MODULAR DESIGN OF THE BI(MULTI ?)FUNCTIONAL PENICILLIN-BINDING PROTEINS

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INTRODUCTION

The penicilloyl serine transferases are proteins or protein domains that catalyse rupture of the ß-lactam amide bond of penicillin and transfer of the penicilloyl moiety to an essential serine. The serine-ester-linked penicilloyl derivatives formed by reaction with the ß-lactamases are hydrolytically labile. Those formed by reaction with the penicillin-binding proteins (PBPs) are hydrolytically inert. Penicilloylation of the essential serine of some PBPs produces a signal that is transmitted to the cytosol. Penicilloylation of other PBPs causes loss of a catalytic function related to wall peptidoglycan synthesis. For a recent review and list of references, see Ghuysen (1991).

The defensive ß-lactamases are secretory proteins. Some of them are produced in maximal amounts only in the presence of ß-lactam antibiotics. The high-Mr PBP BlaR is the penicillin sensory-transducer responsible for the induction of ß-lactamase synthesis in Bacillus licheniformis. The penicillin-binding C-terminal domain, i.e. the sensor, is extracellular and is fused at its amino side to a three-transmembrane segment transducer. The extracellular domain of the transducer is responsible for the reception of the signal generated by penicilloylation of the active-site serine of the sensor and the cytosolic domain is responsible for the generation of an intracellular signal that launches ß-lactamase synthesis.

As with the reactions they catalyse in vitro on D-alanyl-D-alanine-terminated peptides, the monofunctional low-Mr PBPs are peptidoglycan-remodelling DD-peptidases and help control the extent of peptidoglycan crosslinking. Some monofunctional PBPs are secretory proteins. Most of them are membrane-bound. The extracellular penicillin-sensitive DD-peptidase domain has a C-terminal extension the end of which serves as membrane anchor.

The high-Mr PBPs involved in wall peptidoglycan synthesis contain a penicillin-binding C-terminal domain and a several hundred amino acid residue N-terminal domain. Membrane anchoring is at the amino terminus so that the bulk of the protein is extracellular. As with the reactions they catalyse in vitro on lipid-linked disaccharide-(D-alanyl-D-alanine-terminated) pentapeptide precursors, the isolated PBPs 1A and 1B of Escherichia coli (Broome-Smith et al., 1985) are peptidoglycan-synthe-

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sizing enzymes. The N-terminal domain functions as a penicillin-insensitive transglycosylase (performing glycan chain elongation) while the C-terminal domain functions as a penicillin-sensitive DD-transpeptidase (performing peptide crosslinking). The isolated PBP 2 (Asoh et al., 1986) and PBP3 (Nakamura et al., 1983) of E. coli are inert in vitro on lipid-linked peptidoglycan precursors. They play important roles in cell morphogenesis in conjunction with intrinsic membrane proteins. The pair PBP2-RodA is responsible for the rod shape of the cell and the pair PBP3-FtsW is responsible for septum formation and cell division. PBP 2' of Staphylococcus aureus (Song et al., 1987), PBP5 of Enterococcus hirae R40 (El Kharroubi et al., 1991) and PBP3r of E. hirae S185r (Piras et al., 1990, and unpublished data) confer penicillin resistance to the relevant strains. They are thought to be able to take over the functions needed for wall peptidoglycan assembly under conditions in which the other PBPs are inactivated. PBPs 1a (Martin et al., 1992), 2X (Laible et al., 1989) and 2b (Dowson et al., 1989) of Streptococcus pneumoniae, PBP2 of Neisseria meningitidis (Zhang and Spratt, 1989) and PBP2 of Neisseria gonorrhoeae (Spratt, 1988) are also of known primary structure.

Four ß-lactamases and, to a lesser resolution, one monofunctional secretory PBP are of known 3-D structure. They consist of two structural domains : an all-α domain and an α/ß domain whose five-stranded ß-sheet is protected on both faces by additional helices. As a result of the folding of the polypeptide chain, three conserved amino acid groupings are brought close to each other and form part of the active site. Using the numbering of the ß-lactamases of class A, the essential serine of the S70XXK motif is at the N-terminus of helix α2 of the all-α domain, the S130DN motif, on a loop connecting helices α4 and α5 of the all-α domain, occurs on one side of the cavity, and the K234T(S)G motif, on the innermost ß3 strand of the ß-sheet, is on the other side of the cavity.

Based on homology searches and hydrophobic cluster analysis, the ß-lactamases of class C and D, the monofunctional DD-peptidases/PBPs and the BlaR penicillin sensor for which no X-ray data are available, have, in all likelihood, the same folding and active-site defining motifs (or analogues) as the ß-lactamases of class A used as reference for structural modelling (Joris et al., 1991; Palomeque-Messia et al., 1991; Granier et al., 1992). One peculiarity of the ß-lactamases of class A is the presence of a fourth marker, the E166XELN pentapeptide. This marker occurs on a loop connecting helices α7 and α8 of the all-α domain at the entrance of the active site. It is an important component of the catalytic machinery (Lamotte-Brasseur et al., 1991).

In order to shed light on their molecular organization, the twelve high-Mr bifunctional PBPs of known primary structure, mentioned above, have been examined by hydrophobic-cluster analysis.

MATERIAL AND METHODS

Hydrophobic cluster analysis (HCA) is a powerful method for comparing proteins that are weakely related in the primary structure (Gaboriaud et al, 1987). It rests upon a duplicated representation of the amino acid sequences on an α-helical two-dimensional pattern (in which the hydrophobic residues tend to form clusters) and compares the distribution of the hydrophobic clusters along the sequences. In this representation, the hydrophobic residues F, I, L, M, V, W, Y are encircled, the hydrophobic clusters are also delineated and different symbols are used for P (⋇), G (◆), S (▣), T (□) and C (Ⓒ). The shapes of the clusters are usually associated with definite secondary structures. Clusters of similar shapes, sizes and relative positions express similarity in the polypeptide folding. When compared to methods based only on single amino acid property/identity, HCA allows distant information to become visible more readily (since the six residues adjacent to the amino acid i are i-4, i-3,

i-1, i+1, i+3 and i+4) and allows deletions/insertions to be introduced more easily between the secondary structures.

