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Structure-Activity Relations and <latex>β</latex>-Lactamase Resistance

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Structure–activity relations and β-lactamase resistance

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β-Lactam antibiotics resistant to β-lactamase degradation can be produced by many chemical modifications, but often at the expense of antibacterial activity. Substitution onto several positions in the molecule produces different and often selective resistance; for instance, heavily sterically hindered acyl groups give staphylococcal β-lactamase resistance to penicillins, and resistance to some enzymes from Gram-negative pathogens to both penicillins and cephalosporins. 6-α- or 7-α-substituents respectively confer a broad spectrum of resistance (e.g. cefoxitin), but changes at positions 2 or 3 have only a minor influence on enzyme susceptibility.

Changes in the ring condensed with the β-lactam, such as changing ceph-3-em to ceph-2-em may greatly enhance stability. Small improvements can occur when the nuclear sulphur atom is oxidized, but a much better effect is obtained when it is replaced by another atom such as oxygen, as in clavulanic acid. This compound appears to have broad spectrum resistance which is actually due to susceptibility and subsequent product inhibition.

Introduction

The first β-lactamase to be observed clinically was produced by staphylococci; the advent of the cephalosporins and broad spectrum penicillins many years later emphasized the fact that the β-lactamases elaborated by Gram-negative organisms were also of clinical significance. As the numbers of cephalosporins and broad spectrum penicillins increased, more and finer distinctions could be made between such enzymes. They are much more diverse than the staphylococcal enzymes in their substrate and inhibition profiles and physicochemical characteristics (Jack & Richmond 1969; Sykes & Matthew 1976; Richmond & Sykes 1973).

Gradually the structural requirements for resistance to some of the enzymes are becoming apparent, although the resistance attained has often been at the expense of antimicrobial activity. In addition, structural modifications that give resistance to one type of β-lactamase frequently fail to protect the molecule from attack by others. The structural requirements for resistance to some of the clinically more important β-lactamases will be discussed here.

Clinically relevant β-lactamases

A large amount of work has been done on a wide range of β-lactam antibiotics, with a multiplicity of enzymes, much of it with enzymes from organisms of no clinical significance such as *Bacillus cereus* and *B. licheniformis*. With the enzymes from Gram-negative organisms in particular, it has often been difficult to identify the enzymes used by different workers, and whether these were chromosomally or plasmid mediated.

The many different enzymes produced by Gram-negative organisms are not evenly distributed. In a recent survey in Nottingham, 74% of 112 ampicillin resistant organisms, serially isolated from out-patients, produced plasmid-mediated β-lactamases, mainly TEM and PIT-1, with two chromosomally mediated enzymes accounting for almost all of the remainder (O'Callaghan *et al.* 1978).

To simplify the picture, the work described here will be confined to four enzymes of major clinical significance. These are staphylococcal β-lactamase, and three of the most commonly occurring enzymes from Gram-negative organisms, which are representative of three widely differing types, easily distinguishable by their substrate and inhibition profiles; they are the most common plasmid mediated enzyme, TEM, and two chromosomal enzymes, P99 and K1 (table 1).

TABLE 1. ORGANISMS PRODUCING THE β-LACTAMASES

enzymes	producing organisms
staphylococcal	staphylococci
type 1, P99 (chromosomal)	*Enterobacter cloacae*, *Proteus morganii*
type III, TEM (plasmid)	*Escherichia coli*, most Enterobacteriaceae, *Haemophilus influenzae*, *Neisseria gonorrhoeae*
type IV K1 (chromosomal)	*Klebsiella*

There are many degrees of enzyme resistance; the description 'resistant' is used here for compounds undergoing little or no detectable breakdown in the presence of a specific β-lactamase. The structures giving enzyme resistance have evolved over a long period, as it was soon found that combining broad spectrum enzyme resistance and good antibacterial activity in the same molecule was not easy. The structure–activity relationships to be described here may well be modified in future by the discovery of new compounds or enzymes.

