12

Mode of action of β-lactam antibiotics at the molecular level

J. M. Frère,† C. Duez, J. Dusart, J. Coyette, M. Leyh-Bouille and J. M. Ghuysen
(Service de Microbiologie, Faculté de Médecine, Institut de Botanique,
Université de Liège, Sart Tilman, B-4000 Liège, Belgium),
O. Dideberg (Laboratoire de Cristallographie approfondie,
Institut de Physique, Université de Liège, Sart Tilman, B-4000 Liège, Belgium)
and J. Knox (Biological Sciences Group and Institute of Materials Science,
University of Connecticut, Storrs, Connecticut 06268, USA)

INTRODUCTION

In recent years, two main approaches have been utilised for unravelling the mode of action of β-lactam antibiotics. One consists of treating whole bacteria or isolated cytoplasmic membranes with ^{14}C-labelled penicillin G and separating the so-called penicillin-binding proteins (PBPs) by polyacrylamide gel electrophoresis. In some mutants, the absence of one of the PBPs can be correlated with impaired function. Moreover, some β-lactams exhibit a strong preference for one of the PBPs and induce morphological changes similar to those observed with mutants lacking this same PBP (Spratt, 1978). This is mainly a physiologist's approach and, not surprisingly, it has yielded little information about the interaction between the antibiotics and their target(s) at the molecular level. This type of information has been obtained by an alternative approach, which might be thought of as a chemist's approach: it involves the isolation of penicillin-sensitive enzymes (D,D-carboxypeptidases and transpeptidases) (PSEs) and study of the interaction between purified enzymes and antibiotics as bimolecular reactions. Sometimes, for good measure, substrate is also thrown in: this makes the system a little more complicated but still manageable.

†To whom correspondence should be addressed.

In this paper, we will restrict our discussion to results obtained by this latter approach. The long-term goal of these studies is establishing correlations between structure and function so that, ideally, new molecules can be designed for inhibiting a given enzyme. We are still very far away from performing such a *tour de force*, but interesting features are beginning to emerge.

Transpeptidases and D,D-carboxypeptidases are the only bacterial enzymes which seem to be specifically inhibited by β-lactam antibiotics. At high concentrations, penicillins are known to acylate many proteins but, although this phenomenon might be of practical importance (such as in determining the allergenic effect of penicillins), it is not involved in the antibacterial effect itself. Many bacterial D,D-carboxypeptidases and transpeptidases have been described and some purified. All of them are sensitive to penicillins, with the notable exception of the D,D-carboxypeptidase from *Streptomyces albus* G (albus G. enzyme) which, as will be discussed below, displays a very low sensitivity. Are there other enzymes which specifically recognise the β-lactam ring? Only a limited number of enzyme classes catalyse reactions involving β-lactam antibiotics. The amide bond which connects the side-chain to the 6-aminopenicillanic acid (6-APA) or 7-aminocephalosporanic acid (7-ACA) nuclei can be hydrolysed by an amidase (Savidge and Cole, 1975; Vanderhaeghe, 1975) and the group on C_3 of some cephalosporins modified by an esterase (Abraham and Fawcett, 1975; Abbott and Fukuda, 1975) (figure 12.1). These enzymes usually decrease the biological

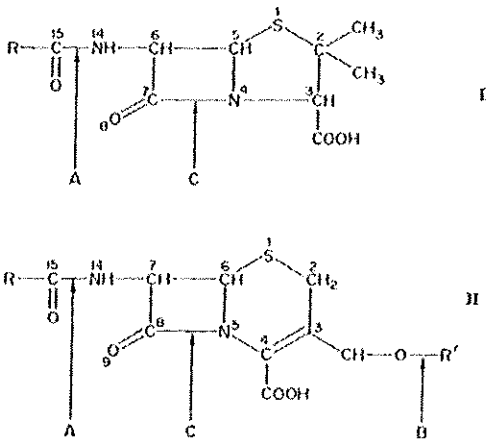


Figure 12.1 General structures of penicillins (I) and cephalosporins (II) showing the site of action of amidases (A), esterases (B) and β-lactamases (C). The group on C_3 of II does not necessarily bear an ester function; compounds such as cephalexin are thus not susceptible to esterases.

