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Molecular dynamics simulation in aqueous solution of *N*-methylazetidinone as a model of *β*-lactam antibiotics

Jesús Pitarch[1], Juan-Luis Pascual-Ahuir[1], Estanislao Silla[1], Iñaki Tuñón[1], Manuel F. Ruiz-López[2], Claude Millot[2], Juan Bertrán[3]

[1] Departamento de Química Física, Universidad de Valencia, E-46100 Burjassot (Valencia), Spain
[2] Laboratoire de Chimie Théorique, URA CNRS 510, Université Henri Poincaré-Nancy I, F-54506 Vandoeuvre-les-Nancy, France
[3] Departament de Química, Universitat Autonoma de Barcelona, E-08193 Bellaterra (Barcelona), Spain

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Abstract. In this article, we analyze the results of a molecular dynamics simulation in aqueous solution of the *N*-methylazetidinone molecule, often used to model *β*-lactam antibiotics. The radial distribution functions (RDFs) corresponding to the most interesting atoms, in terms of reactivity, are presented. We focus our study on the effect of a polar environment on the molecule. The solvent structure around the system is compared to the structure of *β*-lactam-water complexes, as obtained in a previous study of reaction mechanisms for the neutral and alkaline hydrolyses of *N*-methylazetidinone. Two types of complexes have been considered which are related to different hydrolysis mechanisms having similar energy barriers at the rate-limiting step of the reaction path. In the first type, the *β*-lactam-water interaction takes place through the oxygen carbonyl atom and there is agreement between the maxima of the RDFs obtained here and the ab initio structure of the complexes previously reported. In the second type, the interaction takes place through the nitrogen atom and we do not predict a coordination layer around the *β*-lactam nitrogen atom. The results suggest that in aqueous solution hydrolysis of the carbonyl group is the most probable starting point for the overall hydrolysis reaction. Some discussion on the use of cluster models to represent the solvent effect is included.

Key words: *N*-Methylazetidinone – Molecular dynamics – *β*-Lactam hydrolysis – Solvent effects

1 Introduction

For a long time, *β*-lactam chemistry has been a field of active research because of the antibiotic properties of some *β*-lactam derivatives. The antibacterial activity is due to the inhibition of transpeptidase enzymes involved in the synthesis of the peptidoglycan bacterial cell walls, as a consequence of the acylation of a serine residue [1–5].

Many of the theoretical studies developed in this area have focused on conformational analysis, aimed at correlating structural parameters (such as the pyramidal character of the *β*-lactam nitrogen atom or the ring strain) with the chemical and biological reactivity of the molecule [6–11]. Other work has analyzed the reaction mechanisms of these compounds, fundamentally in the case of the hydrolysis reaction [12–17]. The interest in the hydrolysis reaction is mainly due to the presence of *β*-lactamases in some bacteria, which catalyze the hydrolysis of the antibiotic into its inactive amino acid. In addition, the study of ligand-enzyme interactions has received substantial attention [18–24].

In previous theoretical work by some of us [25, 26], a study of the mechanistic aspects of the *β*-lactam hydrolysis reaction was carried out. Both, neutral and alkaline hydrolyses of a *N*-methyl-substituted *β*-lactam (*N*-methylazetidinone) were considered in the gas phase and in aqueous solution. The bulk solvent effect was evaluated by means of a polarizable continuum model. A discrete representation of two water molecules was also taken into account, allowing possible bifunctional catalysis mechanisms in which an ancillary water molecule acts as a proton relay to be studied. The studies raised some questions related to the use of cluster models to represent *β*-lactam-water interactions. According to these studies, the hydrolysis mechanism may be stepwise or concerted. In the stepwise mechanism, a water molecule, initially interacting with *β*-lactam through the carbonyl oxygen, is added to the CO bond to form a diol intermediate. In a subsequent step, a proton is transferred to the nitrogen atom and simultaneously the ring is opened. In the case of the concerted process, a water molecule, initially hydrogen-bonded to the nitrogen atom, directly hydrolyses the CN bond. The difference between the energy barriers of the rate-limiting steps for the concerted and stepwise mechanisms is small and the

