

Penicillin-Sensitive Enzymes of Peptidoglycan Metabolism

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In 1940, for the first time, Chain, Florey, and their associates obtained benzylpenicillin in a solid form and demonstrated its efficiency in higher organisms against various pathogenic bacteria. Nine years later, almost all that is known today on the chemistry of penicillin had been discovered (3). In comparison, the unraveling of the mechanisms by which penicillin kills bacteria and exerts its remarkable selective toxicity has been a slow process.

STRUCTURE OF THE WALL PEPTIDOGLYCAN (16, 28)

It was early recognized that penicillin, when acting on growing bacteria, caused damages to the bacterial wall and more precisely to its peptidoglycan moiety. The wall peptidoglycan is a rigid, netlike heteropolymer which is composed of glycan chains cross-linked by peptide chains. Essentially, the glycan moiety consists of linear strands of alternate pyranoside residues of *N*-acetylglucosamine and *N*-acetylmuramic acid linked together by 1→4,β bonds. All glycans have short tetrapeptide units L-alanyl-D-glutamyl-L-R₃-D-alanine linked to their muramyl carboxyl groups, and the peptide units that substitute adjacent glycan chains are, in turn, covalently linked together by means of interpeptide bridges. These bridges always extend between the C-terminal D-alanine residue of one tetrapeptide and often, but not always, to the ω-amino group of the L-R₃ residue of a second tetrapeptide. The most frequent type of peptidoglycan is that found in the gram-positive bacilli and in the gram-negative bacteria; *meso*-diaminopimelic acid is at the R₃ position in the peptide unit, and the interpeptide bridge is mediated via a direct D-alanyl-(D)-*meso*-diaminopimelic acid linkage.

SYNTHESIS OF NASCENT PEPTIDOGLYCAN (19, 30)

About 30 enzymes are involved in peptidoglycan synthesis. Some of them are cytoplasmic (and soluble). They catalyze the formation of two nucleotide precursors: UDP-*N*-acetyl-

glucosamine and UDP-*N*-acetylmuramyl-L-Ala-D-Glu-L-R₃-D-Ala-D-Ala. The peptide moiety of the latter is not a tetrapeptide as is found in the completed wall peptidoglycan but a pentapeptide ending in a D-Ala-D-Ala sequence. Once formed, the two precursors are assembled by a membrane-bound, multienzyme system which works in conjunction with a specific, undecaprenyl phosphate carrier. The mechanism is such that chains consisting of multiple disaccharide-peptide units grow by addition of new disaccharide-peptide units on the reducing terminal of the lengthening chain (33, 34). The acceptor on which the nascent glycan chains are assembled may be the undecaprenyl phosphate carrier itself or perhaps another membrane component such as, for example, lipoteichoic acid (W. Wong, F. E. Young, and A. N. Chatterjee, in press). Whatever the exact nature of the membrane acceptor, the nascent, uncross-linked peptidoglycan thus formed emerges on the exterior of the plasma membrane, which in fact has been seen by use of isolated protoplasts (5-7).

PEPTIDOGLYCAN CROSS-LINKING REACTION

Insolubilization of the nascent peptidoglycan is achieved by peptide cross-linking and is catalyzed by a membrane-bound transpeptidase (32, 35). The carbonyl group of the penultimate C-terminal D-alanine residue of one pentapeptide (i.e., the donor) is transferred to the ω-amino group of the L-R₃ residue of another pentapeptide (i.e., the acceptor). New peptide bonds are formed, and equivalent amounts of D-alanine residues are liberated from the peptide donors. Such an operation can be performed by cells that, at the onset of the process, completely lack preexisting walls (5-7). The process is slow and appears to be difficult since immobilization of the protoplasts by fixation to the agar in a medium of low fluidity is necessary. In normal cells, the nascent peptidoglycan, probably as its own synthesis proceeds, undergoes insolubilization by attachment to the preexisting wall peptidoglycan. The process is

achieved mainly by transpeptidation although, in some bacteria, addition of the nascent peptidoglycan to the glycan strands of the preexisting wall peptidoglycan by transglycosylation may also be a minor pathway of peptidoglycan expansion (23). The direction of the transpeptidation is not uniform among bacteria. Thus, in *Bacillus licheniformis*, the nascent peptidoglycan is the peptide donor and the preexisting wall peptidoglycan is the peptide acceptor (through its amino groups on the D-center of *meso*-diaminopimelic acids; 33, 34). On the contrary, in *Gaffkya homari*, the ϵ -amino groups of lysine residues in the nascent peptidoglycan function as acceptors whereas D-Ala-D-Ala sequences in the preexisting wall function as donors (20a, 20b).