activity of the antibiotic (see below) but they do not alter its characteristic bicyclic structure. In contrast, however, penicillin-sensitive enzymes (PSEs) appear specifically to break open the four-membered ring. They share this property with the β-lactamases, a class of penicillin-destroying enzymes which, for therapeutic

reasons, has received a lot of attention. Understandably, β-lactamase-producing bacteria exhibit an increased resistance to β-lactam antibiotics. Is there a relationship between the sensitivity of penicillins and cephalosporins to β-lactamase and their ability to inhibit PSEs? At first sight, the answer might be yes. Very generally, β-lactams with high biological activity are substrates for β-lactamases. An evolutionary relationship between β-lactamases and PSEs is thus a distinct possibility.

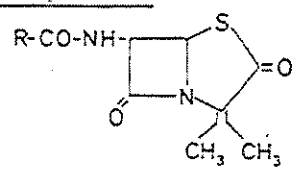
The existence of enzymes the only role of which would be to destroy penicillins has seemed puzzling to some authors (Saz, 1970; Ozer and Saz, 1970). However, some β-lactamases are among the enzymes exhibiting the highest turnover numbers and it seems unlikely that this high β-lactamase activity would only be a 'secondary activity'. Moreover, it was recently demonstrated that many PSEs (if not all of them) are endowed with a low, but significant (clearly a 'secondary activity') penicillin-destroying activity implying in all cases the hydrolysis of the β-lactam amide bond (Frère *et al.*, 1975*b*; Hammarström and Stromhger, 1975; and references on tables 12.2 and 12.3). That penicillinases would be derived from PSEs (by gene duplication, for instance) would thus appear to be a reasonable hypothesis.

CHARACTERISTIC STRUCTURAL FEATURES OF β-LACTAM ANTIBIOTICS

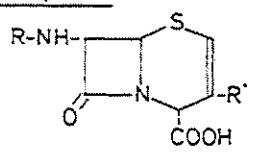
This hypothesis suggests that the 'activity' of penicillins and cephalosporins as substrates of β-lactamases or inhibitors of PSEs could have a common structural basis. Unfortunately, only substances active as antibacterial agents have been tested as substrates for β-lactamases and, more rarely, as inhibitors for D,D-carboxypeptidases and transpeptidases. Several well documented reviews have been published, exploring the relationship between structure and antibacterial activity (Nayler, 1971; Sweet, 1972; Gorman and Ryan, 1972; Jászberényi and Gunda, 1975; Gunda and Jászberényi, 1977). These data are difficult to use in an analysis of the relationship between structure and activity as inhibitor of the target enzyme(s) since the measured value (minimum inhibitory concentration) depends upon at least three phenomena: penetration of the antibiotic into the cell, its sensitivity to the β-lactamase(s) which the cell might produce and, finally, its interaction with the target enzyme(s). However, from the data accumulated so far by X-ray crystallography and infrared and nuclear magnetic resonance spectroscopy, the following features appear to be of major importance:

(1) In active compounds, the β-lactam amide is more unstable than in normal amides. The main reason for this instability is not the strain inherent in a four-membered ring but the fact that the presence of a second, fused ring renders the β-lactam nitrogen pyramidal and not planar as in a normal amide or a peptide bond. The non-planarity of the β-lactam amide bond decreases the amide resonance, which makes the N—C(=O) bond longer and more unstable than in normal amides (Sweet, 1972; Boyd, 1973). Compounds with a monocyclic β-lactam and many other bicyclic systems have a planar nitrogen and are not active. Striking examples of the last group of compounds are the Δ^2-cephems (Figure 12.2). These substances are inactive although, geometrically, they are more closely

