Analysis of a concerted mechanism in β-lactam enzymatic hydrolysis. A quantum mechanics/molecular mechanics study

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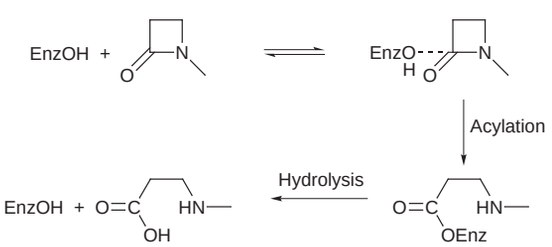
One of the postulated mechanisms for the acylation step in β-lactamase catalyzed hydrolysis of β-lactams, a concerted one, has been explored by means of a quantum mechanics/molecular mechanics approach. Minima and transition structures for the reaction path are reported. The TEM-1 enzyme, a class A β-lactamase, and a penicillanate, a substrate easily hydrolyzed by this enzyme, constitute the system employed in our study. We have also analyzed the effects of the protonation state of Lys73 on the reaction mechanism. The energy barriers obtained, too high for a catalytic process, indicate that a concerted mechanism is not the most probable enzymatic mechanism for the acylation. Useful information is obtained by comparing the enzyme structures corresponding to the protonated and the deprotonated Lys73 residue along the reaction path. In the protonated Michaelis complex the Glu166 residue appears considerably closer to the Lys73 residue than in the deprotonated structure. This fact implies that an initially protonated Lys73 could easily transfer a proton and thus would not be a factor in excluding acylation mechanisms in which Lys73 acts as the general base in the deprotonation of Ser70. On the other hand, the Lys73 deprotonated acyl–enzyme structure is in better agreement with the reported X-ray crystallographic data than that of the protonated case.

Introduction

β-Lactamases are a group of bacterial enzymes that constitute the major cause of bacterial resistance to β-lactam antibiotics. These enzymes catalyze the hydrolysis of the sensitive β-lactam moiety of these kind of antibiotics, rendering the drug biologically inactive.[1] On the basis of sequence relationships, four different classes of β-lactamases, A–D, have been identified. The class A enzymes are the most common and intensively studied, whereas the class C enzymes are the second most common. Both classes A and C are active site serine enzymes.[2] The global catalytic pathway involves the acylation of the serine at the active site by the β-lactam carbonyl group forming an acyl–enzyme intermediate.[3–5] This step is followed by hydrolysis of the ester bond formed in the acylation (see Scheme 1).



Scheme 1

β-Lactamases are able to undergo deacylation easily, regenerating the enzyme and releasing the inactive antibiotic.

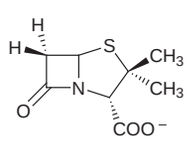
Though the global reaction mechanism in Scheme 1 is well-known, the specific proton transfer steps and the role played by the conserved residues present in the active site region remain the subject of controversy. A clear similarity of the amino acid sequence in this region arises from the analysis of structural data of different β-lactamase enzymes.[6–13] In particular, the class A enzymes contain a set of conserved residues presumably crucial for catalysis, Ser70, Lys73, Lys234, Ser130, Glu166 (the sequence numbering of Ambler *et al.*[14] is used throughout this paper). The acylation implies the deprotonation of the hydroxy group of the active serine, Ser70, and the protonation of the β-lactam nitrogen. Despite the accumulation of kinetic and mutagenesis data,[15] neither the identity of the residue which accepts the proton coming from the Ser70 nor the steps for achieving the β-lactam nitrogen protonation are clear. Different possibilities have been proposed. In one of them the Glu166 residue plays the role of the general base which deprotonates the Ser70 residue prior to nucleophilic attack.[16] Structural data indicate a distance between the carboxylate oxygens of Glu166 and the hydroxy group of Ser70 that is too long for a direct proton transfer. However, a recent molecular dynamics MD study[17] shows a very high mobility for Glu166 in the PC1 enzyme, a class A β-lactamase, which could favour the approach to Ser70. Variants of the previous mechanism assign to a conserved water molecule, existing between Glu166 and Ser70, the role of proton relay for this transfer.[18,19] Another possibility for the acylation has been proposed by Strynadka *et al.*[4] This mechanism is more complicated and involves different residues for an indirect proton transfer from Ser70 to the β-lactam nitrogen through Lys73 and Ser130. Thus, this mechanism would explain not only the deprotonation of Ser70 but also the β-lactam nitrogen protonation. A third proposed possibility consists of a direct proton transfer from the hydroxy group of Ser70 to the β-lactam nitrogen atom.[20,21] The proton transfer, the serine acylation, and the β-lactam ring opening would occur through a single step. Therefore along this paper we will refer to this possibility as the concerted mechanism.