PEPTIDOGLYCAN CROSS-LINKING ENZYME SYSTEM

Basically, three types of enzymes may exist, all of them catalyzing the release of the C-terminal D-Ala residue from D-Ala-D-Ala-ending peptides:

1. Release of D-Ala by transpeptidases is necessarily coupled with the transfer of the residual peptide donor moiety to a proper amino acceptor. These enzymes seem to be exclusively membrane bound.

2. Release of D-Ala by DD-carboxypeptidases is a simple hydrolytic process. These enzymes are often membrane bound, sometimes periplasmic. They probably control the number of peptide donors made available to the transpeptidase activity and, hence, the extent of peptide cross-linking in the wall peptidoglycan. An exact balance between transpeptidase activity and DD-carboxypeptidase activity appears to be a physiologically important parameter (20a, 20b, 24).

3. DD-Carboxypeptidases-transpeptidases also exist that are able to catalyze concomitantly both hydrolysis and transfer reactions. These enzymes are also membrane bound or periplasmic. Strains of actinomycetes, however, excrete such DD-carboxypeptidases-transpeptidases (17). With these enzymes, the channeling of the total enzyme activity into either of these pathways depends upon the environmental conditions (pH, polarity, and acceptor and donor concentrations) (11) as well as upon minor chemical alterations of the peptide substrates (the occurrence of an amide group on the α -carboxyl group of D-glutamic acid; 18). In vivo, a cell-bound DD-carboxypeptidase-transpeptidase might fulfill more than one function, its activity being modulated by the properties of its microenvironment in the cell.

Finally, the situation is made more complex

by the fact that, at least in some bacteria, the cross-linking enzyme system or some of its enzyme constituents may occur in two distinct forms that exhibit largely different penicillin sensitivities and, perhaps, are involved in distinct physiological functions such as, for example, wall septation and wall elongation (2, 22, 22a, 25-27, 31).

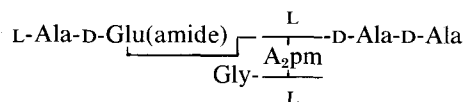
KILLING TARGET OF PENICILLIN

The peptidoglycan cross-linking enzyme system, considered as a whole, is the target specifically attacked by penicillin. Its complete inhibition causes cessation of growth. The question arises, however, whether all enzyme members are physiologically important, i.e., whether the specific inactivation of one of them may sufficiently impair the peptidoglycan cross-linking machinery to cause serious cell abnormalities and cell death. An approach to the problem rests upon the observation that, in one given organism, the various enzymes involved in peptide cross-linking differ, sometimes drastically, with respect to their penicillin sensitivity. With gram-negative bacteria, low dose levels of penicillin selectively inhibit cell septation, leading to filament formation, whereas higher penicillin concentrations are required to block cell elongation and cause loss of rod shape and, eventually, cell lysis. In *Escherichia coli*, a set of membrane-bound proteins that are able to fix penicillin are apparently specifically involved in cell division, cell elongation, and cell shape, but their possible correlation with specific enzymes remains to be established (29; Spratt, p. 182, this volume). Among gram-positive bacteria, it has been shown that both *B. subtilis* (1) and *G. homari* (20a) possess a highly penicillin-sensitive DD-carboxypeptidase. Specific inactivation of this enzyme has no detectable effect on the growth of *B. subtilis* but seems to be lethal for *G. homari*. At present, a unified view cannot be proposed. Furthermore, killing by penicillin causes cell lysis, and therefore peptidoglycan hydrolases (autolysins) must be actively involved in the process. Under normal conditions of growth, hydrolase action is controlled in such a way that it is only permitted in specific cell areas and at given stages of the cell cycle (G. D. Shockman, unpublished data). Penicillin has no direct action on isolated autolysins, but in its presence triggering of hydrolase activity occurs, resulting in cell lysis. Obviously, the functioning, regulation, and coordination of both peptidoglycan cross-linking and peptidoglycan autolyzing enzyme systems are topics of extreme importance (Tomasz and Høltje, p. 209, this volume).