By analogy with the ß-lactamases and secretory monofunctional DD-peptidases/PBPs, the penicillin-binding C-terminal domain of the high-Mr PBPs is assumed to start ≈ 60 residues upstream from the SXXK motif. On this basis, the HCA plots have been divided into two parts which extend upstream and downstream from the amino end of the C-terminal domain, respectively. In these representations, the hydrophobic residues and clusters occurring at equivalent places in pairs or groups of proteins are in bold; the amino acid residues A, C, D, E, G, H, K, N, P, Q, R, S, T occurring as strict identities are marked by scattered points; and the SXXK, SDN and KT(S)G motifs (or analogues) are in dashed spots.

There is a high extent of similarity in the amino acid sequence between the E. hirae PBPs 5 and 3r (73 % and 85 % identities for the N-terminal and C-terminal domains, respectively) and between the N. meningitidis PBP2 and N. gonorrhoeae PBP2 (97 % and 99 % identities). Consequently, the HCA plots shown for the E. hirae PBP5 and N. meningitidis PBP2 apply to the E. hirae PBP3r and N. gonorrhoeae PBP2, respectively.

RESULTS

The twelve high-Mr PBPs under study are proteins of varying molecular masses. The number of constituent amino acid residues range from about 580 (the N. meningitidis PBP2 and E. coli PBP3) to about 850 (the E. coli PBPs 1A and 1B). Hydrophobic cluster analysis leads to the following observations.

Based on the identification of large polypeptide segments that have significant similarity in the distribution of the hydrophobic and hydrophilic clusters, the high-Mr PBPs fall into groups. Members of a given pair or triad possess several homologous segments, i.e. modules, whose conserved clusters align easily and cover a large proportion of the amino acid sequences.

The modules thus defined help solve the molecular organization of the PBPs. The simplest pattern is that of a three-module protein. The membrane anchor, usually 40 to 60 amino acid residues long, is linked to the N-terminal domain which is linked to the C-terminal domain. Moreover, the carboxy end of the C-terminal domain, about 60 to 80 amino acid residues downstream from the KT(S)G motif, is the carboxy end of the protein. This pattern is found in the E. coli PBP3 (Fig. 1a,b), N. meningitidis PBP2 (Fig. 1a,b), S. pneumoniae PBP2b (Fig. 1a,b) and E. coli PBP2 (Fig. 2a,b).

The other PBPs are of a more complex design due to the presence of large inserts. The E. coli PBP1B has the hydrophobic membrane anchor (the G64-L87 transmembrane α-helix) in the middle of a 185 amino acid residue N-terminal extension (Fig. 3a). The S. aureus PBP2' and E. hirae PBP5 have a highly homologous ≈ 100 amino acid residue polypeptide inserted between the carboxy side of the membrane anchor and the amino side of the N-terminal domain (Fig. 2a). Note that the N-terminal domains of the S. aureus PBP2' and E. hirae PBP5 are similar to the N-terminal domain of the E. coli PBP2 (Fig. 2a). The E. coli PBP1A has a 90 amino acid residue intervening sequence that links the carboxy side of the N-terminal domain to the amino side of the C-terminal domain (Fig. 3a). The C-terminal domains of the S. pneumoniae PBP2X (Fig. 1b), E. coli PBP1A, E. coli PBP1B and S. pneumoniae PBP1a (Fig. 3b) bear a tail, 80 to 100 amino acid residues long. Inserts - 40 to 50 amino acid residues long-also occur in the N-terminal domain of the S. pneumoniae PBP2b (Fig. 1a) and in the C-terminal domains of the S. pneumoniae PBP2X (Fig. 1b) and E. coli PBP1A (Fig. 3b).

