physique, B5, B-4000 Sart Tilman (Liège 1), Belgium, and §Institute of Materials Science,

Department of Molecular and Cell Biology, University of Connecticut, Storrs, CN 06268, U.S.A.

Homology searches and amino acid alignments, using the *Streptomyces* R61 DD-peptidase/penicillin-binding protein as reference, have been applied to the β -lactamases of classes A and C, the Oxa-2 β -lactamase (considered as the first known member of an additional class D), the low- M_r DD-peptidases/penicillin-binding proteins (protein no. 5 of *Escherichia coli* and *Bacillus subtilis*) and penicillin-binding domains of the high- M_r penicillin-binding proteins (PBP1A, PBP1B, PBP2 and PBP3 of *E. coli*). Though the evolutionary distance may vary considerably, all these penicillin-interactive proteins and domains appear to be members of a single superfamily of active-site-serine enzymes distinct from the classical trypsin or subtilisin families. The amino acid alignments reveal several conserved boxes that consist of strict identities or homologous amino acids. The significance of these boxes is highlighted by the known results of X-ray crystallography, chemical derivatization and site-directed-mutagenesis experiments.

INTRODUCTION

The active-site-serine DD-peptidases, involved in bacterial cell-wall metabolism, catalyse the attack of the C-terminal D-alanyl-D-alanine peptide bond in peptidoglycan precursors. They are inactivated by the β -lactam antibiotics (penicillins, cephalosporins and monobactams), whose endocyclic amide linkage is equivalent to the scissile peptide bond in the peptidoglycan precursors. In turn, the active-site-serine β -lactamases are defensive enzymes; they hydrolyse the β -lactam antibiotics into biologically inactive metabolites. These two groups of enzymes not only bind similar ligands, they also operate by a common acyl-enzyme mechanism. Central to this mechanism is the transfer of the



electrophilic group R—C of the scissile (peptide, amide) bond to the hydroxy group of the active-site serine residue. The ester-linked acyl-(penicilloyl-, cephalosporoyl-) enzymes formed by reaction between the β -lactam antibiotics and the β -lactamases are usually very short-lived. In contrast, those formed by reaction with the DD-peptidases are usually very long-lived. Consequently, the β -lactam antibiotics are substrates of the β -lactamases and mechanism-based inactivators of the DD-peptidases, which thus behave as penicillin-binding proteins (PBPs) (for reviews, see Cartwright & Waley, 1983; Ghuyesen *et al.*, 1984; Frère & Joris, 1985).

Gene sequencing has yielded the amino acid sequences of fifteen β -lactamases and DD-peptidases/PBPs (for references, see Table 1). In parallel with this, X-ray crystallography has revealed details on the three-dimensional structure and active-site environment of

some of these proteins (for references, see Table 1). On the basis of these advances, the relationship between the penicillin-recognizing enzymes has been analysed and assessed.

MATERIALS AND METHODS

Enzymes (Table 1)

The β -lactamases and the low- M_r DD-peptidase/PBP of *Streptomyces* R61 are water-soluble (periplasmic or extracellular) proteins. The precursor of the *Streptomyces* DD-peptidase, however, possesses in addition to a peptide signal, a cleavable 26-amino-acid C-terminal extension. Should it not be removed during maturation, this C-terminal extension might function as a stop-transfer sequence through which the enzyme would become membrane-bound (Duez *et al.*, 1987).

The low- M_r DD-peptidases/PBPs of *Escherichia coli* and *Bacillus subtilis* are inserted into the plasma membrane by a non-cleaved C-terminal signal-like peptide segment, whereas the bulk of the polypeptide chain is on the periplasmic side of the membrane. Replacement, by genetic engineering, of the C-terminal 21-amino-acid region of the *E. coli* PBP5 by a shorter 9-amino-acid sequence (possessing two arginine and one lysine residues) causes excretion of the PBP in the periplasm in the form of a water-soluble derivative (Ferreira *et al.*, 1988).

The high- M_r PBPs of *E. coli* are bifunctional proteins. They possess a C-terminal penicillin-binding domain that catalyses the penicillin-sensitive peptidoglycan transpeptidase reaction and an N-terminal domain that is assumed to catalyse the penicillin-insensitive peptido-

Abbreviations used: PBP(s), penicillin-binding protein(s); s.d.u., standard-deviation unit.

|| To whom correspondence and reprint requests should be sent.

Table 1. Origin and main characteristics (primary and tertiary structures) of the enzymes studied

Key to reference numbers cited in the Table below: (1) Neugebauer *et al.* (1981); (2) Sloma & Gross (1983); Madonna *et al.* (1987); (3) Sutcliffe (1978); (4) Wang & Novick (1987); (5) Dehottay *et al.* (1987); (6) Jaurin & Gründström (1981); (7) Lindberg & Normark (1986); (8) Dale *et al.* (1985); (9) Duez *et al.* (1987); (10) Broome-Smith *et al.* (1983); (11) Todd *et al.* (1986); (12) Broome-Smith *et al.* (1985a); (13) Asoh *et al.* (1986); (14) Nakamura *et al.* (1983); (15) Kelly *et al.* (1986); (16) Samraoui *et al.* (1986); (17) Knox *et al.* (1976); (18) Herzberg & Moulton (1987); (19) Dideberg *et al.* (1987); (20) Kelly *et al.* (1987).