ISOLATION AND FRACTIONATION OF THE PEPTIDOGLYCAN CROSS-LINKING ENZYME SYSTEM (17)

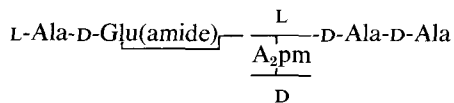
The isolation of the peptidoglycan cross-linking enzyme system and its fractionation into its individual enzyme components is a difficult task. In particular, substrates must be made available which permit the assay of the various enzymes when uncoupled from their natural substrates. DD-Carboxypeptidase is assayed by action either on isolated UDP-N-acetylmuramyl-pentapeptide or on synthetic L-R₃-D-Ala-D-Ala-ending peptides that closely resemble the corresponding C-terminal part of the natural substrates. Artificial systems of donor and acceptor peptides have been developed that allow transpeptidase activity to be estimated directly and independently of the preceding biosynthetic reactions. The following examples are meant to illustrate the point:

1. In *Streptomyces* sp., transpeptidation occurs between hexapeptides

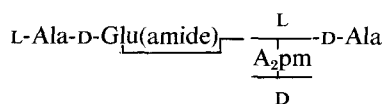


where D-Ala-D-Ala is the donor group and the N-terminal Gly is the acceptor group. Membrane-bound, periplasmic, and exocellular enzyme preparations have been obtained from various strains of *Streptomyces* which hydrolyze Ac₂-L-Lys-D-Ala-D-Ala into D-Ala and Ac₂-L-Lys-D-Ala (DD-carboxypeptidase activity) and perform artificial transpeptidations by using the same tripeptide Ac₂-L-Lys-D-Ala-D-Ala as donor and, for example, Gly-Gly as acceptor. D-Ala and tetrapeptide Ac₂-L-Lys-D-Ala-Gly-Gly are the reaction products.

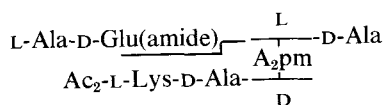
2. In *Actinomadura* strain R39 (formerly *Streptomyces* R39), transpeptidation occurs between pentapeptides



where the acceptor group is the amine located on the D-carbon of *meso*-diaminopimelic acid in α -position to a free carboxyl group. This organism produces an exocellular DD-carboxypeptidase-transpeptidase. The isolated enzyme hydrolyzes Ac₂-L-Lys-D-Ala-D-Ala into D-Ala and Ac₂-L-Lys-D-Ala and carries out transpeptidation with the amidated tetrapeptide



as acceptor. D-Ala and hexapeptide



are the reaction products. Amidation of the carboxyl group on the D-center of *meso*-diaminopimelic acid abolishes acceptor function. Note that in gram-negative bacteria, the transpeptidation reaction is identical to that which occurs in *Actinomadura* except that the α -carboxyl group of D-glutamic acid is not amidated. However, the specificity profile of the *E. coli* peptidoglycan cross-linking enzyme is such that it does not utilize the tripeptide Ac₂-L-Lys-D-Ala-D-Ala for hydrolysis or for transpeptidation. Hence, substrate systems specific for *E. coli* had to be devised.

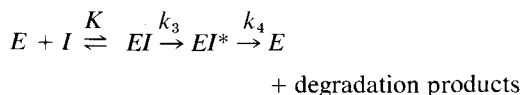
Through the use of artificial substrates such as those described above and others, transpeptidases, DD-carboxypeptidases, and DD-carboxypeptidases-transpeptidases have been isolated from various organisms. Although very few of them have been purified to protein homogeneity, they have been extremely useful for the study of the mechanisms of transpeptidation and penicillin action at the molecular levels.

