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# THE *STREPTOMYCES* DD-CARBOXYPEPTIDASE-TRANSPEPTIDASE SYSTEM AS A MODEL FOR THE STUDY OF PENICILLIN ACTION

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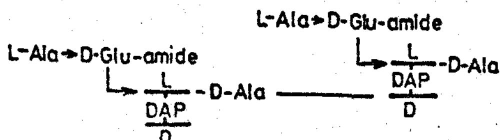
## ABSTRACT

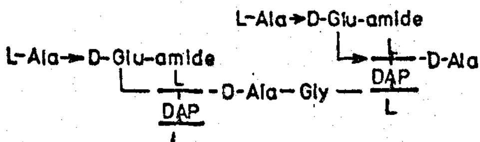
A new model for the transpeptidation reaction involved in the biosynthesis of the bacterial wall peptidoglycan and for its inhibition by penicillin is proposed. This model is in open conflict with the hypotheses previously postulated. It rests upon the demonstration that 1) carboxypeptidase and transpeptidase activities are performed by the same enzyme, 2) inhibition of both activities by penicillin is carried out in the absence of irreversible acylation of the protein, 3) the enzyme contains multiple sites some of which are involved in regulation, 4) penicillin does not act as a structural analogue of the donor peptide involved in transpeptidation but may act at the level of regulatory site(s).

## INTRODUCTION

The bacterial wall peptidoglycan is a single macromolecule which forms a continuous network around the cytoplasmic membrane and provides the cell with a supporting structure of high tensile strength. Basically, this peptidoglycan network is composed of glycan strands which are interconnected through peptide chains. The glycan consists of linear chains of alternating β-1,4-linked pyranoside N-acetylglucosamine and N-acetylmuramic acid residues. The D-lactyl groups of the N-acetylmuramic acid residues are substituted by tetrapeptide units which have the general sequence L-Ala-γ-D-Glu-L-$R_3$-D-Ala. The peptide units are in turn cross-linked through specialized bridges. Considerable species variations in the amino acid composition and the location of peptide cross-linking bridges have been observed. They have been used to divide the bacterial species into four chemotypes (1). In all cases, however, the bridging between two peptide units involves the C-terminal D-alanine residue of one peptide. For example, a peptide dimer of the wall peptidoglycan of *Streptomyces* strain R39 has the following structure :




in which the interpeptide bridge is a direct bond extending between the C-terminal D-Ala residue of one tetrapeptide and the amino group located on the D-center of *meso*-diaminopimelic acid (DAP) of another tetrapeptide (peptidoglycan of chemotype I). By contrast, a peptide dimer of the wall peptidoglycan of *Streptomyces* strains R61, K11 and *albus* G has the following structure :

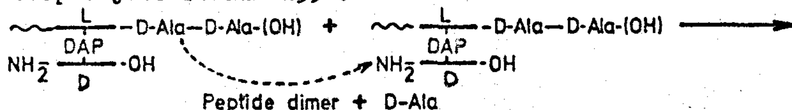



in which an additional glycine residue extends between the C-terminal D-Ala residue of one tetrapeptide and one amino group of the LL-diaminopimelic acid residue of another tetrapeptide (peptidoglycan of chemotype II).

The biosynthesis of the wall peptidoglycan occurs at three different sites in the cell (2). Peptidoglycan precursors made on a uridylic acid *cytoplasmic* carrier are transferred from uridylic acid to an undecaprenyl phosphate *membrane* carrier and from this, to a final acceptor in the expanding *wall* peptidoglycan. The peptide units in the precursors differ from the peptide units in the wall peptidoglycan in that precursors end in a D-alanyl-D-alanine sequence. At some time during the later stages of the biosynthesis, the nascent peptidoglycan undergoes peptide crosslinking and becomes insoluble. The closure of the bridges is achieved through a transpeptidation reaction (3,4) during which the penultimate C-terminal D-alanine residue of a peptide *donor* is transferred to a N-terminal amino group of a peptide *acceptor*. Interpeptide bonds are formed (in the absence of an exogenous source of energy) and equivalent numbers of D-alanine residues are liberated from the peptide donors so that tetrapeptide units are crosslinked.