Enzyme and bacterial origin	Abbreviation used in the following Tables and Figures, and reference	Resolution [nm (Å)] and three-dimensional structure (reference)
β -Lactamases of class A		0.35 (3.5); (15)
<i>Bacillus licheniformis</i>	B.l. (1)	
<i>Bacillus cereus</i>	B.c. (2)	0.35 (3.5); (16)
Plasmid pBR322 (TEM)	pBR322 (3)	0.5 (5); (17)
<i>Staphylococcus aureus</i>	S.a. (4)	0.25 (2.5); (18)
<i>Streptomyces albus</i> G	S.a. G (5)	0.3 (3); (19)
β -Lactamases of class C		
<i>Escherichia coli</i> K12	E.c. (6)	
<i>Citrobacter freundii</i> OS60	C.f. (7)	
β -Lactamase of class D		
<i>Salmonella typhimurium</i>	Oxa-2 (8)	
Low- M_r DD-peptidases/PBPs		
<i>Streptomyces</i> R61	S. R61 (9)	0.225 (2.25); (20)
<i>E. coli</i> (PBP5)	5 (E.c.) (10)	
<i>Bacillus subtilis</i> (PBP5)	5 (B.s.) (11)	
Penicillin-binding domains of the <i>E. coli</i> high- M_r PBPs		
1A	1A (12)	
1B	1B (12)	
2	2 (13)	
3	3 (14)	

glycan transglycosylase reaction. A gene fusion that removes the *N*-terminal 240-amino-acid region of PBP3 and links the *C*-terminal 349-amino-acid region to the *N*-terminal of the β -galactosidase results in a truncated polypeptide that still binds penicillin (Hedge & Spratt, 1984). PBP1B and PBP3 are held in the plasma membrane at their *N*-terminus, with essentially all the protein in the periplasm (Spratt *et al.*, 1987). It is assumed that the same organization applies to PBP1A and PBP2.

Amino acid alignments and homology searches

Alignments of pairs of proteins were made by using the Goad & Kanehisa procedure (1982), itself an extension of the Needleman–Wunsch algorithm (1970). In this procedure, comparisons are made from the smallest unit of significance, i.e. two amino acids, one from each protein. Each pair of amino acids is assigned a score, the value of which is based on the relative amino-acid-substitution frequencies found among families of homologous proteins (the score varies from 0 to -17 ; the more negative the score, the better the homology) (Dayhoff, 1972). All the pair combinations of amino acids are then introduced in a two-dimensional array in which all possible pathways are signified by lines connecting cells of the array. From this, the optimum-match pathway is derived by connecting those partial pathways that maximize the final score (SEQHP program; Kanehisa, 1982). This score is the sum of the individual scores of the connected cells, including a gap penalty factor every time a deletion is made. The significance of the comparison between pairs of aligned

sequences is assessed using the SEQDP program (Kanehisa, 1982). This program gives the score of the best alignment of two entire sequences according to the original algorithm of Needleman–Wunsch (1970). The significance of the score is expressed by the standard-deviation unit (S.D.U.) of the scores of a given number of random sequences (20 in the present study) of the same composition (Dayhoff, 1978). An S.D.U. value of 5 or higher indicates a statistically significant homology. A negative value is obtained when the mean random score is better than the score obtained for the two sequences under comparison.

The McLachlan procedure (Staden, 1982) was also used to present all possible comparisons between portions of pairs of proteins on a matrix in a graphical form, thus giving a rough, but immediate, estimate of the significance of any correlation. For this purpose, the two sequences to be compared are divided into all possible segments of a given length and each segment of one protein is compared with each segment of the other protein. Given that each pair of amino acids is assigned a score (ranging in this case from 2 to 27; the higher the score, the better the homology), only those segments having a total score above a certain threshold value are graphically represented. The threshold value was such that the probability that similarity occurred by chance was lower than 1 in 1000.

Prediction of secondary structures

The computerized Robson's empirical approach described by Garnier *et al.* (1978) served to estimate the