INTERACTION BETWEEN PENICILLIN AND EXOCELLULAR DD-CARBOXYPEPTIDASES-TRANSPEPTIDASES FROM STREPTOMYCES R61 AND ACTINOMADURA R39: THE MODEL (9, 10, 12, 14, 15)

Actinomycetes produce exocellular DD-carboxypeptidases-transpeptidases. Those excreted by *Streptomyces* strain R61 and *Actinomadura* strain R39 were purified to protein homogeneity. They are globular proteins, each consisting of a single polypeptide chain with molecular weights of 38,000 (R61 enzyme) and 53,000 (R39 enzyme). They were selected for the study of the mode of action of penicillin for the following reasons: (i) at present, they are the only enzymes to have been purified to protein homogeneity; (ii) they catalyze artificial hydrolysis and transfer reactions that are almost identical to those which occur *in vivo*; (iii) these two activities are indistinguishable from each other with respect to their penicillin sensitivity; and (iv) the R39 enzyme is much more sensitive to penicillin than the R61 enzyme, thus offering a way to study the mechanisms through which the enzymes of the peptidoglycan cross-linking system may exhibit large differences in their penicillin

sensitivity whether or not they belong to the same organism.

The simplest model which best explains the mode of action of penicillin is



Enzyme (E) and antibiotic (I , for inhibitor) react to form a stoichiometric intermediate complex EI characterized by a dissociation constant K . Complex EI then undergoes isomerization into complex EI^* , in which the enzyme is inactivated and the antibiotic is chemically modified. Depending upon the antibiotic and the enzyme, the EI^* complexes have different half-life values, but in all cases they undergo spontaneous breakdown with regeneration of the enzyme and release of the antibiotic into biologically inactive metabolites. The two last steps are characterized by first-order rate constants, k_3 and k_4 , respectively.

The reaction products arising from benzylpenicillin are phenylacetyl-glycine and N -formyl-D-penicillamine (8, 13). Penicillin is thus split into two fragments. Globally, the reaction consists of the addition of two H_2O molecules and results in the hydrolysis of the amide bond and in the rupture of both C_5-C_6 and C_5-S linkages. Enzyme reactivation, release of phenylacetyl-glycine, and release of N -formyl-D-penicillamine during breakdown of complex EI^* are concomitant events. Boiling of complex EI^* (made with the R61 enzyme) stabilizes it and makes it sensitive to Pronase or thermolysin action. By using [^{14}C]benzylpenicillin for complex formation, a radioactive tripeptide Val-Gly-Ser was thus obtained, suggesting that, in the R61 enzyme, serine is the residue involved in penicillin binding (7a). Cephalosporins are also destroyed by both R61 and R39 enzymes, but the degradation products have not yet been characterized.

PENICILLIN AS SUBSTRATE AND INHIBITOR OF THE EXOCELLULAR DD-CARBOXYPEPTIDASES-TRANSPEPTIDASES (9, 10, 12, 14, 15)

β -Lactam antibiotics are thus substrates of both R61 and R39 enzymes. However, by immobilizing the enzymes, at least temporarily, in the form of complexes EI^* , they also behave as inhibitors. The lower the K value is the higher the k_3 value is, and the higher the k_4 value is the better the antibiotic is as a substrate. The lower the K value is the higher the k_3 value is, but the lower the k_4 value is the more active

the antibiotic is as an "inhibitor." The importance of the K and k_3 constants is obvious since the rate of formation of complex EI^* directly depends on the k_3/K ratio value. The k_4 constant is also important since, at the steady state, the smaller its value, the higher is that part of the total enzyme which is immobilized in the form of complex EI^* . There are two ways to express the efficiency of an antibiotic as inhibitor:

1. A K_i value can be defined as $K_i = k_4 K/k_3$. The lower the K_i is the better the inhibitor is.

2. The level of active enzyme $[E]$ which, at the steady state, remains functional as hydrolase and transpeptidase is given by the equation

$$[E] = \frac{E_0}{1 + \frac{[I]}{K} + \frac{k_3}{K} \frac{[I]}{k_4}}$$

If the k_4 is much smaller than $k_3 [I]/K$, then irrespective of the k_4 value, all the enzyme is, at the steady state, immobilized in the form of couple EI^* . However, if both the k_3 and k_4 values are very small, then at an antibiotic concentration equal to the K_i value, formation of complex EI^* may be so slow that within the time used for the experiment it may not occur; i.e., the enzyme may remain active.

