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Comparison of the Sequences of Class A β-Lactamases and of the Secondary Structure Elements of Penicillin-Recognizing Proteins

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The sequences of class A β-lactamases were compared. Four main groups of enzymes were distinguished: those from the gram-negative organisms and bacilli and two distinct groups of *Streptomyces* spp. The *Staphylococcus aureus* PC1 enzyme, although somewhat closer to the enzyme from the *Bacillus* group, did not belong to any of the groups of β-lactamases. The similarities between the secondary structure elements of these enzymes and those of the class C β-lactamases and of the *Streptomyces* sp. strain R61 DD-peptidase were also analyzed and tentatively extended to the class D β-lactamases. A unified nomenclature of secondary structure elements is proposed for all the penicillin-recognizing enzymes.

In the last few years, many different β-lactamase sequences have become available. Most of them are active serine enzymes, and they have been divided into three major classes (classes A, C, and D) on the basis of their sequences. Crystallographic studies have revealed details of the three-dimensional structures of four class A and one class C β-lactamases and of a related penicillin-recognizing enzyme, the *Streptomyces* sp. strain R61 DD-peptidase. These data indicate, as expected, that most of the major structural features of the four class A enzymes are nearly superimposable and, in addition, that the peptidase and the class C enzyme appear to be built according to very similar patterns. Class A β-lactamases, for which the most detailed structural information is available, have been used as models for the whole group of active serine penicillin-recognizing enzymes. In this study we compared the sequences of class A β-lactamases and searched for corresponding areas in the other serine β-lactamases and the *Streptomyces* sp. strain R61 DD-peptidase.

Origins of compared sequences. (i) Class A β-lactamases. Class A β-lactamase sequences were those from *Bacillus licheniformis* 749/C, *Bacillus cereus* 569/H (type I), TEM-1, *Staphylococcus aureus* PC-1 (1), PSE-3 encoded by plasmid Rms149, and *Rhodopseudomonas capsulata* (8); SHV-1 (also called PIT-2) (5); *Bacillus cereus* 5/B (50); *Bacillus cereus* 569/H (type III) (24); LEN-1 (*Klebsiella pneumoniae*) (3); *Klebsiella oxytoca* E23004 (4); PSE-4 (7); *Actinomadura* sp. strain R39 (22); *Streptomyces albus* G (11); *Streptomyces lavendulae*, *Streptomyces badius*, and *Streptomyces fradiae* (15); and *Streptomyces aureofaciens* (2).

Two different enzymes secreted by *Streptomyces cacaoi* have been described. In this report, to distinguish the two sequences, we added the abbreviation of the institution where the gene was sequenced to the name of the strain, as follows: ULg and NDRE for Lenzini et al. (33) and Forsman et al. (15), respectively. A large number of extended-spectrum β-lactamases (SHV-2 and all the TEM enzymes) have been described recently. Sequencing of the corresponding genes often showed that they were derived from the parent enzymes by a very small number of amino acid substitutions (see, for instance, references 6 and 36). Similarly, the OHIO-1 and the *Citrobacter diversus* ULA-27 β-lactamases are closely related to the SHV-1 (47) and *K. oxytoca* (43) enzymes, respectively. Thus, only the TEM-1, SHV-1, and *K. oxytoca* enzymes were included in the comparison.

Unless otherwise stated, the ABL consensus numbering scheme of class A β-lactamases (2) is used throughout this report.

(ii) Class C β-lactamases. Class C β-lactamase sequences were those from *Escherichia coli* K-12 (26); *Citrobacter freundii* 0S60 (34); *Enterobacter cloacae* P99, Q908R, and MHN1 (16); *Serratia marcescens* SR50 (38); and *Pseudomonas aeruginosa* (35).

(iii) Class D β-lactamases. The aligned sequences of class D β-lactamases were those from OXA-1 (41), OXA-2 (9), and PSE-2 (23) were used. A consensus numbering scheme for class D β-lactamases is proposed in this report. The putative active Ser residue was arbitrarily for class D β-lactamases assigned the number 70 (as in the ABL consensus numbering scheme).

(iv) Penicillin receptor. The penicillin-binding, C-terminal domain of the *blaR* gene has been expressed as a 26,000-M_r soluble protein in *E. coli* (28). It is referred to as the BLAR-CTD protein, and its numbering is according to the class D consensus scheme.

(v) DD-Peptidase. The DD-peptidase was that from *Streptomyces* sp. strain R61 (14). The numbering system used is that of the mature protein.