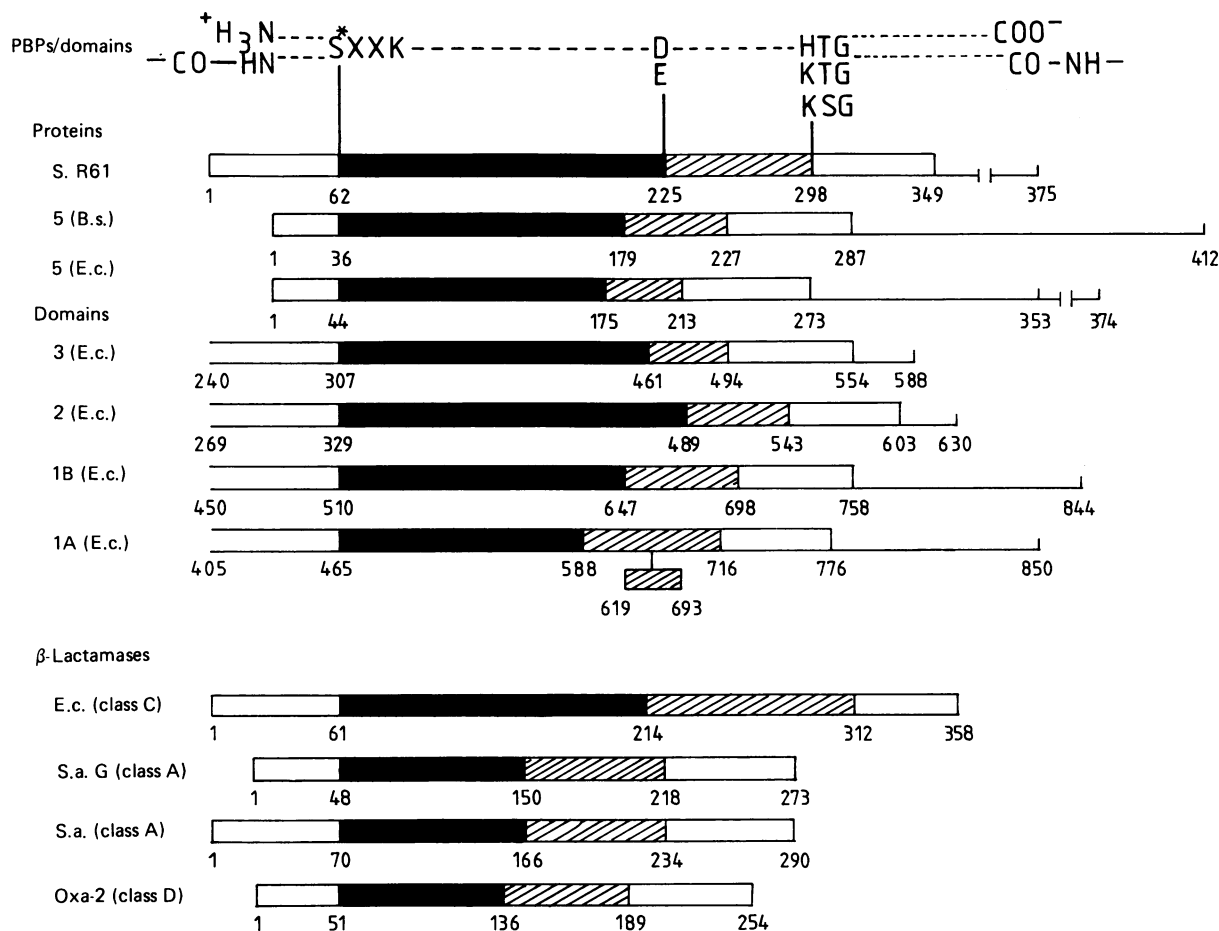


Fig. 1. Definition of the penicillin-interactive proteins and domains

The position of the conserved boxes Ser*-Xaa-Xaa-Lys, where Ser* is the active-site serine residue (box II), Asp or Glu (box V) and His-Thr-Gly, Lys-Thr-Gly or Lys-Ser-Gly (box VII) along the amino acid sequences are shown. For boxes, see also Fig. 4 and, for abbreviations, see Table 1. C-Terminal extensions assumed to be dispensable for activity (—) and peptide segments involved in plasma membrane insertion (—|—) are also shown. The 619–693 insertion in the *E. coli* PBP1A was proposed by Broome-Smith *et al.* (1985a). All the sequences are drawn at the same scale. The black and hatched areas define the segments extending between box II and box V, and between box V and box VII, respectively.

α-helix and β-strand potentials along the sequences. The selected average decision constants, –100 for the β-strands and –80 for the α-helices, were those proposed for an α/β-type structure.

The above computations were carried out on a VAX 11/780 computer.

RESULTS

Definition of the penicillin-interactive proteins and domains

The active-site serine residue (Ser*) in all the enzymes listed in Table 1 is flanked by a lysine residue at the third position on its carbonyl side (conserved sequence Ser*-Xaa-Xaa-Lys). This serine residue is close to the N-terminus of the β-lactamases, the low-M_r DD-peptidases/PBPs and the penicillin-binding (transpeptidase) domain of the *E. coli* PBP3 (at position 67, instead of 307 in the intact PBP). Moreover, all the enzymes listed in Table 1 possess a conserved triad His-Thr-Gly, Lys-Thr-Gly or Lys-Ser-Gly (the substitutions His/Lys or Thr/Ser are known to occur with high frequencies in homologous proteins). In the water-soluble β-lactamases and *Strepto-*

myces R61 DD-peptidase/PBP, this triad occurs about 60 residues upstream of the C-terminal end of the protein. On the basis of these facts, the penicillin-binding (DD-peptidase) domain was assumed to start 60 residues upstream of the active-site serine residue in the high-M_r membrane-bound PBPs and to terminate 60 residues downstream of the aforementioned triad in the low- and high-M_r membrane-bound PBPs. Fig. 1 defines the penicillin-interactive proteins and domains and shows the relative positions of the conserved tetrad Ser-Xaa-Xaa-Lys and triad His-Thr-Gly, Lys-Thr-Gly or Lys-Ser-Gly along the amino acid sequences. The amino acid numbering used for this Figure and throughout the text is that of Ambler (1980) for the class A β-lactamases of *B. licheniformis*, *B. cereus*, pBR322 and *Staphylococcus aureus*, and of Lindberg & Normark (1986) for the class-C β-lactamases. In other cases, the numbering is that given in the original papers (see Table 1). Residue 1 refers to the mature proteins.

