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The Mechanism of Formamide Hydrolysis in Water from Ab Initio Calculations and Simulations

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Abstract: The neutral hydrolysis of formamide in water is a suitable reference to quantify the efficiency of proteolytic enzymes. However, experimental data for this reaction has only very recently been obtained and the kinetic constant determined experimentally is significantly higher than that predicted by previous theoretical estimations. In this work, we have investigated in detail the possible mechanisms of this reaction. Several solvent models have been considered that represent a considerable improvement on those used in previous studies. Density functional and ab initio calculations have been carried out on a system which explicitly includes the first solvation shell of the formamide molecule. Its interaction with the bulk has been treated with the aid of a dielectric continuum model. Molecular dynamics simulations at the combined density functional/molecular mechanics level have been carried out in parallel to better understand the structure of the reaction intermediates in aqueous solution. Overall, the most favored mechanism predicted by our study involves two reaction steps. In the first step, the carbonyl group of the formamide molecule is hydrated to form a diol intermediate. The corresponding transition structure involves two water molecules. From this intermediate, a water-assisted proton transfer occurs from one of the hydroxy groups to the amino group. This reaction step may lead either to the formation of a new reaction intermediate with a marked zwitterionic character or to dissociation of the system into ammonia and formic acid. The zwitterionic intermediate dissociates quite easily but its lifetime is not negligible and it could play a role in the hydrolysis of substituted amides or peptides. The predicted pseudo-first-order kinetic constant for the rate-limiting step (the first step) of the hydrolysis reaction at 25 °C ($3.9\times10^{-10}\,s^{-1}$) is in excellent agreement with experimental data ($1.1\times10^{-10}\,s^{-1}$).

Keywords: ab initio calculations · amide hydrolysis · molecular dynamics · peptide bonds · reaction mechanisms

Introduction

Peptide-bond hydrolysis is a key biochemical reaction in both intracellular and extracellular regions. Aspartic, cysteine, serine or metallo-proteases are the four main classes of enzymes that catalyze this process in vivo. They are essential for the regulation of many physiological processes and also play an important role in disease propagation. For instance, aspartic protease is essential for the replication of the HIV virus so that inhibitors of this protease are some of the most effective drugs in the treatment of AIDS.[1,2] More generally, there is an increasing interest in protease inhibitors as therapeutic agents.[3,4]

Not surprisingly, the literature devoted to peptide-bond hydrolysis is extremely rich. As this reaction covers a large variety of biological domains, the reaction mechanism has been studied from many points of view. Some studies have focused on the uncatalyzed hydrolysis in water in an attempt to quantitatively determine the efficiency of the enzymes.[5–8]

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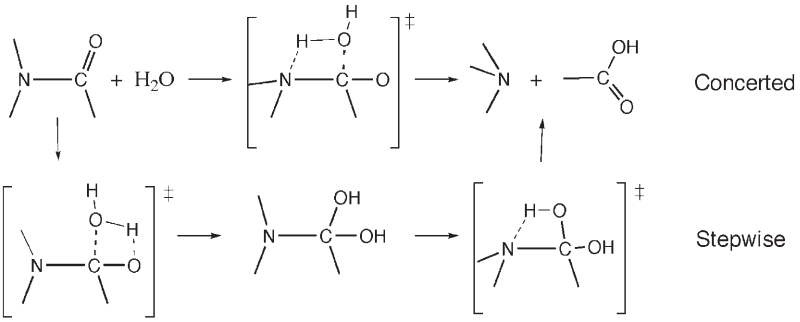
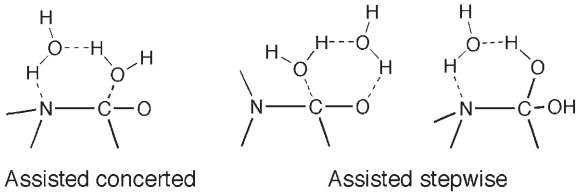
Though there is some experimental evidence that uncatalyzed hydrolysis occurs in water at a neutral pH, the corresponding reaction mechanism is controversial. Some experiments favor a base-catalyzed mechanism even in neutral aqueous solution,[9] but recent experiments[10] have allowed the reaction in water to be isolated from the acid- and base-hydrolysis terms.

