The Crystal Structure of a Penicilloyl-serine Transferase of Intermediate Penicillin Sensitivity

THE DD-TRANSPEPTIDASE OF STREPTOMYCES K15

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The serine DD-transpeptidase/penicillin-binding protein of Streptomyces K15 catalyzes peptide bond formation in a way that mimics the penicillin-sensitive peptide cross-linking reaction involved in bacterial cell wall peptidoglycan assembly. The Streptomyces K15 enzyme is peculiar in that it can be considered as an intermediate between classical penicillin-binding proteins, for which benzylpenicillin is a very efficient inactivator, and the resistant penicillin-binding proteins that have a low penicillin affinity. With its moderate penicillin sensitivity, the Streptomyces K15 DD-transpeptidase would be helpful in the understanding of the structure-activity relationship of this penicillin-recognizing protein superfamily. The structure of the Streptomyces K15 enzyme has been determined by x-ray crystallography at 2.0 Å resolution and refined to an R-factor of 18.6%. The fold adopted by this 262-amino acid polypeptide generates a two-domain structure that is close to those of class A β-lactamases. However, the Streptomyces K15 enzyme has two particular structural features. It lacks the amino-terminal α-helix found in the other penicilloyl-serine transferases, and it exhibits, at its surface, an additional four-stranded β-sheet. These two characteristics might serve to anchor the enzyme in the plasma membrane. The overall topology of the catalytic pocket of the Streptomyces K15 enzyme is also comparable to that of the class A β-lactamases, except that the Ω-loop, which bears the essential catalytic Glu466 residue in the class A β-lactamases, is entirely modified. This loop adopts a conformation similar to those found in the Streptomyces R61 DD-carboxypeptidase and class C β-lactamases, with no equivalent acidic residue.

The bacterial cell wall peptidoglycan assembly machinery comprises, among other components, multimodular (transglycosylase/transpeptidase) membrane enzymes that catalyze the polymerization of lipid-transported disaccharide-pentapeptide units. Cross-linking of peptidyl moieties of adjacent glycan strands is a two-step reaction. The first step involves the rupture of the d-alanyl-d-alanine bond of a peptide unit precursor acting as carbonyl donor, the release of the carboxyl-terminal d-alanine, and the formation of a serine ester-linked peptideidyl enzyme. The second step involves the breakdown of the peptidyl enzyme and the formation of a new peptide bond between the carboxyl of the d-alanine moiety and the α-amino group of another peptide unit acting as amino acceptor. The enzyme is classified as a DD-transpeptidase because the susceptible peptide bond of the carbonyl donor extends between two carbon atoms with the d-configuration.

Penicillins, the most familiar antibacterial agents, exert their effects by inactivating the serine DD-transpeptidase modules of the peptidoglycan-synthesizing enzymes. Because penicillin is a cyclic analogue of the d-alanyl-d-alanine-terminated carbonyl donors, the reaction stops at the level of the serine ester-linked penicilloyl enzyme, and the enzymes behave as penicillin-binding proteins (PBPs). Resistance to β-lactam antibiotics is PBP- and β-lactamase-mediated. PBPs with a reduced affinity for the drug result from local changes in the amino acid sequences of the wild-type forms. Serine β-lactamases hydrolyze penicillin into penicilloate via the formation of a hydrolytically labile serine ester-linked penicilloyl enzyme. The emergence of an increasing number of resistant bacteria has become a threatening public health problem. It has fostered interest in understanding the biochemical and mechanistic features of the penicillin targets.

The Streptomyces K15 serine DD-transpeptidase/PBP (hereafter referred to as the K15 enzyme) is a 262-amino acid monomodular protein with a molecular mass of 27,474 Da (1). Although lacking transmembrane segments, the K15 enzyme is associated with the plasma membrane of the wild-type strain. Overexpression of the encoding gene in Streptomyces lividans results in the secretion of ~30% of the synthesized enzyme in the culture medium. The cloned enzyme has the same enzymatic properties as the membrane-associated form and requires the presence of 0.5 M NaCl to remain water-soluble (2).