MATERIALS AND METHODS

The comparison matrix (Table 1) was constructed by comparing pairwise the sequences of the β-lactamases of class A with the help of two algorithms. The distances algorithm requires aligned sequences and compares them residue by residue (12). The alignments were those of Ambler et al. (2), but the C- and N-terminal portions were deleted so that all compared sequences extended from residues 30 to 285 (ABL consensus numbering scheme). The final score represents the number of matches divided by the length of the shorter sequence, excluding the gaps. In the

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TABLE 1. Comparison of 20 class A β-lactamases

Enzyme	Comparison scores[a]																			
	LEN-1	Kl.o.	SHV-1	Rh.c.	PSE-3	PSE-4	TEM-1	A.R39	B.c.I	B.c.I5	B.c.III	Ba.l.	St.a.	S.au.	S.al.	S.c.Lg	S.c.N	S.ba.	S.fr.	S.la.
LEN-1		43	91	46	51	49	68	41	42	43	41	42	33	41	40	42	44	41	40	40
Kl.o.	−45		42	41	43	43	44	46	47	47	45	45	35	44	43	44	43	47	42	44
SHV-1	−111	−45		46	52	51	70	41	42	42	41	41	34	40	41	42	44	40	41	41
Rh.c.	−54	−45	−54		46	45	47	40	36	36	36	39	29	37	40	36	38	39	37	37
PSE-3	−57	−46	−57	−55		50	49	48	45	47	42	44	36	41	42	46	43	45	44	42
PSE-4	−58	−50	−60	−54	−64		47	40	42	42	39	38	40	37	37	37	41	40	40	38
TEM-1	−92	−48	−92	−55	−58	−56		42	44	43	42	43	36	42	42	45	46	43	42	40
A.R39	−42	−47	−42	−39	−50	−41	−40		66	66	63	64	44	46	47	54	61	65	49	49
B.c.I	−46	−49	−46	−38	−48	−48	−46	−74		90	68	67	47	47	46	56	55	61	45	44
B.c.I5	−46	−52	−46	−38	−50	−50	−47	−76	−103		68	66	47	47	47	56	55	61	47	44
B.c.III	−45	−53	−45	−39	−49	−47	−46	−74	−81	−84		76	49	45	48	53	53	57	49	47
Ba.l.	−47	−52	−47	−41	−46	−44	−48	−75	−81	−83	−94		52	41	47	53	55	62	47	45
St.a.	−33	−36	−35	−24	−33	−36	−36	−45	−50	−52	−54	−57		35	35	44	43	45	35	33
S.au.	−43	−44	−43	−34	−41	−38	−46	−41	−44	−48	−47	−43	−32		59	44	45	46	65	65
S.al.	−44	−49	−45	−34	−42	−42	−44	−44	−51	−52	−53	−50	−33	−74		45	46	49	68	68
S.c.Lg	−40	−38	−41	−32	−39	−37	−43	−52	−57	−60	−55	−54	−45	−40	−46		58	60	46	44
S.c.N	−48	−48	−47	−42	−46	−48	−52	−68	−65	−70	−66	−68	−46	−48	−50	−60		68	44	44
S.ba.	−45	−51	−45	−40	−47	−43	−46	−70	−69	−72	−69	−72	−47	−46	−50	−62	−86		48	46
S.fr.	−47	−46	−48	−33	−46	−44	−50	−48	−49	−51	−54	−52	−34	−81	−88	−47	−52	−50		76
S.la.	−46	−50	−46	−34	−44	−43	−46	−49	−49	−50	−52	−50	−30	−78	−88	−44	−48	−49	−92	

[a] The positive values above the diagonal were calculated on the basis of the distances algorithm. They represent the percentage of very similar or identical residues (see text). The negative values below the diagonal were obtained with the help of the SEQDP algorithm. The gross values were divided by 10. Kl.o., *Klebsiella oxytoca*; Rh.c., *Rhodopseudomonas capsulata*; A.R39, *Actinomadura* sp. strain R39; B.c.I, B.c.I.5, and B.c.III: β-lactamases I, I5b, and III, respectively, of *Bacillus cereus*; Ba.l., *Bacillus licheniformis*; St.a., *Staphylococcus aureus*; S.au., *Streptomyces aureofaciens*; S.al., *Streptomyces albus* G; S.c.Lg, *Streptomyces cacaoi* ULg; S.c.N, *Streptomyces cacaoi* NDRE; S.ba., *Streptomyces badius*; S.fr., *Streptomyces fradiae;* S.la., *Streptomyces lavendulae*.

present analysis, a match was scored when the substitution had a value greater than 1 in the table of Gribskov and Burgess (19), which is a normalized form of the table of Dayhoff (10). The chosen value corresponds to a high degree of structural conservation, since identical residues yield a score of 1.5, while the mean value is zero.

The SEQDP algorithm gives a score for the best alignment of two sequences, as described by Goad and Kanehisa (18) and Needleman and Wunsch (39). The final score is the sum of the individual scores obtained for each pair of residues minus a gap penalty of 8. The original table of Dayhoff (10) was used, and the scores varied from 0 to −17, with the lower value representing identical residues. The sequences that were compared were the same as those above, from residues 30 to 285 (ABL consensus numbering scheme), but the gaps were introduced by the program itself, in contrast to the distances algorithm, in which the gaps were imposed a priori.

Secondary structure predictions were performed as described by Robson and Garnier (44).