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Correspondence to: E. Silla

preference for one or the other reaction channel could be determined by the molecular environment or other factors. In particular, the probability distribution of water molecules around the β-lactam molecule prior to the initial attack is an important factor to be considered and it depends on the ability to form hydrogen bonds through the carbonyl oxygen and nitrogen atoms. Although continuum models are useful for evaluating the solvation free energies and the relative stability of the species involved in the chemical reactions, a detailed structural analysis of solvated systems requires a statistical treatment to be performed.

Within this scope, we have carried out a molecular dynamics (MD) simulation of *N*-methylazetidinone in aqueous solution. Comparison is made with the results derived from ab initio studies on β-lactam-water complexes.

2 Methodology

The MD simulation was carried out in the microcanonical ensemble (NVE) on a cubic box of edge 21.67 Å containing 336 water molecules and the *N*-methylazetidinone molecule. Periodic boundary conditions and a cutoff distance of a half edge for the solute-solvent and the solvent-solvent interactions were used. The SPC/E model potential [27] was selected for the water molecules. For β-lactam we used the HF/6-31G* fixed geometry (previously optimized), Lennard-Jones parameters taken from Ref. [28] and charges computed using the GRID program [29]. The electrostatic interactions are generally described in terms of atom-centered monopole expressions. The direct utilization of Mulliken charges is not effective, since these charges reflect reasonably the polarity of atoms and functional groups, but they are inadequate for describing molecular properties such as the electrostatic potential [30]. A more effective method seem be the fitting of point charges on the atomic sites to the ab initio electrostatic potential evaluated on a grid of regularly spaced points surrounding the molecule. This task was carried out with the GRID program, employing the GEPOL algorithm [31, 32] for the grid construction. The selection of points is achieved by generating a series of concentric layers of van der Waals surfaces around the molecule, separated by a grid step. The ab initio electrostatic potential was evaluated over all these points at the HF/6-31G* level, and the net charges fitted to them. After equilibration, the production run was carried out for a total of 112.5 ps, with a time step of 1.5 fs. For this task the MDpol program [33] was used.

3 Results and discussion

The radial distribution functions (RDFS) for the O5, C4 and N3 atoms of β-lactam are shown in Fig. 1. The numbering of some atoms is presented in Fig. 2. The existence of a well-structured coordination layer around the carbonyl oxygen O5 and carbon C4 atoms can be observed, whereas it is not present around the N3 nitrogen atom. The surprising absence of hydrogen-bonding interactions with the amide nitrogen atom has also been reported in a recent quantum mechanics/molecular mechanics simulation of *N*-methylacetamide in aqueous solution [34]. The maxima of the distribution functions are located at 1.7 and 2.7 Å for the H_W—O5 and the O_W—O5 distances, respectively. These functions were integrated, and a value of 2.7 for the O5 coordination number was obtained. For the C4 atom the maximum of the water oxygen RDF is at a distance of 3.6 Å. These results indicate the presence of a well-defined first solvation shell around the carbonyl group as opposed to around the amide nitrogen atom.