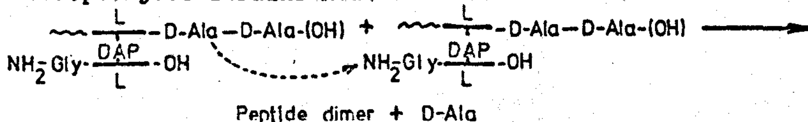
Two examples of transpeptidation which would result in the closure of peptide bridges are as follows :

In *Streptomyces* strain R39 :




Peptide dimer + D-Ala

In *Streptomyces* strains R61, K11 and *albus* G :




Peptide dimer + D-Ala

An important step in the lethal action of penicillin G upon bacteria is the abolition of or the reduction in the efficiency of the membrane-bound transpeptidase involved in peptide crosslinking (3,4). For a long time, it was thought that penicillin action might

rest upon a combination of two features, *i.e.* its resemblance to the nascent peptidoglycan at the level of the D-alanyl-D-alanine donor site (thus favouring the binding of the antibiotic to the transpeptidase) (3) and the acylating capacity of the penicillin molecule through cleavage of its β-lactam amide bond (hence-resulting in the irreversible inactivation of the enzyme) (2). In fact, however, the peptide bond between the two D-alanine residues of the peptide donor is about 25 % longer than the corresponding bond in the β-lactam ring of penicillin, and, furthermore, the angle around the D-Ala-D-Ala peptide bond (180°) is considerably different from the angle around the corresponding bond in the β-lactam ring (135.7°). Accordingly, the initial hypothesis was slightly modified (5). Cleavage and formation of peptide bond by the transpeptidase was assumed to be preceded by the distortion of the D-alanyl-D-alanine peptide bond to an angle of 135.7° and penicillin was assumed to be a structural analogue of this transition state.

A second enzymatic activity which is also sensitive to penicillin has been detected in many bacteria and is catalyzed by a D-alanyl-carboxypeptidase. This activity is similar to that of the transpeptidase in that D-alanine is released from the C-terminal end of the peptide chain. However, peptide crosslinking does not occur. Depending upon the bacterial species, carboxypeptidase activities seem to be reversibly inhibited (in *Escherichia coli*) or irreversibly inactivated (in *Bacillus subtilis*) by penicillins. It has been suggested that transpeptidase and carboxypeptidase are independent activities performed by distinct enzymes (6).

The existence of carboxypeptidases distinct from transpeptidases and the above theories of penicillin action are not supported by recent experiments which were carried out with transpeptidases isolated from strains of *Streptomyces*.

## TRANSPEPTIDASES FROM *STREPTOMYCES* STRAINS R39 AND R61

*Streptomyces* sp have the ability, perhaps unique in the microbial world, to release their membrane-bound transpeptidase to the external culture medium from which they can then be easily isolated. These soluble enzymes when purified act on peptides ending in a C-terminal D-alanyl-D-alanine sequence and can function either as carboxypeptidases or as transpeptidases depending upon the availability of nucleophilic carboxylyl acceptor ($H_2O$ or $NH_2$-R). Attack by water leads to the simple hydrolysis of the D-alanyl-D-alanine peptide bond of the peptide donor (carboxypeptidase activity). Attack by a suitable amino group acceptor leads to the formation of a new D-Ala-R peptide bond with the concomitant release of the terminal D-alanine residue of the peptide donor.