The fate and rate of the two-step transpeptidation reaction catalyzed by the K15 enzyme depend on both the nature of the scissile bond (peptide, ester, thiol ester) of the carbonyl donor and the acceptor activity of an exogenous nucleophile (3). With (R)-d-alanyl-d-alanine-terminated peptide donors and in aqueous media, the released d-alanine is reutilized as an amino acid.
acceptor in the enzyme decacylation step so that the peptide donor is continuously regenerated and the enzyme is seemingly silent, although it turns over one time every 10 s. In the presence of amino acceptors structurally related to cell wall peptidoglycan, the peptidyl (R)-d-alanyl enzyme intermediate is aminolyzed much more effectively than it is hydrolyzed, and under certain conditions, the aminotransferase functions exclusively as a dd-transpeptidase (4). In contrast, the dd-carboxypeptidases/PBPs have a much increased preference for water as the attacking nucleophile of the peptidyl enzyme.

The K15 enzyme is a PBP. The value of the second-order rate constant of enzyme acylation by benzylpenicillin is $150 \text{ M}^{-1} \text{s}^{-1}$, and the serine ester-linked penicilloyl enzyme undergoes hydrolytic breakdown at an extremely slow rate via two pathways that give rise to benzylpenicilloate and phenylacetylglutamine, respectively. Cefoxitin is a better inactivating agent (rate constant of $-850 \text{ M}^{-1} \text{s}^{-1}$), and in this respect, the K15 enzyme differs from many PBPs for which benzylpenicillin is one of the most efficient known inactivators tested (5). Low enzyme acylation rate values confer increased “intrinsic” resistance on the bacterial cell. Thus, for example, the values of the rate constants of enzyme acylation by benzylpenicillin are $10–20 \text{ M}^{-1} \text{s}^{-1}$ for the low affinity PBP2p of the methicillin-resistant Staphylococcus aureus and PBP5 of Enterococcus hirae. In contrast, the rate constant values are 18,000 $\text{ M}^{-1} \text{s}^{-1}$ for the Streptomyces R61 dd-carboxypeptidase/PBP, 58,000 $\text{ M}^{-1} \text{s}^{-1}$ for Streptococcus pneumoniae PBP2x, and 300,000 $\text{ M}^{-1} \text{s}^{-1}$ for Bacillus licheniformis PBP1 (6–9). In comparison with those extreme values, the K15 enzyme may be considered as a PBP of intermediate penicillin sensitivity.

The PBPs and the serine $\beta$-lactamases are members of the penicillloyl-serine transferase family (10). The three-dimensional structural proteins for the class A $\beta$-lactamases of S. aureus PC1 (11), Streptomyces albus G (12), B. licheniformis 749/C (13), and Escherichia coli TEM (14–16) and for the two class $\beta$-lactamases of Citrobacter freundii (17) and Enterobacter cloacae P99 (18). Among the PBPs, only the structure of the monomodular dd-carboxypeptidase of Streptomyces R61 has been determined at high resolution (19), and that of the multimodular PBP2x from S. pneumoniae has been established at low resolution (20). Understanding the structure-function relationships among members of the penicillloyl-serine transferases requires a detailed knowledge of each type of enzyme of this family at the atomic level. The K15 enzyme is the only known DD-transpeptidase/PBP that has been characterized in detail in biochemical terms. In this paper, we describe the high resolution x-ray crystallographic structure of this enzyme, compare its structure with that of the class A serine $\beta$-lactamases, and emphasize the most significant structural features that distinguish these two types of enzymes.

**EXPERIMENTAL PROCEDURES**

**Crystalization and Data Collection**—The K15 enzyme is synthesized in the form of a 291-amino acid precursor possessing a cleavable 29-amino acid signal peptide. The overproduced soluble enzyme was purified to 95% homogeneity, and the mass spectrum revealed a mass corresponding to the theoretical value of 27,474 Da. Large pyramidal crystals with maximum dimensions of $0.6 \times 0.5 \times 0.3 \text{ mm}$ were obtained at 20 °C within a few days in 10-$\mu$l drops containing 1.3 mg/ml protein solution in 50 mM Tris-HCl (pH 7.2), 0.4 mM NaCl, 10 mM Na$_2$SO$_4$, 0.2 mM diethiothreitol, and 7.5% (w/v) polyethylene glycol 4000 or 8000, equilibrated against a 1-ml reservoir well containing 15% (w/v) polyethylene glycol 4000 or 8000 in the same buffer. The crystals belong to the orthorhombic space group $P2_12_12_1$, with cell dimensions of $a = 46.59 \AA$, $b = 54.53 \AA$, and $c = 108.70 \AA$. They contain 51% solvent and one molecule/asymmetric unit (21).

