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Possible binding orientations of β-lactams within *Staphylococcus aureus* PC1 β-lactamase suggest factors involved in β-lactamase resistance

J. Frau[1], S.L. Price[2]

[1] Departament de Química (Química-Física) Edifici Mateu Orfila, Universitat Illes Balears, E-Palma de Mallorca 07071, Spain

[2] Department of Chemistry, University College London, London WC1H OAJ, UK

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Summary. The electrostatic forces within the active site of the β-lactamase *Staphylococcus aureus* PC1 have been used to predict structures for the precatalytic complex with ampicillin, methicillin, clavulanate and imipenem. There are significant differences in the orientation of these β-lactams within the binding site, which explains the differences in their resistance to the lactamase. The electrostatic forces were calculated using a distributed multipole analysis of *ab initio* wave functions for both the lactams and the binding site residues, to ensure a good representation of the orientation dependence of this dominant contribution. The predicted binding orientations are contrasted with those predicted by overlaying the electrostatic extrema around the ligands. The accuracy of the ligand-only-based predictions is limited in some cases because of the subtle steric requirements of the lactamase binding site.

Key words: β-lactam compounds – β-lactamase class A – Binding orientations – Electrostatic forces – Distributed Multipole Analysis

1 Introduction

During the last 40 years, the ability to produce β-lactamases has become widespread amongst the pathogenic bacteria, primarily through the mechanism of plasmid exchange [1]. In recent years, some strains of bacteria cell have been shown to be resistant to even the new generation of β-lactams because they carry β-lactamase mutants of relevant specificity. As a result, the therapeutic effectiveness of many β-lactam antibiotics has been considerably reduced. It is therefore important to know in detail the structure and function of β-lactamases in order to design new, resistant, β-lactam antibiotics.

β-lactamases hydrolyse the antibiotic and thereby prevent it acting against the carboxy and transpeptidase enzymes. It is assumed that the penicillin antibiotic targets enzymes involved in cell-wall biosynthesis (trans and carboxypeptidases) and that the β-lactamases have common origins and function in a similar manner to the trans and carboxypeptidase enzymes [2]. In both cases, the serine group attacks the carbonyl β-lactam carbon and forms an acyl-enzyme intermediate (EA), but the intermediate evolves differently in the carboxy and transpeptidase enzymes

and in the β-lactamase enzymes (Fig. 1). The acyl-enzyme formed with carboxy and transpeptidases is stable, whereas the acyl-enzyme with a β-lactamase degrades the β-lactam.

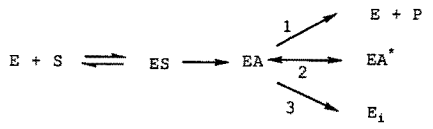
Various classifications of β-lactamases have been proposed [3]. The sequence alignment approach divides β-lactamases into four main classes: A, B, C and D [4, 5]. Many class A β-lactamases from gram-positive organisms are penicillinases. The TEM and SHV class A lactamases, disseminated in gram-negative bacteria, hydrolyse both penicillins and cephalosporins [6]. Class C β-lactamases have a cephalosporinase substrate profile. β-lactamase enzymes which hydrolyse oxacillins more efficiently than penicillin have been grouped into class D. All β-lactamases A, C and D are characterised by a serine residue in the active site. However, class B β-lactamases require a catalytic zinc [7].

Various crystal structures of wild-type class A β-lactamases have been reported. The structure of *Staphylococcus aureus* PC1 enzyme has been determined [8] and refined at 2 Å resolution [9] and that of the *Bacillus licheniformis* β-lactamase at 2 Å resolution [10]. The structures of the *Streptomyces albus* G [11], *Bacillus cereus* β-lactamase I [12] and *Escherichia Coli* TEM1 [13] have been reported. Recently, two independent determinations of the structure of the enzyme of the TEM plasmid [14, 15] and some protein mutant structures: D179N of *S. aureus* [16] and E166A of *B. Licheniformis* [17] have been published.

Despite having X-ray structures of the active sites of the class A β-lactamase enzymes, it is still not established what determines which β-lactams are hydrolysed and which are resistant. As the binding between enzyme and ligand is likely to be dominated by electrostatic forces, the analysis of the electrostatic properties of the β-lactam antibiotics and their complementarity to the electrostatic field in the binding site should reveal differences in how the β-lactams bind within the β-lactamase. The details of these interactions of substrate or inactivators with the active site of β-lactamases is the first step towards an understanding of the β-lactam resistance problem.