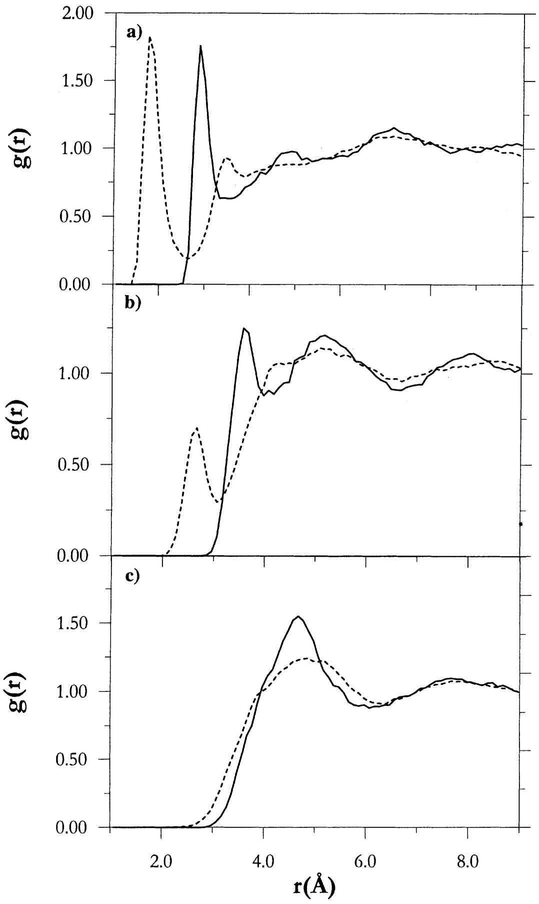

Fig. 1a-c. Radial distribution functions computed for *N*-methylazetidinone in water. **a** *Solid line*: carbonyl oxygen (O5)-water oxygen; *dashed line*: carbonyl oxygen (O5)-water hydrogen. **b** *Solid line*: carbonyl carbon (C4)-water oxygen; *dashed line*: carbonyl carbon (C4)-water hydrogen. **c** *Solid line*: nitrogen atom (N3)-water oxygen; *dashed line*: nitrogen atom (N3)-water hydrogen

It is interesting to relate these MD results to the structural and energetic description of the ab initio complexes, β-lactam-water dimer and β-lactam-water molecule, presented in previous studies on the hydrolysis reaction mechanism [25, 26]. These complexes are reproduced in the Fig. 2 and their relative energies and free energies are given in Table 1. All the structures, except A2, were optimized at the HF/6-31G* level. The A2 structure is only found when the 3-21G basis set is employed. The A1 structure corresponds to the β-lactam-water monomer complex interacting through the O5 oxygen. It has H_W—O5, O_W—O5 and O_W—C4 distances of 2.0, 2.9 and 3.3 Å, respectively. The internal energy of this complex with respect to the separated reactants is −7.10 kcal/mol. When thermal and entropic contributions are considered, this complex has a free energy of 2.03 kcal/mol above the separated reactants. In agreement with the poor nucleophilic character predicted by the simulation study, the equivalent complex interacting through the N3 nitrogen, structure A2, it is not found

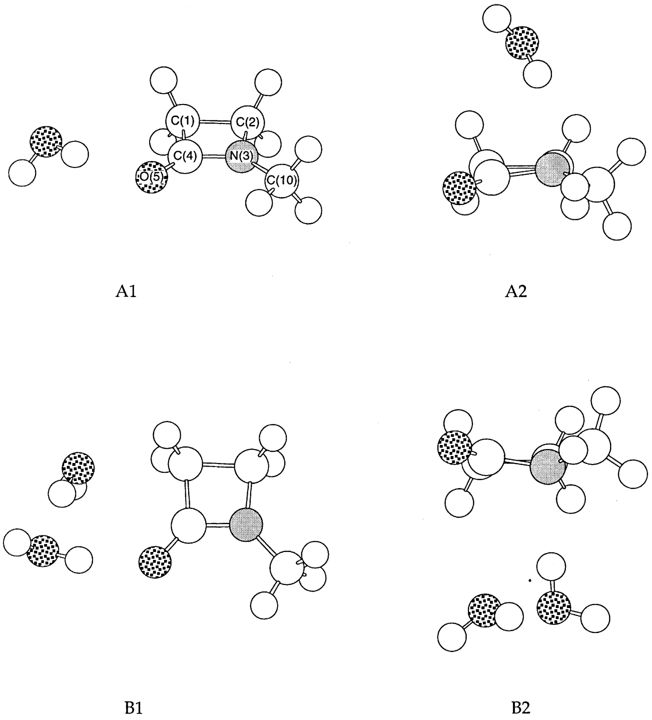

Fig. 2. Stationary structures of the β-lactam-water molecule complexes (*A1* and *A2*) and β-lactam-water dimer complexes (*B1* and *B2*). Note two types of complexes: those interacting through the O5 atom (A1 and B1) and those interacting through the N3 atom (A2 and B2). A1, B1 and B2 have been optimized at the HF/6-31G* level. A2 is only found employing the 3-21 G basis set at the same computational level