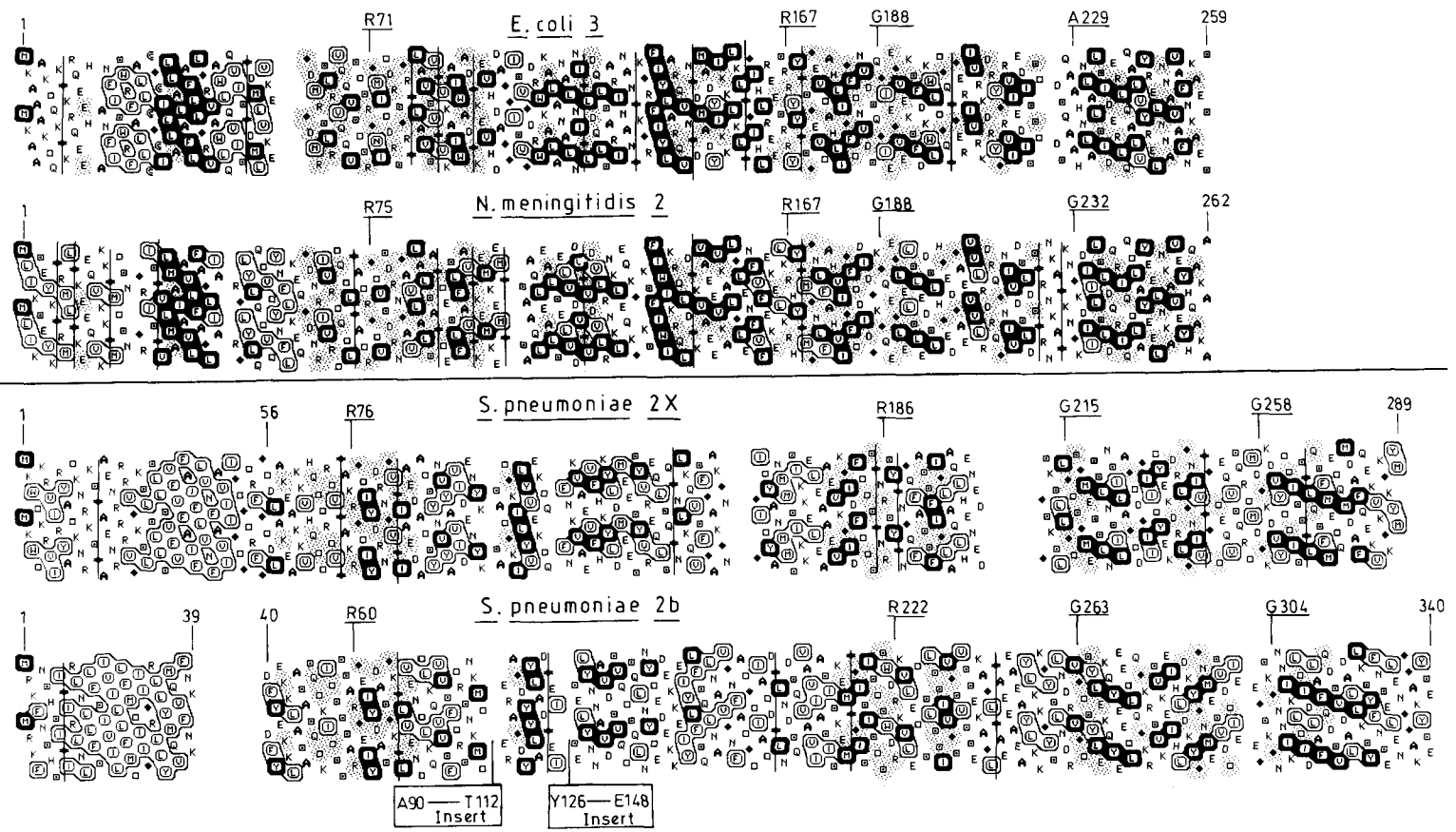

Fig. 1a. HCA plots of the pair E.coli PBP3-N.meningitidis PBP2 and the pair S. pneumoniae PBPs 2X and 2b. Analysed sequences : from the amino end of the PBPs to the carboxy end of the N-terminal domains.

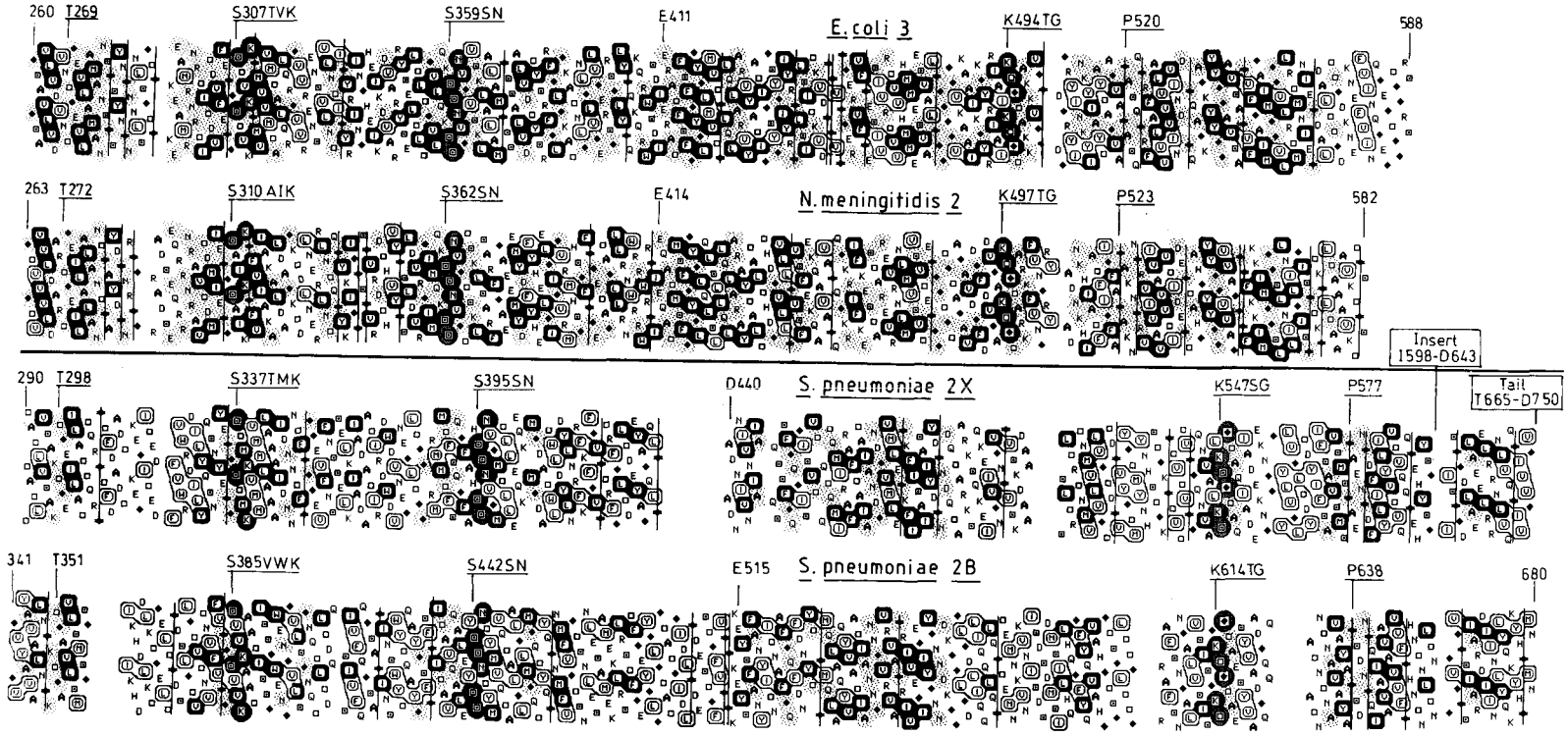

Fig. 1b. HCA plots of the pair E.coli PBP3-N.meningitidis PBP2 and the pair S. pneumoniae PBPs 2X and 2b. Analysed sequences : from the amino end of the C-terminal domains to the carboxy end of the PBPs.