Our approach is to study the electrostatic forces involved in the binding, using a high-quality representation of the *ab initio* charge densities of the ligands and binding site residues. Given the range of functional groups found in the antibiotics, it is essential that realistic models are used for the electrostatic forces. In this work, the charge distribution around each atom is represented by a charge, dipole, quadrupole, etc., derived by a Distributed Multipole Analysis (DMA) [18] of a high-quality *ab initio* wave function of the molecule. The higher multipole moments represent the nonspherical features in the valence electron distribution around each atom, such as lone pairs and π-electrons. Such nonspherical features are often invoked in rationalizing organic reactions mechanisms and hydrogen-bonding geometries. Optimising the electrostatic energy calculated accurately by DMA in accessible orientations has been successful at predicting relative orientations in a variety of molecular complexes ranging from van der Waals complexes to protein side chains and nucleic acids [19].

Our model assumes that the binding site is rigid, although the conformation of the binding site could change as different ligands are bound. However, Chen et al. [20] have pointed out that the overall structure of the acyl–enzyme complex with clavulanate closely resembles that of the native enzyme at room temperature. When both protein structures are superimposed by least squares, the root-mean-square deviation is 0.4 Å for the main-chain atoms and 1.1 Å for all atoms. Most of the differences in side-chain conformations are associated with flexible surface residues, in particular lysine. Only three residues in the vicinity of the active site



E- Enzyme
S- Substrate or Inhibitor
ES- Non covalent enzyme substrate complex (Michaelis-Menten complex)
EA- Covalent acyl-enzyme
P - Inactive products of degradation
EA*-Transiently inhibited enzyme species
E_i- Irreversible inactivated enzyme product

Fig. 1. Scheme of the enzymatic reaction of antibiotics and β-lactamases and carboxy and transpeptidases
Route 1 corresponds to the hydrolysis of a β-lactam antibiotic due to a β-lactamase or transpeptidase, yielding a new compound (P), without antibacterial activity. Routes 2 and 3 are related to β-lactamase inhibitors. Route 2 yields to a temporally inhibition of the enzyme and finally it deacyles and route 3 yields to an irreversible inactivation of the enzyme

show a different side-chain conformation when binding clavulanate in the native enzyme: Tyr105, Glu168 and Tyr171. These changes are associated with the change in unit cell dimensions and the avoidance of side chains not in the binding site, and do not affect the structure of the binding site around the inhibitor clavulanate. Therefore, large-scale conformational changes in the binding site are unlikely, and our assumption of a rigid binding site is a reasonable approximation, which is necessary if accurate electrostatic models are to be used with existing programs. The optimisation of a realistic electrostatic binding energy within the steric constraints of a rigid binding model provides a complementary approach to traditional force field studies with crude electrostatic models.

A second reason for this study is to assess a recently proposed method of determining relative binding orientations of structurally diverse ligands when the binding site is unknown. This method has been applied successfully to the study of the phosphodiesterase III substrate and its inhibitors [21], of adenosine receptors ligands [22] and of the transpeptidase substrate and its inhibitors [23]. The alignment of extrema of the electrostatic potential at fixed distances with the same sign and similar magnitude between a ligand and substrate ensures a similar electrostatic potential around the two molecules in the matched regions. Since the electrostatic extrema correspond to regions where strong interactions such as hydrogen bonds with the binding site are possible, some of the extrema of the ligand and substrate are expected to overlay (to within 1 Å or so) when they correspond to strongly stabilising van der Waals contacts between the ligands and binding site.

The aim of this work is to study the electrostatic interactions between all the residues involved in the binding site of the *Staphylococcus aureus* PC1 (class A β-lactamase) and a set of ligands. The set of β-lactam structures, Fig. 2, includes two penicillins (ampicillin and methicillin), imipenem and clavulanate. Ampicillin is hydrolysed by class A β-lactamases, but the other penicillin, methicillin, is stable against β-lactamases. Imipenem is one of the typical β-lactams resistants to β-lactamase hydrolysis, whereas the contrasting clavulanate forms a sufficiently stable complex with the β-lactamase to be an important lactamase inhibitor.