*Streptomyces* strains R39 and R61 are of special interest for the following reasons. 1) The isolated enzymes have high activity on the synthetic peptide $N^{\alpha}$,$N^{\varepsilon}$-diacetyl-L-lysyl-D-alanyl-D-alanine. By studying their hydrolytic action in water on a series of peptides presenting the general structure ~~L-$R_3$-$R_2$-$R_1$-(OH), their specificity profiles for peptide donor were determined. 2) The amino group acceptor involved in the transpeptidation reaction in strain R39 (see above) is located on the D-carbon of *meso*-diaminopimelic acid so that the interpeptide bond which is synthesized is a D-alanyl-D-*meso*-diaminopimelic acid linkage in α position to a free carboxyl

group. In marked contrast, the amino group acceptor in strain R61 is a N-terminal glycyl-LL-diaminopimelic acid sequence so that the interpeptide bond formed is a D-alanyl-glycyl linkage in an endo-position. The study of the R39 and R61 transpeptidases thus allowed us to examine how closely the differences in the structure of the wall interpeptide bridges are reflected by the requirements of the enzymes for peptide acceptors. 3) Strain R39 was considerably more sensitive to penicillins and cephalosporins than strain R61 and these differences facilitated the study of the relationship between the *in vivo* lethal action of the antibiotics and their *in vitro* action upon the isolated enzymes. 4) The extracellular transpeptidases from *Streptomyces* are proteins of relatively small size. The molecular weight of the R61 enzyme, as determined by sedimentation at equilibrium and diffusion is about 37,000 .

## SUBSTRATE REQUIREMENTS FOR PEPTIDE DONOR

The substrate requirements of the R39 and R61 enzymes for peptide donor were studied with the help of peptides presenting the sequence X-L-$R_3$-$R_2$-$R_1$-(OH) . Reactions were carried out in water and the amount of C-terminal $R_1$ residue released was determined. The R39 enzyme and the R61 enzyme, respectively, differed in their Km and Vmax values with the various peptides studied (7,8). Both enzymes, however, showed considerable specificity for the occurrence of 1) a D-amino acid residue, often preferentially a D-alanine, at the C-terminal $R_1$ position, 2) solely D-alanine at the penultimate $R_2$ position, and 3) a relatively long aliphatic side chain at the L-$R_3$ position. In other words, provided that the peptides have a C-terminal D-alanyl-D-alanine sequence preceded by a L-$R_3$ group with a long side chain (*i.e.* a profile found in all nascent peptidoglycans of chemotypes I and II), both enzymes seemed content, and were able to bind the peptides and to hydrolyze readily the C-terminal D-alanyl-D-alanine bond. For more details see (7,8).

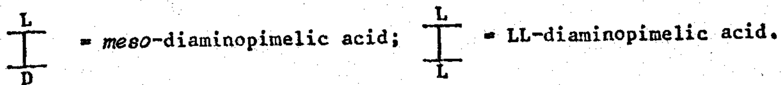
## SUBSTRATE REQUIREMENTS FOR PEPTIDE ACCEPTOR

In the presence of a suitable carboxyl donor — the tripeptide $N^{\alpha}$,$N^{\varepsilon}$-diacetyl-L-lysyl-D-alanyl-D-alanine — and a proper amino acceptor, both R39 and R61 enzymes catalyzed transpeptidations in the absence of an exogenous input of energy and with the concomitant release of the terminal D-alanine of the donor peptide.

Qualitative differences between the two enzymes were not observed when free amino acids were tested as possible acceptors (9). With either [$^{14}$C]-D-Ala, [$^{14}$C]-Gly or [$^{3}$H]-*meso*-diaminopimelic acid as acceptors, both enzymes catalyzed the formation of either diacetyl-L-lysyl-D-alanyl-[$^{14}$C]-D-Alanine, diacetyl-L-lysyl-D-alanyl-[$^{14}$C]-glycine or diacetyl-L-lysyl-D-alanyl-D-[$^{3}$H]-*meso*-diaminopimelic acid. Note that with *meso*-diaminopimelic acid, only the amino group located on the D-center was used as acceptor. L-alanine was not a substrate for transpeptidation. In the presence of saturating concentrations of [$^{14}$C]-D-alanine, the time course of transpeptidation paralleled the time course of hydrolysis of the donor tripeptide when no acceptor other than

