Biochem. J. (1991) 279, 223–230 (Printed in Great Britain)

Amino acid sequence of the penicillin-binding protein/DD-peptidase of *Streptomyces* K15

Predicted secondary structures of the low-M_r penicillin-binding proteins of class A

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The low-M_r penicillin-binding protein (PBP)/DD-transpeptidase of *Streptomyces* K15 is synthesized in the form of a 291-amino acid-residue precursor possessing a cleavable 29-amino acid-residue signal peptide. Sequence-similarity searches and hydrophobic-cluster analysis show that the *Streptomyces* K15 enzyme, the *Escherichia coli* PBPs/DD-carboxypeptidases 5 and 6, the *Bacillus subtilis* PBP/DD-carboxypeptidase 5 and the *spo*IIA product (a putative PBP involved in the sporulation of *B. subtilis*) are structurally related and form a distinct class A of low-M_r PBPs/DD-peptidases. The distribution of the hydrophobic clusters along the amino acid sequences also shows that the *Streptomyces* K15 PBP, and by extension the other PBPs of class A, have similarity in the polypeptide folding, with the β-lactamases of class A, with as reference the *Streptomyces albus* G and *Staphylococcus aureus* β-lactamases of known three-dimensional structure. This comparison allows one to predict most of the secondary structures in the PBPs and the amino acid motifs that define the enzyme active sites.

INTRODUCTION

The low-M_r penicillin-binding proteins (PBPs)/DD-peptidases catalyse rupture of the *C*-terminal peptide bond of the bacterial wall peptidoglycan precursors and other R-D-alanyl-D-alanine-terminated analogues by sequential transfer of the R-D-alanyl moiety to an essential serine residue (with release of the leaving group D-alanine and formation of a serine ester-linked acyl-enzyme) and then to an exogenous acceptor. Penicillin inactivates the DD-peptidase activity by immobilizing the essential serine residue into a stable acyl-enzyme (Ghuysen, 1991).

The low-M_r PBP/DD-peptidase of *Streptomyces* K15 has been purified to protein homogeneity in the presence of cetyltrimethylammonium bromide (Nguyen-Distèche *et al.*, 1982). It is peculiar in at least two respects. (1) At variance with other low-M_r PBPs/DD-peptidases that are anchored into the plasma membrane by a non-cleaved *C*-terminal peptide segment (Spratt & Cromie, 1988), the *Streptomyces* K15 enzyme can be solubilized directly from the mycelium with the help of a high NaCl concentration (M. Nguyen-Distèche & M. Leyh-Bouille, unpublished work). (2) The efficacy with which the low-M_r PBPs/DD-peptidases perform hydrolysis compared with transpeptidation of the carbonyl donor depends on the efficacy with which water, the leaving group D-alanine and an exogenous peptide NH_2–X can attack the acyl (R-D-alanyl-)enzyme. Although all known PBPs/DD-peptidases act mainly as DD-carboxypeptidases, the relative acceptor activity for the acyl-(R-D-alanyl-)(*Streptomyces* K15 enzyme) is water $\ll$ D-alanine $\ll$ H_2N–X. Hydrolysis of the carbonyl donor, in the absence of H_2N–X, is negligible because attack of the acyl-enzyme by the leaving group D-alanine regenerates the original substrate. In the presence of a suitable compound NH_2–X, the carbonyl donor is quantitatively converted into a transpeptidated product, i.e. the *Streptomyces* K15 enzyme functions as a strict DD-transpeptidase (Nguyen-Distèche *et al.*, 1986).

Understanding these peculiar properties needs a precise knowledge of the structure of the *Streptomyces* K15 PBP/DD-peptidase. Gene cloning and sequencing has yielded the amino acid sequence of the protein. Pair-wise comparison with the other low-M_r PBPs of known primary structure has led to the conclusion that (1) the *Streptomyces* K15 PBP, the *Escherichia coli* PBPs 5 and 6 (Broome-Smith *et al.*, 1988), the *Bacillus subtilis* PBP5 (Todd *et al.*, 1986) and the *spo*IIA product, which is thought to be a PBP specifically involved in sporulation (Wu & Piggot, 1991), belong to a same class A of low-M_r PBPs/DD-peptidases, and (2) these PBPs adopt a polypeptide scaffolding comparable with that of the class A β-lactamases of *Staphylococcus aureus* (Herzberg & Moult, 1987) and *Streptomyces albus* G (Dideberg *et al.*, 1987; Lamotte-Brasseur *et al.*, 1991).

MATERIALS AND METHODS

Bacterial strains, plasmids and media

Streptomyces K15 was from this laboratory. Plasmid pBR322 (Bolivar *et al.*, 1977) was used for cloning experiments. *Escherichia coli* HB101 (Boyer & Roulland-Dussoix, 1969) served as host of the recombinant plasmids. *Streptomyces* K15 was grown at 28 °C in tryptone/soya broth (Oxoid) with vigorous orbital shaking. *E. coli* was grown at 37 °C in Luria Bertani (LB) or M9CA medium (Maniatis *et al.*, 1982) with vigorous shaking. Transformants were selected on LB/agar plates containing 25 μg of ampicillin/ml or 12.5 μg of tetracycline/ml.

Abbreviations used: PBP, penicillin-binding protein; HCA, hydrophobic-cluster analysis.

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The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBK Nucleotide Sequence Databases under the accession number X59965.