2 Methodology

Coordinates of the β-lactam structures have been obtained from the Cambridge Structural Database [24] and the coordinates for the β-lactamase have been

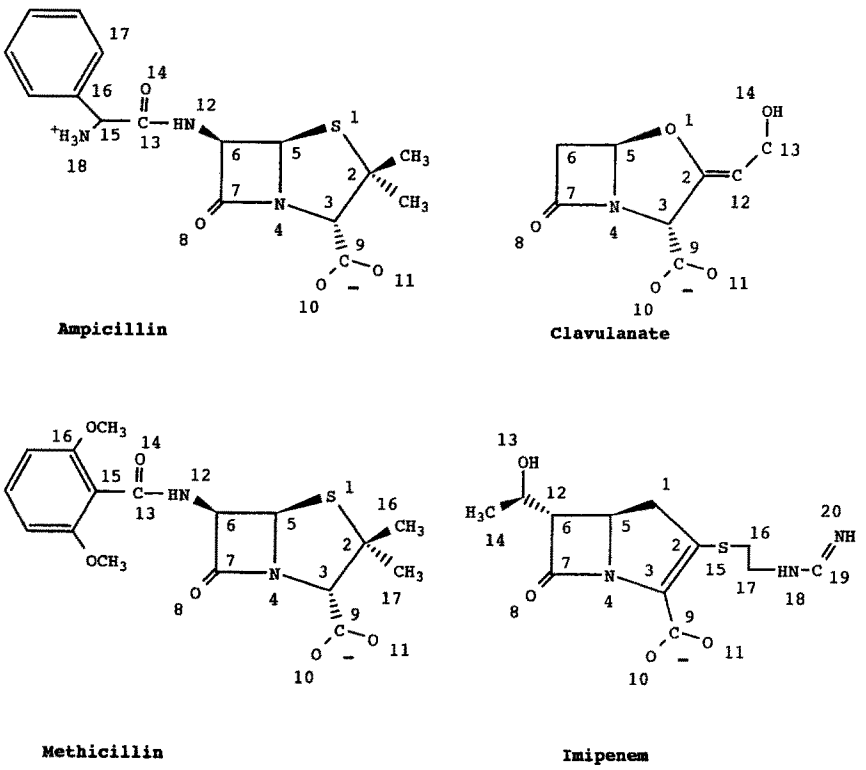


Fig. 2. β-lactam structures studied in this work

obtained from the refined 2.0 Å crystallographic structure of *Staphylococcus aureus* PC1 [9] (3BLM refcode in Brookhaven Protein Data Bank [25]). Only the residues directly implied in the binding site or close to it, have been used. These residues are: Ala69–Ser70–Thr71–Ser72–Lys73–Ala74, Ala104–Tyr105–Ser106, Tyr129–Ser130–Asp131–Asn132–Thr133, Tyr165–Glu166–Ile167–Glu168, Leu169–Asn170–Tyr171, Lys215–Ser216–Gly217–Asp218–Thr219–Leu220, Asp233–Lys234–Ser235–Gly236–Gln237–Ala238–Ile239, Ser243–Arg244–Asn245, Lys273–Pro274–Asn275–Asp276–Lys277. The sequences of residues have been blocked with N-methylacetylamide or methylamino groups. Hydrogen atoms have been added with standard bond lengths and hydrogen atoms in hydroxyl groups, such as serine, have been orientated in the binding site to optimise their hydrogen bonding interactions. The protonation states for charged residues were assigned according to their ionization states at pH = 7.0, i.e. the charges for Glu and Asp were set to -1 and those for Lys and Arg to $+1$.

The electrostatic models of lactam compounds and binding site residues were derived from the SCF wave function of each group of rigid residues calculated with a 3-21G basis set [26] using the CADPAC *ab-initio* program suite [27]. Each wave function was represented by sets of multipoles up to hexadecapole at each atomic site, obtained by a distributed multipole analysis (DMA) [18].

The electrostatic interaction energy between the binding site and β-lactam was evaluated using all the terms in the multipole expression up to R^{-5} within the

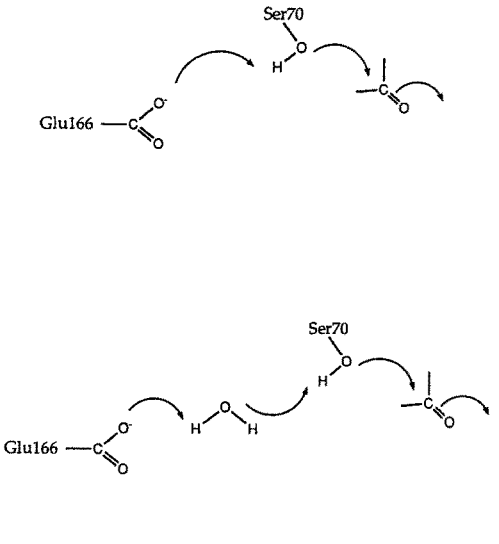


Fig. 3. Proposed mechanisms of catalysis of nucleophilic attack on the substrate by the Ser70 hydroxyl group. a) Direct general base catalysis by the Glu166 carboxylate, proposed by Gibson et al. [33] and Vijayakumar et al. [29] b) Two-stage process with a water molecule proposed by Lamotte et al. [34]