Nowadays, quantum-chemical calculations are a powerful theoretical tool that complement experimental data or may even replace them in experimentally difficult situations. A number of theoretical studies have been devoted to the acid-[11–15] or base-catalyzed[11,15–23] hydrolysis of amides as well as to the enzyme-catalyzed hydrolysis of peptides.[24–34] The catalysis of β-lactam-ring hydrolysis has also been studied,[35,36] in particular by Donoso and co-workers (see refs. [34, 37–39] and references cited therein) because of its relevance to the understanding of bacteria resistance to antibiotics. Nevertheless, some authors have studied the neutral hydrolysis reaction by assuming that gas-phase processes operate. The hydrolysis of formamide has previously been studied by Krug et al.[11] and by Antonczak et al.[12,40] The inverse reaction was also considered by Oie et al.[41] and by Jensen et al.[42] Further calculations have been reported since.[15,17,43–46] Here, we briefly summarize the computations made on the uncatalyzed reaction, although the original references should be consulted for further details on the mechanisms of amide hydrolysis. The two mechanisms that have been described are represented in Scheme 1. In the concerted mechanism, the addition of the water molecule and breaking of the NC amide bond occur in a single step. In the stepwise mechanism, water first adds to the CO bond to form an amino-*gem*-diol intermediate and subsequently proton transfer from one of the OH groups to the nitrogen atom provokes the dissociation of the system and formation of the products. Both reaction mechanisms may undergo water catalysis[12,43] in which two water molecules (or more) participate in the reaction coordinate and cooperate to decrease the activation energy significantly. The corresponding transition states are represented in Scheme 2.



Scheme 1.



Scheme 2. Transition states for the concerted and stepwise mechanisms of the water-catalyzed hydrolysis of formamide.

Table 1. Summary of theoretical calculations reported in the literature of the energies of the transition structures in the neutral hydrolysis of formamide in the gas phase.[a]

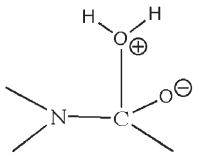
Theoretical level	Relative energy [kcal mol^{-1}]	Ref.
concerted non-assisted		
MP2/6-31G**//HF/3-21G	$\Delta E = 42.0$	[41]
	$\Delta G = 53.0$	
MP2/6-31G**//HF/4-31G	$\Delta E = 44.3$	[11]
MP2/6-311G**//HF/6-31G*	$\Delta E = 42.5$	[42]
	$\Delta G = 57.2$	
MP2/6-31G**	$\Delta E = 40.9$	[40]
B3LYP/6-31G**	$\Delta E = 32.7$	[40]
	$\Delta G = 44.6$	
stepwise non-assisted		
MP2/6-31G**//HF/3-21G	$\Delta E = 42.1, 38.9$	[41]
	$\Delta G = 52.7, 50.4$	
MP2/6-31+G**	$\Delta H = 45.1, 45.4$[b]	[44]
MP2/6-311G**//HF/6-31G*	$\Delta E = 41.1, 38.4$	[42]
	$\Delta G = 54.2, 53.1$	
concerted assisted		
MP2/6-31G**//HF/3-21G	$\Delta E = 25.8$	[12]
	$\Delta G = 49.6$	
MP2/6-31G**	$\Delta E = 31.7$	[40]
B3LYP/6-31G**	$\Delta E = 12.6$	[40]
	$\Delta G = 36.5$	
stepwise assisted		
MP2/6-31+G**	$\Delta H = 37.2, 32.5$[b]	[44]

[a] Values are given relative to separated reactants unless otherwise noted. The reactants in non-assisted (assisted) processes involve formamide and one (two) water molecules. [b] Values given relative to the formamide•water complex.

The estimated gas-phase activation energies are rather high at standard temperatures and pressures. The reported values are presented in Table 1. For non-assisted processes the activation enthalpies roughly lie in the range of 40–50 kcal mol^{-1} and the activation free energies in the range of 50–60 kcal mol^{-1}.[11,41,42] In the case of the assisted processes, the activation enthalpies are typically reduced by about 15–20 kcal mol^{-1} when two water molecules are considered.[12] The decrease in the activation energy may be more pronounced with a larger number of water molecules.[43] The free energies of activation in the assisted mechanisms are also lower than the non-assisted ones,[12] although the effect is much smaller than for the activation enthalpies. Indeed, entropy diminution along the reaction coordinate in the gas

phase is substantial and may be estimated to be -40 ± 10 cal mol^{-1} K^{-1} per water molecule.