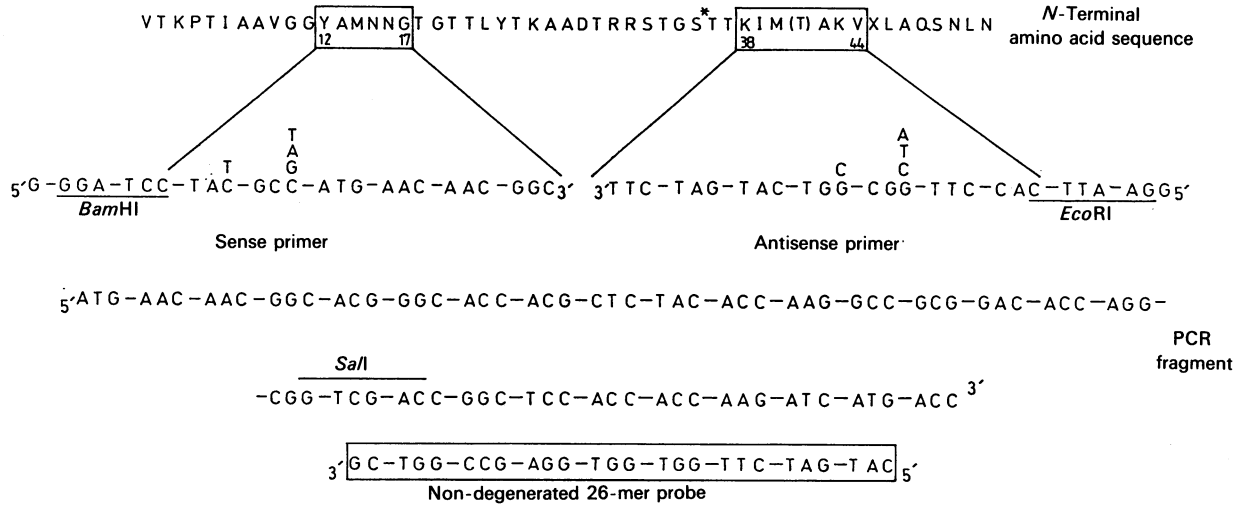

Fig. 1. DNA probe construction

Only part of the sequence of the PCR fragment is shown (see the text).

Enzymes, antibiotics, oligonucleotides and recombinant DNA techniques

Restriction endonucleases and T4 DNA ligase were from Boehringer (Mannheim, Germany) and Bethesda Research Laboratories (Gaithesburg, MD, U.S.A.); lysozyme and tetracycline were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sequenase was from United Biochemical Corp. (Cleveland, OH, U.S.A.), bacterial alkaline phosphatase was from Amersham International (Amersham, Bucks., U.K.) and ampicillin was from Beecham (Brussels, Belgium). Oligonucleotides (see below) were synthesized by Eurogentec (Liège, Belgium). The *Streptomyces* chromosomal DNA was prepared as described by Hopwood *et al.* (1985). Preparation of plasmid DNA, transformation of *E. coli*, digestion of DNA with restriction enzymes, treatment with bacterial alkaline phosphatase, ligation, agarose-gel electrophoresis of plasmids and digested DNAs and elution of separated DNA fragments were performed essentially as described in Maniatis *et al.* (1982).

Radioactive oligonucleotide probe and screening of gene libraries

On the basis of the known amino acid sequence of the *N*-terminal region of the mature *Streptomyces* K15 PBP (Leyh-Bouille *et al.*, 1989) and the known *Streptomyces* codon usage, two nucleotide primers were synthesized (Fig. 1). The sense oligonucleotide 1 had a *Bam*HI site at the 5'-end and encoded the sequence Y12AMNNG17 of the protein and the antisense oligonucleotide 2 had an *Eco*RI site at the 5'-end and was complementary of the nucleotide sequence encoding the sequence K38IMTAKV44. Polymerase chain reaction (PCR) was performed on 100 μl samples containing the *Streptomyces* K15 chromosomal DNA (1.5 μg), the primers (1 μM each), the dNTPs (200 μM each), the *Taq* DNA polymerase (2.5 units; Perkin–Elmer–Cetus, Norwalk, CT, U.S.A.) and gelatin (10 μg). The buffer was 10 mM-Tris/HCl buffer, pH 8.3, containing 50 mM-KCl and 1.5 mM-$MgCl_2$. Samples were covered with mineral oil and submitted to 30 amplification cycles: 1 min denaturation at 94 °C, 1.5 min annealing at 55 °C and 2 min polymerization at 72 °C (Saiki *et al.*, 1988; Lee *et al.*, 1988). The 110 bp-DNA PCR product was partially sequenced and the sequence translated exactly into the expected peptide segment (Fig. 1). From this nucleotide sequence, a non-degenerated 26-mer probe (Fig. 1) was synthesized, labelled with [γ-^{32}P]ATP (Maxam & Gilbert, 1980) and used to screen gene libraries by hybridization [according to the procedure described in Focus (1984); BRL, Bethesda, MD, U.S.A.]. Southern blotting was performed as described in Maniatis *et al.* (1982).

Nucleotide sequence

DNA segments cloned into bacteriophages M13mp18 and M13mp19 were sequenced by the dideoxynucleotide chain-termination method (Sanger *et al.*, 1977, 1980). The sequencing reactions were conducted with the universal M13 sequencing primer or the two synthetic oligonucleotides shown in Fig. 2. Zones of base compression due to high G+C content were resolved by using dITP instead of dGTP (Sequenase kit). For each possible reading frame the codon usage was analysed with Staden's program (Staden & McLachlan, 1982; Staden, 1982), with the gene encoding the *Streptomyces* R61 PBP/DD-peptidase (Duez *et al.*, 1987) as reference.