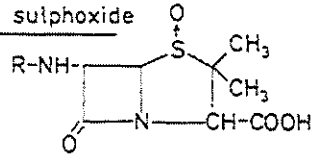
Anhydro penicillin



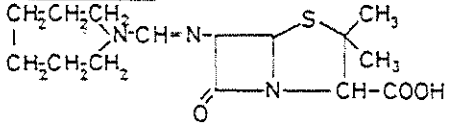
Δ²- Cephalosporins



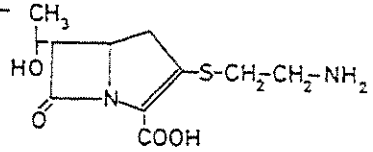
Penicillin sulphoxide



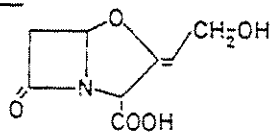
Mecillinam



Thienamycin



Clavulanic acid



Nocardicin A

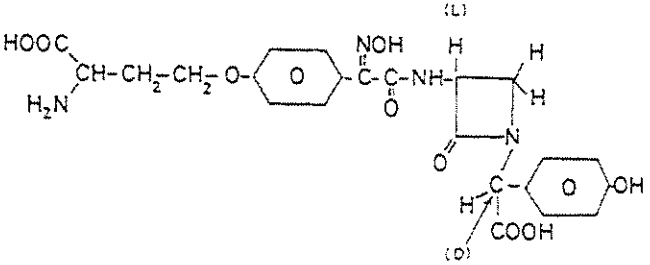


Figure 12.2 Structures of some 'non-classical' β-lactams

related to penicillins than are active cephalosporins. In contrast, the non-planarity of the β-lactam nitrogen does not appear to be a sufficient condition for activity: anhydropenicillin and penicillin sulphoxide have a pyramidal nitrogen atom but no antibacterial activity.

(2) The four atoms of the β-lactam ring do not lie exactly in the same plane: C_6 (C_7 in cephalosporins) is displaced and the lactam O_8 (O_9) is bent towards the S_1. The same bending is observed in anhydropenicillin and Δ^2-cephems. Again, the non-coplanarity of the four atoms of the β-lactam ring is not sufficient to determine antibiotic activity (Sweet, 1972).

(3) The R–NH– group on C_6 of penicillins (C_7 of cephalosporins) must be on the β-face of the β-lactam ring. The β-face is the upper face when the β-lactam amide is viewed in a direction parallel to the O_8, C_7, N_4 plane, with the O on the front left, the C on the back left and the N on the right. Inversion of the substituents on C_6 (6-epipenicillins) yields inactive compounds. R must be an acyl group.

(4) In general, replacement of the hydrogen atom on C_6 of penicillins or C_7 of cephalosporins by a larger group strongly decreases antibacterial activity. Only if the substituent is a methoxy group might activity be retained or even increased (Ho *et al.*, 1973).

(5) In cephalosporins, the presence of a good leaving group on the substituent of C_3 further decreases the stability of the β-lactam amide (Boyd *et al.*, 1975). Antibacterial activity is generally also increased by the presence of a better leaving group (Gorman and Ryan, 1972).

(6) Although esterification of the carboxyl on C_3 of penicillins strongly decreases the activity, lactonisation of the carboxyl on C_4 with an alcohol function on the substituent on C_3 of cephalosporins yields active compounds (Jászberényi and Gunda, 1975).

(7) No active compound has yet been found in the crystals of which the β-lactam C=O was engaged in a hydrogen bond (Sweet, 1972). The meaning of this observation is still obscure.

(8) Recently, various β-lactam antibacterial agents have been isolated or synthesised which do not present the structure of classical penicillins and cephalosporins: mecillinam, an amidino penicillin, still presents the usual fused-ring system of penicillins but nocardicin (Aoki *et al.*, 1976), clavulanic acid (Brown *et al.*, 1976), thienamycin and other derivatives of olivanic acid (Corbeth *et al.*, 1977) are quite different (figure 12.2). The mode of action of these substances is under current study in various laboratories. Thienamycin and clavulanic acid compete with penicillin G for the PBPs of *E. coli* (Spratt *et al.*, 1977) and mecillinam, nocardicin and clavulanic acid compete for the penicillin binding site of the D,D-carboxypeptidase-transpeptidase from *Actinomadura* R39 (J. Kelly, M. Noël, and J. M. Frère, unpublished results).