X-ray diffraction data were collected at 20 °C using a Siemens X1000 area detector. The x-ray source was graphite-monochromated Cu radiation produced by a Rigaku RU-200 rotating anode generator operating at 50 kV and 90 mA with a 0.3-mm fine-focus cathode. Indexing, integration, scaling, and merging of the intensity data were carried out using the XENGEN Version 2.0 (Native 1 and 2 and heavy atom derivatives) (22) and the SAIMT (Native 3 and 4) (23) software packages. To improve the completeness of the native data, the four native data sets were merged with the BIOMOL package provided by the Groningen-CF/BIOMOL Group (Department of Chemistry and Biophysical Chemistry, Crystallography Group, Rijksuniversiteit Groningen) (Table I).

**Structure Determination and Refinement**—The structure was solved by the method of multiple isomorphous replacement with anomalous scattering and heavy atom derivatives (22) and the SAINT (Native 3 and 4) (23) software packages. To improve the completeness of the native data, the four native data sets were merged with the BIOMOL package provided by the Groningen-CF/BIOMOL Group (Department of Chemistry and Biophysical Chemistry, Crystallography Group, Rijksuniversiteit Groningen) (Table I).

Table I gives the refinement parameters of the heavy atom derivatives. Of the two cysteine residues present in the K15 enzyme at positions 38 and 223, respectively, only one is labeled by $p$-chloromercurobenzoate and mersalyl mercury derivatives showed binding sites. Of the two cysteine residues present in the K15 enzyme at positions 38 and 223, respectively, only one is labeled by $p$-chloromercurobenzoate in aqueous solution, resulting in a drastic decrease in the peptidase activity and penicillin-binding capacity (25). As long as the model was incomplete, the MIRAS phases were used and combined with those determined by the model in order to improve the quality of the electron density map and to avoid model bias. The structure was refined to 2.0-Å resolution with a conventional $R$-factor of 18.6%, and the statistics of refinement are summarized in Table I.

**Calculation of the Molecular Hydrophobic Potential (MHP)**—The hydrophobicity of a molecule is usually defined by a single parameter representing its partition coefficient between water and octanol. For the analysis of complex interactions between lipids and proteins, this parameter was extended to the most elaborate concept of helical hydrophobic moment (29). The calculation of the MHP optimal superimposition of the K15 enzyme and class A $\beta$-lactamases (35), the first domain of the PHASES program (24). Of the 30 heavy atom derivatives tested, only $p$-chloromercurobenzoate and mersalyl mercury derivatives showed binding sites. Of the two cysteine residues present in the K15 enzyme at positions 38 and 223, respectively, only one is labeled by $p$-chloromercurobenzoate in aqueous solution, resulting in a drastic decrease in the peptidase activity and penicillin-binding capacity (25). Table I gives the refinement parameters of the heavy atom derivatives at 3.0-Å resolution. The quality of the 3.0-Å MIRAS electron density map was improved by solvent flattening using the SQUASH program (26).

The protein structure was built stepwise using TURBO-FRODO (27) and refined by simulated annealing using X-PLOR (28). Fourteen cycles of refinement (positions and temperature factors) and model refinement were carried out. Each step, the structure was constructed on the basis of $2F_o - F_r$ and $F_r - F_o$ maps computed at a 20-Å lower resolution limit. As long as the model was incomplete, the MIRAS phases were used and combined with those determined by the model in order to improve the quality of the electron density map and to avoid model bias. The structure was refined to 2.0-Å resolution with a conventional $R$-factor of 18.6%, and the statistics of refinement are summarized in Table I.

**RESULTS**

**Overall Structure**—The overall three-dimensional structure of the K15 enzyme consists of a single polypeptide chain organized into two domains. One domain contains mainly $\alpha$-helices, and the second one is of $\beta$-type. The K15 enzyme bears the signature fold topology of the penicilloyl-serine transferase superfamily, but it exhibits more overall similarity to the class A $\beta$-lactamases (Fig. 1). Using the standard secondary structure numbering of the class A $\beta$-lactamases (36), the first domain contains a central helix (a2) that is surrounded by four helices (a4, a5, a6, and a9) and, in addition, a four-stranded antiparallel $\beta$-sheet (b2a to b2d) and helix a9. The a4$\beta$-domain consists of a five-stranded antiparallel $\beta$-sheet (b1 to b5) that is covered on one side by the short helix a10 and the long carboxyl-terminal helix a11 and on the other face by helix a8 and one turn of a 3-$\gamma$ helix (a0).