Amino acid analysis

Amino acid analysis was performed on a Multichrom B analyser (Beckman, Palo Alto, CA, U.S.A.). Before hydrolysis the samples were oxidized either in the gas phase or in the liquid phase and then hydrolysed *in vacuo* with 6 M-HCl at 106 °C for 24 h.

Similarity searches

The Goad & Kanehisa (1982) procedure expresses the extent of similarity between pairs of amino acid sequences by a score, the more negative the score, the better the similarity. The significance of the extent of similarity found between two aligned amino acid sequences was estimated by the SEQDP program. Scores with at least five deviations above that expected from a run of 20 randomized pairs of proteins having the same amino acid compositions as the proteins under consideration indicate significant similarity. Pair-wise amino acid sequences alignment was also optimized using the local homology algorithm of Smith & Waterman (1981) (Bestfit program in the GCG package; gap weight, 5.0; length weight, 0.3).

Prediction of secondary structures by the hydrophobic-cluster analysis

Hydrophobic-cluster analysis (HCA) was performed and the HCA sequence-similarity scores were estimated as described in

Gaboriaud *et al.* (1987). HCA is a powerful method for analysing proteins that are weakly related in the primary structure. It rests upon a representation of the protein sequences on an α-helical two-dimensional pattern (in which the hydrophobic residues tend to form clusters that usually correspond to the secondary structure elements) and compares the distribution of the clusters along the sequences. Clusters of similar shapes, sizes and relative positions express similarity in the polypeptide folding of the proteins. HCA offers the following advantages: (1) the two-dimensional plots allow distant information to become visible more readily than with methods based only on single amino acid property/identity; (2) deletions or insertions are easily introduced between the secondary structures (Henrissat *et al.*, 1990). Proteins with very close polypeptide folding have HCA score > 75%. Scores of 55–65% indicate similarity in the secondary-structure topology (Gaboriaud *et al.*, 1987; Henrissat *et al.*, 1990).

RESULTS

Gene cloning and sequencing

The genomic DNA of *Streptomyces* K15 was partially cleaved with *Bam*HI, with *Bgl*II and with *Bcl*I, and the DNA fragments were cloned in pBR322 previously cut with *Bam*HI and dephosphorylated. Of the 2990 ampicillin-resistant and tetracycline-sensitive *E. coli* transformants obtained, two clones from the *Bam*HI library and one clone from the *Bcl*I library gave a strong positive reaction with the radioactive probe after washing the filters at 75 °C (T_m − 10 °C). Two clones, one from each library, were analysed. Plasmids pDML220 from the *Bam*HI library and pDML221 from the *Bcl*I library had acquired respectively a 10.7 kb and 9.8 kb insert. They were digested with restriction endonucleases, and the DNA fragments (separated by gel electrophoresis and transferred to nitrocellulose) were submitted to hybridization with the radioactive probe. Among the smallest hybridizing fragments, the 1.3 kb *Sph*I DNA fragments originating from both pDML220 and pDML221 were subcloned into pBR322, yielding pDML223 and pDML224 respectively. As derived from the restriction maps (Fig. 2*a*), these two plasmids contained the same insert, but in opposite direction.

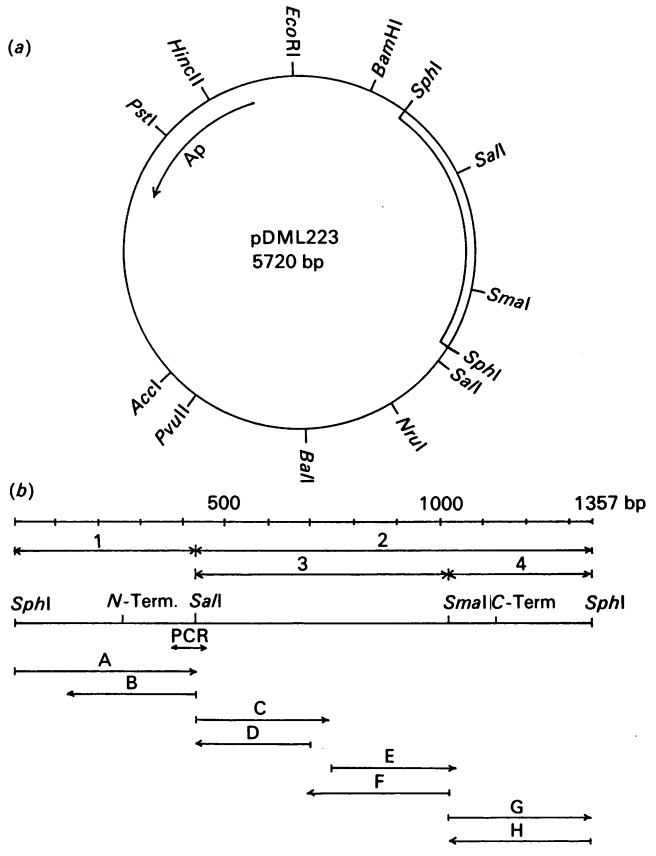

Fig. 2. Restriction map of pDML223 (*a*) and strategy of nucleotide sequencing (*b*)

The bacteriophage vectors M13mp18, M13mp19, M13tg130 and M13tg131 were used to clone the various subfragments. The sub sequences A, B, C, F, G and H were obtained by using the universal primer and the subsequences D and E by using the synthetic nucleotides corresponding to nucleotide residues 742 to 723 of the sequence and nucleotide residues 710 to 729 (see Fig. 3). The positions corresponding to the *N*-terminal and *C*-terminal residues of the DD-peptidase precursor are indicated.