program ORIENT [28]. The minima in the electrostatic interaction energy were found, within sterically accessible orientations, using a pseudo-hard sphere model. The van der Waals surface of the molecules has been defined according to the Pauling radii: 1.5 Å for N, 1.4 Å for O, 2 Å for C bonded to hydrogen, 1.6 Å for C not bonded to hydrogen, 1.85 Å for S and 0 Å for H, as the hydrogen atom is included in a united atom methyl radius, and polar hydrogen atoms effectively have no radius when involved in hydrogen bonding.

Prior to minimising the enzyme–ligand electrostatic energy, the antibiotics were first manually docked in the binding site according to the scheme proposed by Moews et al. [10], in which the α-face of the β-lactam is presented to the nucleophilic Ser70, the β-lactam's carbonyl oxygen atom is in the oxyanion hole formed by NH70 and NH237, and the carboxylic acid group is near Ser130, Lys 234, Ser235 and Arg244 residues. The β-lactam's acylamide side chain hydrogen bonds with the backbone carbonyl of the residue 237 and with Asn132.

Two models have been used in the analysis of the interactions. Firstly, the reactive Ser70 was protonated and the Glu166 deprotonated, according to the protonation states of these residues at pH = 7.0. However, as both the proposed catalytic processes [29] (Fig. 3) imply that the Ser70 is anionic and the Glu166 protonated, just prior to the nucleophilic attack on the β-lactam carbonyl carbon atom, this combination of protonation states was also used.

To test the use of electrostatic extrema to determine relative binding orientations of the β-lactams, we only consider the positions and strengths of the minima in the electrostatic potential energy at the distance that would be sampled by a hydrogen bonding proton of the binding site. These were determined by minimising the interaction energy of a single positive point charge radius 0.5 Å with each lactam, using pseudo-hard-sphere repulsion between sites with non-zero van der Waals radii. We compared the electrostatic potential around methicillin, clavulanate and imipenem with that of the natural substrate ampicillin, by minimising the RMS separation of the corresponding minima. It is hoped that these overlays

predict the orientation of the β-lactam compounds relative to the orientation of the good substrate, ampicillin, in the binding site. In this work, we test this method by comparing its predictions of the relative binding orientations, with those found by minimisation of static energies in the binding site. This essentially tests how well optimising the overlay of possible strong interactions with a point charge mimics the optimum electrostatic interaction with the binding site. The assumption that the binding site is essentially rigid and that the electrostatic forces dominate the orientation dependence of the binding are inherent in any method of comparing the electrostatic similarity of ligands.

3 Results

In the first step of the enzymatic reaction, the substrate or inhibitor recognises the active site of the β-lactamase, in order to form a Michaelis–Menten complex (Fig. 1). This complex is non-covalent and fully reversible: the enzyme is not activated and the chemical structure of substrate remains unchanged. In all the compounds studied, the Michaelis complex where Ser70 is anionic and Glu166 is protonated produce a significantly closer approach by the susceptible carbonyl carbon to the nucleophilic serine oxygen, and therefore only the distances for this protonation state set of the ligand–enzyme complexes are presented in the following results (Table 1 and Fig. 4). The serine must at some instant, prior to the formation of the Michaelis–Menten complex, become deprotonated for acylation to occur, supporting the previously proposed mechanism [29].