CHEMICAL AND β-LACTAMASE-CATALYSED OPENING OF THE β-LACTAM RING

As stated above, only a very limited number of substances not active as antibacterial agents have been tested as substrates for β-lactamases. From the scant information available, the following points appear to be of importance in relation to the preceding paragraphs.

(1) Δ^2-Cephalosporins are not substrates of the β-lactamases (O'Callaghan *et al.*, 1968). In contrast, penicillin G sulphoxide is a good substrate for β-lactamase I from *Bacillus cereus* (Davies *et al.*, 1974) and penicillin G sulphone, a good substrate for the exocellular β-lactamase from *Staphylococcus aureus* (Cartwright and Coulson, 1979). The sulphone of penicillanic acid inhibits various β-lactamases (English *et al.*, 1978) while that of 6-α-chloropenicillanic acid is a good inhibitor of the *S. aureus* β-lactamase (Cartwright and Coulson, 1979), but the real inactivator could be the β-chloro isomer, as in the case of the bromopenicillanic acids (Knott-Hunziker *et al.*, 1979*a, b*).

(2) 6-Epipenicillin G is not a substrate for the β-lactamase of *Pseudomonas aeruginosa* (Furth, 1975).

(3) 6-α-Methylpenicillin G is not a substrate for β-lactamases I and II from *B. Cereus*; 7-phenoxyacetamido-7-α-methyldesacetoxycephalosporanic acid and 7-methoxycephalothin are not substrates for β-lactamase II from *B. Cereus* (Davies *et al.*, 1974), an enzyme which is usually a good cephalosporinase.

(4) A cephalosporin with a good leaving group on the substituent on C_3 is generally a better substrate than the corresponding substance with a methyl group.

(5) The lactone from desacetoxycephalosporin C is a good substrate for β-lactamase I but not a substrate for β-lactamase II from *B. cereus* (Kuwabara and Abraham, 1969).

(6) The new, non-classical β-lactams do not appear to be substrates for the β-lactamases. However, some of them behave as rather specific inhibitors of these enzymes (Brown *et al.*, 1976; Maeda *et al.*, 1977).

(7) If one ignores the sulphoxide and the sulphone, the four-membered β-lactam ring must be condensed with a thiazoline or a dihydrothiazine ring (Richmond and Sykes, 1973).

Discounting two important exceptions (the sulphoxide–sulphone group and 7-methoxycephalothin), a relationship between antimicrobial activity and sensitivity to β-lactamases might exist with a common set of requirements responsible for the 'activity' of a β-lactam as antibacterial agent and as substrate (or inhibitor) of β-lactamases. However, as will be seen below, the diversity of β-lactamases is so great that it is extremely imprudent to draw conclusions from the sparse experimental data presented above.

In the microbial world, β-lactamases are very common enzymes. In Gram-negative bacteria, enzymes with so many different activity profiles have been described that Richmond and Sykes (1973) identified five classes and 15 subclasses. Class I enzymes are mainly cephalosporinases. They hydrolyse some cephalosporins up to 80 times faster than penicillin G. Some are active against ampicillin, others are not. Class II enzymes are predominantly active against penicillins. They have little or no activity against cephalosporins. Enzymes from classes III, IV and V hydrolyse both penicillins and cephalosporins but can be distinguished on the basis of their sensitivity to *p*-chloromercuribenzoate (PCMB) and interaction with penicillins, such as cloxacillin, which present a bulky, sterically-hindered side-chain directly attached to the exocyclic amide C atom (C_{15} in figure 12.1). Class IV enzymes are sensitive to PCMB and resistant to cloxacillin – some hydrolyse it, others simply do not recognise it. Classes III and V enzymes are resistant to PCMB. Cloxacillin is an inhibitor for class III enzymes, but a substrate for class V. In Gram-positive strains, the situation is also very

Table 12.1 Rate of hydrolysis of β-lactam antibiotics by OH^- ions and β-lactamases from various Gram-positive strains†