Establishment of the nucleotide sequence of 90% of each strand of the 1357 bp *Sph*I DNA fragment (by using the strategy shown in Fig. 2*b*) revealed an 876-nucleotide-residue open reading frame (Fig. 3). This open reading frame starts with a GTG codon, presents the biased pattern of codon usage typical to *Streptomyces* genes (the percentage of G·C being 49, 45 and 98 at the first, second and third positions of the triplets respectively), terminates with an amber stop TAG codon and is followed by a putative terminator sequence. The 5′-ATGAAGGG-3′ segment at position −9 to −2 upstream of the translation start codon (GTG) complements five bases out of eight of the 3′-end of the 16 S rRNA of *Streptomyces lividans* (Bibb & Cohen, 1982) and resembles a ribosome-binding site (Hopwood *et al.*, 1986).

Primary structure

The 876-nucleotide-residue open reading frame translated into a 291-amino acid-residue protein precursor (Fig. 3). The 29-amino acid-residue *N*-terminal region has the features of a signal peptide; it contains three arginine residues at positions −28, −26 and −25, a long hydrophobic stretch from Ala-25 to Pro-4 and the leader peptidase-cleavage site Ala−−3-Thr-Ala−−1. The amino acid sequence of the protein from Val-1 to Asn-52 is exactly that of the *N*-terminal region of the purified membrane-bound *Streptomyces* K15 enzyme (as determined by Edman degradation; Leyh-Bouille *et al.*, 1989) and the amino acid composition of the polypeptide chain from Val-1 to Leu-262 is in excellent agreement with that of the mature protein (Table 1).

Similarity searches

Both the Goad & Kanehisa (1982) procedure (Table 2) and Bestfit program (results not shown) revealed high similarity, in the primary structure, between the *Streptomyces* K15 enzyme, the *E. coli* PBPs 5 and 6 (Broome-Smith *et al.*, 1988) and the *B. subtilis* PBP5 (Todd *et al.*, 1986) and *spo*IIA product (Wu & Piggot, 1991). No or marginal similarity was observed with the *Streptomyces* R61 PBP/DD-peptidase (Duez *et al.*, 1987), the serine β-lactamases of class A, C and D (Dehottay *et al.*, 1987; Ambler, 1980; Jaurin & Gründström, 1981; Dale *et al.*, 1985) and the penicillin-binding domains of the bifunctional high-M_r PBPs (Broome-Smith *et al.*, 1985; Asoh *et al.*, 1986; Nakamura *et al.*, 1983).

Hydrophobic cluster analysis and amino acid alignment

The upper part of Fig. 4 compares the distribution of the hydrophobic clusters along the amino acid sequences (see the Materials and methods section) of the *Streptomyces* K15 PBP, the *E. coli* PBP5 (and by extension PBP6; not shown) and the *B. subtilis* PBP5 (and by extension, the *spo*IIA product; not shown). Alignment of the conserved clusters requires very few deletions.