3.1 Clavulanate

Clavulanate was the first compound studied because it does not have a side chain on C_6. As a mechanism based inactivator, it is recognised as a substrate by the enzyme and forms the covalent acyl–enzyme complex EA, but instead of hydrolysis and regeneration of the enzyme (route 1, Fig. 1), the acyl–enzyme transforms into an effectively irreversibly covalently bound enzyme product (route 3, Fig. 1). The potential surface for the crystallographic structure of clavulanate within the binding site is quite flat, so several minima were obtained, but only the minimum where the distance between the hydroxyl oxygen of Ser70 and the β-lactam carbonyl carbon (C_7) is close enough (< 3.0 Å) for possible hydrolysis has been considered. As we can see from Table 1, the hydroxyl group of Ser130 is strongly hydrogen bonded with the carboxylate of clavulanate. This carboxylate also hydrogen bonds to the lactamase-invariant residues Ser235, Arg 244 and Lys234. The existence of at least three possible interactions between the carboxylate group and enzyme residues indicates that the loss of a hydrogen bond (relative to the initial position as proposed by Moews et al. [10]) at either position 244 or 235 is compensated by strengthening the other existing hydrogen bonds in the active site. However, Ser130 and Lys234 seem to play a role in stabilizing the enzyme, independently of any electrostatic interaction with the ligand [30]. The strongest interaction is between NH237 and the carbonyl group of clavulanate. Another hydrogen bond has been detected between the amide of Ser70 and the carbonyl group. However, the model does not have Ser130 close to the nitrogen of the β-lactam ring. The oxygroup of clavulanate is exposed at the opening of the active site and appears to be free of any interactions with the active site residues. Gln237 comes close to the alkene

Table 1. Calculated intermolecular distances [Å] in the minimized *Staphylococcus aureus* PC1-β-lactam complexes

Distance	Clavulanate	Ampicillin[a]	Imipenem[b]	Methicillin[c]
A Ser130 O..O_{10}	3.10	3.33 (3.41)	4.16	3.44
B Ser235 O..O_{11}	3.43	2.87 (2.87)	3.00	2.86
C Ser235 O..O_{10}	3.95	3.47 (3.34)	2.96	3.86
D Lys234 N..O_{10}	3.66	3.34 (3.31)	3.83	3.96
E Arg244 N..O_{11}	3.51	3.01 (3.00)	3.00	2.98
F Ser70 N..O_8	3.47	3.27 (3.30)	4.15	4.96
G Gln237 N..O_8	2.99	2.99 (3.00)	3.01	3.47
H Gln237 O..N_{12}	–	2.99 (3.95)	–	4.90
I Asn132 N..O_{14^x}	–	4.03 (4.69)	3.06	5.74
J Ile167 O..N_{18}	–	4.58 (4.97)	–	–
K Asn170 O..N_{18}	–	4.10 (3.79)	–	–
L Ser70 O..C_7	2.81	2.85 (2.77)	3.45	4.28
Elect. Energy (kJ mol^{-1})	−224.8	−319.2 (−331.9)	−287.8	−225.3
K_m(μM)[e]		17		10000

Dihedral angles of the conformations used in the binding site.
[a] $C_{13}N_{12}$–C_6C_7 = 150° (125°); $N_{18}C_{15}$–$C_{13}N_{12}$ = −70° (−25°); $C_{17}C_{16}$–C_{15} C_{13} = 90° (90°)
[b] $C_{14}C_{12}$–C_6C_7 = 55°
[c] $C_{13}N_{12}$–C_6C_7 = 150°; $C_{16}C_{15}$–$C_{13}C_{12}$ = 150°
[d] In imipenem, Asn132 N..O_{13}
[e] Ref. [32].

functionality of clavulanate, however no interaction between this residue and the clavulanate can be found. This set of interactions shows that the carbonyl group of the β-lactam ring mainly determines the orientation of the antibiotic in the binding site. The strong interaction of the residue NH237 with the carbonyl oxygen (O_8) and the close approach (2.81 Å) of the oxygen atom of the Ser70 and the carbonyl carbon is consistent with the occurence of acylation process to form the Michaelis–Menten complex.

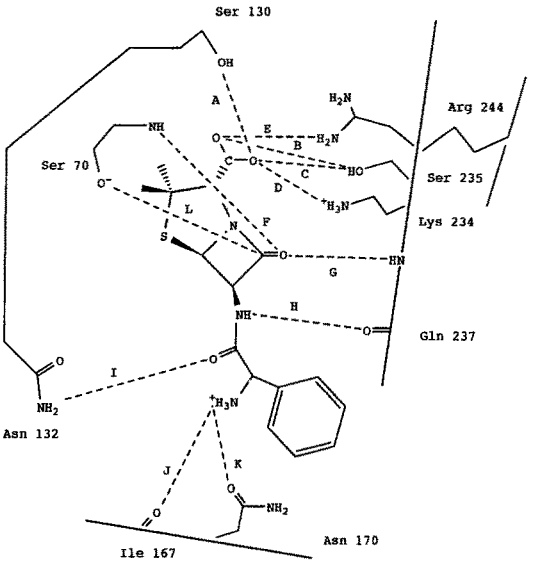


Fig. 4. Diagram defining the distances in Table 1 for the preacylation complex between ampicillin and *Staphylococcus aureus* PC1. The same labels are used for corresponding distances in the complexes of the other β-lactams