Table 1. Relative energies and free energies (kcal/mol) at the HF/6-31G* level for the β-lactum-water complexes (A1 and A2) and the β-lactam-water dimer complexes (B1 and B2). Note two possible energy references for this latter case. The values in parentheses correspond to the HF/3-21G results for A1 and A2

	A1	A2	B1	B2
ΔE	−7.10 (−11.02)	(−6.13)	−16.45[a] −10.83[b]	−9.79[a] −4.17[b]
ΔG	2.03 (−0.15)	(4.35)	3.36[a] 1.42[b]	8.86[a] 6.92[b]

[a] Energy reference: β-lactam + two isolated water molecules
[b] Energy reference: β-lactam + water dimer

with this basis set. However, it is possible to obtain it using the 3-21G basis set, which is known to overestimate the hydrogen bonds. Here the H_w—N3 and O_w—C4 distances are 2.2 and 3.2 Å. For comparison purposes we also optimized the A1 structure at the HF/3-21G level, giving the values for the energy listed in Table 1. The values indicate that A1 is considerably more stable than A2 by 4.89 kcal/mol.

Structures B1 and B2 correspond to the β-lactam-water dimer complexes. A hydrogen atom of the dimer is oriented toward the O5 oxygen atom in B1, and toward the N3 nitrogen atom in B2. For clarity, the water molecule to which this hydrogen atom belongs is denoted as w1, and the other one as w2. In B1, the β-lactam structure is not affected by interaction with water, keeping the ring planarity. The H_{w1}—O5, O_{w1}—O5 and O_{w1}—C4 distances are 1.9, 2.9 and 3.6 Å respectively. In B2, the nitrogen atom is pyramidalized and the ring planarity is lost. The H_{w1}—N3, O_{w1}—N3 and O_{w1}—C4 distances are 2.2, 3.2 and 3.7 Å, respectively. Also, in both complexes the second water molecule is located in a region relatively close to the C1 carbon atom. The O_{w2}—C4 distance is 3.4 and 3.5 Å in B1 and B2, respectively, whereas the O_{w2}—C1 values are 4.3 and 4.1 Å. The preferred solvation of the O5 atom with respect to the N3 atom is also reflected in a greater stability of the B1 complex. The relative internal energies with respect to the separated β-lactam and two water molecules, are −16.45 and −9.79 kcal/mol for B1 and B2, respectively. The two complexes have a positive free-energy (with respect to separated reactants), as a consequence of the entropic decrease caused by the cluster formation. It is necessary to note that in B1 and B2 two types of interactions are considered, β-lactam-water and water-water. This fact can lead to overestimation of the B1 and B2 stabilization with respect to the reactants. It seems more correct to calculate the relative energy of these complexes with respect to β-lactam and a water dimer, instead of considering two isolated water molecules. This consideration increases the internal energy of B1 and B2 by 5.62 kcal/mol, whereas the free energy diminishes by 1.94 kcal/mol. These values are the same for both complexes, as corresponds to the common origin of this correction, i.e., the interaction between the two water molecules.