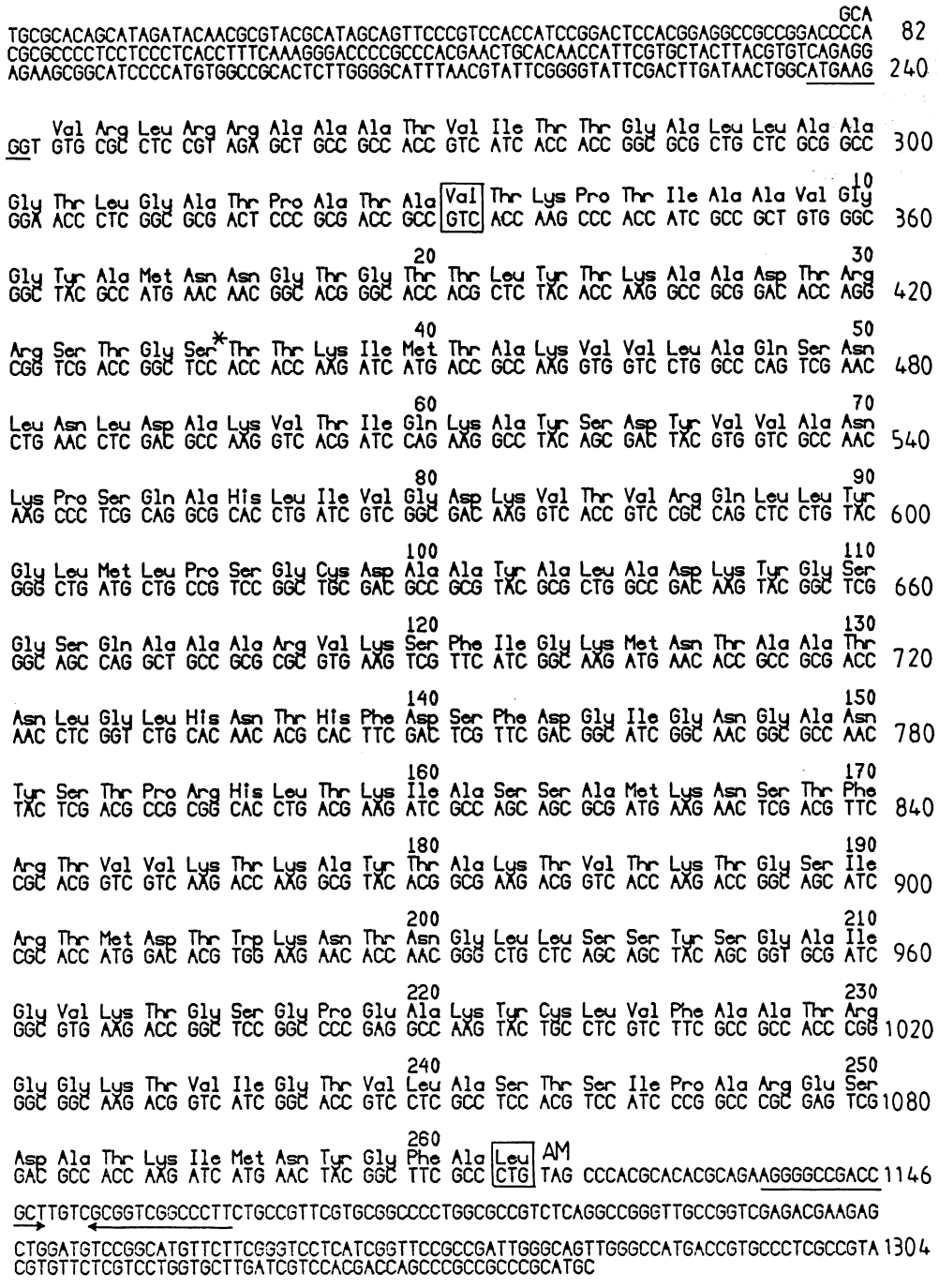

Fig. 3. Nucleotide sequence of the gene encoding the *Streptomyces* K15 PBP/DD-peptidase precursor and deduced amino acid sequence of the protein

The *N*-terminal valine residue and *C*-terminal leucine residue of the mature enzyme are boxed. The putative ribosome-binding site is underlined. The inverted repeats of the putative transcription termination signal are shown by horizontal arrows. Ser* represents the active-site serine residue.

The conserved clusters cover approx. 30% of the proteins. Of the total 14 aligned clusters, ten for the pair *Streptomyces* K15–*E. coli* (pair I) and eight for the pair *Streptomyces* K15–*B. subtilis* (pair II) have HCA scores higher than 65%. Within the conserved area, the overall HCA scores are 80% for pair I and 71% for pair II. Many charged and additional hydrophobic residues are also conserved around the clusters showing considerable sequence similarity. The lower part of Fig. 4 shows the amino acid sequence alignment as derived from the hydrophobic-cluster distribution. The cost of this comparison, expressed in percentage of residues of the original sequences that are not effectively aligned, is 8% for both pairs. The alignment generates 26% strict identities and 34% similarities for pair I, and 21% strict identities and 29% similarities for pair II. Note that, at variance with *Streptomyces* K15 PBP, the *E. coli* and *B. subtilis* PBPs have a long *C*-terminal extension. These extensions are excluded from the present analysis.

As shown above, the Goad & Kanehisa (1982) and Bestfit procedures did not highlight similarity, in the primary structure, between the three PBPs and the class A β-lactamases. HCA, however, revealed similarity, in the polypeptide folding, between the PBPs and the β-lactamases of *Staph. aureus* (Herzberg & Moult, 1987) and *Strep. albus* G (Dideberg *et al.*, 1987; Lamotte-Brasseur *et al.*, 1991). Thus pair-wise comparison (upper part of

Table 1. Amino acid composition of the *Streptomyces* K15 DD-peptidase

The amino acid composition is given as number of residues per protein molecule as derived from chemical analysis (A) and from the nucleotide sequence of the encoding gene (B).

Amino acid	Amino acid composition (residue/molecule) A	B
Lys	20	22
His	4	4
Arg	10	9
Cys	2	2
Asp	28	10
Asn		14
Met	8	7
Thr	34	34
Ser	21	22
Glu	8	2
Gln		5
Pro	7	6
Gly	28	25
Ala	34	33
Val	17	18
Ile	12	12
Leu	19	18
Tyr	10	12
Phe	6	6
Trp	–	1
Total	268	267

Fig. 5) of the *Streptomyces* K15 PBP with the *Strep. albus* G β-lactamase (pair III) and with the *Staph. aureus* β-lactamase (pair IV) shows that the conserved areas represent 25% (pair III) and 30% (pair IV) of the proteins. Within the conserved areas, the overall HCA scores are 70% (pair III) and 73% (pair IV). The HCA scores for the individual clusters vary from 50% to 89% (pair III) and from 22% to 100% (pair IV). The cost of the amino acid alignment (lower part of Fig. 5) does not exceed 12%. The sequences effectively aligned contain 15.6% strict identities and 23% similarities for pair III, and 11.5% strict identities and 19% similarities for pair IV. The significance of the similarity is expressed by a low but significant standard deviation of 9 units (pair III) and 6 units (pair IV).

DISCUSSION

The *Strep. albus* G and *Staph. aureus* β-lactamases of class A are of known three-dimensional structure. These proteins consist of an all-α domain (helices α2–α9) and an α/β domain (helices α1, α10, α11 and strands β1–β5). Their active sites are defined by four conserved amino acid motifs. Using the ABL numbering scheme, the S70VFK motif in the *Streptomyces* enzyme or S70TSK in the staphylococcal enzyme is central to the enzyme cavity with the essential S70 at the *N*-terminus of helix α2. The S130DN motif (in both enzymes), on a loop connecting helices α4 and α5, occurs on one side of the cavity. The E166PELN motif in the *Streptomyces* enzyme or E166IELN motif in the staphylococcal enzyme is on a loop at the entrance of the cavity. Finally, the K234TG motif in the *Streptomyces* enzyme or K234SG motif in the staphylococcal enzyme occurs on the innermost β3 strand of the β-sheet on the other side of the cavity.