Table I  Substrate requirements of two *Streptomyces* DD carboxypeptidases-transpeptidases acting as transpeptidases. Transfer of diacetyl-L-lysyl-D-alanyl from diacetyl-L-lysyl-D-alanyl-D-alanine tripeptide donor to various peptides acceptors. + + + , + + , + : high, good and low yield of product of transpeptidation, respectively. 0 : no detectable product.

| Acceptor used | Product of transpeptidation | Yield of product with enzymes | |
|---|---|---|---|
| | | R61 | R39 |
| Gly-Gly | $Ac_2$-L-lys-D-Ala-Gly-Gly | + + + | 0 |
| Gly-Gly-Gly | $Ac_2$-L-lys-D-Ala-Gly-Gly-Gly | + + | 0 |
| Gly-L-Ala | $Ac_2$-L-lys-D-Ala-Gly-L-Ala | + + + | 0 |
| Gly- L<br>Ac- L | $Ac_2$-L-lys-D-Ala-Gly- L<br>Ac- L | + + | 0 |
| Gly-D-Ala | $Ac_2$-L-lys-D-Ala-Gly-D-Ala | + | 0 |
| D-Ala-Gly | $Ac_2$-L-lys-D-Ala-D-Ala-Gly | + | 0 |
| D-Ala-L-Ala | $Ac_2$-L-lys-D-Ala-D-Ala-L-Ala | + | 0 |
| L-Ala-L-Ala | | 0 | 0 |
| L-Ala-D-Glu<br>L -D-Ala<br>D -(OH) | L-Ala-D-Glu<br>L -D-Ala<br>$Ac_2$-L-lys-D-Ala D -(OH) | + + | + + |
| L-Ala-D-Glu<br>L -D-Ala<br>D -(amide) | L-Ala-D-Glu<br>L -D-Ala<br>$Ac_2$-L-lys-D-Ala D -(amide) | + + | 0 |




= *meso*-diaminopimelic acid; = LL-diaminopimelic acid.

water was present. In some cases, transpeptidation was shown to occur at a molar ratio of amino acid acceptor : water as low as 1:1.8 × $10^6$ (assuming that the concentration of water in the active site region of the enzyme is 55 M). This observation demonstrated the exceedingly high efficiency of the enzymes as transpeptidases.

Striking differences between the two enzymes were observed when peptides instead of free amino acids were tested as possible acceptors in transpeptidation reactions (unpublished data). As shown in Table I, the R39 transpeptidase only catalyzed the synthesis of interpeptide bonds that are α to a free carboxyl group whereas the R61 enzyme catalyzed the synthesis of peptide bonds either in an endo- or at a C-terminal position. As discussed above, the interpeptide bond in strain R61 is a D-alanyl-glycyl linkage in an endo-position whereas the interpeptide bond in strain R39 is a D-alanyl-(D)-*meso*-diaminopimelic acid linkage at a C-terminal position. Hence, the differences in the requirements of the enzymes for peptide acceptors reflected the differences in structure of the wall peptidoglycan of the corresponding strains.

## REGULATORY SITES ON THE ENZYMES

The use of peptides as acceptors also revealed the existence of control mechanisms in both R61 and R39 transpeptidases (unpublished data).