Hydrolysing agent / Antibiotic	OH^- ions	β-Lactamases: *Bacillus cereus* 569/H I	*Bacillus cereus* 569/H II	*Bacillus licheniformis* 749/C	*Bacillus licheniformis* 6346/C	*Staphylococcus aureus* A+B	*Staphylococcus aureus* C	*Staphylococcus aureus* E_3	Actinomycetes: *Actinomadura* R39	*Streptomyces albus* G	*Streptomyces* K11
Penicillin G	100	100	100	100	100	100	100	100	100	100	100
Penicillin V	170	153				110	100	110	220	80	116
Carbenicillin	100	22							33	10	27
Ampicillin	115	194	47	68	120	185		198	520	210	82
Methicillin	90	4	120	0.5	1.0	1.5	0.6	1.0	28	1.5	0
Oxacillin	100	5	89			4.5	1		320	19	0
Cloxacillin	100	0.5	89	0		2	1		30	2	0
6-APA		12	10	5	13	10		1	76	75	111
Cephalosporin C	47	0.1	14	1.1	1.0	1.1	1.2		6	6	5
Cephalothin	150	< 0.1	89						55	2.4	116
Cephaloglycine	180	< 0.1	41	1	42				215	7.5	88
Cephalexin	20		1.3						17	1.2	0
Nitrocefin	2000			20					230	23	
References‡		a, b	a, b	c	c	d		e	f	f	f

‡In each case, maximum velocities were calculated with penicillin G = 100. The rate of hydrolysis by OH^- ions was measured by incubating the substance at 37 °C (at a 1 mM concentration) in 1 M potassium phosphate buffer, pH 12.0 and estimating the product by the iodine method. The concentration of nitrocefin was different (10^{-4} M), and the product was estimated by direct spectrophotometric reading at 482 nm. The value of the first-order rate constant for the hydrolysis of penicillin G by OH^- ions was 0.5×10^{-3} s^{-1}. For the cephalosporins, similar values were also obtained at lower concentrations (10^{-4} M) by following the variation of absorbance at 260 nm (Waley, 1974; Fuad *et al.*, 1976; J. M. Frère, unpublished).

‡References: a, Thatcher, 1975*a*; b, Kubarawa and Abraham, 1967; c, Thatcher, 1975*b*; d, Richmond, 1975; e, Citri, 1971; f, Johnson *et al.*, 1973.