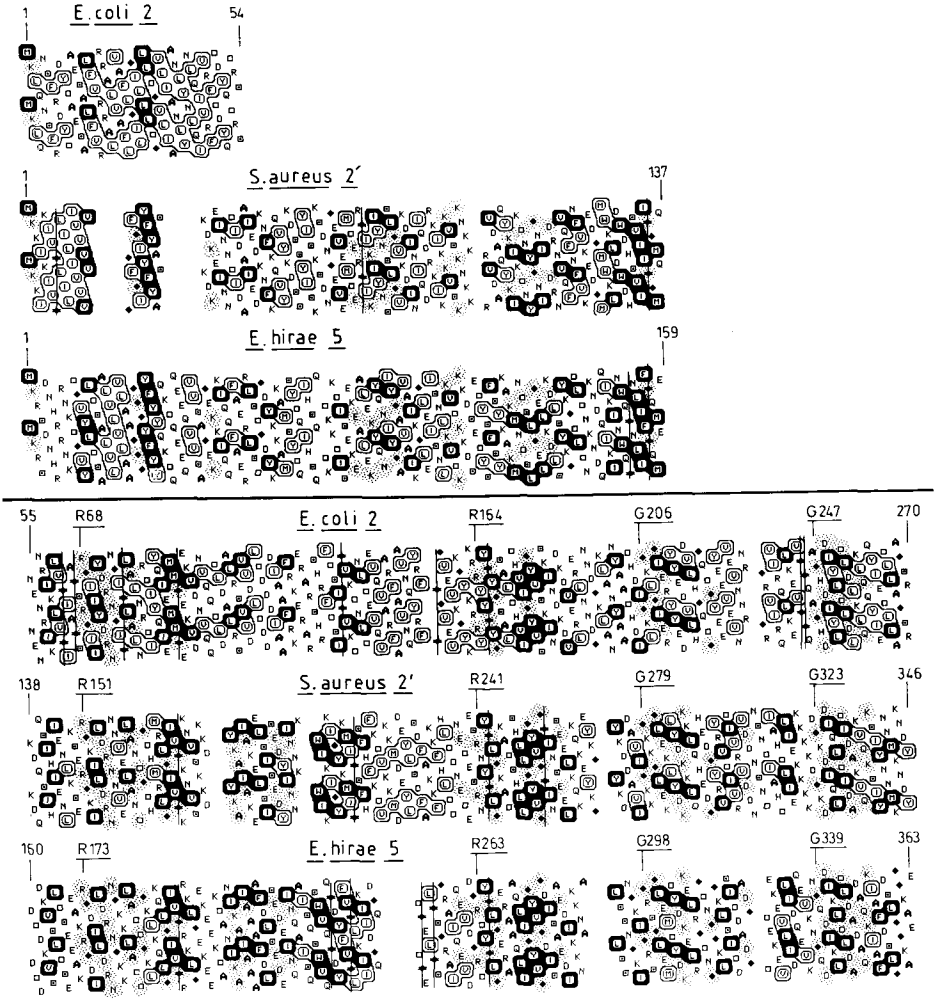

Fig. 2a. HCA plots of the pair S.aureus PBP2'-E.hirae PBP5 and the E. coli PBP2 used as reference. Analysed sequences : from the amino end of the PBPs to the carboxy end of the N-terminal domains.