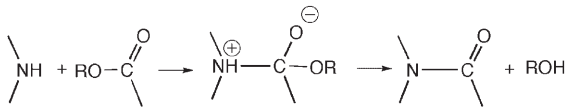
The role of the solvent in the neutral hydrolysis reaction has been less studied. Antonczak et al.[12] reported the first calculations to use a dielectric continuum solvent model. The computations suggested that electrostatic interactions with the continuum should not significantly modify the energetics of the process. Kallies and Mitzner[43] carried out a similar study also using a dielectric continuum model with explicit treatment of one or three discrete water molecules. The authors did not report gas-phase free energies, but from their results it is clear that the effect of explicit water molecules is much more important than that arising from pure electrostatic interactions with the continuum. Very recently, a much more sophisticated approach was used by Cascella et al.[17] and by Zahn[45] who carried out Car–Parrinello molecular dynamics (CPMD) simulations.[47] The main interest of such an approach is that all the solvent molecules are treated explicitly at the quantum level and that statistical averages are considered. Nevertheless, in MD simulations of chemical reactions in solution, the free-energy variation during the process is most often obtained by computing the potential of the mean force along a selected reaction coordinate (note that some approaches do not presuppose a reaction coordinate, see for instance ref. [13]). In both the papers mentioned, the authors chose to study the C(amide)–O(water) bond distance but their computations led to different products. Cascella et al.[17] described a process in which the intermediate species shown in Scheme 3 is



Scheme 3. Zwitterionic intermediate obtained in the CPMD simulations carried out by Cascella et al.[17]

formed. In contrast, Zahn[45] described a process very similar to the concerted water-assisted one reported by Antonczak et al.[12] in the gas phase. The stepwise mechanism has not been described by these CPMD simulations, although, as we show below, it is found to be the most favorable mechanism in aqueous solution. Clearly, a more elaborate definition of the reaction coordinate would be required to discriminate between the concerted and the stepwise mechanisms. It cannot be limited to the C(amide)–O(water) bond length since this coordinate is involved in both processes. In fact, as the C–O bond length decreases, proton transfer from the water molecule to the formamide nitrogen or oxygen atom may occur and this will determine whether the reaction proceeds through the concerted or stepwise mechanisms. Hence, the reaction coordinate should include other internal parameters chosen after a careful analysis of the potential-energy surface. This can only be achieved by using quantum mechanical calculations and simplified solvation models that allow rigorous location of stationary points. Once a realistic approximation of the reaction coordinate has been obtained, the free energies may be accurately computed by using sophisticated techniques like ab initio or combined quantum mechanics and molecular mechanics (QM/MM) molecular dynamics simulations.

To complete this introduction, the relationship between amide hydrolysis and the aminolysis of alkyl esters should be stressed. The latter reaction has attracted much attention since it might account for the synthesis of peptides from amino acids (or their esters) in prebiotic conditions. Experimental work reported by Jencks[48] supported a stepwise mechanism with the formation of the zwitterionic intermediate, as shown in Scheme 4. At a high pH, the formation of



Scheme 4.

the intermediate would be rate-determining, whereas at a lower pH, the rate-determining step would be the breakdown to the amide. This reaction mechanism has been the object of some recent controversy.[49,50] Theoretical studies of the model reaction $NH_3+HCOOH$[41,42] which is also of interest in astrochemistry, have also been reported.[44] In general, the calculations were carried out in the gas phase in which the existence of a zwitterion is not expected. Simulations in aqueous solution have been recently reported[51] and suggest the possible formation of the zwitterion though its lifetime is quite dependent on solvation dynamics. Quantum mechanical calculations have shown[50] that at least four water solvation molecules are needed to stabilize the species and avoid spontaneous dissociation into reactants. However, the stability of the intermediate and its role in amide hydrolysis reactions deserves further study.