RESULTS AND DISCUSSION

Amino acid sequence comparisons. (i) Class A β-lactamases. The alignment of the class A β-lactamase sequences has been described by Ambler et al. (2). Table 1 presents the comparison matrices which were obtained with residues 30 to 285 of the ABL numbering scheme. It should be stressed that the deletion of the N- and C-terminal residues increased the similarities between the various enzymes, since the alignments show that these areas exhibit higher degrees of variation (2).

Both comparison methods yielded very similar results (Table 1). The SEQDP algorithm generally yields higher scores, since it uses a replacement matrix, while the other procedure counts matches only above a certain level. Two pairs of enzymes, SHV-1–LEN-1 and *B. cereus* I–*B. cereus* 5b, exhibited particularly high scores (greater than 90). They were not considered to be different proteins in the following analysis. The enzymes of *S. aureus* and *R. capsulata* consistently exhibited poor scores. Moreover, they were extremely different from each other; indeed, the score obtained by comparing them with each other was the lowest score of all. To position each enzyme in the complete population, we computed an average score as the mean of all the scores (the distances program was used) for a given enzyme, with the standard deviation representing the heterogeneity of its behavior. Those averages ranged from 39.5 ± 5 for the *R. capsulata* and *S. aureus* enzymes to 51 ± 10 for the *B. licheniformis* enzyme and were generally higher for the *Streptomyces* and *Bacillus* β-lactamases than for those originating from gram-negative species. Those scores were probably somewhat biased because of the presence of seven *Streptomyces* and four *Bacillus* enzymes in the sample. As a consequence, we tried to group the various enzymes into a limited number of enzyme families. The basic idea was that a given enzyme should exhibit a significantly higher average score against its own enzyme family than against the others. This resulted in the classification shown in Table 2 comprising four enzyme families and three "loners," in which each enzyme nicely fulfills that condition. The major surprise was that the *Streptomyces* enzymes fell into two distinct enzyme families and that one of them was much closer to the enzyme family of the bacilli than to that of the other *Streptomyces* spp. Moreover, the *Streptomyces* I enzyme family could not even be considered as an intermediate between the bacilli and the *Streptomyces* II enzyme families since the average score of the latter enzyme family was higher with the bacilli than with the other *Streptomyces* spp. enzyme families (46.5 versus 45.6, although the difference is probably not meaningful). The three loners were the *S. aureus*, the *K. oxytoca*, and the *R. capsulata* enzymes. Although the *S. aureus* and

TABLE 2. Classification of the enzymes and score for each family

Enzyme	Score for enzyme family:			
	1 (gram-negative bacteria)	2 (bacilli)	3 (*Streptomyces* I)	4 (*Streptomyces* II)
LEN-1 (SHV-1)	56.8	42.2	41.5	40.5
TEM-1	58.5	42.8	44.7	41.5
PSE-3	50.5	45	44.7	42.3
PSE-4	49.8	40	39.3	37.8
Mean	54 ± 4.4	42.5 ± 2	42.6 ± 2.6	40.5 ± 2
Actinomadura sp. strain R39	42.4	64	60	47.8
BcI (BcI 5b)	43.2	72.6	57.3	45.9
BcIII	41	69	54.3	47.3
B. licheniformis	41.6	68	56.7	45
Mean	42.1 ± 1	68.4 ± 3.5	57 ± 2.3	46.5 ± 1.3
S. cacaoi ULG	42.4	54.4	59	44.8
S. cacaoi NDRE	42.8	55.8	63	44.8
S. badius	41.8	61.2	64	47.3
Mean	42.3 ± 0.5	57 ± 3.6	62 ± 2.6	45.6 ± 1.4
S. aureofaciens	40.6	45.2	45	63
S. albus G	40.2	47	46.7	65
S. fradiae	41.4	47.4	46	69.7
S. lavendulae	40.2	45.8	44.7	69.7
Mean	40.6 ± 0.6	46.4 ± 1.0	45.6 ± 1.0	66.9 ± 3.4
K. oxytoca	43 ± 1	46 ± 1	44.7 ± 2.1	43.3 ± 1
S. aureus	35.8 ± 2.7	47.8 ± 3	44 ± 1	34.5 ± 1
R. capsulata	46 ± 1	37.4 ± 2	37.7 ± 1.5	37.8 ± 1.5
ROB-1	42.2 ± 2.5	53.8 ± 1.5	48.7 ± 1.5	44.2 ± 0.5
LIPEN	41 ± 2.3	69 ± 8	55 ± 1.7	44 ± 1

the *K. oxytoca* enzymes appeared to be closer to those of the bacilli and the *R. capsulata* enzyme appeared to be closer to those of the gram-negative bacteria, because of their low scores, they could not be included in those families without destroying the homogeneity of the bacilli and gram-negative bacteria enzyme families. As noted above, the three loners were also extremely different from each other. It should be noted, however, that the *K. oxytoca* enzyme was not a real loner, since the enzyme of *Citrobacter diversus* certainly belongs to the same enzyme family. The *Actinomadura* sp. strain R39 enzyme was much closer to the bacilli than to the *Streptomyces* enzymes, which is a somewhat surprising behavior for a member of the *Actinomycetales* taxonomic family. Finally, in the gram-negative bacteria, the enzymes LEN-1, SHV-1, and TEM-1 formed a rather homogeneous group. On the contrary, the enzymes PSE-3 and PSE-4 could not be considered to be more related to each other than to the enzymes from other gram-negative bacteria.