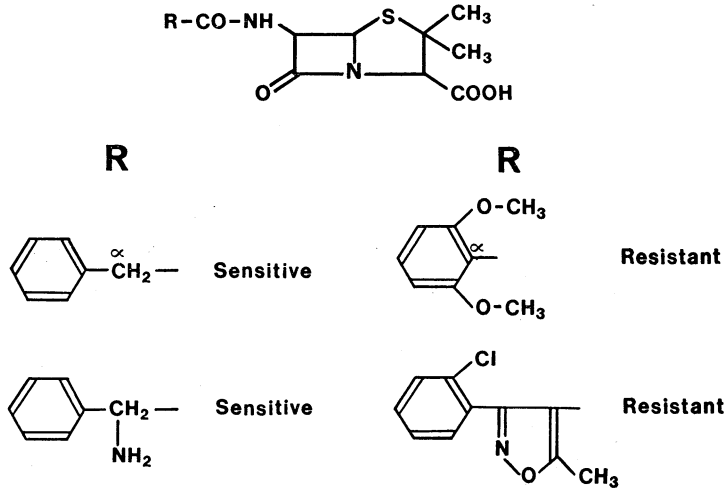

FIGURE 1. Effect of some penicillin side chains on resistance to staphylococcal β-lactamase.

β-LACTAMASES FROM STAPHYLOCOCCI

Many analogues of benzylpenicillin have been made in efforts to improve and widen its antibacterial spectrum, mostly by changes in the group attached at position 6-β. The main type of substituent giving penicillins resistance to staphylococcal penicillinase has the alpha carbon atom, next to the amide link incorporated into an aromatic system, as in methicillin, nafcillin or cloxacillin (figure 1).

The penicillin nucleus can be modified in many ways, some of which considerably increase resistance to staphylococcal β-lactamase, such as the presence of a side chain directly linked through carbon, replacement of sulphur by carbon (thienamycin; Tally *et al.* 1978) or enlargement of the fused ring to the 6-membered ring of a cephalosporin. At first sight, replacement

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of sulphur by oxygen (clavulanic acid) appears to give stability, and also enables the compound to inhibit the enzyme. However, it now seems likely that the enzyme attacks it initially and is then inhibited by the product in the way that Charnas *et al.* (1978) and Fisher *et al.* (1978) have described for the interaction of clavulanic acid with TEM enzyme.

Thus, the requirements for resistance suggest that there are several points of attachment between the substrate and the enzyme. One of these is the amide link; if it is sterically hindered, then attachment is prevented. Attachment at position 6 is prevented if the substituent is attached by a C–C link, or if two groups are attached at position 6. Attachment is also prevented in the absence of a sulphur atom at position 1, or if its configuration is modified because it is in a 6-membered ring (table 2).

TABLE 2. STRUCTURAL FEATURES IN β-LACTAM COMPOUNDS GIVING RISE TO SUSCEPTIBILITY TO STAPHYLOCOCCAL β-LACTAMASE

feature	intrinsic susceptibility	intrinsic resistance
nucleus	penam clavam	cephem 1-carbapenam (e.g. thienamycin olivanic acid) nocardicin
6-β-	R–C(XY)–CO–NH–, where X, or Y, or both are H	Ar–CO–NH–
6-α-		–OMe
2 or 3 side chain	apparently of subsidiary importance	

STRUCTURAL CHARACTERISTICS GIVING RESISTANCE TO β-LACTAMASES FROM GRAM-NEGATIVE ORGANISMS

(*a*) 6- *or* 7-*substituents in penicillins and cephalosporins*

(i) *Aromatic carboxamido penicillins and cephalosporins*

All analogues with simple side chains of the Ar–CH_2–CO–NH– type are generally susceptible to attack; some enzymes may attack penicillins or cephalosporins preferentially, while others can attack them equally. When the alpha carbon atom in the side chain is incorporated in an aromatic ring, resistance to all three enzymes is improved, but not to the same extent (table 3). In general, this type of compound has the greatest resistance to P99, with high affinity and ability to inhibit the enzyme, irrespective of the type of nucleus. Their resistance to TEM is intermediate, with the penicillin analogues having higher affinity and better inhibiting ability than the cephalosporins. With K1, the resistance is less complete, and the affinity of the compounds depends on the steric configuration of the side chain, planar groups such as naphthyl having a much greater affinity than three-dimensional groups, although they have similar amounts of resistance (table 4) (O'Callaghan & Morris 1972). This type of compound has no antibacterial activity against Gram-negative organisms.

(ii) *Substitution on the alpha carbon atom in the side chain*

While groups such as carboxy, amino or hydroxy give a smaller range and amount of enzyme resistance than the aromatic carboxamido side chains, they often give good antibacterial activity; this compromise between resistance and activity has resulted in the introduction of several clinically useful compounds. An acidic group gives good resistance to type I enzymes,

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which partly accounts for the activity of compounds like carbenicillin and cefsulodin against *Pseudomonas aeruginosa*. An amino group also enhances resistance, which is better for cephalexin than for ampicillin (Sykes & Matthew 1976). The hydroxy group in cefamandole primarily gives resistance to type I enzymes (table 5) (Richmond & Wotton 1976). Compounds with an acidic or a hydroxy group have high affinity for P99 enzyme, and can inhibit it.