We can compare some structural parameters of these complexes with the results obtained in the simulation study. In the complexes oriented toward the O5 atom (A1 and B1), the O_w—O5 distance is 2.9 Å. This value is close to the maxima of the corresponding distribution function, which is 2.7 Å. On the other hand the H_w—O5 distance is 2.0 and 1.9 Å in A1 and B1, respectively. The maximum of the corresponding RDF is 1.7 Å. Finally, for the O_w—C4 distance the value is 3.3 and 3.6 Å in A1 and B1, whereas the RDF maximum is found at 3.6 Å. In these two later cases it is observed that the B1 value, corresponding to the β-lactam-water dimer interaction, is slightly more close to the MD result than the A1 value, corresponding to the β-lactam-water monomer complex. With respect to the complexes oriented toward the N3 nitrogen atom (A2 and B2), a structural comparison between the MD results and the cluster model cannot be made in the same way. The simulation indicates the absence of a coordination layer around the N3 atom, and this fact is manifested in the cluster model in different ways. On the one hand, a β-lactam-water molecule complex interacting through the N3 atom is not found with the 6-31G* basis set. It is necessary to appeal to the 3-21G basis set to find it. On the other hand, when water dimers are considered, it is possible to find such a complex, B2, but it has a considerably lower stability than B1.

The net Mulliken charges on β-lactam and the water molecules are listed for the A1, A2, B1 and B2 ab initio structures in Table 2. The practical absence of a net charge transfer among β-lactam and the water molecules or between the water molecules themselves can be seen.

Table 2. Mulliken net charge (a.u.) on the β-lactam and water molecules in the A1, A2, B1 and B2 complexes. A1, B1 and B2: HF/6-31G*; A2: HF/3-21G

	A1	A2	B1	B2
β-Lactam	0.02	−0.01	0.02	0.00
Water 1	−0.02	0.01	0.00	0.01
Water 2	–	–	−0.02	−0.01

The maximum charge transfer is only 0.02 a.u., i.e., all the molecules preserve their identity in the complexes. This fact justifies the previous comparisons between the ab initio structures and the MD results.

A more detailed analysis about the solvation shell can be carried out on the simulation results. We have analyzed 750 configurations homogeneously distributed along the MD trajectory searching for structures resembling the previous complexes. We have found a set of 318 configurations with at least one water molecule presenting both a O_w—C4 distance less than 3.5 Å and a H_w—O5 distance less than 2.5 Å. These distances have been chosen to discern structures where the carbonyl group is hydrated. However, among the 750 configurations analyzed, there are only two configurations that fulfill similar requirements with respect to the β-lactam nitrogen, i.e., an O_w—C4 distance less than 3.5 Å and a H_w—N3 distance less than 2.5 Å.

The previous statements refer to the interaction between the β-lactam molecule and water monomers. We can extend this analysis to the water dimers, but it is necessary to introduce some different search requirements. For each configuration we have obtained a set 1 of water molecules for which the H_w—O5 distance is less than 2.5 Å. Independently, a set 2 where the O_w—C4 distance is less than 4.5 Å has been also obtained. Note that when we search for structures resembling B1 or B2 structures the O_w—C4 distance is increased with respect to the previous analysis (A1 and A2 search). This is due to the fact that in both the B1 and B2 structures the second water molecule is found in a region relatively distant from the C4 atom, at 4.1 Å in B1 and at 4.3 Å in B2, therefore more relaxed search requirements are needed. The water dimers oriented to the O5 oxygen correspond to those molecules of sets 1 and 2 that mutually form hydrogen bonds. There are 385 configurations where a dimer fulfills these requirements. When we apply this analysis to the search for dimers interacting through the nitrogen atom (set 1 is selected according to the H_w—N3 distance; set 2 is the same as before) only one configuration satisfying the criteria was found.

We have analyzed the relative orientation of the water molecules in the dimers, evaluating a series of geometrical parameters on each of them. The angles α and β are defined in Fig. 3; the dihedral angle between dipoles, γ, has also been considered. Its value is defined as the angle between planes P_1 and P_2, P_1 being the plane that contains the dipole vector of the first water molecule ($\mathbf{d}_1$) and the O1—O2 vector. P_2 is the equivalent plane for the second water molecule. For the three angles we have considered different intervals and evaluated the number of dimers found for each of them: the corresponding histograms are shown in Fig. 4. A certain structural order for the α and β angles is observed. The most probable value is found between 8° and 12° for β and between 50° and 58° for α. However, the γ angle exhibits random behavior. Because of the different nature of the models, these values are not directly comparable with those corresponding to the B1 and B2 ab initio structures. However, the closeness of the α (52° for B1 and