Amidation of the tetrapeptide L-Ala-γ-D-Glu-(L)-*meso*-DAP-(L)-D-Ala either on the D-center of *meso*-diaminopimelic acid (DAP-amidated tetrapeptide) or on the α-carboxyl group of D-glutamic acid (Glu-amidated tetrapeptide) exerted drastic and specific influences on the activity of the R39 enzyme. For example : 1) When increasing amounts of the non-amidated tetrapeptide were used, the yield of transpeptidation with diacetyl-L-lysyl-D-alanyl-D-alanine as donor, increased until it reached a maximal value and then remained constant. 2) Amide substitution of the (D)-COOH of *meso*-diaminopimelic acid completely prevented the DAP-amidated tetrapeptide from being recognized by the enzyme. In its presence, transpeptidation did not occur and hydrolysis of the tripeptide donor was unaffected. 3) Transpeptidation with the Glu-amidated tetrapeptide — *i.e.* the natural peptide which undergoes transpeptidation in *Streptomyces* R39 — was maximal within a narrow range of relative concentrations of enzyme and substrates. At high concentrations of acceptor, both transpeptidation and hydrolysis were inhibited until, eventually, they were completely abolished. Under such latter conditions, the tripeptide donor present in the reaction mixtures remained unused. 4) The interpeptide bond D-alanyl-(D)-*meso*-diaminopimelic acid which is synthesized by the R39 enzyme is at a C-terminal position and therefore is susceptible to be hydrolyzed through the carboxypeptidase activity of the same enzyme. These antagonistic activities of the R39 enzyme readily explain the fact that under conditions of prolonged incubation in time course experiments, the product initially formed by transpeptidation is slowly reconverted into peptide monomers. 5) Finally, a peptide dimer formed of two tetrapeptides (in which the D-alanyl-(D)-*meso*-diaminopimelic acid interpeptide linkage was protected against hydrolysis by amidation

of the α-carboxyl group) was found not to be a substrate for transpeptidation. However, when added to the system containing diacetyl-L-lysyl-D-alanyl-D-alanine as donor and Glu-amidated tetrapeptide as acceptor, the above peptide dimer strongly inhibited both hydrolysis of the donor and transpeptidation.

The R39 enzyme thus exhibited many properties that were likely to be involved in the regulation of its hydrolyzing and synthesizing activities *in vivo*. The R61 enzyme has not yet been extensively studied. However, this latter enzyme was also inhibited by an excess of acceptor peptide. At concentrations higher than 0.01 M, the peptide acceptor glycyl-L-alanine inhibited the total amount of D-alanine liberated from diacetyl-L-lysyl-D-alanyl-D-alanine by the R61 enzyme and decreased the yield of tetrapeptide diacetyl-L-lysyl-D-alanyl-glycyl-L-alanine formed by transpeptidation.

ACTION OF PENICILLINS AND CEPHALOSPORINS ON *STREPTOMYCES IN VIVO*, ON ISOLATED TRANSPEPTIDASES AND ON MEMBRANE-BOUND TRANSPEPTIDASES

Several penicillins and cephalosporins were tested as inhibitors of both the transpeptidase and carboxypeptidase activities of the purified R39 and R61 enzymes (unpublished data) (Table II). Inhibition of carboxypeptidase activity was estimated as the amount of antibiotic which reduced by 50 % the release of D-alanine from diacetyl-L-lysyl-D-alanyl-D-alanine by the enzyme, in the absence of any amino acceptor group. Inhibition of transpeptidase activity was estimated as the amount of antibiotic which reduced by 50 % the amount of diacetyl-L-lysyl-D-alanyl transferred by the enzyme from the tripeptide donor to a glycine acceptor. With both R39 and R61 enzymes, inhibition of the transpeptidase activity always occurred at those concentrations of antibiotics (ID50; Table II) which inhibited the carboxypeptidase activity, thus providing additional evidence that the same enzyme performed both carboxypeptidase and transpeptidase activities. The ID50 values greatly varied according to the antibiotics and the sensitivity of the R39 enzyme was greater than that of the R61 enzyme (Table II).

The ID50 values were compared with the LD50 values on the corresponding *Streptomyces* strains. The LD50 values were determined as the concentrations of antibiotics which reduced by 50 % the number of single cell colonies. In these experiments, suspensions of conidia were spread on agar media containing various concentrations of antibiotics and incubated at 28° for several days, at which time the colonies were counted. There appeared to be no correlation between the ID50 and LD50 values (Table II). For the R39 strain, the LD50 values were usually much higher than the ID50 values on the isolated R39 enzyme. For the R61 strain, the LD50 values were either similar to, considerably higher or lower than the corresponding ID50 values.