Correlation between amino acid sequences

The amino acid sequences of the penicillin-interactive proteins and domains were compared pairwise by using

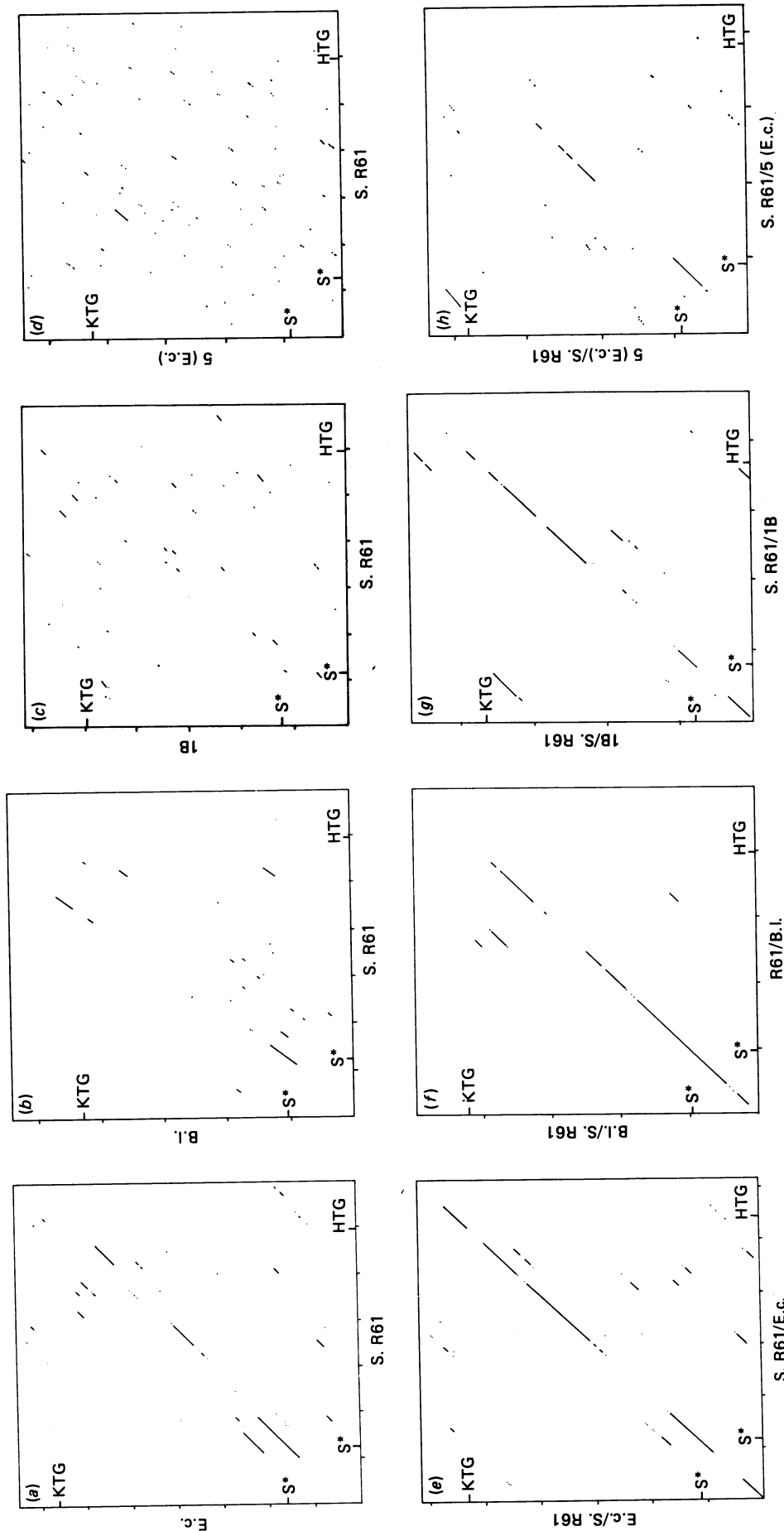


Fig. 2. Comparison matrices using McLachlan's (1971) method

Intersequence comparisons between the *Streptomyces* R61 DD-peptidase and the *E. coli* K12 β -lactamase (a, e), the *B. licheniformis* β -lactamase (b, f), the *E. coli* PBP1B (the penicillin-binding domain) (c, g), and the *E. coli* PBPs (d, h). (a), (b), (c) and (d) compare the original amino acid sequences; (e), (f), (g) and (h) compare those portions of the original sequences as they are aligned in Fig. 4. For abbreviations, see Table 1. In all cases, the span length is fixed to 25 and the selected threshold score is such that the probability that similarity between pairs of segments occurs by chance is lower than 1 in 1000. The positions of the active-site serine residue (Ser*) and the conserved box His-Thr-Gly or Lys-Thr-Gly are shown. The axes are divided into 50 amino acid segments.