3.2 Ampicillin

The calculations with ampicillin are slightly more complicated as different conformations of the side chain of this antibiotic in position 6 had to be considered. Therefore, the $C_{13}N_{12}$–C_6C_7, $N_{18}C_{15}$–$C_{13}N_{12}$ and $C_{17}C_{16}$–$C_{15}C_{13}$ dihedral angles were varied from $-125°$ to 125° (in steps of 25°), $-100°$ to 170° (step 30°) and 0° to 135° (step 45°). The repulsion of the side chain with the enzyme was analysed in all structures, based on the relative orientation obtained with clavulanate. The ligand conformations which were sterically plausible for this binding orientation were then fixed, and the ligand orientation in the binding site optimised. AM1 semiempirical calculations of these conformations of ampicillin showed that they are sufficiently low energy to be accessible.

Table 1 shows the main interactions of two conformations of the ampicillin with the enzyme, both with a β-lactam carbonyl carbon-O Ser70 distance about 2.8 Å. As we can see, the carboxylate group interactions are similar to those of the clavulanate binding orientation, with hydrogen bonds with Ser235 (2.87 Å), Arg244 (3.0 Å), Ser 130 and Lys234, but many of the distances are shorter, most notably with Ser235, which will contribute to the greater electrostatic binding energy of ampicillin. Of the two hydrogen bonds involving the β-lactam carbonyl function (F and G), the one with the backbone NH237 group on B3 β strand (G) is stronger than the one with NH70 on the A2 helix (F). The enzyme's two hydrogen bonds with the acylamide linkage (H and I) are of moderate strength. It seems to be that the bond to CO237 (H) is somewhat shorter than that to Asn132 (I). The protonated D-amine group of ampicillin is in the vicinity of the backbone carbonyl group of Ile167 and the side chain of Asn170; however, these interactions are not optimal in our rigid model and some minor conformational changes in the enzyme could increase side chain-enzyme binding in the preacylation complex. The benzyl group remains relatively exposed to solvent, though is shifted towards Ala238 in the B3 β-strand, a position which in all class A β-lactamases is generally non-polar

or hydrophobic. This binding orientation is consistent with the hydrolysis of ampicillin by β-lactamase.

3.3 Imipenem

Imipenem is a compound with a small side chain on C_6, which can easily be accommodated in the binding site of the enzyme. The $C_{14}C_{12}$–C_6C_7 dihedral angle has been modified manually from $-15°$ to 115°, every 10°, and all the structures generated were docked in the binding site in the relative orientation obtained in the clavulanate. Fitting this structure into the active site leads to short contacts between the carboxylate group and the side chain of residue Gly236. The electrostatic forces move the imipenem away from the reactive group Ser70 and catalytic Glu166, resulting in the distance between OSer70 and β-lactam carbonyl carbon being greater than 3.40 Å. It is difficult to believe that the serine could attack the lactam compound at this distance. The lowest energy complex is shown in Table 1, which shows that there are five strong hydrogen bonds: Ser235 interacts with both oxygen atoms in the carboxylate group, NH237 with the oxygen atom in the carbonyl group, Arg244 with the carboxylate group and the Asn132 with the side chain of imipenem. This new relative orientation of the imipenem shows a significative displacement of the compound towards the B3 β-strand, which is compatible with its resistance to β-lactamase.

3.4 Methicillin

We have studied different orientations of the side chain as a function of the dihedral angles $C_{13}N_{12}$–C_6C_7 and $C_{16}C_{15}$–$C_{13}N_{12}$, but we could not find an orientation of the antibiotic in the binding site with a distance O Ser70-β-lactam carbonyl carbon lower than 3.4 Å. If we compare this structure with ampicillin, it is clear that the different length of side chain at C_6 means that the bulky aromatic side chain of methicillin will occupy different regions of the enzyme. Table 1 shows the most important interactions obtained in the minimised complex, where the bulky side chain does not allow a closer approach of the serine group. As we can see, the two most important interactions in this minimum energy structure, are those involving residues Ser235 and Arg244 with the carboxylate group. These results strongly suggest that the bulky side chain at C_6 could only fit in the binding site with a significantly shorter O Ser70-β-lactam C separation with such considerable conformational changes in the enzyme that the binding site is likely to be deactivated. Thus, either a long O-Ser70 C distance or a grossly distorted binding site would explain the stability of methicillin.