Table 1 gives the k_3/K and k_4 values for both R61 and R39 enzymes and a series of β -lactam antibiotics. The k_4 values range from 0.3×10^{-6} to $1.4 \times 10^{-4} \text{ s}^{-1}$, which correspond to half-lives for the complexes EI^* of 40,000 to 80 min. These variations are important. Nevertheless, even those complexes which have the highest k_4 value are still rather stable. The k_3/K values range from about 100,000 to 3,000 $M^{-1}s^{-1}$ for the R39 enzyme and from 14,000 to 22 $M^{-1}s^{-1}$ for the R61 enzyme. Because of the relatively high stability of all the complexes EI^* , differences between antibiotics with respect to their efficiency as inhibitors should be mainly related to the k_3/K values. If enzyme inhibition is expressed in terms of ID_{50} value (i.e., the antibiotic concentration required to inhibit enzyme activity by 50% under standard conditions), then, irrespective of the antibiotics, the ratio value of k_3/K for the R39 enzyme to k_3/K for the R61 enzyme should be equal to the ratio value of ID_{50} for the R61 enzyme to ID_{50} for the R39 enzyme. Table 2 shows that, within the limits of experimental error, this conclusion is well supported by the experimental data.

Table 3 gives the individual K and k_3 values for various enzyme-antibiotic systems characterized by k_3/K values ranging from 66,000 to

TABLE 1. Interaction between β -lactam antibiotics and the exocellular R61 and R39 enzymes

Interaction: antibiotic—enzyme	k_3/K ($M^{-1}s^{-1}$)	k_4 at 37°C (s^{-1})	Half-life of complexes EI^* at 37°C (min)	ID_{50} at 37°C (10^{-8} M)
Benzylpenicillin—R39	90,000 (20°C)	2.8×10^{-6}	4,100	(5) ^a
Ampicillin—R39	74,000 (20°C)	4.4×10^{-6}	2,600	(3) ^a
Cephaloglycine—R39	74,000 (20°C)	0.8×10^{-6}	14,000	(6) ^a
Cephalosporin C—R39	66,000 (20°C)	0.28×10^{-6}	40,000	(5) ^a
Benzylpenicillin—R61	13,700 (25°C)	1.4×10^{-4}	80	14
Carbenicillin—R39	2,900 (20°C)	5.4×10^{-6}	2,125	200
Cephalosporin C—R61	1,150 (37°C)	1×10^{-6}	11,200	45
Carbenicillin—R61	820 (37°C)	1.4×10^{-4}	80	800
Ampicillin—R61	107 (37°C)	1.4×10^{-4}	80	2,600
Cephaloglycine—R61	22 (37°C)	3×10^{-6}	3,700	6,900

^a In these cases, the k_3/K ratio values are so high and the k_4 values are so low that these determinations are probably equivalent to a simple titration of the enzyme used in the experiments.

TABLE 2. Relation between the rates of formation of complexes EI^* with the exocellular R61 and R39 enzymes and the corresponding ID_{50} values

Antibiotic	a		b		a/b
	k_3/K (R39)	k_3/K (R61)	ID_{50} (R61)	ID_{50} (R39)	
	Benzylpenicillin	7.3	2.8	2.8	
Carbenicillin	3.6	4	4	0.9	
Cephalosporin C ...	31	9	9	3.4	
Ampicillin	626	867	867	0.7	
Cephaloglycine	3,523	1,150	1,150	3.1	

$22 M^{-1}s^{-1}$. In no case is the recognition of the antibiotic by the enzyme (as expressed by the K value) exceedingly good, but there is a 20,000-fold variation between the highest and the lowest k_3 value. Note that values of $180 s^{-1}$ and $13 s^{-1}$ for the k_3 constant are similar to the k_{cat} value of a true enzymatic reaction (the hydrolysis of a good peptide donor by the R61 enzyme is about $50 s^{-1}$).