On the basis of the principle that similarity in the hydrophobic-cluster distribution expresses similarity in the polypeptide folding of the proteins, the present study leads to the following conclusions.

(1) The *Streptomyces* K15 PBP, the *E. coli* PBP5 (and 6) and the *B. subtilis* PBP5 (and *spo*IIA product) are structurally related (Fig. 4). It is proposed that they belong to a same class A of low-M_r PBPs/DD-peptidases.

(2) The secondary structures in the *Streptomyces* K15 PBP (and the other PBPs of class A) have a spatial disposition comparable with that found in the β-lactamases of class A (Fig. 5). Identification in the PBPs of strands β1, β2, β3, β4 and β5 and of helices α2, α4, α5, α6, α8 and α11 is not a matter of controversy. Identification in the PBPs of clusters equivalent to helices α3, α7, α9 and α10 in the β-lactamases is hypothetical. Note, however, the presence of one glycine residue in helix α7 and of two asparagine residues on the *N*-terminal side of helix α10 in all PBPs.

(3) Three of the four motifs that define the active site of the β-lactamases (see above) have homologues in the *Streptomyces* K15, *E. coli* and *B. subtilis* PBPs. Thus the STTK, SLTK or SMTK tetrad of the PBPs (as they are listed) are homologues of the S70XXK motif of the β-lactamases, the SGC, SGN or SAN

Table 2. Search for sequence similarity between the amino acid sequences of several low-M_r PBPs and β-lactamases by using the Goad & Kanehisa (1982) method

Comparison scores (below the diagonal line) and significance in SDU (above the diagonal line) are shown.

	1. *Streptomyces* K15 PBP	2. *E. coli* PBP5	3. *E. coli* PBP6	4. *B. subtilis* *Spo*IIA	5. *B. subtilis* PBP5	6. *Streptomyces* R61 PBP	7. *Staph. aureus* β-lactamase	8. *Strep. albus* G β-lactamase
1. *Streptomyces* K15 PBP		22	16	28	14	−0.5	6	3
2. *E. coli* PBP5*	−230		106	29	12	0.9	0.4	−0.7
3. *E. coli* PBP6*	−225	−1270		24	21	2	0.6	0.8
4. *B. subtilis* *Spo*IIA*	−290	−460	−500		25	−0.3	0.3	−0.6
5. *B. subtilis* PBP5*	−180	−170	−300	−390		3	2	4
6. *Streptomyces* R61 PBP	−40	−50	−70	−30	−80		3.6	0.01
7. *Staph. aureus* β-lactamase	−110	−50	−40	−70	−70	−60		61
8. *Strep. albus* G β-lactamase	−50	−25	−45	−20	−70	−35	−330	

* The *C*-terminal extensions of the *E. coli* PBP5 (from P267 to G374), PBP6 (from T262 to S373), the *B. subtilis* PBP5 (from E283 to W412) and *Spo*IIA (from P284 to K389) have been eliminated for the comparison.