From the above comments, one may conclude that the reaction mechanism for the neutral hydrolysis of amides in water has not yet been definitively established. The aim of this work was to obtain a mechanistic scheme for this process that is as complete as possible. Specifically, we have considered the following aspects: 1) the explicit treatment of the first solvation shell of formamide in water, which has been estimated to consist of five molecules,[52] 2) a detailed description of the transition structures, reaction coordinates, and reaction intermediates of the concerted and stepwise mechanisms, 3) the potential presence and role of zwitterionic intermediates (Scheme 3 and Scheme 4), 4) a comparison of non-assisted and water-assisted reactions, and 5) the structure, stability, and dynamics of possible reaction intermediates. Of course, the experimental and theoretical data reported in the literature will be compared. The results of this work will provide a new insight into the reaction as well as an appropriate framework for future ab initio or QM/MM simulations.

Computational Methods

Models and level of computation: Three solvent models were used in this work. The first one (Model 1) is a discrete model in which a complex formed by the reactants and a few solvent water molecules is treated quantum mechanically. The initial complex corresponds to formamide and the five water molecules in its first solvation shell. The choice of this structure was based on the results of QM/MM MD simulations of the formamide hydration performed in reference [52] and detailed below. The final product is a complex formed by ammonia, formic acid, and four water molecules. Explicit consideration of at least these solvent molecules is crucial to describe some of the reaction intermediates, as shown below. The geometries of the complexes were optimized and the potential-energy surface explored to locate transition structures and reaction intermediates. Each stationary point (energy minimum or saddle point) was characterized by vibrational frequency calculations. Minima linked by transition structures have been checked by analysis of the corresponding imaginary frequency normal mode and by a limited number of intrinsic reaction coordinate calculations.[53] Zero-point energy corrections and free energies were computed by using standard statistical mechanics expressions for an ideal gas. Energy values are given relative to the initial complex (formamide + five water molecules), which is the appropriate choice for modeling a process in solution. Note that for such a multimolecular system accurate computation of the free energy might require a statistical analysis that takes into account configuration averages. While this is fundamental for very large systems, in small complexes like those considered here, the neglect of configuration averages is expected to be a reasonably good approximation. Errors introduced in this way will be estimated below.

The second solvent model (Model 2) is a discrete-continuum model. The complexes in Model 1 are allowed to interact with a polarizable dielectric medium having the static dielectric constant of water ($\varepsilon = 80$). The complex is placed in a molecular-shaped cavity[54] created within the dielectric continuum. Its charge distribution polarizes the medium which in turn creates an electric field that interacts with the electrons and nuclei. The wavefunction of the solute was computed through the so-called self-consistent reaction field equations.[55–57] This model allows solute–solvent interactions beyond the first solvation shell to be taken into account.

Model 3 is based on the so-called QM/MM approach.[58–61] It is a pure discrete model in which the solute is described quantum mechanically and the solvent is described through a simple force field used in molecular mechanics. In this way, a large number of solvent molecules in the system can be considered in the calculations. Molecular dynamics simulations were carried out for a simulation box with periodic boundary conditions.

The computation level and some other details of the methods used here are as follows:

Model 1: Geometry optimization calculations were carried out by using density functional theory (DFT), the 6-31G(d) basis set,[62–65] and the B3LYP hybrid functional.[66,67] This computational level was chosen as a compromise between accuracy and computational cost. It has already been used for this reaction[43] and is quite similar to that used in CPMD simulations.[17,45] Previous calculations have shown that B3LYP/6-31G(d)-derived geometries are suitable although the interaction energies may include significant errors[40] (see Table 1). For this reason, single-point energy calculations on DFT geometries have been performed at the MP2/6-311+G** level of theory. Some calculations at the MP4/6-311+G** level of theory have also been carried out for the most important structures. An important point must be stressed here. Differences between DFT and correlated ab initio calculations are expected to be large when one compares interaction energies, that is, the energy of the complex with respect to the separated molecules (as was done in the studies summarized in Table 1). These energies are expected to include errors arising from BSSE (basis set superposition error) and from some intermolecular interaction terms, in particular from the dispersion term. When one compares the energies of two complexes, as done in this work, errors are expected to be largely cancelled out so that the differences between the DFT and MP2 or MP4 calculations should be smaller. This actually applies in this work, as shown below. Computations have been performed using the Gaussian 03 program.[68]

Model 2: Bulk solvent effects on free energies have been incorporated through calculations with the PCM model of Tomasi and co-workers[69,70] using the integral equation formalism.[71] The electrostatic and non-electrostatic solvation energies were added to the free energies obtained in Model 1 without further geometry optimization of the structures. The Gaussian 03 program[68] was also employed in this case.