We further tested our classification with two other enzymes which were inadvertently omitted during the first analysis. Data for those two enzymes, ROB-1 (29) and LIPEN (30), appear at the bottom of Table 2, where they are compared with the various enzyme families. Clearly, LIPEN presents all the characteristics of a member of the bacilli enzyme family, including a good similarity with the *Streptomyces* I enzyme family. Although the ROB-1 enzyme was more similar to the bacilli enzyme family, it cannot be included in that family without drastically decreasing its average internal score. It could be considered a distant relative, but it also exhibits the same preference for the *Streptomyces* I enzyme family as do the true members of the bacilli enzyme family.

At present, it is not possible to build a phylogenetic tree with the proteins compared here. Pastor et al. (42) have recently proposed a tree, some aspects of which are at variance with our classification. The reasons for those differences are not clear, but it remains difficult to understand how the *K. oxytoca* and *S. albus* G enzymes, which, when compared by our criteria, exhibited a very low score of 43, could be considered to be closely related by those investigators.

(ii) Class C β-lactamases and the *Streptomyces* sp. strain R61 DD-peptidase. Alignment of the five known distinct sequences of class C β-lactamases (16, 35, 38) reveals 93 invariant residues (26%), and this does not allow an easy pinpointing of functionally important conserved residues. However, by introducing the *Streptomyces* sp. strain R61 DD-peptidase into the comparison, a more significant identification of such conserved areas could be performed (27). Recent three-dimensional data show that Y159 in the DD-peptidase superimposes on Y150 in the β-lactamases, which suggests that the YSN sequence of the peptidase corresponds to the YAN sequence of the class C β-lactamase enzymes. Moreover, the hydroxyl group of the Y residue superimposes on that of S130 in class A β-lactamases.

(iii) Class D β-lactamases and BLAR-CTD. A surprisingly

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                                                                      40
OXA1        ....MKNTIH INFAIF.LII ANIIYSSASA STDISTVASP LFEGTE...G
OXA2        ......MAIR IFAILFSIFS LATFAHAQEG TLERSDWRK. FFSEFQA.KG
PSE2        .........M KTFAAYVIIA CLSSTALAGS ITENTSWNKE .FSA.EAVNG
BLAR-CTD    .......... .......... .......PGT NVEYEDYST. FFDKFSAS.G
Consensus   ---------- ---------- ---------- --E------- -F-----A--G

                                                70                    90
OXA1        CFLLYD.AST NAEIAQFNKA KCATQMAPDS TFKIALSLMA FDAEII.DQK
OXA2        TIVVADERQA DRAMLVFDPV RSKKRYSPAS TFKIPHTLFA LDAGAVRDEF
PSE2        VFVLCKSSSK SCATN..DLA RASKEYLPAS TFKIPNAIIG LETGVIKNEH
BLAR-CTD    GFVLFN.SNR KKYT.IYNRK ESTSRFAPAS TYKVFSALLA LESGIITKND
Consensus   -FVL------ ---------- -------PAS TFKI---L-A L--G-I----

                                                                     140
OXA1        TIFKWDKTPK GMEIWNSNHT PKTWMQFSVV WVSQEITQKI RLNKIKNYLK
OXA2        QIFRWDGVNR GFAGHNQDQD LRSAMRNSTV WVYELFAKEI GDDKARRYLK
PSE2        QVFKWDGKPR AMKQWERDLT LRGAIQVSAV PVFQQIAREV GEVRMQKYLK
BLAR-CTD    SHMTWDGTQY PYKEWNQDQD LFSAMSSSTT WYFQKLDRQI GEDHLRHYLK
Consensus   --F-WDG--- ----WN-D-- L--AM--S-V WV-Q-----I G------YLK

                                                                     190
OXA1        DFDYGNQDFS GDKERNNGLT EAWLESSLKI SPEEQIQFLR KIINHNLPVK
OXA2        KIDYGNADPS TSNG...... DYWIEGSLAI SAQEQIAFLR KLYRNELPFR
PSE2        KFSYGNQNIS GG......ID KFWLEGQLRI SAVNQVEFLE SLYLNKLSAS
BLAR-CTD    SIHYGNEDFS VP.......A DYWLDGSLQI SPLEQVNILK KFYDNEFDFK
Consensus   ---YGN-D-S ---------- --WLEGSL-I S--EQ--FL- K-Y-N-L---

                                                                     240
OXA1        NSAIENTIEN MYLQDLDNST KLYGKTGAGF TANRT..... LQNGWFEGFI
OXA2        .VEHQRLVKD LMIVEAGRNW ILRAKTGWEG RMG....... WWVGWVE...
PSE2        K.ENQLIVKE ALVTEAAPEY LVHSKTGFSG VGTESNPGVA WWVGWVE...
BLAR-CTD    QSNIE.TVKD SIRLEESNGR VLSGKTGTSV INGELHAG.. WFIGYVE...
Consensus   --------VK- -----E----- -L--KTG--- ---------- W--GWVE---