A last point deserves to be discussed. Both penicillin and D-Ala-D-Ala-ending peptide donor are substrates of the DD-carboxypeptidases-transpeptidases, and therefore one would like to know whether fixation of peptide donor and fixation of penicillin on the same form of enzyme are mutually exclusive. Kinetically, enzyme inhibition by penicillin is competitive with regard to the peptide donor in Lineweaver-Burk plots. However, in a noncompetitive inhibition, linear plots would also be obtained if the dissociation constant for the ternary complex ESI would be roughly equal to that of the binary complex EI . They would then be indistinguishable from those obtained from a classical competitive inhibition. It is thus impossible to make a choice

between the two alternatives, and a structural analogy between penicillin and donor substrate cannot be justified on the basis of a "competitive" inhibition.

INTERACTION BETWEEN PENICILLIN AND CELL-BOUND ENZYMES

The above model applies to cell-bound enzymes. Formation of an inactive enzyme-antibiotic complex is followed by spontaneous breakdown, enzyme reactivation, and release of antibiotic degradation product(s). For technical reasons, it has not yet been possible to show whether or not complex formation is a two-step reaction, but the bimolecular rate constant k for the interaction $E + I \xrightarrow{k} EI^*$ has been measured in several cases (k is also expressed in $M^{-1}s^{-1}$ and, for $[I] \ll K$, is equivalent to the k_3/K ratio value in a two-step reaction).

Isolated membranes of *Streptococcus faecalis* possess a DD-carboxypeptidase which, apparently, also performs simple transfer reactions (J. Coyette and J. M. Ghuysen, unpublished data). The half-life values of all the complexes formed with various penicillins exceed the generation time of this organism, but the k values exhibit large differences, from $560 M^{-1}s^{-1}$ with

TABLE 3. Effect of K and k_3 constant values on the formation of complexes EI^* with the exocellular R61 and R39 enzymes

Interaction: antibiotic—enzyme	k_3/K ($M^{-1}s^{-1}$)	K (mM)	k_3 (s^{-1})
Cephalosporin C—R39 ..	66,000	0.19	12.5
Benzylpenicillin—R61 ..	13,700	13	180
Carbenicillin—R61	820	0.11	0.09
Ampicillin—R61	107	7.2	0.77
Cephaloglycine—R61 ..	22	0.4	0.009

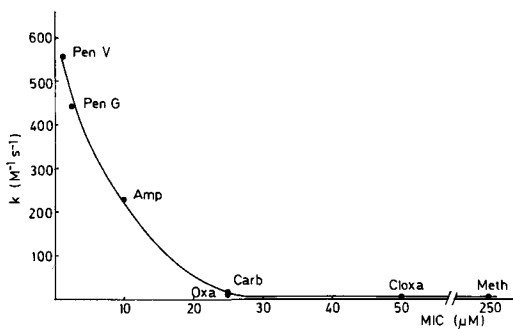


FIG. 1. Effects of penicillins on *Streptococcus faecalis* ATCC 9790. Relationship between the minimal inhibitory concentration (MIC) values of penicillins and the bimolecular rate constant (k) values for their interaction with the membrane-bound DD-carboxypeptidase. Pen V = phenoxymethylpenicillin; Pen G = benzylpenicillin; Amp = ampicillin; Oxa = oxacillin; Carb = carbenicillin; Cloxa = cloxacillin; Meth = methicillin.

phenoxymethylpenicillin to $0.7 \text{ M}^{-1}\text{s}^{-1}$ with methicillin. There exists a relationship (Fig. 1) between the k values and the in vivo activity of various penicillins (expressed as minimal inhibitory concentrations), demonstrating that this membrane-bound enzyme is physiologically important and may be the killing target of penicillin in *S. faecalis*.