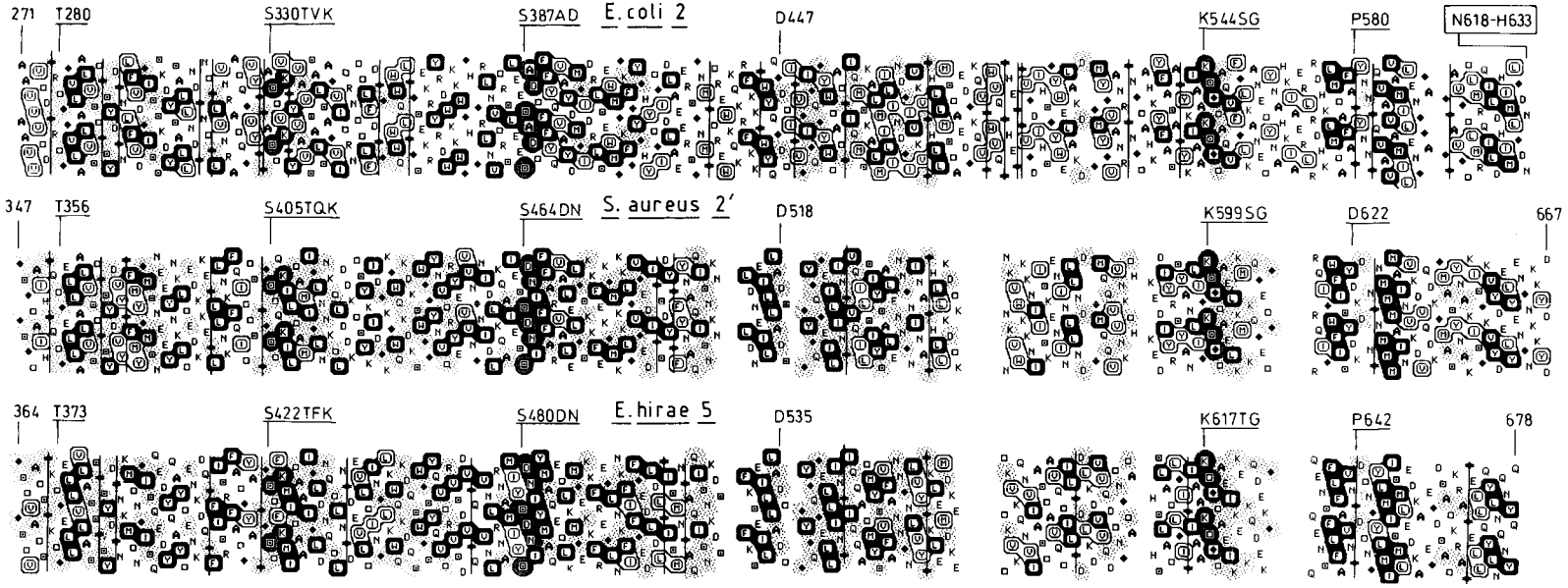

Fig. 2b. HCA plots of the pair S.aureus PBP2'-E.hirae PBP5 and the E. coli PBP2 used as reference. Analysed sequences : from the amino end of the C-terminal domains to the carboxy end of the PBPs.

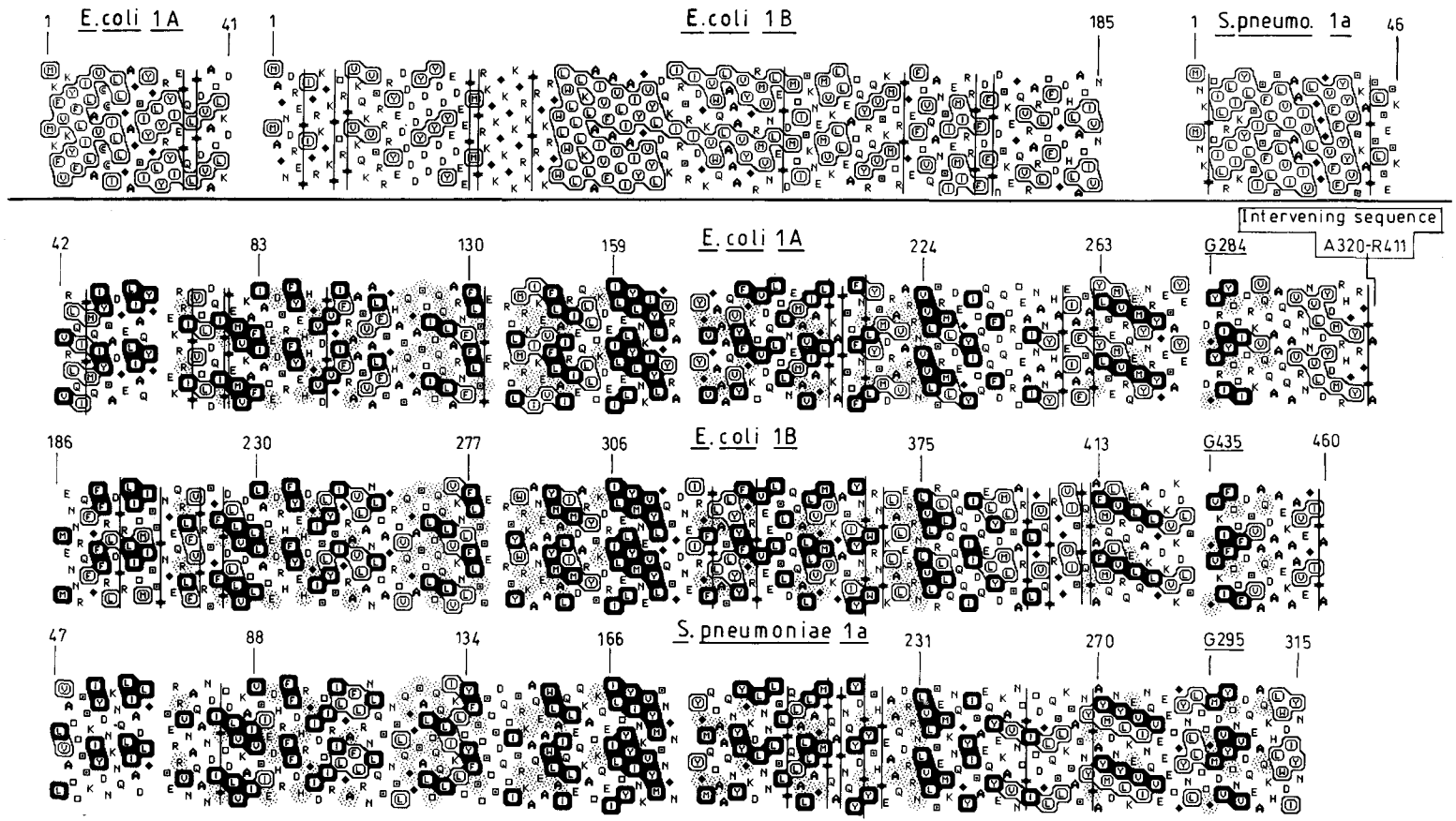

Fig. 3a. HCA plots of the triad E.coli PBP1A-E.coli PBP1B-S.pneumoniae PBP1a. Analysed sequences : from the amino end of the PBPs to the carboxy end of the N-terminal domains.