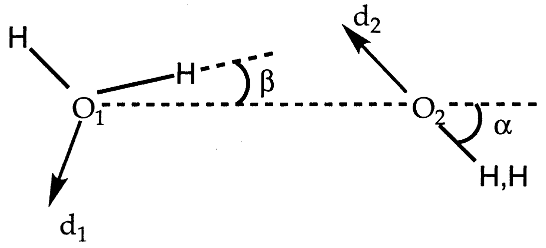

Fig. 3. Definition of the α and β angles

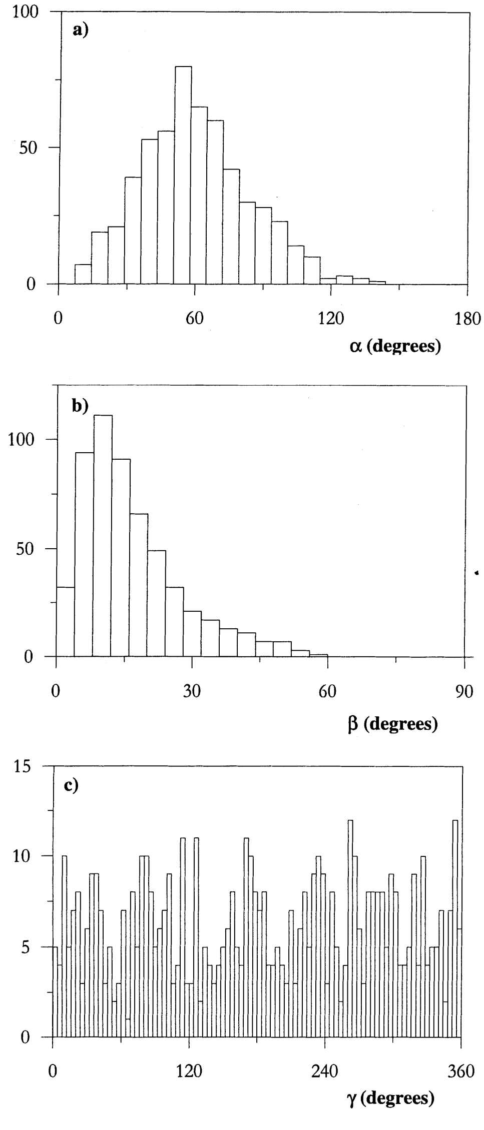

Fig. 4. Histogram of the **a** α, **b** β and **c** γ angles for the 386 structures considered

51° for B2) and β values (7° for B1 and 10° for B2) to the maximum of the α and β distributions is surprising. The random behavior of γ contrasts with the closeness to 180° for this angle in both structures (170° for B1 and 181° for B2). The γ values can reflect the lack of interactions of the water molecules in B1 and B2 with other solvent molecules. Both structures have practically the same values for α, β and γ, (i.e., the same orientation of the two water molecules in the dimer) and they are close to the values of the isolated water dimer ($\alpha = 62.5°$, $\beta = 5.2°$, $\gamma = 180°$). Thus the relative orientation of the water molecules in B1 and B2 seems to be specially adapted to optimize the interactions among the water molecules and can lead to complexes artificially more stable than those actually existing in solution. In fact, we have previously seen the decrease in stability obtained when the energy reference is taken with respect to a dimer instead of two isolated water molecules (Table 2). In solution, with a far greater number of solvent-solvent interactions, it is not difficult to think that this effect will be amplified.