Cytoplasmic membranes of *Streptomyces* strain R61 were prepared which were able to utilize diacetyl-L-lysyl-D-alanyl-D-alanine and various amino acids and peptides in transpeptidation reactions, as does the purified exocellular enzyme. The ID50 values of each antibiotic for the membrane-bound transpeptidase (ID50 - membrane) were determined as above. They were found to be very similar to the *in vivo* LD50 values on the R61 strain (Table II). Hence, the

**Table II** Relationship between ID50 (soluble enzyme) ID50 (membrane-bound enzyme) and LD50 (on single cell colonies)

| Antibiotics | *Streptomyces* R39 | | | *Streptomyces* R61 | | | | |
|---|---|---|---|---|---|---|---|---|
| | ID50 (a) | LD50 | ID50/LD50 | ID50 (a) | ID50 (b) membrane | LD50 | ID50/LD50 | ID50 membrane/LD50 |
| 6-aminopenicillanic acid | 575 | 5,100 | 0.113 | 130,000 | 8,000 | 1,850 | 70 | 4.34 |
| Penicillin G | 4.4 | 70 | 0.063 | 14 | 215 | 560 | 0.025 | 0.38 |
| Penicillin V | 4 | 140 | 0.025 | 190 | 1,850 | 510 | 0.37 | 3.6 |
| Ampicillin | 3.4 | 240 | 0.014 | 2,900 | 800 | 540 | 5.4 | 1.48 |
| Carbenicillin | 197 | 830 | 0.240 | 800 | 3,400 | 3,310 | 0.24 | 1.03 |
| Oxacillin | 22 | 710 | 0.017 | 1,900 | 8,800 | 4,710 | 0.40 | 1.87 |
| Cloxacillin | 38 | 530 | 0.074 | 7,000 | 3,900 | 5,040 | 1.4 | 0.78 |
| Methicillin | 47 | 730 | 0.058 | 14,000 | 3,500 | 4,410 | 3.2 | 0.79 |
| Cephalosporin C | 5.3 | 9,000 | 0.0006 | 52 | 51,000 | 11,220 | 0.0046 | 4.55 |
| Cephaloglycine | 6.4 | 120 | 0.053 | 7,100 | 6,200 | 2,860 | 2.5 | 2.17 |
| Cephalexin | 21 | 15 | 1.40 | 55,000 | 6,000 | 1,900 | 27 | 3 |
| Cephalothin | 4.2 | 90 | 0.047 | 92 | 7,000 | 1,400 | 0.066 | 5 |

ID50, LD50 and ID50 membrane : see text. All results are expressed in $10^{-8}$ M of antibiotic.

(a) the same values are for both carboxypeptidase and transpeptidase activities;

(b) for transpeptidation.

carboxypeptidase-transpeptidase is the target of penicillins and cephalosporins but the sensitivity of the exocellular enzyme towards the antibiotics and that of the membrane-bound enzyme may be very different. An increased resistance of the membrane-bound enzyme, compared to that of the exocellular enzyme, can be easily explained since non-specific binding sites for penicillins and cephalosporins are likely to occur on the membrane. An increased sensitivity of the membrane-bound enzyme is much more difficult to interpret. It suggests conformational changes of the enzyme as the result of its integration in the lipophilic environment of the membrane.

## PENICILLIN BINDING SITES

The mechanism of inhibition by penicillin of the hydrolysis of the tripeptide donor diacetyl-L-lysyl-D-alanyl-D-alanine, in the absence of amino acceptor, has been studied. Kinetically, inhibition of the R39 enzyme was noncompetitive. Penicillin appeared to combine with the enzyme at a site that was not identical with the substrate binding site. Increasing the penicillin concentration caused disproportionate decreases in the catalytic rate of hydrolysis, suggesting a conformational response of the enzyme towards the inhibitor (8).