Theoretical chemistry provides valuable tools for studying a system at a molecular level. Until recently only models of limited size have been studied quantum mechanically. In a recent work, Wladkowski *et al.*[22] studied the initial acylation step in the enzymatic hydrolysis of β-lactams using an *ab initio* quantum mechanical approach. The model used incorporates a simple β-lactam substrate and essential fragments of the key residues needed to analyse the mechanism proposed by

Strynadka *et al.* Moreover, several studies employing molecular orbital calculations have been devoted to the study of the reaction mechanism in non-enzymatic β-lactam hydrolysis.[21,23–25] The azetidinone molecule or some substituted derivatives have been used as the β-lactam system, although full antibiotic molecules have also been studied in semiempirical calculations. The nucleophile agent is normally represented by a hydroxide anion,[23–25] a water molecule[21a,25] or methanol.[21a] These kinds of non-enzymatic studies have supplied useful information and increased our understanding of these systems, but they cannot consider the specific enzyme–substrate interactions.

Recently, a new procedure based on a mixed quantum/classical (QM/MM) approach has been developed.[26] This allows the identification of transition structures. The substrate and the desired key residues (or part of them) are treated quantum mechanically whereas the rest of the protein is treated by a classical force field. The details are given in the next section. This new methodology is suitable for studying reaction mechanisms in an enzymatic environment: the characteristic ability of the hybrid methods for treating systems of the size of a protein is combined with the possibility of obtaining both minima and transition structures along a reaction path (and therefore energy barriers). This constitutes an essential new tool to differentiate between postulated reaction mechanisms.

We have previously commented on some of the several proposed mechanisms for the acylation process. All of them have both critical points and supporting features and new contributions are necessary to better understand this process. In this paper we have applied the quantum mechanics/molecular mechanics (QM/MM) methodology to study the concerted mechanism for the acylation step of the enzymatic hydrolysis of β-lactam compounds. This mechanism has only one transition structure that connects the reactant complex and the acyl–enzyme intermediate and thus it is the simplest case for applying this new methodology. Furthermore, some of us have previously studied the neutral and alkaline hydrolysis of the *N*-methylazetidinone molecule, a model for β-lactam.[25] In the neutral case a similar concerted mechanism has been studied, which allows us to compare the enzymatic and non-enzymatic processes. The clinically relevant TEM-1 β-lactamase, a prototypic class A enzyme, and the penicillanate **1**, a



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substrate easily hydrolyzed by this enzyme, constitute the system employed in our study.

A second objective of this paper is to analyse the effects of the protonation state of Lys73 on the reaction mechanism. There is considerable controversy on the protonation state of this residue. It is possible to find studies supporting an initially protonated state[19] and studies that propose a deprotonated Lys73 caused by a shift of the pK_a from 8 to 14 as the substrate binds.[27] The mechanism proposed by Strynadka for the acylation requires an initially deprotonated Lys73 as the general base for activating the essential Ser70. The other possible mechanisms do not present this crucial dependence, but their respective energy profiles could be modified by the presence or not of a positive charge on the lysine. To analyse this aspect we have obtained the energy profile of the concerted mechanism for the two possible situations. The observed differences along the reaction path between the enzyme structures corresponding to the protonated and the deprotonated lysine are discussed.