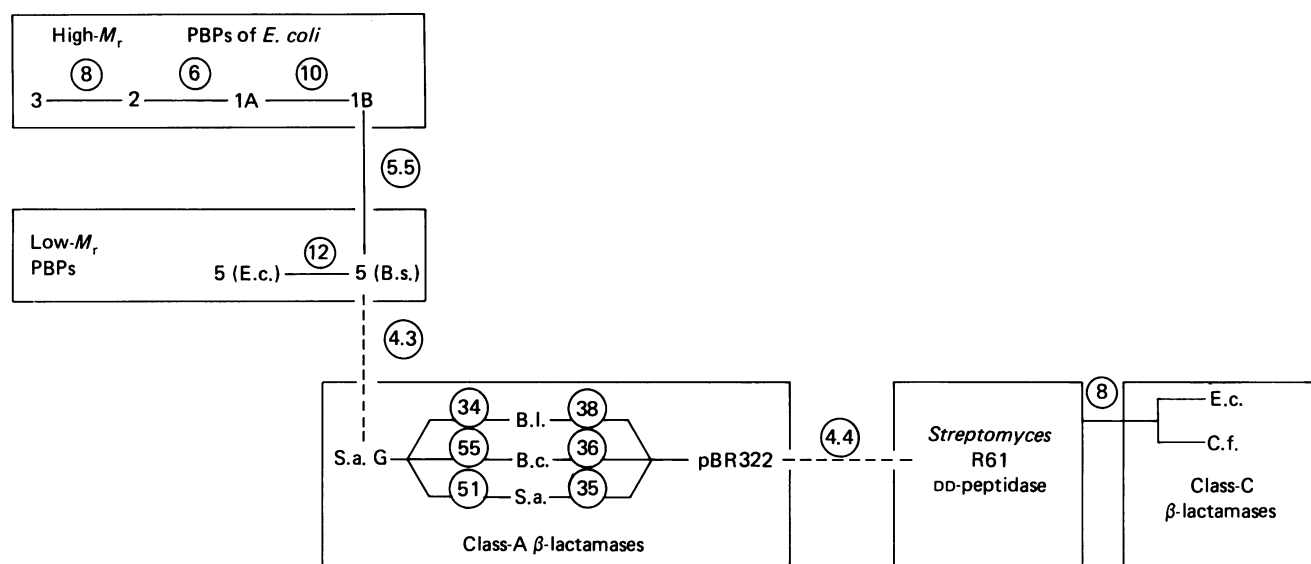


Fig. 3. Tentative family tree of the penicillin-recognizing enzymes

Note that the Oxa-2 β -lactamase of class D is not represented. Significances (in S.D.U.) are encircled. For organism abbreviations, see Table 1.

the Goad-Kanehisa (1982) algorithm and a uniform gap penalty of +8. The significance of the pair combinations was assessed by using the SEQDP program. The homology indexes, S.D.U., thus generated (Table 2) revealed that several groups of enzymes matched well or at least significantly through the amino acid sequences (S.D.U. > 5). However, when the pairs or groups thus defined were compared with each other, the overall similarity became marginally significant or completely vanished. Figs. 2a–2d illustrate the significance of the comparisons in the form of McLachlan graphs.

Reference structure and calibration marks

The above procedures are known to give consistent results only with closely related proteins. In particular, they failed to reveal homology between the *Streptomyces* R61 DD-peptidase on the one hand and the β -lactamases of class A (from *B. licheniformis*, *B. cereus*, *Staphylococcus aureus*, and *Streptomyces albus* G) on the other, in spite of the fact that, on the basis of X-ray-crystallographic studies, these proteins are very similar in terms of the spatial arrangement of secondary structures (Kelly *et al.*, 1986; Samraoui *et al.*, 1986; Herzberg & Moulton, 1987; Dideberg *et al.*, 1987). Moreover, as shown in Fig. 3, the groups of homologous enzymes highlighted by the data of Table 2 (i.e. the penicillin-binding domains of the high- M_r PBPs, the low- M_r PBPs, the β -lactamases of class A and class β -lactamases of class C) could be linked to each other through particular pairs of enzymes characterized by S.D.U. values ranging from 4.3 to 8. In this family tree, from which the Oxa-2 β -lactamase was excluded, the *Streptomyces* R61 DD-peptidase served as a bridge between the β -lactamases of class A and C.

Consequently, any possible correlation between the penicillin-interactive proteins and domains was re-examined. For this purpose, the conserved tetrad Ser*-Xaa-Xaa-Lys and triad His-Thr-Gly (in the *Streptomyces* R61 DD-peptidase/PBP), Lys-Thr-Gly or Lys-Ser-Gly (in the other penicillin-interactive proteins and domains)

were used as calibration marks. When this research was initiated, these two groups of amino acids were known to occupy critical positions in the three-dimensional structure of the *Streptomyces* R61 DD-peptidase/PBP (Kelly *et al.*, 1987). Ser*-62 was at the N-terminal end of one of the helices of the 'all- α ' region, so that, after one turn of the helix, the side chains of Lys-65 was brought back within the active-site area. In turn, the triad His²⁹⁸-Thr²⁹⁹-Gly³⁰⁰ was on the other side of the pocket on the innermost strand of the five-stranded β -sheet, with the imidazole ring also pointing to the active site.