Biosynthesis of chemically normal (but functionally defective) peptidoglycan in the unstable spheroplast L-form of *Proteus mirabilis* continues during growth in the presence of 200 to 1,000 units of penicillin/ml. Current studies (22, 22a; H. H. Martin, W. Schilf, and P. Gruss, L-Form Symp., INSERM, Montpellier, 1976) suggest that in normal *Proteus* bacteria at least two isoenzymes of transpeptidase and DD-carboxypeptidase are involved in peptidoglycan synthesis, and that conversion to the L-form by penicillin causes permanent inactivation of the isoenzyme with high penicillin sensitivity. L-form growth and peptidoglycan synthesis in the presence of penicillin would thus continue with the isoenzyme of low penicillin sensitivity. A DD-carboxypeptidase has been isolated from the L-form which interacts with benzylpenicillin with a k value of about $3,500 \text{ M}^{-1}\text{s}^{-1}$ (unpublished data) and a k_4 value of $4.16 \times 10^{-3}\text{s}^{-1}$. Hence, the half-life value of the complex thus formed is of 2.8 min! Such a rapid breakdown may well be sufficient to maintain a high level of functional DD-carboxypeptidase in the continuous presence of penicillin. Another interesting feature of this enzyme is that, kinetically, its inhibition by penicillin is noncompetitive, strongly suggesting that the ternary complex ESI can be formed.

Streptomyces sp. have a membrane-bound transpeptidase. Cetyltrimethylammonium bromide has been used to solubilize it directly from mycelia of strains K15 and *rimosus* (as well as from other strains (4, 21; unpublished data). With strain K15, the same treatment also solubilizes a DD-carboxypeptidase (its location in the cell is still uncertain). These partially purified enzymes interact with benzylpenicillin to form complexes of rather long half-lives (40 to 900 min at 37°C , depending upon the enzyme). The k values for complex formation are $340 \text{ M}^{-1}\text{s}^{-1}$ for the transpeptidase of *S. rimosus* and 30 and $5,000 \text{ M}^{-1}\text{s}^{-1}$ for the transpeptidase and the DD-carboxypeptidase of strain K15, respectively. Hence, in strain K15, the inactivation of the DD-carboxypeptidase is considerably faster than the inactivation of the transpeptidase, a situation reminiscent of that of *G. homari* and *B. subtilis*, where DD-carboxypeptidase activity is more sensitive than transpeptidase activity (see above). *S. rimosus* has no detectable DD-carboxypeptidase activity, and its isolated transpeptidase has a penicillin sensitivity intermediate to those of the transpeptidase and DD-carboxypeptidase of strain K15. The physiological significance of these observations is still obscure.

Phenylacetyl glycine is the main degradation product arising from benzylpenicillin by interaction with the cetyltrimethylammonium bromide-solubilized *Streptomyces* enzymes, the membranes of *S. faecalis*, and those of *B. stearothermophilus* (20). However, splitting of the antibiotic molecule into two fragments does not always occur. After interaction with the membranes of *Streptomyces* sp. and the isolated DD-carboxypeptidase of the L-form of *P. mirabilis* (unpublished data), the main degradation product is penicilloic acid (and perhaps other degradation products with intact $\text{C}_5 - \text{C}_6$ linkage; unpublished data). In this respect, one should note that breakdown of the complex formed between benzylpenicillin and the exocellular enzyme from *Streptomyces* R39 yields phenylacetyl glycine when breakdown occurs in a medium of high ionic strength under conditions of enzyme reactivation. In a medium of low ionic strength where the enzyme does not recover its activity as DD-carboxypeptidase-transpeptidase, breakdown of the complex apparently yields penicilloic acid (unpublished data). The mechanism of these reactions remains to be discovered.

CONCLUSIONS

Qualitatively, penicillin is, at the same time, a substrate and an inhibitor of the pep-

tidoglycan cross-linking enzyme system. Quantitatively, this double property can be expressed by the values of the kinetics constants involved in the interaction.

Comparison of the "penicillin sensitivity" of the exocellular R61 and R39 enzymes that have been used as models in the present study (Tables 1-3) and of other enzymes demonstrates that "intrinsic" resistance of the penicillin target may result either from a lack of recognition (a high K value) or from a rapid degradation of the antibiotic molecule (a high k_3/K ratio value and a high k_4 value). When considered as penicillin-degrading agents, the enzymes of the peptidoglycan cross-linking system catalyze either the complete splitting of the antibiotic molecule into two fragments with rupture of the C_5-C_6 linkage or the simple hydrolysis of the β -lactam ring, thus suggesting a possible relationship with classical penicillinases.

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