TABLE 3. ACTIVITY OF P99, K1 AND TEM AGAINST CARBOXAMIDO PENICILLINS AND CEPHALOSPORINS

		percentage enzyme susceptibilities relative to cephaloridine		
acylamido type	3-substituent	P99	K1	TEM
thienylacetamido†	pyridiniummethyl	100	100	100
cloxacillin (p)	—	0	25	1
cloxacillin (c)	acetoxymethyl	0	5	2
nafcillin (p)	—	0	5	2
α-naphthylamido (c)	acetoxymethyl	8	10	10

†, cephaloridine; (p), penicillin; (c), cephalosporin. Arbitrary value of 100% susceptibility given for comparison purposes.

TABLE 4. EFFECTIVENESS OF THE CARBOXAMIDO PENICILLINS AND CEPHALOSPORINS AS INHIBITORS OF P99, K1 AND TEM

		I_{50} concentration/M		
acylamido type	3-substituent	P99	K1	TEM
cloxacillin (p)	—	3×10^{-9}	$>10^{-4}$	2×10^{-5}
cloxacillin (c)	acetoxymethyl	4×10^{-9}	$>10^{-4}$	$>10^{-4}$
nafcillin (p)	—	3×10^{-8}	4×10^{-6}	2×10^{-8}
α-naphthylamido (c)	acetoxymethyl	3×10^{-7}	8×10^{-5}	5×10^{-6}

Susceptible substrate = cephaloridine at 10^{-4} M. I_{50} is the concentration required to produce 50% inhibition of enzyme activity against cephaloridine.

TABLE 5. EFFECT OF ALPHA SUBSTITUENTS ON THE β-LACTAMASE RESISTANCE OF PENICILLINS AND CEPHALOSPORINS

	relative rates of hydrolysis		
compound	P99	K1	TEM
cephaloridine	100	100	100
carbenicillin†	0	70	8
ampicillin†	10	243	130
cephalexin‡	13	0.5	0.5
cefamandole‡	0.1	20	60
cefuroxime‡	0	0.1	1.3
cefoxitin‡	0	0	0

† Sykes & Matthew (1976). ‡ Richmond & Wotton (1976).

Larger substituents such as the complex substituted ureido groups in, for example, azlocillin (Stewart & Bodey 1977) or the cephalosporin T1551 (Mitsuhashi *et al.* 1978) also give resistance to P99 and other type I enzymes, but little resistance to the others. A second substituent on the alpha carbon atom does not increase resistance further, and tends to have adverse effects on antibacterial activity.

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The spectrum of resistance is considerably broadened when the alpha substituent is attached by a double bond, as in cefuroxime, (O'Callaghan *et al.* 1976) and other oximinocephalosporins such as cefotaxime (Fu & Neu 1978) of both of the general formula ArC(=N–OMe)CO–NH–. These compounds have high affinity only for P99 and inhibit it effectively. As the size of the ether group increases, the degree of resistance to many enzymes also increases (O'Callaghan & Gregory 1977).

(*b*) *Introduction of another group onto the* 6- *or* 7- *carbon atom*

Penicillins and cephalosporins have the β-configuration of attachment of the side chain to the 6- or 7- carbon atom. An additional substituent such as methoxy at 6- or 7-α greatly increases the scope and degree of enzyme resistance of penicillins and most cephalosporins, but frequently at the expense of antibacterial activity. However, compounds like cefoxitin (table 5) (Neu 1974) and cefmetazole (Nakao *et al.* 1976) combine excellent enzyme resistance with broad spectrum antibacterial activity.

Again, resistance to TEM and K1 appears to be due to low affinity, with the resistant compounds being unable to inhibit these enzymes. In contrast, cefoxitin has high affinity for P99 and is a good inhibitor for type I enzymes. It thus appears that P99 has high affinity for both 7-α and 7-β side chains, whereas changes away from Ar–CH_2–CO–NH– side chains and the addition of a 7-α group both appear to reduce the affinity of the compounds for K1 and TEM.