Computational details

Initial coordinates for the system have been obtained from the crystallographic structure of an acyl–enzyme intermediate recently reported.[5] The intermediate is formed by the TEM-1 β-lactamase enzyme and the 6α-(hydroxymethyl)penicillanate, a novel inhibitor for this enzyme. The structure is available in the Protein Data Bank (ID code, 1TEM). The hydroxymethyl moiety was manually removed to obtain the penicillanate **1**.

The hybrid QM/MM treatment was performed by means of the CHARMM 24b2 program,[28] using the semi-empirical AM1 hamiltonian[29] with the CHARMM 24b2 protein parameter set.[30,31] The entire molecular system, containing 4820 atoms, was divided into QM and MM regions. The substrate and the entire residue implied in the studied mechanism (Ser70) were treated quantum mechanically, while the rest of the protein and the water molecules present in the crystallographic structure were treated by the classical force field. Other residues, such as Glu166, Lys73 and Ser130, should be included in the QM region in order to explore other possible mechanisms. This will be the subject of future studies based on the conclusions of the present study. The entire TEM-1 protein was considered in the calculations and the position of all the atoms of the system were allowed to relax. Two link atoms[32] were inserted where the QM/MM boundary intersected covalent bonds: these were placed (a) along the C–N bond between Met69 and Ser70, and (b) along the C–N bond between Ser70 and Thr71. The QM region contained a total of 36 atoms, including the quantum link atoms. Although the sulfur atom does not actively participate in the reaction we have tested the AM1 parametrization to describe carbon–sulfur bonds, which appear in our present QM system. In the case of dimethyl sulfide, the AM1 values for the CS bond length and CSC angle (1.717 Å and 102.95 degrees respectively) compare quite well to the MP2/6-31G* values (1.804 Å and 98.49 degrees). We have also optimized an isolated penicillanate molecule at the AM1 and HF/6-31G* levels. At the AM1 level, the two CS bond lengths and the CSC bond angle are 1.747 Å, 1.783 Å and 96.25 degrees respectively while at the HF level the converged values are also quite similar (1.830 Å, 1.867 Å and 94.39 degrees).

QM/MM energy minimizations were performed in order to obtain the potential energy surface. A guest structure from the quadratic region of the saddle point was used as the input in the transition state structure search carried out with GRACE software.[26] A partial-rational-function-operator/adopted-basis-Newton–Raphson method was employed, using a Hessian matrix of 108 × 108 order, describing the curvature of the QM/MM energy hypersurface for a sub-set of the system, together with a diagonal Hessian plus updates for the rest of the system. The rms residual gradient on the 36 atoms in the sub-set is less than 0.001 kcal mol^{-1} Å^{-1} in the optimised structure, while on the remaining atoms it is less than 0.005 kcal mol^{-1} Å^{-1}. Finally, the IRC (Intrinsic Reaction Coordinate) path[33] was traced from the refined transition structure in each direction using the GRACE capabilities. From the last point in each direction we started a minimization leading to a reactant complex (the Michaelis complexes described in the Results section) and an acyl–enzyme intermediate (the product of this process).

Results and discussion

As stated in the Introduction, two possibilities were studied depending on the protonation state of the Lys73 residue. In Figs. 1, 2 and 3, we show the obtained structures for the Michaelis complexes, transition structures and acyl–enzyme complexes respectively. For clarity reasons only some key residues are shown.