Amino-acid-alignment editing

The *Streptomyces* R61 DD-peptidase (used as a template) and each of the other penicillin-interactive proteins and domains were aligned pairwise by selecting, among the possible partial pathways of the Goad-Kanehisa (1982) comparison matrices, those having a score of at least -30 (including the uniform gap penalty of +8). From this starting point, adjustments were made such that (i) the two calibration marks defined above were effectively aligned; (ii) the deletions/insertions were restricted to stretches possessing residues known to favour loop or turn formation (Pro, Gly, Asp); (iii) the helix and β -strand potentials [as predicted by the Robson-Garnier (Garnier *et al.*, 1978) procedure] were not, or only slightly, affected; and (iv) the alignments previously proposed for pairs or groups of homologous proteins (the class-A β -lactamases, class-C β -lactamases and high- M_r PBPs) were not, or only slightly, modified. Finally, for each pair combination, the sequence of the *Streptomyces* R61 DD-peptidase, used as reference, was kept unbroken, and the deletions and insertions required for an optimal match were introduced in each of the sequences under comparison. The results shown in Fig. 4 led to the following observations. (1) The alignments highlighted seven conserved regions or boxes (marked I–VII in Fig. 4) consisting of strict identities or homologous residues. (2) The 'cost' of the editing, in

Table 3. Search for homology between the aligned amino-acid-sequence portions (Fig. 4) of the penicillin-recognizing enzymes

The cost of editing is expressed as a percentage of residues eliminated from the original sequences. For abbreviations, see Table 1. For further explanation, see the text.

	Enzyme	Comparison score	Significance (S.D.U.)	Number of residues in the:			
				Aligned sequences	Original sequences	Cost of editing (%)	
β -Lactamases	C	E.c.	-313	40.38	309	358	13
	A	B.1.	-228	20.81	250	273	8
		B.c.	-143	9.19	243	257	6
		pBR322	-153	8.14	244	259	6
		S.a. G	-123	11.0	254	273	7
		S.a.	-127	10.92	239	254	6
D	Oxa-2	-86	10.30	207	254	18.5	
<i>E. coli</i> high- M_r PBPs	1A	-106	9.62	232	372	37 (or 23*)	
	1B	-156	11.35	235	309		
	2	-119	9.25	282	335		
	3	-197	11.45	248	308		
Low- M_r PBPs	5 (E.c.)	-77	4.13	218	273	20	
	5 (B.s.)	-181	14.27	234	287	18	

* If the large insertion occurring between positions 619 and 693 (see Fig. 4) is not included in the calculation.

terms of the percentage of amino acids eliminated from the original sequences (Table 3), ranged from 6 to 13% for the β -lactamases of class A and C, 18% for the Oxa-2 β -lactamase and did not exceed 24% for the low- M_r and high- M_r PBPs. In the case of PBP1A, the 74-residue stretch 619-693 was excluded from the calculation, as previously proposed by Broome-Smith *et al.* (1985a). (3) The portions of the original sequences that were effectively aligned generated s.d.u. values (Table 3) and gave rise to McLachlan graphs (Figs. 2e-2h) indicating a significant homology between the *Streptomyces* R61 DD-peptidase and each of the other penicillin-recognizing enzymes, including the Oxa-2 β -lactamase.

Comparison of primary and tertiary structures

Structural data (that were not available when the work described here was carried out) on the β -lactamases of *S. aureus* (Herzberg & Moulton, 1987) and *Streptomyces albus* G (Dideberg *et al.*, 1987) allowed one to position (i) the secondary structures along the amino acid alignments of Fig. 4, and (ii) the conserved 'boxes' I-VII in the known three-dimensional structures. The polypeptide 'scaffolding' and active-site configuration of these two β -lactamases are virtually identical. However, too small one-turn helices, α_3 and α_7 , in the staphylococcal β -lactamase were not numbered in the *Streptomyces* β -lactamase. Consequently, helices α_1 , α_2 , α_4 , α_5 , α_6 , α_8 , α_9 , α_{10} and α_{11} in the staphylococcal protein are equivalent to helices H₁, H₂, H₃, H₄, H₅, H₆, H₇, H₈ and H₉ respectively in the *Streptomyces* protein.

As shown in Fig. 4, the alignments made by reference to the *Streptomyces* R61 DD-peptidases/PBP did not introduce any gap in the secondary structures of the

Streptomyces and staphylococcal β -lactamases except in helix H₃ (α_4). Essentially, the deletions that were introduced in the sequences did affect the loops between helices H₂ and H₃ (α_3 and α_4) and between helices H₄ and H₅ (α_5 and α_6). Obviously, the *Streptomyces* DD-peptidase/PBP and the class-A β -lactamases have the same pattern of secondary structures, except that helix H₃ (α_4) in the *Streptomyces* DD-peptidases/PBP might be about two turns longer than the corresponding helix in the β -lactamases.