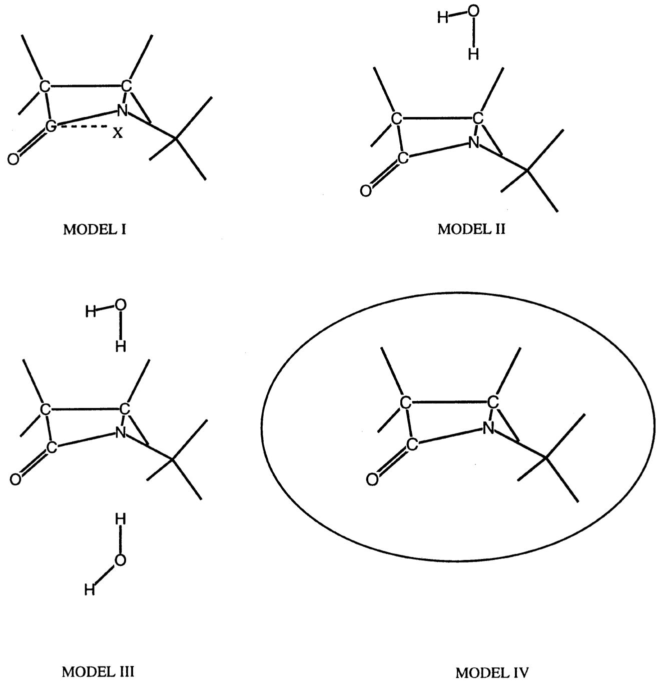

Fig. 5. Models used for studying β-lactam nitrogen pyramidalization in solution. *Model I*: β-lactam molecule in vacuo. *Model II*: β-lactam molecule and a water molecule. *Model III*: β-lactam molecule and two water molecules placed one on each side of the β-lactam ring plane. *Model IV*: β-lactam molecule in an ellipsoidal cavity continuum model

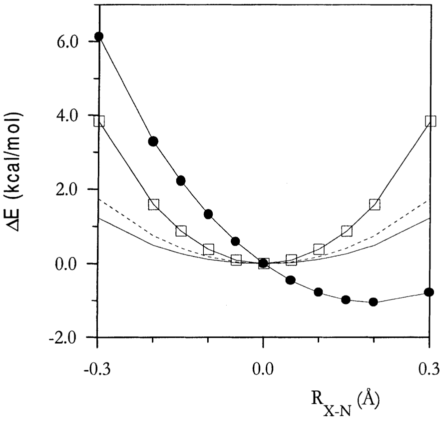

Fig. 6. Relative energies (kcal/mol) obtained along the pyramidalization coordinate (distance of the nitrogen atom to the β-lactam ring plane) for model I (*solid line*), model II (*filled circles*), model III (*squares*) and model IV (*dashed line*). See Fig. 5 and text for definition of the model systems

