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REVIEW

Membrane Topology, Structure, and Functions of the Penicillin-Interactive Proteins

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The establishment of the mode of action of penicillin on susceptible bacteria has been a long process which stems back to the early 1950s, at which time the bacterial cell wall had emerged as a new field of research thanks to the pioneering work of Salton (1). Over the years, the following were successively demonstrate.

1. The rigid matrix of the bacterial cell wall, i.e., the peptidoglycan, is a network structure in which glycan strands of alternate β-1,4-linked *N*-acetylglucosamine and *N*-acetylmuramic acid pyranoside residues are substituted through the D-lactyl group of *N*-acetylmuramic acid by L-Ala-γ-D-Glu-L-Xaa_3-D-Ala peptide units, where L-Xaa_3 is most often a diamino acid, occasionally a neutral one. Peptide units substituting adjacent glycan strands are linked together by means of bridges that involve the carboxyl group of the terminal D-Ala of one peptide and a side-chain amino group of another. Depending on the location and composition of the bridges, the wall peptidoglycans fall into four main chemotypes (2).

The direct precursors of the wall peptidoglycan are lipid-transported disaccharide pentapeptide (β-1,4-*N*-acetylglucosaminyl-*N*-acetylmuramyl-L-Ala-γ-D-Glu-L-Xaa_3-D-Ala-D-Ala) units. Consequently, wall peptidoglycan assembly is made by transglycosylation and peptide crosslinking (3). This latter step is inhibited by penicillin, which immobilizes the relevant enzymes in the form of adducts that are sufficiently stable to be analyzed by SDS[1]–gel electrophoresis. On the basis of this property, a convenient procedure which allows detection of the penicillin-sensitive enzymes as penicillin-binding proteins (PBPs) has been developed (4). This method, used universally, has led to the following observations. All the bacteria possess multiple membrane-bound PBPs, each of them occurring in a small number of copies per cell. Each bacterial species has its own assortment of PBPs. The PBPs show widely varying affinity to penicillin and other β-lactam antibiotics. They range from M_r

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[1] Abbreviations used: SDS, sodium dodecyl sulfate; PBP, penicillin-binding proteins.

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25,000 to M_r 100,000, thus falling into two major groups: the low-M_r PBPs and the high-M_r PBPs.

2. The low-M_r PBPs are serine DD-peptidases. In analogy with the enzymes of the trypsin and subtilisin families, they operate via formation of a serine ester-linked acyl–enzyme intermediate.

Reaction with noncyclic R-L-Xaa-D-alanyl-D-alanine-terminated peptidoglycan precursors proceeds by sequential transfer of the R-L-Xaa-D-alanyl electrophilic moiety to an essential serine (with formation of an ester-linked acyl enzyme and release of the leaving group D-alanine) and, from this, to the exogenous acceptor (5–10). The efficacy with which the enzyme performs carboxypeptidation versus transpeptidation of the carbonyl donor depends on the efficacy with which water (which is 55.5 M), the endogenously released D-alanine, and an exogenous H_2N-Y amino compound attack the acyl enzyme. When the relative acceptor activity is $H_2O \ll$ D-alanine $\ll H_2N$-Y, hydrolysis is negligible because the released D-alanine successfully competes with H_2O and is reutilized in a transfer reaction that maintains the concentration of the carbonyl donor at a constant level. In the presence of the amino acceptor H_2N-Y, the carbonyl donor is quantitatively converted into the transpeptidated product; i.e., the enzyme functions as a strict DD-transpeptidase (10).

Reaction with cyclic β-lactam antibiotics is a suicide process. Because the scissile amide bond in the β-lactam ring is endocyclic, the leaving group formed by acylation of the essential serine remains part of the acyl enzyme and cannot diffuse away from the active site. Consequently, the acyl enzyme is highly inert toward nucleophilic attack and the reaction stops at this abortive level at least for a long time (6, 9, 11). Slow breakdown of the penicilloyl enzyme, and concomitant enzyme recovery, may occur. It then proceeds through a rate-limiting, pH-independent, intramolecular rearrangement of the penicilloyl moiety and/or produces penicilloate, which is the degradation product of β-lactamase action (12–18).