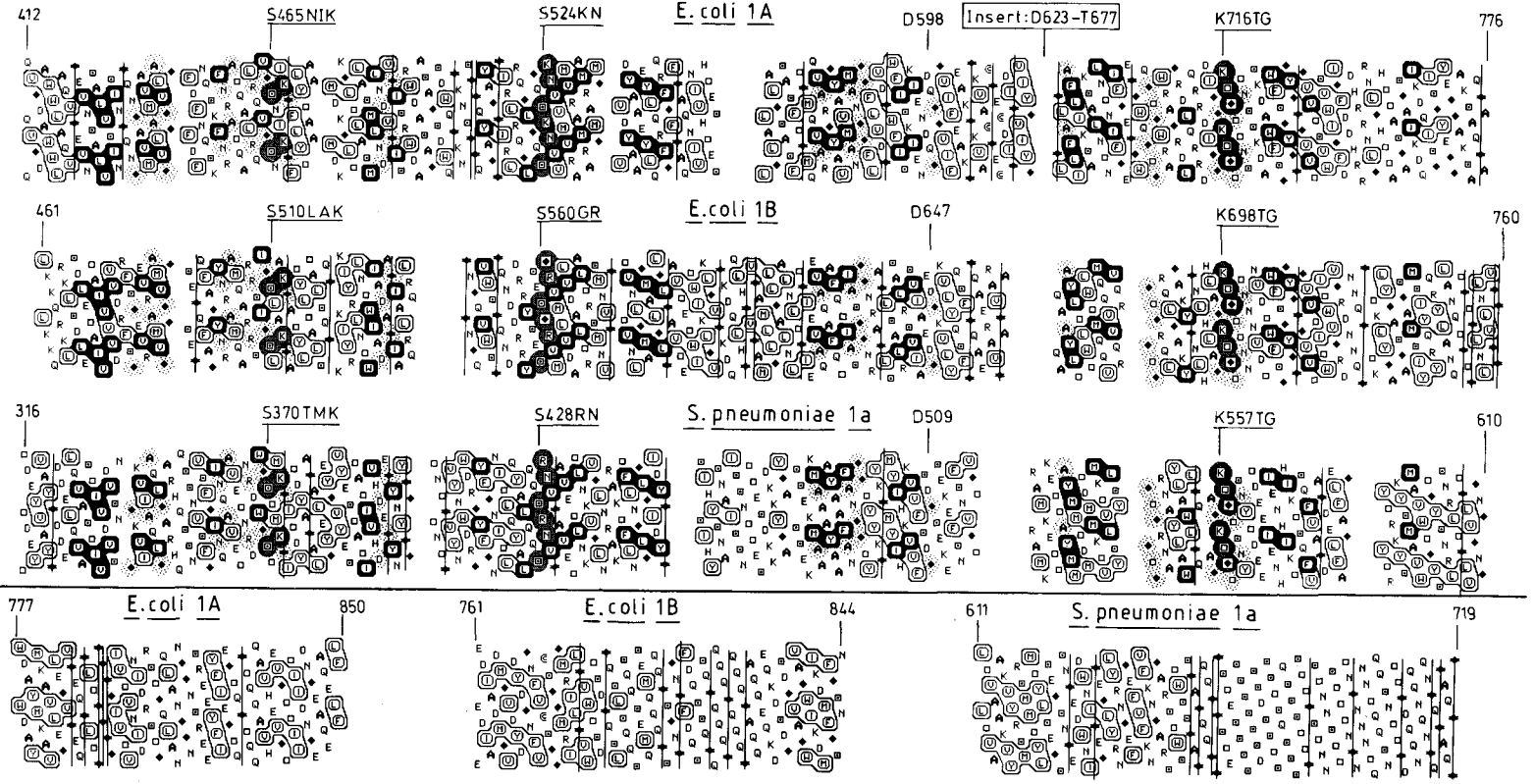

Fig. 3b. HCA plots of the triad E.coli PBP1A-E.coli PBP1B-S.pneumoniae PBP1a. Analysed sequences : from the amino end of the C-terminal domains to the carboxy end of the PBPs.

Depending on the markers borne by the N-terminal domains, the high-Mr PBPs fall into two classes. The E. coli PBPs 1A and 1B and S. pneumoniae PBP1a belong to class A. The E. coli PBPs 2 and 3, N. meningitidis PBP2 (and N. gonorrhoeae PBP2), S. aureus PBP2', E. hirae PBP5 (and PBP3r) and S. pneumoniae PBPs 2X and 2b belong to class B.

The markers of the N-terminal domains of the PBPs of class B are four amino acid groupings or boxes (Figs 4 and 5). Altogether, they provide several conserved amino acids with potentially important functional side-chains : one arginine residue and one aspartic acid residue in box 1, another arginine residue in box 2, one glutamic acid residue in box 3, one glutamine residue in box 4, and one serine or threonine residue also in box 4. Using the numbering of the E. coli PBP2, R68 and D73 of box 1, R164 of box 2, E211 of box 3 and Q259 of box 4 may well be within the active site of the domain.

The N-terminal domains of the PBPs of class A have some amino acid groupings which show remote similarity to boxes 1, 3 and 4 (Fig. 5). These groupings may provide a diamino acid residue, R or H, and a dicarboxylic amino acid, E or D, equivalent to the conserved arginine and aspartic acid residues of box 1.

The C-terminal domains of all the high-Mr PBPs possess the three SXXK, SXN and KT(S)G markers (or analogues) characteristic of the penicilloyl serine transferases (boxes 6, 7 and 8 in Fig. 5). E. coli PBP1B, however, has the unusual triad S560GR (Fig. 3b). This triad aligns perfectly with S524KN of the E. coli PBP1A and S428RN of the S. pneumoniae PBP1a, and the three markers have comparable hydrophobic environments. Note, however, that the E. coli PBP1B possesses a S572MN triad 12 residues downstream from the S560GR motif.