Michaelis complexes

In general, except for some particular aspects, the structures

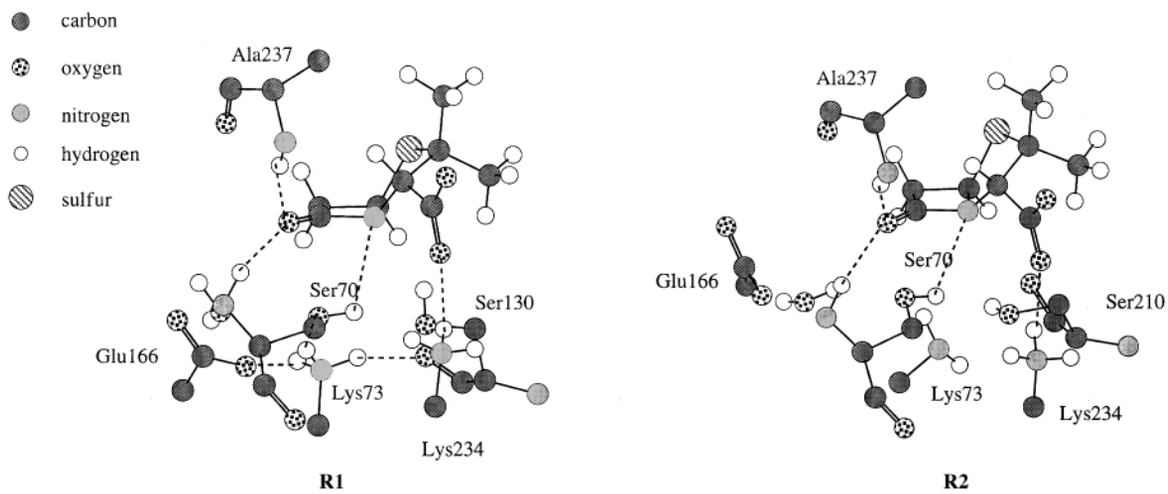

Fig. 1 Structures of the Michaelis complexes obtained after QM/MM minimization. **R1**: protonated Lys73 structure; **R2**: deprotonated structure. For clarity, only some key residues are shown.

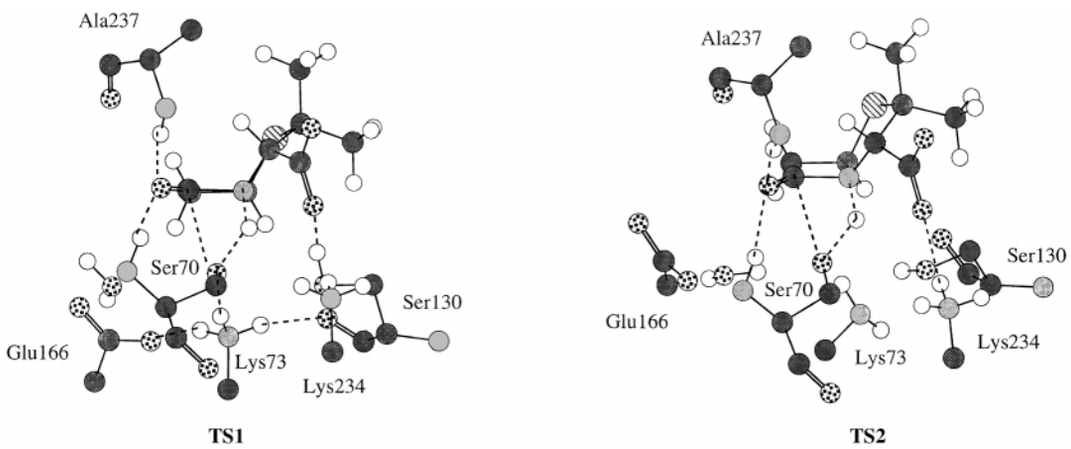

Fig. 2 Transition structures obtained for the concerted mechanism. **TS1**: protonated Lys73 structure; **TS2**: deprotonated structure.