**Comparison of the K15 Enzyme and the Class A $\beta$-Lactamases**—Optimal superimposition of the K15 enzyme and class A $\beta$-lactamases was performed using a maximum distance cutoff of 1.2 Å and, as fitted atoms, the equivalent C-α atoms of the
catalytic residues Ser35, Lys38, Ser96, Lys213, and Gly215 for the K15 enzyme and Ser70, Lys73, Ser130, Lys234, and Gly236 for the class A β-lactamases. The structurally superimposable regions comprise 170 amino acids, and the root mean square deviation for the 170 pairs of Cα atoms varies from 2.1 to 2.5 Å depending on the β-lactamase being compared. As shown in Fig. 2, one may note that pairwise comparison between the amino acid sequences reveals only 15% of strict identity between the K15 enzyme and each of the class A β-lactamases.

In comparison with the class A β-lactamases, the K15 enzyme exhibits four main structural differences (Fig. 3A): (i) the absence of the NH2-terminal helix α1; (ii) the insertion of 11 residues after helix α5, resulting in the extended helix α6; (iii) the absence of helix α7, which contains the class A β-lactamase-specific EXELN motif; and (iv) the occurrence of a large insert between helices α9 and α10. This insert contains strands β2c and β2d of the four-stranded β-sheet, and the stability of this β-sheet on the surface of the α-domain is reinforced by two salt bridges involving Asp134–Arg191 and Asp100–Arg184.

The MHP analysis (32) of the K15 enzyme and class A β-lactamases (Fig. 4) reveals an increase in the hydrophobic isopotential areas in the K15 enzyme, generated by the N- and C-terminal regions that cover the five-stranded β-sheet and the loop corresponding to the 20-amino acid insert. This marked

| Table I |
| Diffraction data, phasing, and refinement statistics |

| Derivatives | Riso | Site | x' | y' | z' | B | q | Phasing power |
|-------------|------|-----|----|----|----|---|---|-------------|
| pCMB        | 21.1 | 1   | 0.85 | 0.76 | 0.90 | 12.3 | 0.87 | 1.96 |
| Mersalyl    | 19.2 | 1   | 0.36 | 0.69 | 0.09 | 18.2 | 0.57 | 1.43 |
|             |      | 2   | 0.50 | 0.12 | 0.33 | 15.4 | 0.54 |

| Refined Model |
|---------------|
|             |
| No. of reflections (1 > 3σ) | 17,859 |
| Completeness (%) | 93.9 |
| R-factor (%) | 18.6 |
| Rfree (%) | 24.1 |
| No. of residues (of atoms) | 262 |
| No. of protein atoms | 1927 |
| No. of solvent atoms | 149 |
| Estimated coordinate error (low resolution cutoff of 5.0 Å) | 8.0 to 2.0 |
| From Luzatti plot (Å) | 0.20 |
| From Sigma (Å) | 0.19 |

### FIG. 1
Stereo view of the ribbon diagram of the Streptomyces K15 DD-transpeptidase structure. The helices are colored red, the 3-10 helix α0 is blue, and the β-sheets are green. The active Ser35 residue of the K15 enzyme is identified in Corey-Pauling-Koltun. The figure was made with MolScript Version 1.4 (34). Ct, COOH terminus; Nt, NH2 terminus.

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hydrophobic characteristic, which is very different from the homogeneous distribution of the hydrophilic/hydrophobic iso-potential areas observed in soluble exocellular proteins, could lead to the interaction of the K15 enzyme with the plasma membrane.

In comparison with the *Streptomyces* R61 carboxypeptidase/PBP (Protein Data Bank code 3PTE) and the class C β-lactamase of *E. cloacae* P99 (Protein Data Bank code 1BLS), the K15 enzyme shows a much higher extent of divergence (Fig. 3B). The only common secondary structures are the central helix α2 and the five-stranded β-sheet. Helices α5, α6, α8, and α11 are also present, but they have different lengths and orientations.