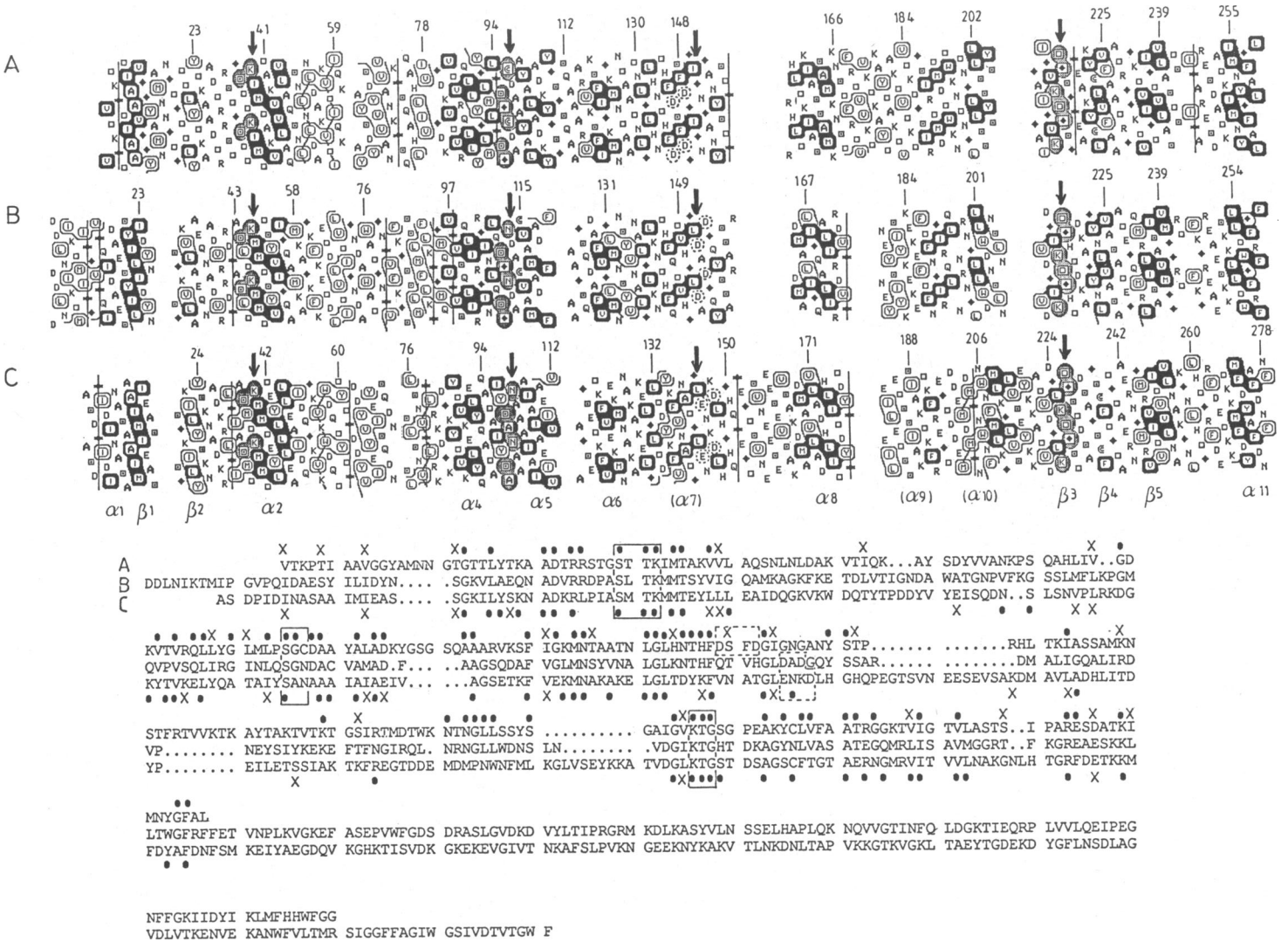

Fig. 4. Pair-wise comparison between the *Streptomyces* K15 PBP (A), *E. coli* PBP5 (B) and *B. subtilis* PBP5 (C): hydrophobic-cluster distribution (upper part) and amino acid sequence alignment (lower part)

Upper part. The *C*-terminal extensions of the *E. coli* PBP5 (from E263 to G374) and *B. subtilis* PBP5 (from S280 to F412) are not shown. Hydrophobic residues are encircled and hydrophobic clusters are also delineated. Proposed homologous hydrophobic residues are in bold. Motifs defining the active sites are shaded and indicated by vertical arrows (for more details see the text). Proline is symbolized by *, glycine by ◆, cysteine by ⋐, serine by ⊡ and threonine by □. The indicated α-helices and β-strands are derived from the comparison between the *Streptomyces* K15 PBP and the class A β-lactamases (see Fig. 5). Amino acid numbering refers to the mature proteins. Lower part. ●, Strict identities; ×, similarities between pair A–B (above) and pair A–C (below). Motifs defining the active-sites are boxed.