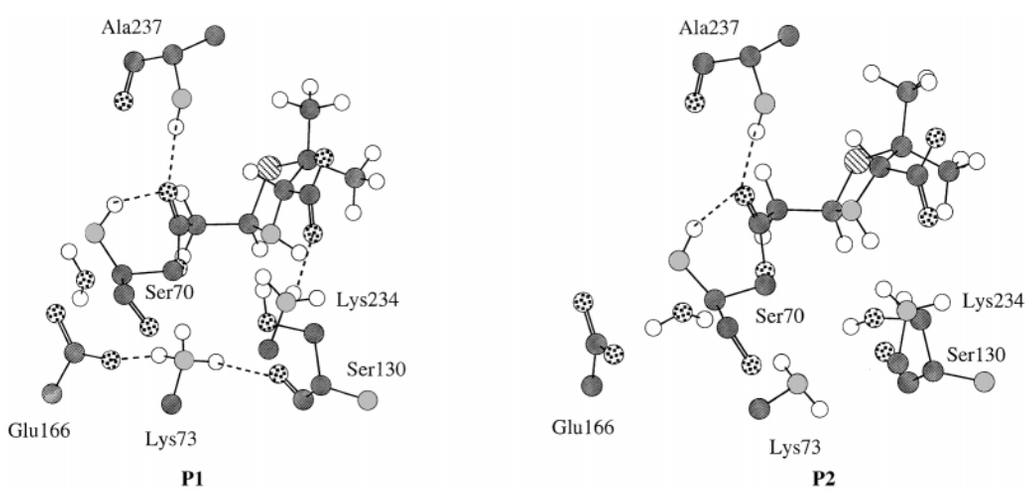

Fig. 3 Structures of the acyl–enzyme intermediate. **P1**: protonated Lys73 structure; **P2**: deprotonated structure.

obtained for the protonated, **R1**, and deprotonated Lys73 enzyme, **R2**, are similar. For this reason a large part of the following discussion is common for both cases. In the following, we will usually give the geometrical parameters as two slash-separated values (A/B). The number on the left will correspond to the protonated Lys73 description and the number on the right to the deprotonated one.

The enzyme substrate hydrogen bonds are structural features of great interest, in particular those related to the β-lactam carbonyl, carboxylate and nitrogen atom. In the Michaelis complexes the β-lactam carboxylate oxygen atoms form strong hydrogen bonds with all the closest residues, the distances to the proton donor atoms being, Arg244 $N\eta_1$ (2.77/2.74 Å), Ser235 Oγ (2.80/2.82 Å), Lys234 Nξ (2.78/2.75 Å) and Ser130 Oγ (2.73/3.47 Å). From the previous values we observe that **R2** keeps the same interactions as **R1**, except for that corresponding to the Ser130. In this case, the hydroxy hydrogen of Ser130 is now pointing toward the Lys73 Nξ atom. This hydrogen bond between Lys73 Nξ and Ser130 Oγ is not very strong, as reflected in the large donor–acceptor distance (3.12 Å) and quite a large

deviation from linearity. In **R1** and **R2**, the Lys234 Nξ also forms strong hydrogen bonds with Ser235 O (2.75/2.79 Å) and with Ser130 Oγ (3.00/2.88 Å). It is not difficult to suppose that all these strong interactions (the lysine employs all three of the Nξ hydrogens) will lead to a relatively fixed position for the Lys234 residue. Effectively, in the above mentioned MD study of the PC1 β-lactamase,[17] the Lys234 residue was found to remain essentially fixed at its crystallographic position during the whole simulation.

The reactant complex structures described here present, as expected, hydrogen bonding interactions between the β-lactam carbonyl oxygen and the amide group of Ser70 N (3.01/3.13 Å) and Ala237 N (2.87/2.84 Å). These interactions form the so called oxyanion hole in the analogous serine proteases.[34] It is believed that these components exercise a stabilizing role on the negative charge developed on the carbonyl oxygen with the Ser70 nucleophilic attack. This effect would be of particular importance when the reaction mechanism involves an initial tetrahedral adduct at the first reaction step, *i.e.*, the acyl-bond is formed prior to the β-lactam ring opening. In this case the negative charge of the nucleophilic agent is transferred to the β-lactam molecule and, at least partially, located on the carbonyl group. In the concerted mechanism studied here, a tetrahedral adduct does not appear along the reaction pathway (the ring is opened as the serine is acylated), therefore a minor role is expected.