                                                                     290
OXA1        ISKSGHKYVF VSALTGNLGS NLTSSIKAKK NAITILNTLN L..... ..
OXA2        ..WPTGSVFF ALNIDTPNRM DDLFKREAIV RAILRSIEAL PPNPAVNSDA AR
PSE2        ..KETEVYFF AFNMDIDNES KLPLRKSIPT KIMESEGIIG G..... ..
BLAR-CTD    ..TADNTFFF AVHIQGEKRA AGSSAAEIAL SILDKKGIYP SVSR..... ..
Consensus   --------FF A--------- ---------- ---------- ---------- --
```

FIG. 1. Aligned sequences of BLAR-CTD and class D β-lactamases. Strictly invariant residues are underlined. A residue was considered as a consensus when it was present in at least three of the proteins.

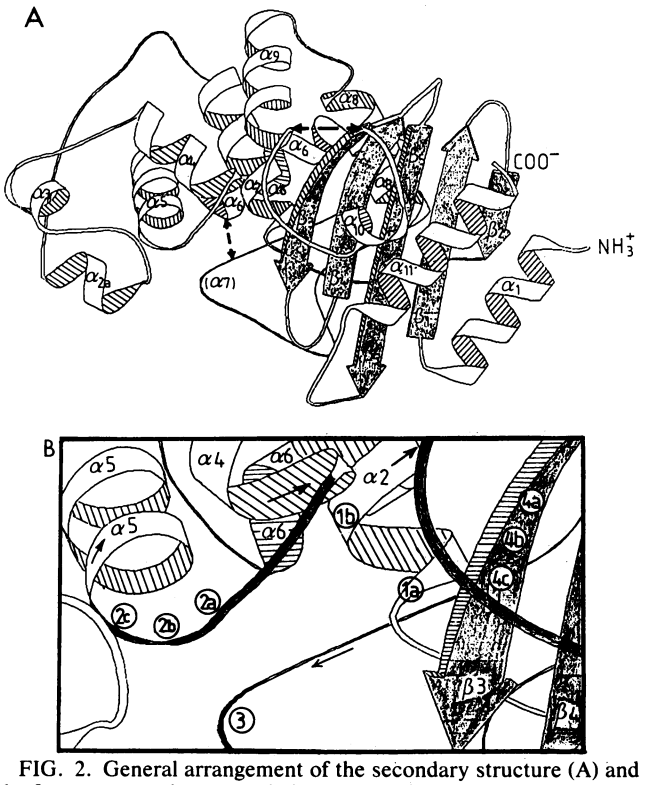

FIG. 2. General arrangement of the secondary structure (A) and the four conserved structural elements (B) in class A β-lactamases. (A) A ribbon representation of the polypeptide chain of the *S. albus* β-lactamase. The dashed lines indicate possible direct connections (see text) in class D enzymes. (B) The circles indicate the positions of the α carbon of the conserved residues composing the four structural elements described in the text. 1a is Ser-89 and ABL-70 and 1b is Lys-92 and ABL-73 of structural element 1. 2a is Ser-153 and ABL-130, 2b is Asp-154 and ABL-131, and 2c is Asn-155 and ABL-132 of structural element 2. 3 is Glu-191 and ABL-166 of structural element 3. 4a is Lys-259 and ABL-234, 4b is Thr-260 and ABL-235, and 4c is Gly-261 and ABL-236 of structural element 4. The numbering of the *S. albus* G residues includes the signal peptide. It should be noted that two errors were found in the sequence published by Dehottay et al. (11): residue Gly-128 should be eliminated and the tripeptide Ala-Gln-Leu at residues 140 to 142 should be replaced by the dipeptide Gly-Met. The complete sequence is thus two residues shorter (312 residues). Taking into account these corrections, the secondary structure assignments are as follows (numbers are residue numbers): α1, 48 to 60 (ABL 28 to 40); β1, 63 to 70 (ABL 43 to 50); β2, 76 to 79 (ABL 56 to 60); α2, 89 to 102 (ABL 70 to 83); α2a, 118 to 124 (ABL 99-103); α3, 133 to 138 (ABL 111a to 115); α4, 142 to 151 (ABL 119 to 128); α5, 155 to 165 (ABL 132 to 142); α6, 168 to 177 (ABL 145 to 154); α8 206 to 217 (ABL 185 to 194); α9, 224 to 235 (ABL 201 to 212); α10, 244 to 247 (ABL 221 to 224); β3, 253 to 260 (ABL 230 to 237); β4, 266 to 273 (ABL 244 to 251); β5, 280 to 287 (ABL 259 to 269); α11, 297 to 310 (ABL 276 to 289).

high degree of similarity has been found between the OXA-2 β-lactamase and the C-terminal domain of the product of the *blaR* gene (BLAR-CTD) (51), although BLAR-CTD exhibits only penicillin-binding properties and an exceedingly low hydrolytic activity. Figure 1 shows the aligned sequences, and Table 3 provides results of the comparisons that were performed as described above for the class A enzymes. It was remarkable to observe that the BLAR-CTD fragment exhibited higher scores with OXA-2 and PSE-2 than OXA-1. In fact, those scores characterize by far the highest degree of similarity ever found between a β-lactamase and a penicillin-binding protein. By contrast, by using the SEQDP algorithm and the same scale, the comparison between the *Streptomyces* sp. strain R61 DD-peptidase and a class C β-lactamase yielded a score of −12.4 (27) which, in that previous analysis, was the highest inter-enzyme family score.

TABLE 3. Copmparison matrix for the class D enzymes

Enzyme	Comparison scores[a]				
	β-Lactamase			BLAR-CTD	
	OXA-1	OXA-2	PSE-2	*B. licheniformis*	Tn*552*
OXA-1		30	30	33	31