The C-terminal domains of all the high-Mr PBPs also possess several dicarboxylic acid residues in the polypeptide stretches that extend between the SXN (or equivalent) and KT(S)G motifs. Among these, E411 and E414 in the pair E.coli PBP3-N.meningitidis PBP2 (Fig. 1b), D447, D518 and D535 in the triad E.coli PBP2-S.aureus PBP2'-E.hirae PBP5 (Fig. 2b), and D598, D647 and D509 in the triad E.coli PBP1A-E.coli PBP1B-S.pneumoniae PBP1a (Fig. 3b) occur as strict identities in the aligned amino acid sequences. D440 in the S. pneumoniae PBP2X is two residues upstream from E515 in the S. pneumoniae PBP2b (Fig. 1b). Each of these dicarboxylic amino acid residues may be equivalent to E166 of the E166XPELN pentapeptide that forms part of the immediate boundary of the active site of the class A ß-lactamases. As shown by site-directed mutagenesis, D447 is important for the functioning of the E. coli PBP2 (Adachi et al., 1992).

The C-terminal domains of the PBPs of class B possess two additional boxes, 5 and 9 (Fig. 5). These boxes are not found in the PBPs of class A. Box 5 is located upstream from the SXXK motif close to amino end of the C-terminal domain. It contains a vertical hydrophobic cluster that is reminiscent of strand ß1 of the ß-lactamases (Fig. 4). Box 9 is located downstream from the KT(S)G motif, close to the carboxy end of the domain. Box 9, probably a turn, is sandwiched between two vertical hydrophobic clusters that are reminiscent of strands ß4 and ß5 of the class A ß-lactamases (Fig. 5).

DISCUSSION

The high-Mr PBPs illustrate the principle that protein molecules frequently are constructed from modules that are linked together in a single polypeptide chain. Each module is folded into one domain that is responsible for a particular function.

High-Mr PBPs exist which consist of a hydrophobic membrane anchor and two domains, the N- and C-terminal domains, suggesting bifunction-

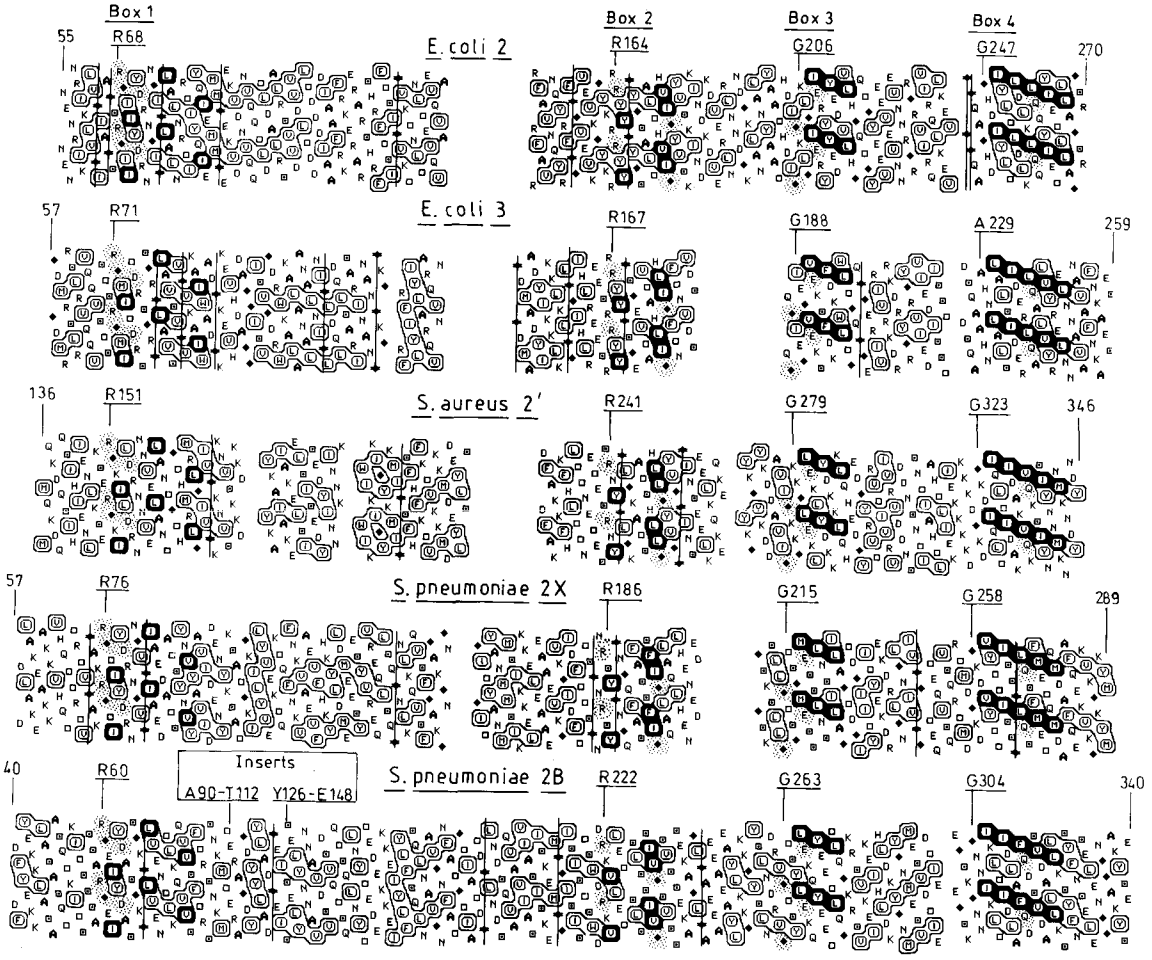

Fig. 4. HCA plots of the N-terminal domains of high-Mr PBPs of class B. Identification of the domain-specific boxes 1 to 4.