The Ser70 Oγ is 2.69/2.52 Å from the carbonyl carbon atom and the hydroxy proton points towards the β-lactam nitrogen. Thus this complex seems to be especially suitable for the concerted mechanism. In **R1**, the amine protons of the protonated Lys73 Nξ are hydrogen bonded to Ser70 Oγ (2.85 Å), Ser130 O (2.85 Å) and to Glu166 $O\varepsilon_2$ (2.60 Å), respectively. This last interaction with the Glu166 residue is of particular interest as we shall discuss below. Obviously, due to the different protonation state of Lys73 in **R2**, the interactions of this lysine with its environment form one of the points where the structural descriptions for **R1** and **R2** differ. Effectively, in **R2** the Lys73 Nξ does not present any hydrogen bond interaction. Ser70 Oγ is located at 3.36 Å and Ser130 O at 3.22 Å from Lys73 Nξ, and no Lys73 hydrogen points towards these atoms. The most interesting feature corresponds to the absence of interaction with the Glu166 residue which is the principal difference between the protonated and deprotonated structures studied here. In the protonated case, one of the carboxylate oxygens of Glu166 is found very close to the Lys73 Nξ, 2.60 Å. This distance is suitable for a low barrier proton transfer. In fact, this distance seems too short and the possible spontaneous proton transfer is prevented by the classical description of both residues. In the deprotonated reactant complex the Glu166 residue is found at a longer distance, about 5 Å. This large difference can be explained on the basis of the large mobility of the Glu166 residue which has been reported in a molecular dynamics simulation study.[17] As was said before, this mobility has led to the impossibility of rejecting the hypothetical role of Glu166 as the general base in the acylation step, despite the long distance between the Glu166 carboxylate and the hydroxy group of Ser70. From the results presented here, a new possibility now emerges. It seems that a protonated Lys73 can easily transfer a proton to Glu166 and thus its initial protonation state would not be decisive for excluding a mechanism in which Lys73 acts as the general base in the deprotonation of Ser70 such as that proposed by Strynadka *et al.*[4]

Transition structures

As in the previous section, we have named the transition structure corresponding to the protonated Lys73 enzyme **TS1** and the deprotonated one **TS2**. Both structures are shown in Fig. 2.

In both **TS1** and **TS2** structures the CN distance, 1.54 Å, is slightly lengthened with respect to that of the Michaelis complexes, 1.45 Å, but the ring still remains essentially closed at this point on the reaction coordinate. On the other hand, the proton transfer from Ser70 Oγ to the β-lactam nitrogen atom is quite advanced (the NH and OH distances are 1.14/1.17 and 1.55/1.53 Å, respectively). The same features were found in previous studies on an equivalent mechanism for the neutral hydrolysis of the *N*-methylazetidinone molecule.[25] However, in both **TS1** and **TS2** structures the Ser70 Oγ is located at 2.56/2.49 Å of the β-lactam carbonyl carbon. These distances seem to be too long for a favorable serine addition to the β-lactam carbonyl group. In fact, in the previous studies on non-enzymatic hydrolysis the position and orientation of the water[21a,25] or methanol[21a] molecules in the transition structures are better adapted for addition to the β-lactam carbonyl, and the distance from the nucleophilic oxygen to the carbonyl carbon is never longer than 2.0 Å. In the **TS1** and **TS2** transition structures the serine appears displaced towards the β-lactam nitrogen atom. However, this displacement does not lead to an advancement in the acyl–enzyme bond formation The displacement is forced by the requirement of a direct proton transfer from the Ser70 Oγ to the β-lactam nitrogen. The Ser70 Oγ distance to the β-lactam carbonyl carbon is reduced with respect to the Michaelis complexes by only 0.13/0.03 Å in **TS1** and **TS2** respectively. Thus, the formation of the new NH and CO bonds takes place in a very asynchronous way, compared with the gas phase or solution mechanisms. As we will show below this fact leads to a large negative charge appearing on the Ser70 Oγ.

In general, the hydrogen bond interactions present in the Michaelis complexes are kept in the transition structures. In the oxyanion hole a slightly weakening of these interactions is observed. The Ala237 N is now 2.92/2.90 Å, and the Ser70 N is 2.98/3.09 Å from the β-lactam carbonyl oxygen. Though the distances to the Ser70 N are shorter than those of the reactant complexes, the carbonyl oxygen–amide proton distance is slightly increased as a consequence of a larger deviation from linearity in this hydrogen bond.