**Catalytic Pocket**—As observed with the other penicilloyl-serine transferases, the active site of the K15 enzyme is located at the interface between the two domains and is mainly defined by three conserved structural elements (Fig. 5). The center of the catalytic cleft is occupied by the Ser16-Thr36-Thr27-Lys38 tetrad, which includes the nucleophilic Ser35 residue at the amino-terminal end of helix α2. One side of the cavity is defined by the Ser36-Gly37-Cys38 loop connecting helices α4 and α5. The Lys213-Thr214-Gly215 triad lies on strand β3 on the opposite side of the cavity. The backbone NH group of the essential Ser35 residue and that of Ser216 downstream from the motif Lys213-Thr214-Gly215 occupy positions that are compatible with the oxyanion hole function required for catalysis.

With X denoting a variable amino acid, the amino acid sequence signature of the penicilloyl-serine transferases is defined by the three active-site structural elements: the invariant S-XXK tetrad, the SX(N/C/S) or YXN triad, and the (K/H/R)(T/S)G triad. Divergence at the level of the second element might have given rise to the “tyrosine” and “serine” enzyme subgroups, respectively (37). All known PBPs, including the K15 enzyme and the class A β-lactamases, belong to the so-called serine subgroup, whereas the *Streptomyces* R61 dd-carboxypeptidase/PBP and the class C β-lactamases belong to the tyrosine subgroup. Superimposition of the active sites reveals that the g-OH of the serine residue of the first subgroup and the phenolic OH of the tyrosine residue of the second subgroup occupy equivalent positions with respect to the nucleophilic serine and strand β3, respectively (Fig. 6A). As the side chain of a tyrosine residue is bulkier than that of a serine residue, this spatial equivalence must be the result of a reorganization of both the corresponding loops and the interconnecting secondary structures of all the α-domains. Accordingly, the serine and tyrosine subgroups show a larger extent of divergence at the level of the α-domains than at the level of the β-domains.

The top of the catalytic cleft of the K15 enzyme is defined mainly by the Thr199-Asn200-Gly201 triad (immediately upstream from helix α10), and the bottom of the cleft by the backbone of the Phe142-Asp143-Gly144 triad. The Thr199-Asn200-Gly201 triad adopts the same topology as in the class A β-lactamases (Fig. 6B) despite large structural differences between the helix α9/strand β3-connecting polypeptide chains.

In the class A β-lactamases, the EXXXN motif of the Ω-loop
(which connects helices α6 and α7) lies at the bottom of the active site, and the side chain of Glu^{166} plays an important role in the catalyzed hydrolysis of penicillin. The K15 enzyme has no motif similar to the Ω-loop, and the fold defined at this position by the Phe^{142}-Asp^{143}-Gly^{144} triad is structurally equivalent to that defined by the Gln^{219}-Ala^{220}-Tyr^{221} triad in the E. cloacae class C β-lactamase and by the Ser^{236}-Ala^{237}-Gly^{238} triad in the Streptomyces R61 dd-carboxypeptidase (Fig. 6A).

Solvent Structure—In the crystal structure, the K15 enzyme is solvated by 149 ordered water molecules mainly located in the first coordination shell, directly hydrogen-bonded to protein atoms. In the active site, where a dense hydrogen bond network prevails, the solvent molecules that interact with the essential amino acids (Table II) are the most buried ones and, accordingly, exhibit the lowest temperature factor values. As the charge distribution induced by the side chains varies among the different classes of enzymes, it is obviously difficult to make a rigorous comparison. The unique water contact with the hydroxyl group of the active Ser^{35} residue is made with Wat^{336}. This water molecule could be compared with the one found in the oxyanion hole of the class A (Wat^{295} for the TEM1 enzyme) and class C (Wat^{1087} for the P99 enzyme) β-lactamases. However, Wat^{336} is not buried so deeply in the catalytic pocket and does not make any interaction with the backbone amide groups of Ser^{35} and Thr^{214}. In comparison with the other penicilloyl-serine transferases, the side chain of the nucleophilic serine of the K15 enzyme is displaced through the $\chi_1$-dihedral angle rotation. The values of $\chi_1$ are 52.3°, −71.5°, −16.3°, and 56.6° in the K15, TEM1, R61, and P99 structures, respectively. The shift of the hydroxyl group of Ser^{35} toward strand β3 and away from the bottom of the active site can certainly be related to the displacement of Wat^{336}.