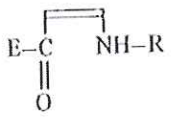
complicated. Table 12.1 summarises data obtained with the most widely studied of these Gram-positive β-lactamases. The antibiotics have been divided into seven groups, according to their chemical structure: penicillins V and G have uncharged, sterically unhindered side-chains; carbenicillin and ampicillin have charged side-chains while methicillin, oxacillin and cloxacillin present a sterically hindered side-chain; 6-APA, with no side-chain at all, but an uncharged amino group at neutral pH, is in a group by itself. Cephalosporins are distinguished by the nature of the substituent on C_3: cephalosporin C, cephalothin and cephaloglycine have a $-CH_2-O-CO-CH_3$ group; cephalexin, a methyl group, and nitrocefin, a chromogenic cephalosporin, a 2, 4-dinitrostyryl group. The inhibitory effect of cloxacillin and of related compounds on some of these enzymes has received a lot of attention. In the presence of an antibiotic from this group (type A substrates), β-lactamase I from *B. cereus* undergoes a slow transconformation into a less active configuration (Citri, 1973). Addition of a saturating amount of penicillin G (or another type S substrate) restores the more active conformer of the enzyme. This mechanism thus appears to follow Frieden's hysteresis model (Frieden, 1970) and the two forms clearly differ by several of their physical or chemical properties (Csanyi *et al.*, 1970; Samuni and Citri, 1975; Kiener and Waley, 1977; Citri *et al.*, 1976; Strom *et al.*, 1976). A similar behaviour has been described for the β-lactamase from *S. aureus* (Sagai and Sato, 1973). An attractive hypothesis can be built on the basis of these data: β-lactamases use the side-chain of penicillins and cephalosporins as a handle further to destabilise the β-lactam amide. This deformation of the antibiotic is only possible if the side chain has a certain degree of rotational freedom. With sterically hindered side-chains, the enzyme itself must 'adapt' to the substrate and change into a less active conformation (P. Blanpain, G. Laurent, B. Nagy and F. Durant, in preparation). The hypothesis can easily be modified to account for enzymes which do not 'recognise' oxacillin (the side-chain just does not fit into the enzyme site) or for enzymes which hydrolyse it (they are somehow capable of overcoming the steric hindrance). More surprising is the fact that some enzymes hydrolyse 6-APA rather well although they may have no 'handle' to use. After examination of numerous specificity profiles, one might wonder whether it is reasonable to search for a unified hypothesis of β-lactamases activity. It is somewhat disappointing to realise that, although a large amount of information is available about the chemistry of these enzymes (four sequences are known (Meadway, 1969; Thatcher, 1975*c*; Ambler and Scott, 1978; Ambler, 1975) and X-ray crystallographic data have been obtained on three of them (Aschaffenburg *et al.*, 1978; Knox *et al.*, 1976)), very little is known about their respective active sites. The involvement of a tyrosine and a histidine residue has been suggested (Scott, 1973) but little direct experimental evidence has been accumulated in favour of this hypothesis. Recently, a serine residue of β-lactamase I from *B. cereus* has been shown to react with β-bromopenicillanic acid. This Ser residue is conserved in the amino acid sequences of all known β-lactamases. The same reagent effectively inactivates the β-lactamases from *S. aureus, E. coli* W3310, *B. licheniformis* and *Pseudomonas aeruginosa* but not β-lactamase II from *B. cereus*. The fact that treatment with dilute alkali results in the release of the label suggests the possibility of an ester or ether linkage (Knott-Hunziker *et al.*, 1979*a, b*). Recent experiments (J. F. Fisher and J. R. Knowles, chapter 13 in this volume) indicate the possibility of formation of a transient acyl-enzyme, involving a serine residue, in the catalytic pathway of β-lactamases.

The reactivity of the β-lactam amide bond in a given compound determines its properties as an acylating agent. Approximate relative values of the acylating power of various antibiotics were obtained by measuring their susceptibility to hydroxyl ions (see table 12.1). In addition, cefoxitin, a cephalosporin with a methoxy group on C_7, was found to be twice as stable as benzylpenicillin. It is not a substrate for various β-lactamases (from *E. coli, S. aureus, Klebsiella, Pseudomonas*, . . .)

Examination of table 12.1 shows that, with the exception of the particularly unstable nitrocefin, the range of 'chemical' stability of the β-lactam antibiotics barely spans one order of magnitude. Interestingly the most stable (cephalexin) and the most unstable (cephaloglycine) differ only by the nature of the substituent on C_3: cephalexin has a methyl group which contains no leaving group, while cephaloglycine has a $-CH_2-O-CO-CH_3$ group, containing the acetoxy moiety, a good leaving group. In contrast, sensitivity to β-lactamases sometimes extends over more than three orders of magnitude, and in all cases, over two orders. In consequence, there does not appear to be any clear indication of a correlation between sensitivity to OH^- ions and to β-lactamases although, in the particular case of the pair cephaloglycine-cephalexin, the chemically less stable compound (cephaloglycine) consistently behaves as a better substrate (with the exception of Gram-negative class I β-lactamases which hydrolyse cephalexin faster than cephaloglycine!).

In the absence of steric hindrance, the attack of the β-lactam ring by OH^- ions can occur on the β- or the α-face (Boyd *et al.*, 1975). The fact that epipenicillins and β-lactams with hindered α-faces are poor substrates for β-lactamases indicates that enzymatic hydrolysis is probably limited to an attack on the α-face.

Inhibition of the β-lactamases from *E. coli* (RTem factor) and *S. aureus* by clavulanate involves the formation of a 1:1 complex (Fisher *et al.*, 1978; Cartwright and Coulson, 1979). Spectroscopic properties of the complex are in agreement with an intermediate of structure



where E is the enzyme. In further steps, reactivation of the enzyme can occur, but the interaction appears to be rather complicated and, for the *E. coli* enzyme, a branched pathway has been proposed (Charnas *et al.*, 1978).