Acyl–enzymes

The acyl–enzyme structures corresponding to the protonated (**P1**) and deprotonated (**P2**) Lys73 are shown in Fig. 3. In the acyl–enzyme structures the CN bond is completely broken (2.69/2.58 Å for **P1** and **P2** respectively) and the β-lactam nitrogen is protonated. The β-lactam ring, practically planar in the Michaelis complexes and transition structures, increases its dihedral angle up to 12.7 and 17.1 degrees in **P1** and **P2** respectively. As in the transition structures the hydrogen bond interactions present in the reactant complexes are in general kept in the acyl–enzyme structures. The β-lactam carbonyl oxygen reinforces its interaction with the oxyanion hole components, Ala237 N (2.86/2.81 Å) and Ser70 N (2.86/2.89 Å). Larger differences appear around the β-lactam carboxylate. In particular, in **P1** the carboxylate has lost one of the four strong hydrogen bonding interactions present in **R1** and **TS1**. The hydroxy hydrogen of the Ser130 residue is now pointing to the β-lactam nitrogen, which is placed 2.91 Å from the Ser130 Oγ.

The deprotonated structure, **P2**, has also lost some of the hydrogen bonds between the enzyme and the β-lactam carboxylate, specifically those formed with Lys234 and Ser130. The carboxylate of the deprotonated acyl–enzyme complex (**P2**) is stabilized just by the Arg244 (2.74 Å) and Ser235 (2.87 Å) residues, whereas in the protonated structure (**P1**) the hydrogen bond with Lys234 is also retained. We can compare both **P1** and **P2** structures with the X-ray crystal data of TEM1 acyl–enzyme intermediates reported by Strynadka *et al.*[4] and Maveyraud *et al.*[5] In both cases, the obtained structures show hydrogen bond interactions of the β-lactam carboxylate with the Arg244 and Ser135 but not with Lys234 and Ser130. Thus, at least in this aspect, the structure of the Lys73 deprotonated

Table 1 Relative energies (kcal mol^{-1}) and Mulliken charges (au) on some selected atoms for the Michaelis complex, transition structure and acyl–enzyme adduct of the protonated and deprotonated Lys73 concerted mechanisms

	Protonated Lys73			Deprotonated Lys73		
	R1	**TS1**	**P1**	**R2**	**TS2**	**P2**
ΔE	0.00	42.06	−31.89	0.00	45.85	−41.23
q_C	0.33	0.37	0.40	0.33	0.36	0.39
q_O	−0.35	−0.24	−0.44	−0.36	−0.25	−0.45
q_N	−0.31	−0.19	−0.31	−0.27	−0.19	−0.32
q_{O_γ}	−0.36	−0.73	−0.30	−0.33	−0.68	−0.22
q_H	0.24	0.34	0.17	0.24	0.36	0.20

acyl-enzyme (**P2**) of the present study is in better agreement with the experimental information than that corresponding to a protonated Lys73 acyl–enzyme. In any case, the rms deviations over protein backbone atoms of **P1** and **P2** with respect to the crystallographic complex[5] are very similar (0.53 and 0.54 Å respectively).

Energy profile

In Table 1 are reported the relative energies for the protonated and deprotonated concerted mechanisms, 42.06 and 45.85 kcal mol^{-1} respectively. They are smaller than the energy barrier found for the equivalent addition step in vacuum using water as a nucleophilic agent and *N*–methylazetidinone as the β-lactam model,[25] 57.12 kcal mol^{-1} at the MP2//HF/6-31G* level. Wolfe *et al.*[21a] obtained for the same water–N-methylazetidinone system a much lower value, 42.651 kcal mol^{-1} (MP2/6-31G*//3-21G), optimizing the geometry with the 3-21G basis set. These facts reveal the great dependence of the energy barrier of the concerted process on the level of calculation. Employing the AM1 method, the energy barrier becomes 55.02 kcal mol^{-1}. Moreover, if the *N*-methylazetidinone is substituted by penicillanate the energy barrier decreases to 45.10 kcal mol^{-1}. This last value is comparable to those obtained in the enzymatic environment.[35]