Whatever the case, reaction of the low-M_r DD-peptidases/PBPs with the antibiotic may be depicted as

$$E + D \overset{K}{\rightleftharpoons} E \cdot D \xrightarrow{k_{+2}} E - D^* \xrightarrow[H_2O]{k_{+3}} E + P,$$

where E = PBP; D = antibiotic carbonyl donor; E · D = Michaelis complex; E-D* = acyl enzyme; and P = reaction product. Hence, the inactivating potency of an antibiotic can be quantitatively expressed—using the conditions precisely described (9, 11)—by the values of the second-order rate constant k_{+2}/K of enzyme acylation and the first-order rate constant k_{+3} of enzyme deacylation. The higher the k_{+2}/K and the smaller the k_{+3}, the lower the antibiotic concentration that is able to completely immobilize the PBP as acyl enzyme at the steady-state of the reaction.

This mode of action of penicillin on its protein targets has stood the test of time and is general: it applies to all the PBPs irrespective of their M_r.

3. Hydrolysis of penicillin into penicilloate by the majority of the defensive β-lactamases also involves formation but rapid breakdown of a serine ester-linked acyl enzyme (high k_{+3} value) (19, 20). Moreover, the serine β-lactamases catalyze acyl transfer reactions on noncyclic depsipeptides, such as diacetyl-L-Lys-D-Ala-D-lactate, with a facility comparable to that shown by the low-M_r DD-peptidases/PBPs (21). The β-lactamases, however, lack DD-peptidase activity.

Gene cloning and sequencing, structural studies, and homology searches have led to the conclusion that the serine β-lactamases, the low-M_r PBPs, and the high-M_r PBPs (at least their penicillin-binding domains; see below) form a superfamily of serine peptidases (amidases, esterases). Though in the course of their divergent evolution, they have acquired different amino acid sequences and distinct functionalities and specificities, they all consist of or contain a "penicillin-interactive domain" that has conserved the same basic three-dimensional structure (22).

Such a domain has a unique signature (23–27). It consists of two regions: one is of the "all-α" type structure and the other has a central core of a five-stranded β-sheet protected by α-helices on both faces. About 60 residues downstream of the amino terminus of the domain, there occurs the active-site serine. This serine belongs to a conserved tetrad Ser-Xaa-Xaa-Lys and is located at the amino terminus of one α-helix of the all-α region. In turn, at about 60 residues upstream of the carboxy terminus of the domain, there occurs a conserved triad His-Thr-Gly, Lys-Thr-Gly or Lys-Ser-Gly. This triad is on the innermost strand of the β-sheet, a strand that forms one side of the active site and, presumably, provides hydrogen bond interactions with substrates and/or inhibitors (25).

These advances have given a major impetus to the study, at the molecular level, of the development of resistance among important bacterial pathogens, in particular, whether the acquired resistance has occurred by emergence of "new" β-lactamases or by emergence of altered PBPs that are less susceptible to inactivation by the antibiotics. The observed changes in the specificity profiles have been translated into alterations in the primary structure, affecting amino acids that occupy critical positions in the three-dimensional structure, often in the direct environment of the active site (28, 29).

4. The β-lactamases are water-soluble proteins. They obey the definition of a penicillin-interactive domain: the active-site serine is close to the amino terminus, and the conserved triad is close to the carboxy terminus of the protein. The penicillin-interactive domain in the low-M_r PBPs may be lengthened by a substantial carboxy terminal extension and in the high-M_r PBPs, it is preceded by a very long (several hundred amino acids) amino terminal extension. In several cases (but not always) short peptide stretches located at the end of these extensions serve to anchor the proteins in the plasma membrane (30, 31). Genetic engineering has allowed the overexpression and isolation of PBPs in the form of water-soluble derivatives (31, 32).

The high-M_r PBPs 1A, 1B, 2, and 3 of *Escherichia coli* have specific functions in cell division, cell elongation, and determination of cell shape. PBPs 1A and 1B are bifunctional enzymes catalyzing peptidoglycan transglycosylation (presumably by their amino terminal domain) and peptidoglycan transpeptidation (by their penicillin-binding carboxy terminal domain) (33). The exact functions of the amino and carboxyl terminal domains of PBPs 2 and 3 have not yet been clearly established.

High-M_r PBPs that have no function in wall peptidoglycan metabolism also exist. Thus, the BLAR 601-amino-acid PBP of *Bacillus licheniformis* is a sensory transducer protein involved in the inducible synthesis of the β-lactamase (34). Penicilloylation of the 255-amino-acid, penicillin-binding domain which protrudes on the outer face of the membrane, presumably induces a conformational change which spreads throughout the 346-amino-terminal transmembrane domain of the protein and produces a signal that interacts with some intracellular regulatory protein (35). Other high-M_r PBPs, in conjunction with specific life cycle proteins (36), may also act

as signal transducers in regulatory mechanisms related to the synthesis of various essential polymers.

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