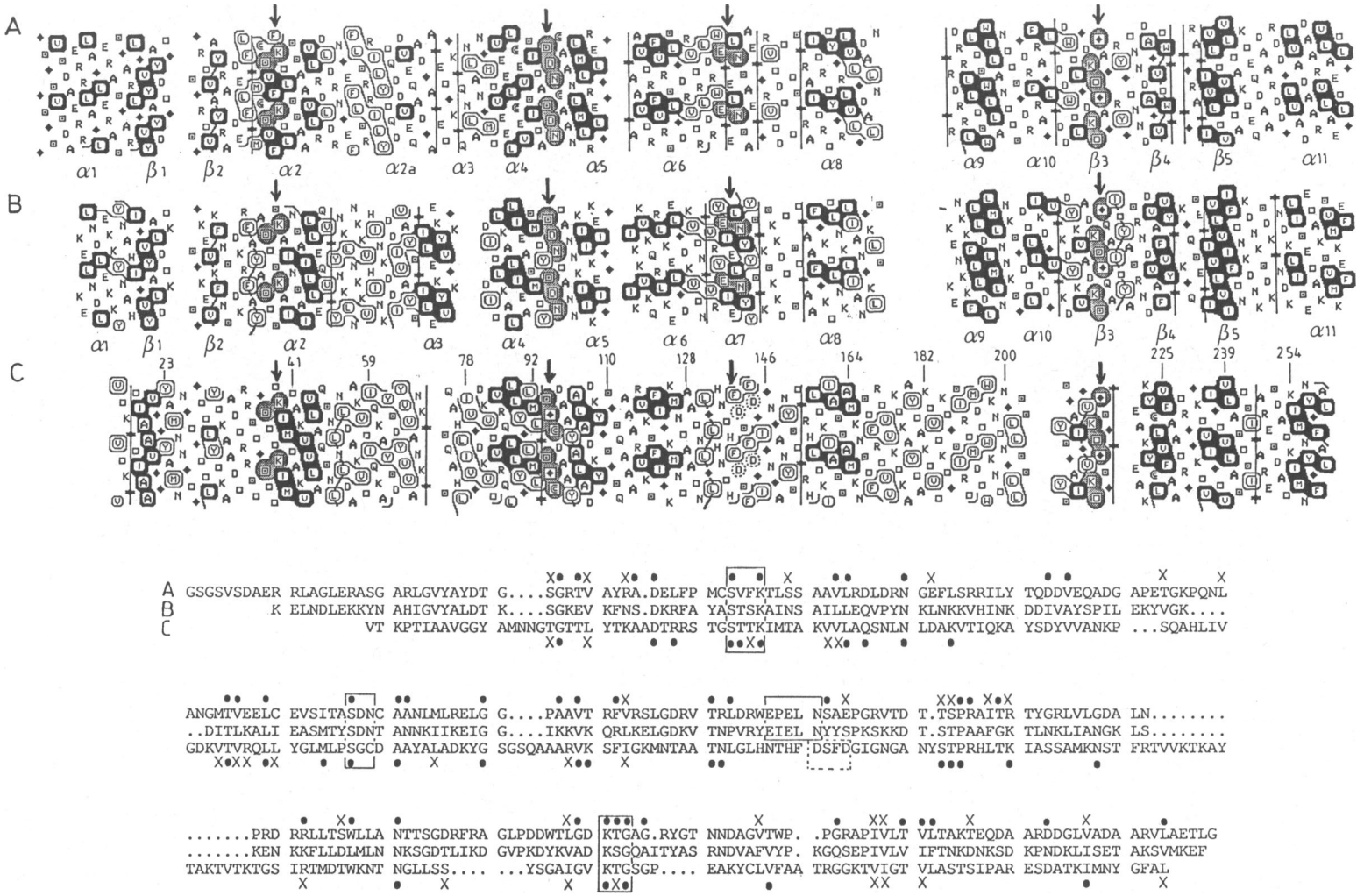

Fig. 5. Pair-wise comparison between the *Streptomyces* K15 PBP (C) and the class A β-lactamases of *Strep. albus* G (A) and *Staph. aureus* (B) used as reference: hydrophobic-cluster distribution (upper part) and amino acid sequence alignment (lower part)

Upper part. For details see the legend of Fig. 4 except that the hydrophobic residues shown in bold in A and B belong to the indicated secondary structures as determined by X-ray crystallography of the β-lactamases. The residues shown in bold in C are homologues of those of the β-lactamases. The amino acid numbering is that of the ABL scheme. Localization of secondary structures is as follows. A: α1, S27–S40; β1, R43–D50; β2, T56–Y60; α2, S70–D83; α2a, Q99g–A105; α3, P112–N115b; α4, V119–T128; α5, C133–L142; α6, P145–R153; α8, P183–V194; α9, P201–L212; α10, R222–L225; β3, T230–A237; β4, N245–W251; β5, I259–T265; α11, D276–T289. B: α1, L33–Y40; β1, R42–D50; β2, G54–F60; α2, T71–E82; α3, P107–V113; α4, L119–M127; α5, N132–I142; α6, I145–L155; α7, E166–Y171; α8, P183–L193; α9, K201–N213; α10, D218–V225; β3, K230–R238; β4, R242–P252; β5, P258–T268; α11, L279–F290. Lower part. The amino acids marked ● or × indicate identities or similarities between pair A–C (above) and pair B–C (below).

triad are homologues of the S130DN motif, and the KTG motif is the homologue of the K234T(S)G motif. In addition, the DSFD, DAD or ENKD groupings of the PBPs might be homologues of the E166XELN motif of the β-lactamases. The conserved motifs play essential roles in the catalytic mechanism of the β-lactamases. Their homologues may fulfil similar functions in the PBPs.

(4) At variance with the β-lactamases and the *Streptomyces* K15 PBP whose polypeptide chain terminates about 60 residues downstream from the KT(S)G motif, the *E. coli* and *B. subtilis* PBPs have long additional *C*-terminal extensions themselves each terminated by an amphiphilic α-helical membrane anchor (Ferreira *et al.*, 1988). With reference to the β-lactamases, the *Streptomyces* K15 PBP appears to have much shorter α1 and α11 helices and thereby its β-sheet is partially uncovered on one face, thus exposing a zone of hydrophobicity potential. This zone might be the site of interaction between the protein and the plasma membrane.

(5) The *Streptomyces* K15 PBP (Leyh-Bouille *et al.*, 1987) and *E. coli* PBP5 (Curtis & Strominger, 1978) are susceptible to thiol-specific reagents. In the *Streptomyces* K15 PBP, one cysteine residue (the SGC motif) occurs within the active site on the loop connecting helices α4 and α5 and another occurs ten residues downstream of the lysine residue of the KTG motif, i.e. on strand β4. Which of these two cysteine residues is the site of reaction with *p*-chloromercuribenzoate can be definitely established only by site-directed mutagenesis. The *E. coli* PBP5 possesses one single cysteine residue at the third position immediately downstream from the SGN motif, i.e. in the immediate vicinity of the active site. Finally, one single cysteine residue, ten residues downstream from the KTG motif (i.e. on strand β4) is also found in the *B. subtilis* PBP5 and in the *Spo*IIA protein. Whether or not these two proteins are susceptible to thio-specific reagents is unknown.

The work in Liège was supported in part by the Fonds de la Recherche Scientifique Médicale (Contract n° 3.4537.88), an Action Concertée with the Belgian Government (Convention 86/91-90), the Fonds de Recherche de la Faculté de Médecine ULg and a Convention Tripartite between the Région Wallonne, SmithKline Beecham, U.K., and the University of Liège. This text presents research results of the Belgian programme on Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister's Office, Science Policy Programming. The scientific responsibility is assumed by its authors. P.P.-M. was a fellow of the European Communities (Training Contract n° 010197). Some of the work described in this paper forms part of a dissertation presented by P.P.-M. as partial fulfilment for a Ph.D. degree at the University of Granada (Spain). The work in Ghent was supported by the National Incentive Program of Fundamental Research in Life Sciences initiated by the Belgian Science Policy Programming Department (Bio22). We thank D. Vaira (University of Liège) for assistance in PCR and D. Allaer (Eurogentec, Liège) for helpful biochemical discussion.

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Received 4 March 1991/24 April 1991; accepted 8 May 1991