These high energy barriers seem to indicate that a concerted mechanism is not the most probable enzymatic mechanism for the β-lactam acylation. In fact, we have seen that this mechanism requires constrained transition structures, with the Ser70 residue appearing at a very different position from that occupied in both reactant and product structures. Moreover, the distribution of charge developed on these structures is not the most favorable for the stabilizing role of some key residues: going from reactants to the acyl–enzyme, the atoms that present larger variations in their charges are, as expected, the carbonyl oxygen, carbonyl carbon and nitrogen atom of the β-lactam antibiotic and the hydroxy group of Ser70. The charges of all these atoms are given in Table 1. The most significant aspect is the large negative charge developed on the serine hydroxy oxygen in both transition structures, as a consequence of the advanced proton transfer. However, the charge of the carbonyl oxygen atom does not increase, in absolute value, with respect to that of the Michaelis complexes. Indeed, it is less negative in the transition structures than in the reactants. Probably, the long distance between the Ser70 Oγ and the carbonyl carbon atom is responsible for this effect because the acyl–enzyme bond is not even partially formed. These facts imply that the oxyanion hole components (amide group of Ala237 and Ser70) have no stabilizing effect in the concerted transition structures. In fact, we have seen in the previous structural descriptions a slight lengthening of the hydrogen bond distances between the β-lactam carbonyl oxygen and the amide nitrogen of these residues in the transition structures. Along the reaction path, the stationary point where the charge on the carbonyl oxygen is at a maximum is in the acyl–enzyme complex, where the hydrogen bonds of the oxyanion hole are shorter.

Conclusions

From the previous results and with the limitation of the methods used in this work, a semiempirical description of a quantum core composed of the substrate and Ser70 and using molecular mechanics for the rest of the system, the following conclusions can be summarized. A mechanism based on a concerted sequence of events, acylation of Ser70 and simultaneous hydroxy proton transfer to the β-lactam nitrogen, has been analysed taking into account a protonated and a deprotonated state for the Lys73 residue. For both cases a high energy barrier has been obtained, 42.06 and 45.85 kcal mol^{-1} respectively. Though the concerted transition structures cannot correspond to the real mechanism, the Michaelis and acyl–enzyme complexes are true minima structures which should be interconnected in any proposed mechanism. Useful information has been obtained by analysing them. The hydrogen bond interactions between substrate and active site residues have been described. One of the most interesting aspects found in this analysis is the dependence of the position of the Glu166 residue on the protonation state of the Lys73 residue. The great mobility of Glu166 has been reported in previous molecular dynamics studies. We have found that in the deprotonated Lys73 study the Glu166 is quite distant from the Lys73 (about 5 Å). However, the Glu166 is very close to Lys73 in the protonated Lys73 Michaelis complex, keeping the proximity in the corresponding transition structure and acyl–enzyme. The proximity between Glu166 and the protonated Lys73 would favor proton transfer between them. In this way, an initial protonated Lys73 would not be enough to discard a catalytic mechanism where this residue acts as general base. Another insight comes from the analysis of the hydrogen bonding interactions around the β-lactam carboxylate in the acyl–enzyme complex. Previous works[4,5] have reported X-ray crystal structures of acyl–enzyme intermediates of the TEM1 enzyme. In these works the carboxylate hydrogen bonds to both Arg244 and Ser235, but not to Lys234 and Ser130. In the present paper we have found that the description of the deprotonated Lys73 structure is in better agreement with the mentioned experimental findings.

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35 Instead of considering the addition of water to the penicillinate, it would be more correct to consider serine as a nucleophilic agent. The calculations in the gas phase lead to senseless structures for the process for which we are interested, even when the positions of some atoms are fixed.

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