(*c*) *Oxidation of the nuclear sulphur atom*

Conversion of a cephalosporin to either of its sulphoxides or the sulphone enhances already existing resistance to some extent (M. H. Richmond, personal communication; C. H. O'Callaghan, unpublished), but this is often insufficient to compensate for the tendency of sulphoxides and sulphones to have less intrinsic antibacterial activity than their parent compounds.

The recently described penam sulphone (English *et al.* 1978), which has no side chain, appears to be highly resistant, but Fisher *et al.* (1978) postulate that some enzymes hydrolyse it, their further action then being prevented by product inhibition. In this case, therefore, the TEM and K1 enzymes have a high affinity for the substrate, but P99 has none.

TABLE 6. EFFECT OF VARIATIONS IN THE SIDE CHAIN ON RESISTANCE TO β-LACTAMASES FROM GRAM-NEGATIVE ORGANISMS

	side chain type			
enzyme	R—CH_2–	R—CH(X)–	R—C(=Y)–	Ar–
P99				
p	±	++	+++	+++
c	−	+, ++	+++	+++
K1				
p	−	−	+	++
c	−	+	++	+++
TEM				
p	−	−	++	+++
c	−	+	+++	+++

p, penicillin; c, cephalosporin.

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(*d*) *Changes at* C_2 *and* C_3

These do not usually affect the enzyme resistance characteristics of penicillin or a cephalosporin which have already been established by its other groups. An exception is the strongly electron withdrawing group 2,4-dinitrostyryl at position 3 in cephalosporins, which renders the β-lactam ring susceptible, despite the presence of groups such as 7-α-methoxy which would normally protect it (M. H. Noble, personal communication).

The effects of structural modification (*a*) to (*d*) are summarized in tables 6 and 7).

TABLE 7. EFFECT OF STRUCTURAL MODIFICATIONS, OTHER THAN IN THE ACYL GROUP, ON RESISTANCE TO β-LACTAMASES FROM GRAM-NEGATIVE ORGANISMS

	modifications with R—CH_2– type acyl group			
enzyme	6 or 7 α-OMe	sulphoxide	sulphone	position 2 or 3
P99				
p	+++	+	+	–
c	+++	+	+	–
K1				
p	+++	–	–	–
c	+++	–	–	–
TEM				
p	+++	+	+	–
c	+++	+	+	–

Abbreviations: p, penicillin; c, cephalosporin.

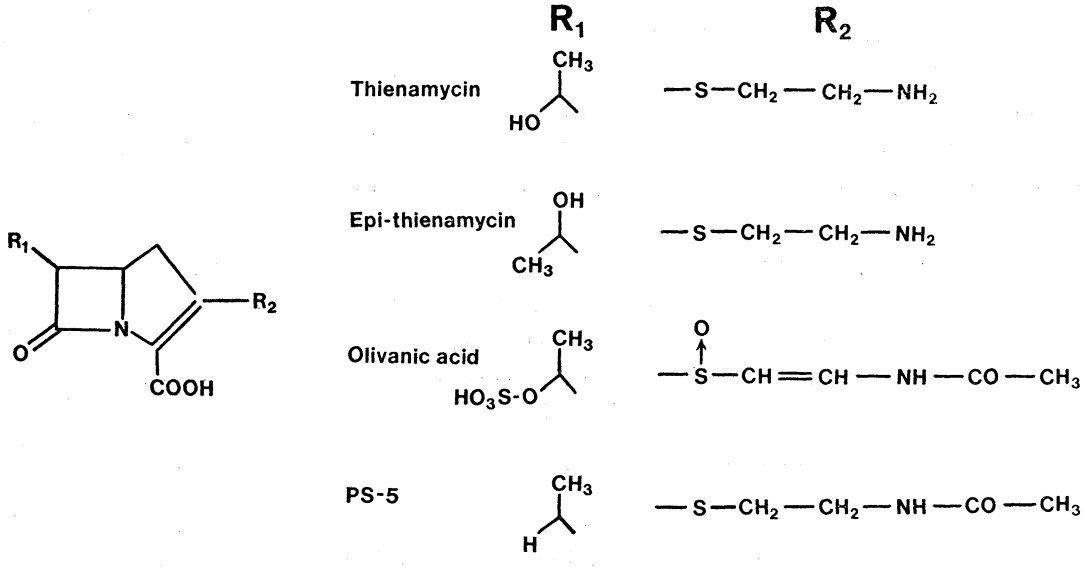

FIGURE 2. Structures of thienamycin, epi-thienamycin, olivanic acid and PS-5.