PENICILLIN-SENSITIVE ENZYMES: D,D-CARBOXYPEPTIDASES AND TRANSPEPTIDASES

As stated above, penicillin-sensitive enzymes exhibit D,D-carboxypeptidase activity, transpeptidase activity, or both (figure 12.3). Some transpeptidases never use water as acceptor, some D,D-carboxypeptidases only utilise water. Many enzymes can utilise both water and an aminated molecule as acceptors but, in most cases, this latter substance must be a simple amino acid, such as glycine. The D,D-carboxypeptidases–transpeptidases excreted by *Streptomyces* R61 and *Actinomadura* R39, on the contrary, catalyse reactions which really mimic the

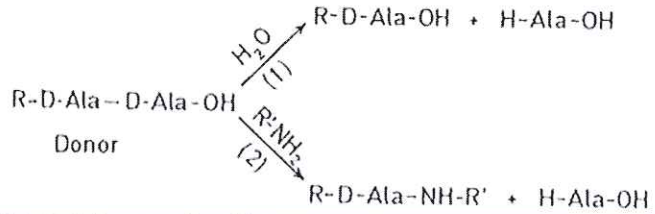


Figure 12.3 D,D-Carboxypeptidase (1) and transpeptidase (2) activities depend upon the nucleophilic acceptor on to which the R-Ala moiety of the donor is transferred: water in the first case, an amino group in the second

cross-linking of the peptide subunits of the nascent peptidoglycan (Ghuysen *et al.*, 1974; Zeiger *et al.*, 1975). In this article, we will use the term transpeptidase only for two classes of enzymes: those which do not use water as acceptor and those which can catalyse the dimerisation of donor-acceptor substrates.

The three-step mechanism which has been proposed for the interaction between the exocellular D,D-carboxypeptidase-transpeptidase from *Streptomyces* R61 and β-lactam antibiotics (Frère *et al.*, 1975*a*) appears to be valid for most penicillin-sensitive enzymes. This mechanism involves the reversible formation of a first stoichiometric complex, EI, which irreversibly transforms into a second, rather stable complex, EI*. This second complex irreversibly decays into a fully reactivated enzyme and a degraded, biologically inert antibiotic:

$$E + I \underset{}{\overset{K}{\rightleftharpoons}} EI \xrightarrow{k_3} EI^* \xrightarrow[\text{Nucleophilic agent}]{k_4} E + P(s)$$

The constants K, k_3 and k_4, respectively, represent the dissociation constant of EI, and the first-order rate constants for the two irreversible steps. The structure of the degradation product(s), P(s), is a function of three variables: the enzyme E, the antibiotic I and the nucleophilic agent involved in the degradation of EI*. For instance, with the exocellular D,D-carboxypeptidase-transpeptidase from *Streptomyces* R61, benzylpenicillin and water, the antibiotic is fragmented into phenylacetylglycine and an unidentified intermediate which spontaneously decays into *N*-formyl-D-penicillamine. In the presence of oxygen, this latter product can in turn give rise to the corresponding disulphide (Frère *et al.*, 1975*b*, 1976*a*). Hammarström and Strominger (1976) reported a similar mechanism for the membrane-bound D, D-carboxypeptidase from *Bacillus stearothermophilus* and claimed they had identified D-5,5-dimethyl-Δ^2-thiazoline-4-carboxylate as the possible intermediate, but it was later shown that the compound had been mischaracterised (Adriaens *et al.*, 1978). With the R61 enzyme (i.e. exocellular D,D-carboxypeptidase-transpeptidase from *Streptomyces* R61), a suitable amine ($R'-NH_2$), if present, can also accept the phenylacetylglycyl moiety (Figure 12.4). However, in the presence of various alcohols, the corresponding penicilloyl esters are also formed but penicilloic acid has never been detected (Marquet *et al.*, 1979). This enzyme similarly fragments penicillin V but only hydrolyses the β-lactam