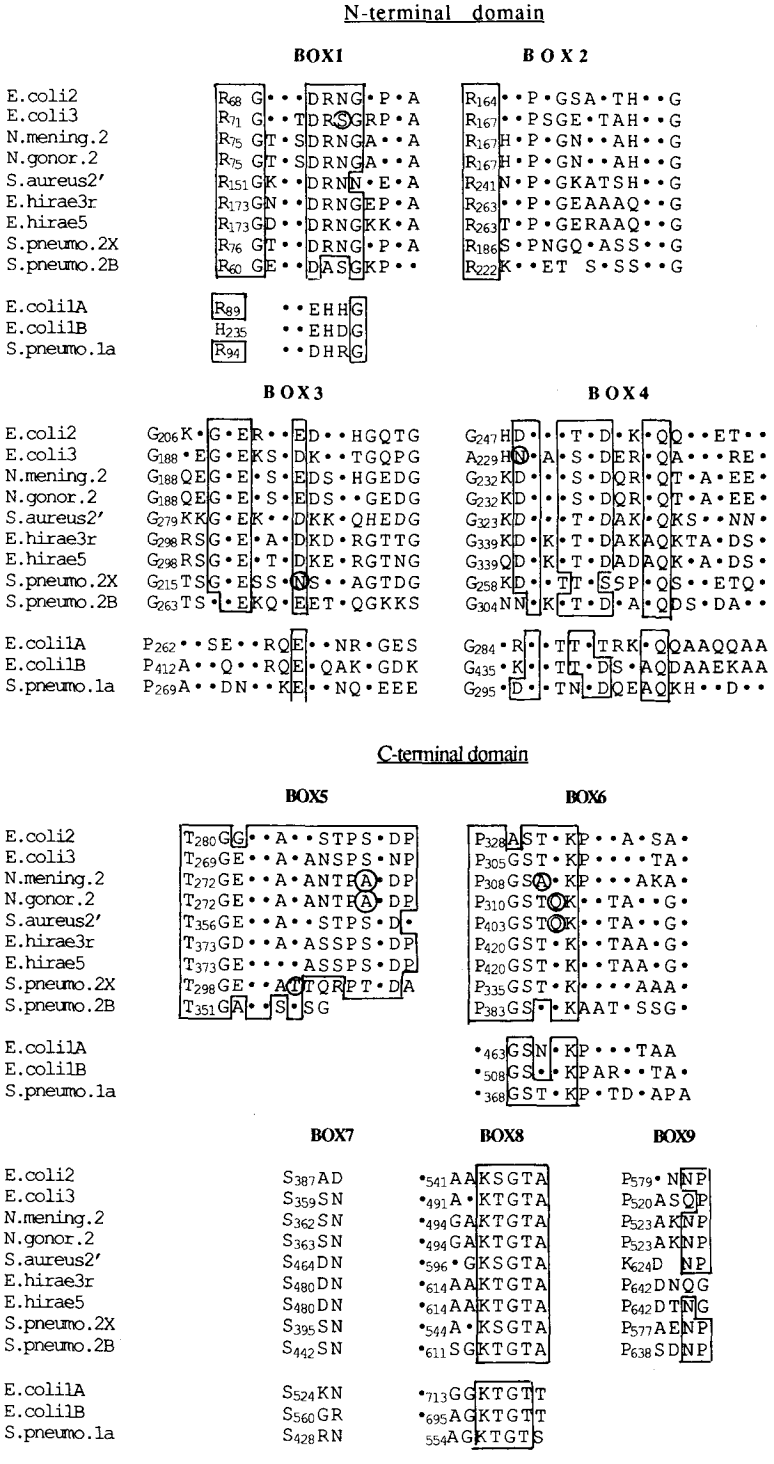

Fig. 5 Domain-specific boxes of the hight-Mr PBPs. Hydrophobic residues are shown in black dots.

ality. Other high-Mr PBPs have additional inserts, 80 to 100 amino acid residues long. These inserts may occur upstream from the N-terminal domain, between the N- and C-terminal domains or downstream from the C-terminal domain. They are large enough to give rise to additional domains each of which possessing a particular folding and performing a separate function. These high-Mr PBPs are probably multifunctional.

The penicillin-binding C-terminal domains of all the high-Mr PBPs studied possess, at the expected relative positions along the amino acid sequences, the active-site defining markers of the penicilloyl serine transferases. However, by reference to the ß-lactamases (and the monofunctional PBPs and BlaR penicillin sensor), the C-terminal domains of the high-Mr PBPs have diverged so far that other traces of homology in the primary structure have almost completely disappeared.

The N-terminal domains of the high-Mr PBPs of class B have markers in the form of four conserved amino acid groupings occurring in distinct hydrophobic environments and possessing amino acid residues that are potentially important for catalytic function. The N-terminal domains of the high-Mr PBPs of class A do not possess this type of fingerprint, except, perhaps, for the presence of a conserved diamino acid, R or H, and a dicarboxylic acid, D or E, close to the amino end of the domain. Whether or not the N-terminal domains of the high-Mr PBPs of class A and B have different foldings and functionalities remains an open question.

The question of which functions the high-Mr PBPs perform remains a major challenge and a major stumbling-block in the field. The E. coli PBPs 1A and 1B are the only known PBPs which catalyse in vitro transglycosylation and transpeptidation reactions and are able, as isolated proteins, to carry out peptidoglycan assembly (see the Introduction). Water-soluble derivatives of the E. coli PBP3 (and other high-Mr PBPs) catalyse acyl transfer reactions on acyclic thioester compounds such as C6H5-CO-D-Ala-S-CH2-COOH in a way that mimics transpeptidation (Adam et al., 1991) but they are devoid of activity in terms of peptidoglycan assembly. High-Mr PBPs may be inactive unless they associate with other host proteins such as RodA for the E. coli PBP2 and FtsW for the E. coli PBP3. By analogy with the high-Mr PBP BlaR which provides an example of how domains with different functions can combine and give rise to a new function, i.e. control of gene expression, high-Mr PBPs may be components of multi-molecular systems in which they function as signalling devices of one kind or another.

Acknowledgements

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