4 Comparison of relative binding orientations obtained by docking with those predicted by electrostatic similarity

Figure 5 shows the predicted relative orientations of ampicillin and the other β-lactams within the energy minimised complex as described in Table 1. Ampicillin and clavulanate have a very similar orientation in the binding site, with the β-lactam carbon atoms superimposed within 0.11 Å. However, the relative orientation of the carboxylate group is slightly different due to the different geometries of

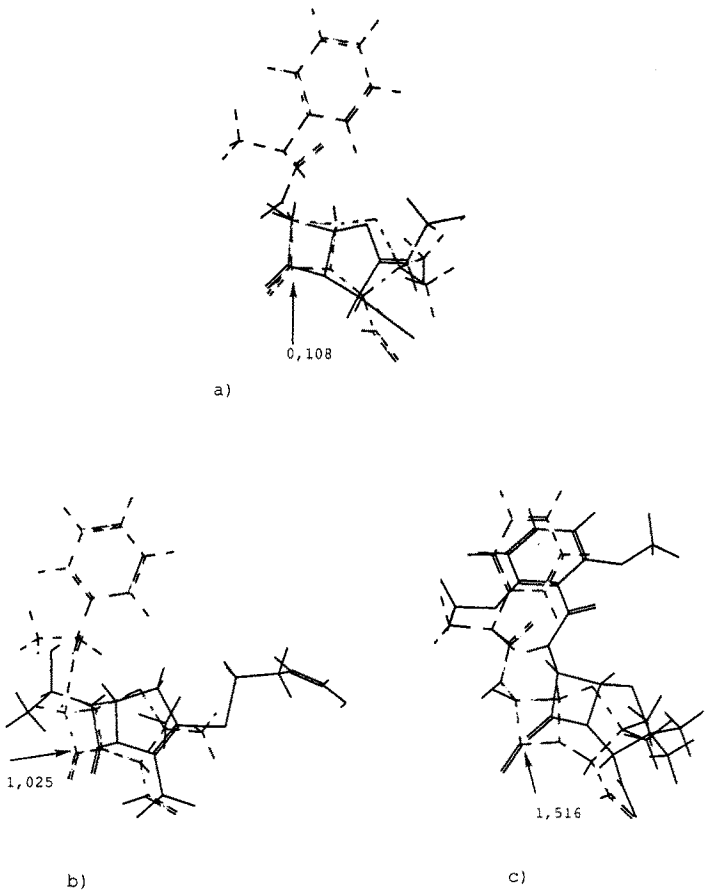


Fig. 5. Relative orientations of the ampicillin (– – –) with respect to a) clavulanate, b) imipenem and c) methicillin, in the minimised Michaelis–Menten complexes (Table 1). Values show the distances between the two β-lactam carbonyl carbon atoms in this relative orientation

the five membered rings. Nevertheless, the four different interactions of the carboxylate group with the enzyme and the absence of a side chain at C_6, do allow a very similar orientation of the carbonyl group in front of the serine residue. On the other hand, the relative orientation in the binding site of imipenem and methicillin is quite different due to the different orientation of the carboxylate group in the first case and to the bulky side chain in the second one.

These results can be complemented with those obtained by the electrostatic overlay of the different β-lactam compounds (Fig. 6). The electrostatic potential surfaces of lactam compounds were compared both visually and through the overlay of the minima for these surfaces. Two of these minima are located very close to the carboxylate group, another near the β-lactam carbonyl oxygen and the last minimum near the carbonyl oxygen of the side chain [23]. The electrostatic similarity of the overlaid structures was quantified by the RMS separation of the four electrostatic minima around the ampicillin and the corresponding minima of the other lactam structures.