In turn, Fig. 5 shows the positions of boxes I-VII in the three-dimensional structure of the *Streptomyces albus* G β -lactamase (Dideberg *et al.*, 1987). Box I defines strand S₁, and box VII is on strand S₃. Strand S₁ is adjacent to the β -meander structure formed by strands S₃, S₄ and S₅, and strand S₃ forms one side of the active-site area (with the ϵ -amino group of lysine pointing to the active-site serine residue). Boxes II and VI are on helices H₂ (α_2) and H₇ (α_9) respectively. H₂, with the active-site serine residue at the N-terminal end, forms the back of the active-site area, and H₇ is at the surface of the 'all- α ' region. Finally, box III, on a loop connecting helices H₂ and H₃ (α_2 and α_4), and box V, on a loop connecting helices H₅ and H₆ (α_6 and α_8), are at the entrance of the cavity. Box IV occurs a few residues on the carbonyl side of box III.

DISCUSSION

The algorithms presently available for the search of correlation between amino acid sequences give consistent results only with closely related proteins. Yet, when applied to a large number of active-site-serine β -lactamases and DD-peptidases (PBPs), these procedures

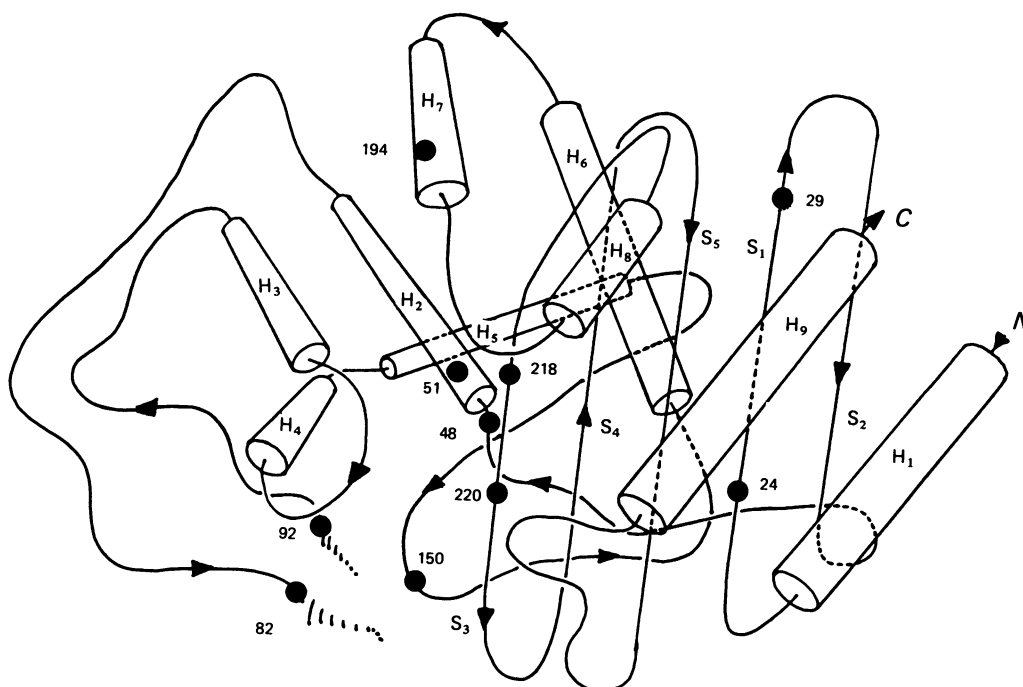


Fig. 5. Positions of boxes I-VII in the *Streptomyces albus* G β -lactamase molecule

I, Gly²⁴-Asp²⁹; II, Ser⁴⁸-Lys⁵¹; III, Gln⁸²; IV, Lys⁹²; V, Glu¹⁵⁰; VI, Trp¹⁹⁴; VII, Lys²¹⁸-Gly²²⁰.

suggest that these enzymes, except the Oxa-2 β -lactamase, behave as members of a single family tree (Fig. 3). Moreover, when aligned with reference to the *Streptomyces* R61 DD-peptidase, the β -lactamases, including the Oxa-2 β -lactamase, the low- M_r DD-peptidases/PBPs and the penicillin-binding domains of the *E. coli* high- M_r PBPs all show significant homology with the *Streptomyces* R61 DD-peptidase through major portions of the amino acid sequences (Table 3). In all likelihood these penicillin-interactive proteins and domains are related in an evolutionary sense and form a superfamily of active-site-serine enzymes. Depending on the evolutionary distance, they may have very different sequences and distinct functionalities and specificities. Yet they would share the same type of polypeptide scaffolding (distinct from that of the classical trypsin and subtilisin families). Predictional (the present paper) and structural studies support this view. Thus the 349-amino-acid DD-peptidase/PBP of *Streptomyces* R61 and the 280-amino-acid β -lactamases of class A, though lacking, at first sight, relatedness in primary structure, are very similar in the extent and distribution of the regions of secondary structures (Kelly *et al.*, 1986; Samraoui, 1986; Herzberg & Moutl, 1987; Dideberg *et al.*, 1987). In addition, all the penicillin-interactive proteins and domains possess several conserved boxes that consist of strict identities or homologous amino acids. Five of these boxes (II, III, IV, V and VII in Fig. 5) occupy critical positions in the three-dimensional structure of the *Staphylococcus aureus* and *Streptomyces albus* G β -lactamases. In parallel with this, and as discussed below, amino acid replacements in each of these boxes affect or abolish the activity of several β -lactamases and low- M_r PBP5 and high- M_r PBP3 of *E. coli*.