OXA-2	−25		43	44	33
PSE-2	−28	−43		39	35
B. licheniformis	−32	−42	−40		47
Tn*552*	−31	−30	−31	−57	

[a] For details regarding the scores, see footnote *a* of Table 1.

A gene homologous to the *blaR* gene has been found in Tn*552*, a transposable element from *S. aureus* (45). Its sequence has been determined, and its C-terminal portion is included in the comparison presented on Table 3.

General comparison. The presently available X-ray structures show that four class A β-lactamases (13, 21, 37, 46) and one class C β-lactamase (40) and the R61 DD-peptidase (31) are composed of two domains, one α/β and one all α (Fig. 2).

TABLE 4. Four structural elements that limit the active site[a]

Enzyme	Sequence and residue no.			
	Element 1	Element 2	Element 3	Element 4
S. albus G	S-V-F-K	S-D-N	E-P-E-L-N	D-K-T-G
	70	130	166	233
S. aureus	S-T-S-K	S-D-N	E-I-E-L-N	D-K-S-G
B. licheniformis	S-T-I-K	S-D-N	E-P-E-L-N	D-K-T-G
C. freundii	S-V-S-K	Y-A-N	D-A-E-A-Y[b]	H-K-T-G
	64	150	217	314
Streptomyces sp. strain R61	S-V-T-K	Y-S-N	D-S-T-E-Q	G-H-T-G
	62	159	225	297
Class D[c]	S-T-F-K	Y-G-N	E-X-X-L-X	X-K-T-G
	70	144	175	214
BLAR-CTD[c]	S-T-Y-K	Y-G-N	D-G-S-L-Q	G-K-T-G
	70	144	175	214

[a] The numbering of the various proteins is as follows: class A β-lactamases, ABL (2), *C. freundii*, consensus class C (16); *Streptomyces* sp. strain R61: mature protein sequence (14); class D and BLAR-CTD, see Fig. 1.

[b] The identification of element 3 in the class C β-lactamase of *C. freundii* is tentative since the C_α coordinates of the enzyme are not available.

[c] For the class D β-lactamases and the BLAR-CTD, the identification of elements 1 and 4 cannot be easily challenged, but that of elements 2 and 3 is based only on sequence alignments.

The penicillin-binding site is in a crevice at the interface between the two domains. In the six enzymes, the active site is limited by four structural elements which occupy similar positions (Table 4).

(i) Element 1. Element 1 is the sequence -S*-X-X-K-, where S* is the active serine, at the N terminus of the α2 helix.

(ii) Element 2. Element 2 is the SDN loop of class A β-lactamases, corresponding, respectively, to YAN and YSN sequences in class C enzymes and the DD-peptidase. This loop is between helices α4 and α5 in class A β-lactamases. However, in class A β-lactamases, that serine appears to be involved in maintaining the functional positioning of the two domains (25) and in the protonation of the substrate's leaving group (32), roles which might be very different from that of the tyrosine in class C β-lactamases, which could act as a general base in the catalytic phenomenon (40).

(iii) Element 3. Element 3 is an acidic residue situated just before or at the N terminus of a one-turn helix or of a loop whose first three residues present helix-like characteristics. The residue is E in class A β-lactamases and D in class C β-lactamases and the peptidase. In class A β-lactamases, site-directed mutagenesis indicates that E166 (ABL consensus numbering scheme) plays an important role in catalysis (17). Accordingly, its side chain points into the substrate-binding cavity. In class C β-lactamases and the *Streptomyces* sp. strain R61 DD-peptidase, the side chain does not point into the active site, and modifications of that residue do not appear to alter the enzyme activity (20, 49). Even if, in those cases, it plays no functional role in catalysis, the reproducible presence of that residue might still represent another element that is common to all the structures.