Another aspect that must be emphasized is that in the B2 cluster optimization the nitrogen atom is piramidalized and the ring planarity is lost. This deformation of the β-lactam monomer requires only 1.17 kcal/mol and it allows better interaction between the β-lactam and the water dimer through the formation of hydrogen bonds. A small frequency value corresponding to an out-of-plane motion of β-lactam (99.26 cm^{-1}) that leads to piramidalization confirms this point. This deformation of a very flexible system is probably an artifact of the small size of the cluster. From an adequate starting point and not allowing the β-lactam molecule and the water dimer to interact with other water molecules a local and an artificial minimum may be obtained. In order to investigate the reliability of these structures in solution we have studied the reduced potential energy surface corresponding to nitrogen piramidalization. For this purpose we have defined the distance of the nitrogen atom to the β-lactam plane (defined by the three carbon atoms C1C2C4) as the distinguished piramidalization coordinate and we have calculated the energy at the HF/6-31G* level for fully relaxed geometries (except for the selected coordinate) in four different β-lactam models (Fig. 5). Model I is the β-lactam molecule in vacuo. Model II corresponds to the β-lactam molecule and a water molecule hydrogen-bonded to the N3 nitrogen atom. This water molecule is placed at a fixed nitrogen-oxygen distance of 2.9 Å linearly hydrogen-bonded to the nitrogen atom along the line perpendicular to the β-lactam C1C2C4 plane. In model III two water molecules are placed one on each side of the β-lactam ring plane, with the same requirements as in model II. Finally, model IV corresponds to the β-lactam molecule in an ellipsoidal cavity continuum model [35–39]. The potential energy surfaces constructed for each model are shown in Fig. 6. Only model II exhibits a minimum for the piramidalized nitrogen. The presence of one water molecule interacting with the nitrogen favors piramidalization because in this way the nitrogen lone pair is localized toward the water hydrogen. Consequently, the carbon-nitrogen (C4—N3) distance increases from 1.365 Å in the planar geometry to 1.373 Å in the piramidalized one. However, if we place two water molecules, one on each side of the β-lactam plane, the planar structure is now the energy minimum. If we piramidalize the nitrogen atom, and thus localize the nitrogen lone pair toward one of the water molecules, the energy of the system increases. Moreover, the force constant associated with the nitrogen piramidalization is larger than in the gas phase. The same result is found when using a continuum model. This can be rationalized by taking into account the fact that the planar structure has a dipole moment larger than the piramidalized one and that bulk solvent effects increase the double-bond character of the carbon-nitrogen bond [40]. In fact the carbon-nitrogen (C4—N3) bond length decreases from 1.355 Å in the gas phase to 1.340 Å in the continuum model. In our cluster models, B1 and B2, water molecules keep a dimer-like structure, and thus they can interact with the *β*-lactam molecule only on one side of the ring plane. In this way piramidalized structures can be obtained if hydrogen bonds are formed with the nitrogen atom. It seems that in real solutions, with water molecules interacting with *β*-lactam from both sides of the ring plane, we have the opposite situation and planar geometries are stabilized both by specific and bulk solvent effects. The MD simulation seems to validate this conclusion, showing the problems of optimizing a cluster in an easily deformable system when a limited number of water molecules is taken into account.

4 Conclusions

We have presented the results of a MD simulation carried out on the *N*-methylazetidinone molecule in aqueous solution. Some relationships between these results and the features of *β*-lactam-water ab initio complexes have been stated. Two type of complexes have been considered, those where the *β*-lactam-water interaction takes place through the oxygen carbonyl atom, A1 and B1, and those where the interaction takes place through the nitrogen atom, A2 and B2. These complexes constitute the starting point in the corresponding reaction paths obtained in previous studies on the hydrolysis reaction mechanism of this compound. The most evident result of the simulation is the presence of a well-defined first solvation shell around the carbonyl group as opposed to the result found for the nitrogen atom. When we compared the RDFs of these atoms with the corresponding distances in the ab initio complexes we obtained good agreement for the first type of complexes, i.e., A1 and B1. Along the trajectory it is possible to find a considerable number of structures resembling them. However, for A2 and B2, the situation is drastically different. The MD results indicate the absence of a coordination layer around the nitrogen atom and practically no structures resembling A2 or B2 are found along the trajectory. These facts lead to two conclusions: on the one hand, in aqueous solution it seems quite probable to find situations where the orientation and position of solvent water molecules are favorable for attacking the carbonyl oxygen, i.e., a stepwise mechanism in the hydrolysis reaction; on the other hand, the importance of A2 and B2 structures could be magnified by the use of one or two water molecules to represent the solvent effect. In a cluster of limited size the lack of interactions with other solvent molecules leads to relative orientations of the molecules forming the cluster especially adapted to optimizing the interactions among them and with the solute. These orientations can be different from those found in solution where each water molecule interacts with a greater number of solvent molecules. These conformational aspects were considered in the previous section when the distributions of some specific angles related to the orientation of the molecules forming a dimer were evaluated. In addition, the use of such limited clusters can lead to overestimation of the importance of nitrogen piramidalization in solution.

In spite of these differences, the two approaches give us complementary information, coincident on many occasions. In fact, in the ab initio study B2 is considerably less stable than B1 and A2 is not found with the 6-31G* basis set. Furthermore, a preference for one or the other reaction channel on the basis of energy barriers was not found for the hydrolysis reaction. However, the simulation results complement this information showing the poor coordination layer around the *β*-lactam nitrogen, an aspect necessary for one of the possible mechanisms.

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