Box II

The importance of box II is, of course, well-established, since it contains the active-site serine residue. With the pBR322 β -lactamase it has been shown that (i) inversion of the Ser^{*}-Thr dyad to Thr-Ser provides *E. coli* with an ampicillin-sensitive phenotype (Dalbadie-McFarland *et al.*, 1982); (ii) replacement of Ser^{*} by Cys generates a thiol β -lactamase whose substrate specificity is distinct from that of the wild-type enzyme (Sigal *et al.*, 1982, 1984); (iii) alteration of Thr to many other residues has little effect, yet cells with β -lactamase mutants having Tyr, Trp, Asp, Lys or Arg at this position have no observable resistance to ampicillin (Schultz & Richards, 1986). In turn, studies carried out with *E. coli* PBP3 have shown that (i) replacement of Ser^{*} by Ala or Thr results in a protein that does not bind penicillin (Houba-Herlin *et al.*, 1985) and (ii) alteration of Thr to Pro produces an *E. coli* mutant that has high level of resistance to cephalixin (Hedge & Spratt, 1985). Note that alteration of Ser^{*} to Cys has also been examined, but has yielded conflicting results (Houba-Herlin *et al.*, 1985; Broome-Smith *et al.*, 1985*b*). Finally, a chimaeric mutant protein containing a 30-amino-acid insert which comprises box II of *E. coli* PBP5 in place of the equivalent 29-amino-acid region of the pBR322 β -lactamase, does not confer an antibiotic-resistance phenotype. This mutant has acquired detectable DD-peptidase activity towards the substrate analogue Ac₂-L-Lys-Ala-D-Ala (Richards, 1986).

Box III

Nitration of the staphylococcal β -lactamase shows that Tyr-105 of box III is readily derivatized and must occupy a particular site with a steric hindrance such that

it is prevented from participating in intermolecular cross-linking (Bristow & Virden, 1978).

Box IV

Alteration of Val-Ala-Arg of box IV to Gly-Ala-Arg results in another type of modified PBP3 with high-level resistance to cephalixin (Hedge & Spratt, 1985). Similarly, alteration of Gly, following immediately box IV, to Asp in the *E. coli* PBP5 produces a mutant protein that still binds penicillin but cannot undergo deacylation (Broome-Smith & Spratt, 1984).

Box V

Glu-168, which occurs two positions downstream of box V, is the main site of derivatization of the *B. cereus* β -lactamase by 1-(3-dimethylaminopropyl)-3-ethylcarbodi-imide. Though Glu-168 is not conserved (and probably not essential), it has been proposed that its chemical conversion might destroy catalytic activity by influencing the nearby conserved Glu-166 of box V (Little *et al.*, 1986). The fact that, as shown by X-ray-crystallographic studies (Herzberg & Moulton, 1987; Dideberg *et al.*, 1987), the Glu of box V in the *Staphylococcus aureus* and *Streptomyces albus* G β -lactamases has its side chain pointing to the enzyme's active site, strongly supports the view that this carboxylate may be important.

Box VII

The increased cephalosporinase activity of the mutant H1 of the pBR322 β -lactamase, obtained by directed selective pressure on the host cells (Hall & Knowles, 1976), can now be understood. An independent isolate having the same substrate activity spectrum as mutant H1 carries a change just after box VII, whereby Ala is replaced by Thr (W. Blattler & J. R. Knowles, personal communication). Also, alterations to Ile of either one of the two Thr residues of box VII or immediately following box VII give rise to a physiologically non-functional *E. coli* PBP3 which still, however, binds penicillin (Hedge, 1985).

The active-site-serine penicillin-interactive enzymes of the *Streptomyces* R61 DD-peptidase family differ from the peptidases of the trypsin and subtilisin families in having carboxypeptidase as against endopeptidase activity and in preferring D as opposed to L configuration in their ligands. The proposed alignments of Fig. 4 show that histidine is not a conserved residue in the fifteen penicillin-recognizing enzymes studied. Moreover, the β -lactamase of *Streptomyces albus* G, when cloned in *Streptomyces lividans*, is excreted by the host cells in the form of multiple molecular species (due to multiple cleavage sites of the signal peptide), and one of these species is histidine-free (Dehottay *et al.*, 1987). These observations also contrast the penicillin-interactive enzymes with the peptidases of the trypsin and subtilisin families, where histidine is an invariant element of the catalytic machinery. The fact remains, however, that whatever the family to which an active-site peptidase belongs, the mechanism of the catalysed rupture of the scissile peptide (amide) bond in a susceptible carbonyl donor is basically identical. In support of this view, cephalosporins, which for a long time were considered exclusively as antibacterial agents targeted against the DD-peptidases/PBPs, can be remodelled into mechanism-based inactivators of the (LL)-endopeptidases of the trypsin family (Doherty *et al.*, 1986).

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