OTHER β-LACTAMS

The closely related new β-lactam compounds produced by *Streptomyces* spp. recently reported (Okamura 1978; Tally *et al.* 1978; Brown *et al.* 1977) have neither oxygen nor sulphur in the ring fused with the β-lactam ring (figure 2). They are highly resistant to several β-lactamases,

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but cannot inhibit them. However, they do appear to have a high affinity for type I enzymes like P99, and inhibit them, which means that compounds like thienamycin, olivanic acid, and PS-5 have inhibition profiles that are complementary to clavulanic acid (table 8).

TABLE 8. β-LACTAMASE RESISTANCE OF OTHER β-LACTAM COMPOUNDS, COMPARED WITH THEIR ENZYME-INHIBITING PROPERTIES AND DEDUCED AFFINITIES

	clavulanic acid	thienamycin	olivanic acid
PC1			
r	−†	++	++
i	+++†	−	−
a	high	poor	poor
P99			
r	++	++	++
i	−	(+)	+
a	low	slight	moderate
K1			
r	−	++	++
i	+++	−	(+)
a	high	poor	slight
TEM			
r	−	++	++
i	+++	−	(+)
a	high	poor	slight
B. fragilis			
r	−		
i	+++	n.t.	n.t.
a	high		

Abbreviations: r, resistance; i, inhibition; a, affinity; n.t., not tested.
† Degree of resistance or inhibition: −, none; +, ++, +++, increasing.

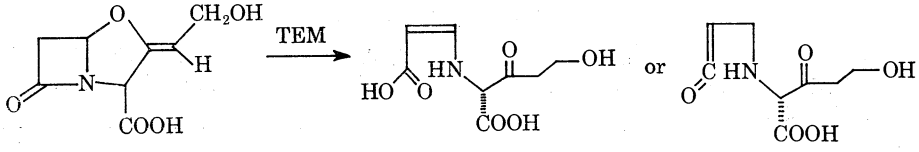

FIGURE 3. Structure of clavulanic acid and its postulated breakdown product.

Clavulanic acid has moderate antibacterial activity; as this is the same against enzyme-producers and non-enzyme-producers, it might be thought to be resistant to β-lactamases. However, recent work (Fisher *et al.* 1978; Charnas *et al.* 1978) has shown this not to be so. Clavulanic acid is highly susceptible to hydrolysis by a variety of enzymes, but their effects are quickly arrested because very small amounts of the hydrolysis product(s) (figure 3) inhibit most enzymes. P99 does not attack clavulanic acid and undergoes no inhibition; it is possible that this implied lack of affinity is due to the absence of a side chain at position 6.

Replacement of the nuclear sulphur atom in cephalosporins by oxygen to give oxacephem compounds increases the chemical reactivity of the β-lactam ring, making them more susceptible to many β-lactamases. They can be stabilized by the addition of a 7-α-methoxy group, as in 6059-S (Yoshida, this symposium).

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Summary and conclusions

(*a*) Staphylococcal β-lactamase appears to have comparatively narrow structural requirements for its substrates. These are a 5-membered ring containing either oxygen or sulphur fused with the β-lactam ring, with a side chain derived from a substituted acetic acid. Groups conferring resistance all act by steric hindrance, which appears to prevent the enzyme approaching the amide bond, or C_5 and C_6, or the 1-position.

(*b*) TEM and K1 can attack a wider range of substrates; it is also more difficult to produce analogues with absolute resistance to them. However, structures that improve resistance to these enzymes have many features in common with those giving resistance to the staphylococcal enzymes. The bulky carboxamido side chains, 6- or 7-α-methoxy groups, absence of sulphur or oxygen in the fused ring, and carbon-linked side chain appear to act by reduction of affinity and probably steric hindrance. These features would all prevent the approach of the enzyme to the amide, carbon atoms 5 and 6, or 6 and 7 (cephalosporins), and position 1, suggesting that these are, separately or together, the important points of attachment of the substrate to the enzyme.

(*c*) The situation is quite the opposite with P99. Although similar groups in the side chain and at 6- or 7-α confer resistance, they appear to do this by increasing the affinity of the substrate for the enzyme. As there seems to be little affinity between substrate and enzyme in the absence of a side-chain, or absence of an amide link, it would seem that the side chain is much the most important determinant of resistance to P99, and might mean that this enzyme attaches differently to its substrates.

However, although the enzymes may differ in the ways they approach and attach themselves to their substrates, their ultimate effects are the same, suggesting that they are all very much alike in the structures of their active centres (Ambler, this symposium).

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