Kinetically, the inhibition of the R61 enzyme was competitive (7). However, competitive kinetics do not necessarily exclude an inhibitory mechanism other than a direct competition between substrate and inhibitor for the same site on the free enzyme. Binding of penicillin G to the R61 enzyme was found to cause quenching of its fluorescence (unpublished data). The amount of enzyme required to bind one mole of penicillin G, as measured by fluorescence quenching, was 35 to 40,000 g , a value which was in agreement with the molecular weight as determined by sedimentation and diffusion. Similarly, the dissociation constant of the enzyme-penicillin complex as measured by fluorescence quenching ($10^{-8}$ M) was also in close agreement with the Ki value as determined by kinetic measurements. The dipeptide glycyl-L-alanine acceptor, but not the diacetyl-L-lysyl-D-alanyl-D-alanine peptide donor, was found to decrease the affinity of the R61 enzyme for penicillin. A 1000 fold decrease of the dissociation constant of the enzyme-penicillin complex was observed in the presence of 24 mM glycyl-L-alanine. On the other hand, glycine, when used at a concentration that should saturate the acceptor site on the enzyme, did not affect the association constant of the enzyme for penicillin. It should be borne in mind that excess of glycyl-L-alanine inhibits the enzyme, whereas excess of glycine does not.

The penicillin molecule has a highly reactive CO-N bond in its lactam ring causing it to be a powerful acylating agent. Using different techniques (8 and unpublished data), the inhibition of both R61 and R39 exocellular enzymes was found to be completely reversible and to occur in the absence of detectable acylation of the protein by the antibiotic.

## CONCLUSION

*Streptomyces* sp have provided an experimental approach for the study of the mechanism of the transpeptidation reaction in the

biosynthesis of the bacterial walls and of its inhibition by penicillin. The results obtained with *Streptomyces* strains R39 and R61 are in open conflict with the hypotheses previously proposed (2-6) in that they show that 1) carboxypeptidase and transpeptidase activities are performed by the same enzyme (M.W. : 37,000 for the R61 enzyme); 2) inhibition of the isolated enzyme is not carried out by irreversible penicilloylation of the enzyme; 3) penicillin does not act as a structural analogue of the donor peptide involved in transpeptidation.

Both R39 and R61 enzymes show considerable specificity in their substrate requirements for donor and acceptor peptides. The profile for the donor peptide is that found in all nascent peptidoglycans of chemotypes I and II, but the profile for the acceptor peptide is much more species-specific. Both catabolic (carboxypeptidase) and anabolic (transpeptidase) activities of the R39 enzyme are deeply influenced by seemingly minor alterations in the structure of the peptide acceptor such as the presence and the location of amide groups. Inhibition of enzyme activity by excess of peptide acceptor and by the products of transpeptidation is also of prime interest for the understanding of the functioning of these enzymes *in vivo*. All these observations point to the existence of multiple sites on the enzymes which function to control the rates of the two reactions. Thus, the relationship between hydrolysis of the peptide donor and its utilization for transpeptidation could be used to control the size of the peptide moiety of the completed wall peptidoglycan. Penicillin appears to act on such control site(s). It may be that either minute and so far undetected amounts of "penicillin" or penicillin like compounds are present in all bacteria or penicillin mimics a natural allosteric modifier in the control of the transpeptidase activity in the growing cells.

There is no relationship between the *in vitro* sensitivity of the isolated *Streptomyces* enzymes to penicillins and cephalosporins and the *in vivo* sensitivity of the corresponding strains. However, the *in vivo* sensitivity of strain R61 is closely reflected by the *in vitro* sensitivity of the membrane-bound enzyme. This observation shows that components of the membrane other than the transpeptidase are involved in the sensitivity (or resistance) to the antibiotics and/or that the conformation of the enzyme is modified by its integration within the membrane.

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