(iv) Element 4. Element 4 is the KT(S)G sequence on the β3 strand of class A β-lactamases facing the SDN loop on the other side of the active serine. The same KTG sequence is found on the β7 strand of the class C enzyme and HTG on the β3 strand of the DD-peptidase, in similar positions.

Finally, it is interesting to remember that the SXXK and KTG elements are also found in all penicillin-binding proteins that have been described so far. Moreover, a SXN sequence, which might correspond to element 2 is also found in all penicillin-binding protein sequences (48).

Those four elements can serve as markers to compare the folding of the four classes of enzymes discussed here. By using the numbering of the helices and β strands as they occur in the sequence in class A β-lactamases (Fig. 3), the following conclusions can be made.

(i) The residues preceding the active Ser contribute one helix and two β strands to the α/β domain.

(ii) The active serine is at the N terminus of the hydrophobic helix α2, which is followed by a loop containing one short helix (α3) in the *B. licheniformis* and *S. aureus* class A β-lactamases and in the *Streptomyces* sp. strain R61 DD-peptidase. The *S. albus* G β-lactamase contains one additional helix (α2a), and the class C enzyme contains two additional short helices. This is also a hydrophilic part of the molecule where a short (12-residue) insertion occurs in class C β-lactamases and the DD-peptidase.

(iii) Thereafter, six helices occur successively in the β-lactamases (α4 to α9). When it is present, the fourth helix (α7) is very short (one turn) and sometimes reduces to a loop where two or three residues present helix-like angle values. Active-site element 2 is between α4 and α5 in all the enzymes, and element 3 is just at the beginning of the short helix α7. Helix α8 is at the border between the all α and the α/β domains. It is in contact with the β sheet and might play an important role in the stability of the whole structure. In this all-α part of the molecules, two insertions appear to occur in the class C enzyme and the DD-peptidase, one between α4 and α5 and the other between α6 and α7. This might explain why the orientation of the α4 helix is somewhat different in the class A enzyme and the DD-peptidase.

(iv) The C-terminal part of the polypeptide chain belongs to the α/β domain. Structures common to all the enzymes are, in the class A nomenclature, helices α10 and α11; β strands β3, β4, and β5; and loops between α9 and α10 and between α10 and β3. In addition to these structures, the class C enzymes contain three short β strands between α9 and α10 and another one between α10 and β3. This could

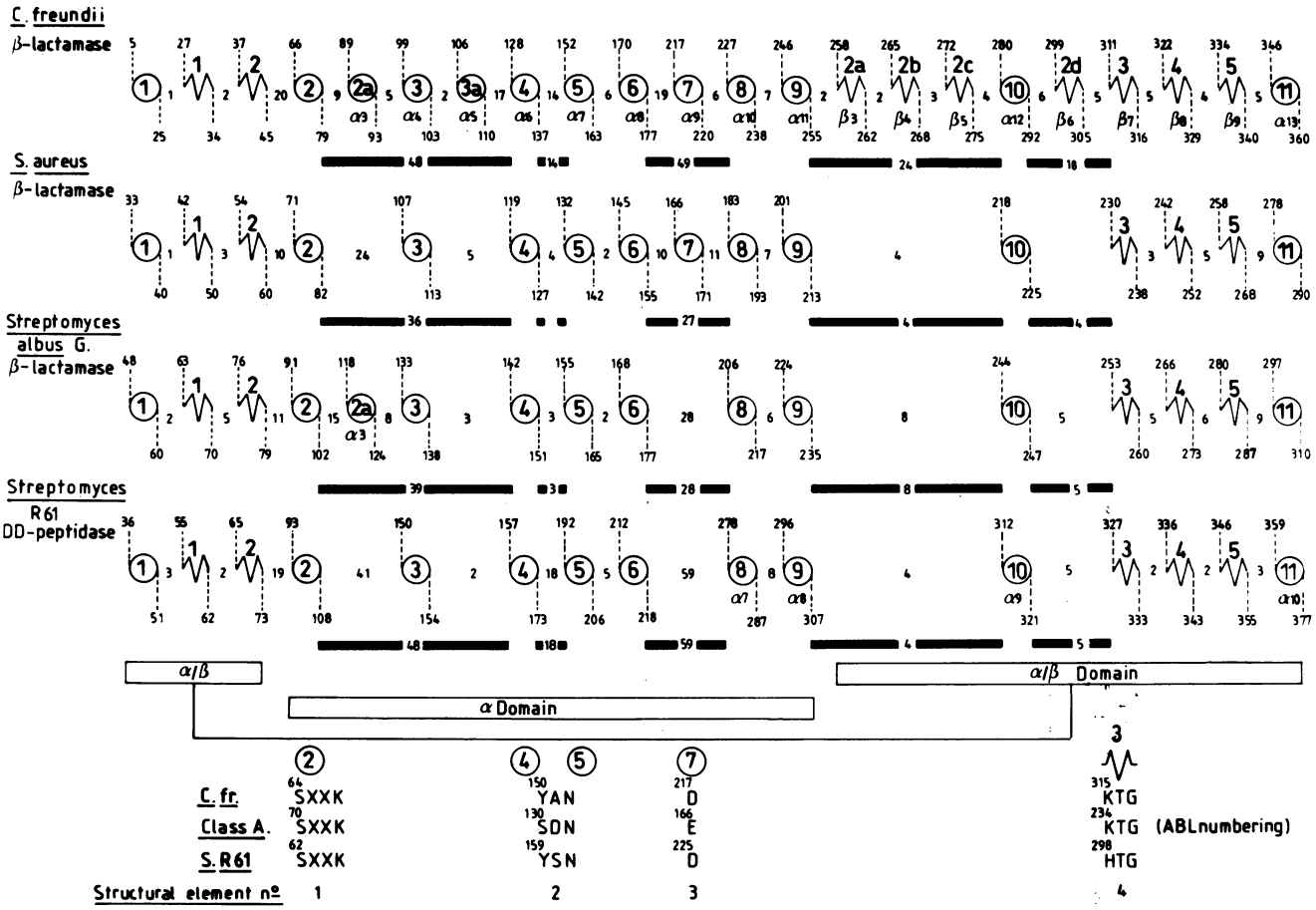

FIG. 3. Analysis of the secondary structures of the *Streptomyces* sp. strain R61 DD-peptidase and the class A and C β-lactamases. The distances (in residues) between each secondary structure element are given. The circles represent α helices, and the jagged lines represent the β strands. Heavy bars indicate the areas where important insertions are observed in the *Streptomyces* sp. strain R61 (S. R61) and class C enzymes. The consensus elements bordering the active site are highlighted in the lower part of the figure. The proposed ABL numbering of the secondary structure elements, corresponding to that of the *S. aureus* enzyme, is used for the four enzymes. When different, the original numbering is also shown under the corresponding value of the ABL consensus numbering scheme. In the proposed unified nomenclature, a secondary structure