In the same way, the so-called hydrolytic water molecule found in all class A β-lactamase structures is not really conserved in the K15 enzyme. This water molecule (Wat^{292} in the TEM1 enzyme) forms a bridge between the nucleophilic serine hydroxyl group and the Glu^{166} carboxylate side chain. Without the Ω-loop, the K15, R61, and P99 enzymes cannot exhibit such
a water molecule that is tightly bound and a fortiori for the K15 enzyme, which has a different conformation for the active serine. One can only remark that as in the R61 and P99 enzymes, there is in the K15 structure a water molecule (Wat381) that neighbors the class A β-lactamase hydrolytic water molecule, but it does not interact with the essential amino acids and has a high temperature factor (40.3 Å2).

As found in all penicilloyl-serine transferases possessing a lysine residue in the conserved (Lys/His/Arg)-(Thr/Ser)-Gly triad, a water molecule (Wat303 in the K15 enzyme) is located at the top of the catalytic pocket and plays a structural role in stabilizing the Lys213 side chain. One may note that the Streptomyces R61 DD-carboxypeptidase is the only known enzyme of the family that has a histidine residue instead of a lysine or arginine residue in this triad.

Accessible Surface Area—The accessible surface area of the K15 enzyme crystal structure has a value of 10,960 Å² as computed with the DSSP program (36), and its distribution

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**FIG. 5.** Stereo view of the catalytic cleft of the K15 enzyme. The water molecules are in black and denoted by x.

**FIG. 6.** Stereo views of the superimposed active sites of serine transferase enzymes. A, the K15 enzyme (colored by atom type) and the no-carboxypeptidase/PBP of Streptomyces R61 (cyan); B, the K15 enzyme (colored by atom type) and the TEM1 class A β-lactamase of *E. coli* (magenta).
along the polypeptide chain is quite similar to that observed in the class A β-lactamasess (38). The central helix α2 and the five-stranded β-sheet are inaccessible to solvent, except strand β2, which is more exposed. Helices α4 and α5 are also buried, and the other α-helices have an amphiphilic profile. Strands β2a and β2d of the small β-sheet are very well exposed, strand β2c is amphiphilic, and strand β2b is more buried. The more accessible regions (Val1-Lys5, Asn30-Leu51, Asn70-Asn141, Ile145-Ala149, and Gly201-Gly232) correspond to the highest temperature factor values (>30 Å²).

The analysis also reveals very low accessibility values for several charged residues of the K15 enzyme. Among these, Lys38 and Lys213 are directly involved in the dense hydrogen bond network of the catalytic cleft. Asp99 interacts with the α-domain and probably stabilizes the position of the Ser96-Gly97-Cys98 motif, as does Asp131 in the class A β-lactamasess (39). Asp143 and Asn150, at the bottom of the active pocket, interact with protein core atoms, allowing the positioning of the carbonyl group of Asp143 in the catalytic cleft. Arg245, near strand β3, may play the same role as Arg220 or Arg244 in the class A β-lactamasess (40). Two polar residues, Asn198 and Asn200, above the catalytic pocket, are also quite buried. They may be compared with the Asp214 (Asn214)/Asp233 pair in class A β-lactamasess and the Glu272/His272 pair in class C β-lactamasess. These latter residues are involved in specific interactions, in particular with a water molecule equivalent to Wat203 in the K15 enzyme (16).

DISCUSSION

Several scenario may be evoked through which the present day penicilloyl-serine transferases of different groups and classes have evolved from a protein ancestor similar to the K15 enzyme in structure and catalytic properties. One evolution pathway is loss of peptidase activity, loss of membrane association site, and acquired catalyzed hydrolysis of the penicilloyl enyme. The resulting exocellular β-lactamases hydrolyze penicilloyl-serine transferase (20). The structurally related K15 β-transpeptidase/PBP, β-carboxyl-peptidase/PBPs, and serine β-lactamasess fulfill different functions and catalyze distinct reactions (albeit exhibiting a similar catalytic mechanism). They illustrate the concept according to which proteins unrelated in sequence and function may diverge from a common ancestor and new enzyme functions may evolve by localized structural changes while retaining the same basic fold. The question of which structural features determine the different functionalities (peptide bond synthesis versus hydrolysis, penicillo binding versus hydrolysis) and vastly different kinetic properties of the monofunctional penicillo-serine transferases remains open.

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