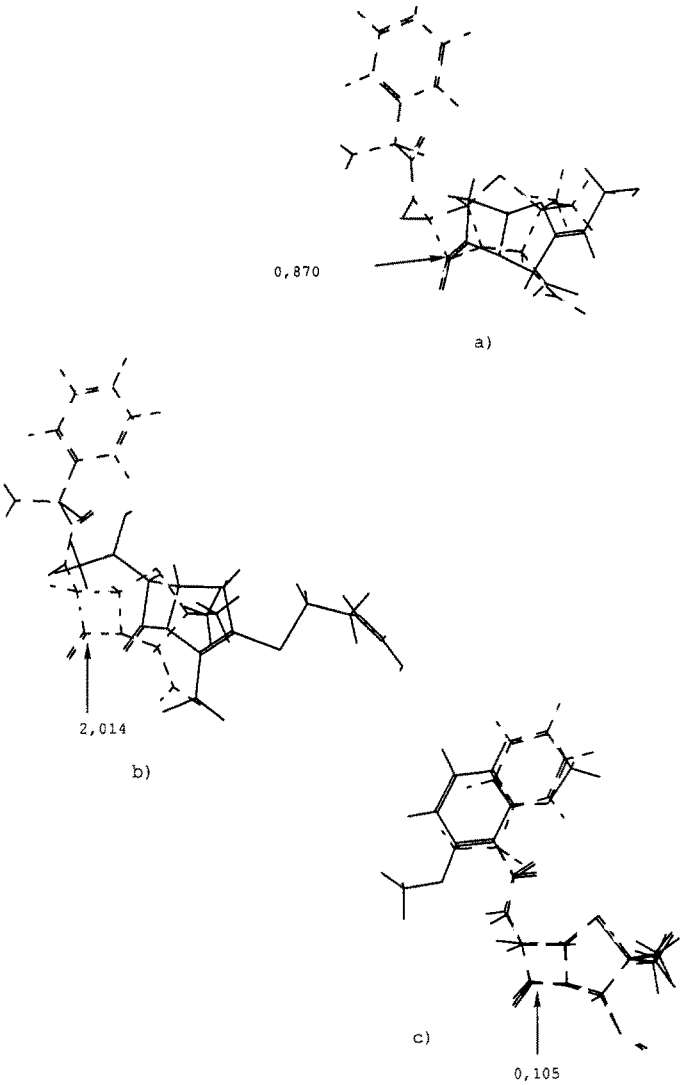


Fig. 6. Relative orientations of a) clavulanate, b) imipenem and c) methicillin, in the electrostatic matching with ampicillin (– – –). Four minima have been used in the electrostatic matching, except in clavulanate. Values show the distances between two equivalent atoms in the relative orientation obtained

The relative orientations produced by optimising overlap of the electrostatic extrema are only crudely similar to those predicted using the binding site. Methicillin gives the best electrostatic overlay with ampicillin (Fig. 6c, RMS 0.220 Å) whereas the steric restrictions of the side chain prevent such a close overlay occurring in the binding site. This steric restriction could not be reliably predicted without a binding site structure.

The static overlays of clavulanate and imipenem with ampicillin (Fig. 6a, b) are both only moderately good (RMS values are 0.829 and 0.909 Å, respectively) and

both overestimate the relative separation of the β-lactam rings that is induced by the binding site. However, it does correctly predict that the relative separation of the carbonyl carbon is much larger for imipenem than clavulanate, consistent with its resistance.

Thus, it appears that the electrostatic overlay method is certainly more informative about relative binding orientation than a simple steric or chemical overlay. However, in some case the position of the carboxylate group (related to the fusioned ring to the β-lactam system) and the steric requirements of the C_6 side chain play such a major role in determining the binding orientation within this particular binding site, that no modelling without a structural knowledge of the binding site could predict the relative orientations.

5 Conclusions

A study of the docking of four β-lactams within the binding site of the β-lactamase *Staphylococcus aureus* PC1 has produced plausible binding orientations for all four molecules. These binding orientations do distinguish clearly between good substrates and inhibitors (which have the same mechanism until the formation of the covalent acyl–enzyme intermediate) and the poor substrates in the initial recognition of the active site. The resistance of methicillin and imipenem to the β-lactamase results from the steric and electrostatic forces preventing their β-lactam rings approaching the Ser70 residue sufficiently closely.

The predictions of the relative strength of binding of these lactams (in contrast to their resistance) could be validated, in principle, by comparison with the experimental affinity of the enzymes for the substrate. The dissociation constant of the enzyme–substrate complex K_s is proportional to the Michaelis parameter K_M [31] which is inversely proportional to the affinity of the enzyme for the substrate. Unfortunately, K_M values are only available for ampicillin and methicillin, preventing any quantitative analysis. However, the values in Table 1 show qualitatively that the most strongly bound complex, ampicillin, has a much higher affinity than the more weakly bound, low-affinity methicillin.

This study suggests that two factors which could impart β-lactamase resistance are the prevention of close approach to the Ser70 through either the steric interactions of either the C_6 side chain or the carboxylate group.

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