which is absent in the reference protein is assigned the number of the preceding helix or strand with an additional letter. To illustrate this point, two class A β-lactamases are included. In the *S. albus* G enzyme, an additional helix is found between *S. aureus* helices 2 and 3. It is referred to as helix α2a. Conversely, in the *S. albus* G enzyme, helix α7 reduces to a loop and cannot be considered to be a helix. The additional β strands in the class C β-lactamase are accordingly numbered β2a, β2b, β2c, and β2d, since they are inserted between β2 and β3. In the upper part of the figure, the numberings of the S.R61 DD-peptidase and of the *S. albus* β-lactamase include the signal peptides.

explain one major difference between the class C β-lactamases and the enzymes of the other classes. In class C β-lactamases, helix α10 appears to run parallel to α1 and antiparallel to α_{11}, while it has the opposite orientation in the DD-peptidase and in class A (Fig. 4). The α/β domain contains only one active-site element (KTG), which is on β3 in all cases.

At this point, we wish to stress again that, on the basis of the available knowledge, the role of structural elements 2 and 3 might be quite different in class A and C β-lactamases. Starting with the rather safe assumption that a general base is necessary for activating the hydroxyl of the active Ser residue, that general base would be E166 (element 3) in class A β-lactamases and the anion of Y150 (element 2) in class C β-lactamases.

One can also attempt to fit the sequence of class D β-lactamases to the general model, on the basis of the conserved active-site elements and secondary structure pre-

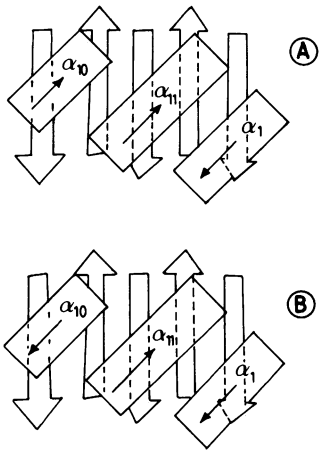

FIG. 4. Organization of the α/β domains in the class A and *Streptomyces* sp. strain R61 enzymes (A) and in class C enzymes (B).

dictions. These predictions show that the active serine is at the end of an α/β area and at the beginning of a hydrophobic helix. There are 74 residues between the active serine and the YGN element; thus, between those elements there are 30 more residues in the class D β-lactamases than there are in class A β-lactamases, but this is also an insertion area in both the DD-peptidase and the class C β-lactamases. By contrast, the distance between the YGN sequence and the third element (E140) is only 15 residues, and no secondary structure is predicted in that area. The class A structure shows that a direct passage from α5 to α7 involving the deletion of α6 might be possible. Similarly, the distance between E140 and the KTG sequence is only 49 residues, i.e., 19 residues shorter than in class A β-lactamases. Assuming that helix α8 is necessary for the stability of the molecule, one might predict, again on the basis of the class A structure, a direct passage from the C-terminal end of α9 to the N terminus of β3, thus involving the deletion of α10. Between the active Ser and residue 187, just before KTG, only helices are predicted. In the C-terminal portion (residues 187 to 255), predictions indicate an α/β domain. The proposed shortcuts in class D enzymes are shown in Fig. 2A.

In conclusion, the class A β-lactamase model can be used as a reference for all serine β-lactamases and probably for many DD-peptidases. We propose that, in addition to the ABL consensus numbering scheme (2), the numbering of class A secondary structure elements also be adopted for all active serine penicillin-recognizing enzymes, as we have done in Fig. 3.

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