Model 3: MD simulations using a combined quantum mechanics and molecular mechanics (QM/MM) potential were carried out using the DFMM program.[72,73] The solute was described at a slightly different DFT level to that used in Model 1. In this case, we used the BP functional[74–76] and a double-zeta basis set with polarization functions having a contraction for heavy (621/41/1) and hydrogen atoms (41/1). This basis set is a standard basis set in the deMon code[77,78] and was used here to obtain the wavefunction. The solvent water molecules were described using the TIP3P force field.[79] Solute–solvent interactions involve both electrostatic and non-electrostatic terms. Electrostatic contributions were evaluated by taking into account the presence of classical TIP3P water charges in the computation of the solute wavefunction. Non-electrostatic contributions were evaluated through a Lennard-Jones potential using TIP3P parameters for classical water molecules and OPLS parameters[80] for the solute atoms. Molecular dynamics simulations were performed using a box with a side length of 18.8 Å containing 216 TIP3P water molecules and the quantum solute molecule. Only reaction intermediates have been described with this method. Periodic boundary conditions and a cut-off of 9 Å were assumed. Simulations were carried out in the NVE ensemble with a target temperature of 298 K and an integration time step of 0.5 fs. The system was thermalized over 20 ps. Afterwards, data were averaged over 25 ps. The mass of deuterium was used for hydrogen atoms.

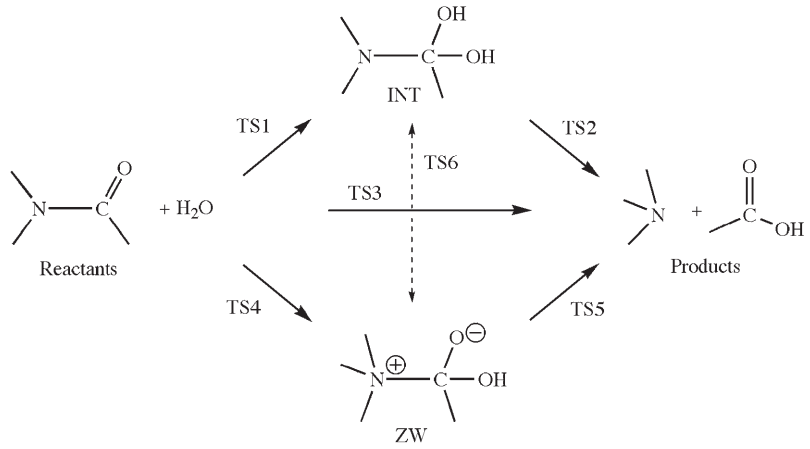
Kinetic constants were computed using transition-state theory. Proton tunneling effects were not considered here although they are expected to be small for transfer processes through water bridges at 300 K.[81]

Results and Discussion

The presentation of the results is organized as follows. First, we summarize all the reaction paths found using solvation Model 1. Next, we describe in detail the corresponding optimized structures (reactants, intermediates, products, and transition states) as well as some dynamic aspects of the reaction intermediates derived by using solvation Model 3. We then present the predicted energy profiles. For this purpose, we use solvation Model 1 (discrete) and Model 2 (discrete-continuum). Both DFT and MP2 results are reported. In subsequent sections, we compare our results with those obtained in previous theoretical and experimental studies of the neutral hydrolysis reaction.

Reaction pathways: The reaction pathways described in this work and the notation used to describe the intermediates and transition states are summarized in Scheme 5. All the transition structures (TSs), except TS5, involve proton transfer and therefore water molecules may assist the processes. Therefore, non-assisted and water-assisted TSs have been computed (except for TS6). Assistance by several water molecules may be envisaged too, but this will not be considered in this work.

In spite of several trials, the reaction intermediate shown in Scheme 3 has not been located. It spontaneously dissoci-



Scheme 5.

ates into formamide and water or into ammonia and formic acid depending on the initial conditions. A labile intermediate of this type was noted in a previous CPMD study,[17] which in part may be a consequence of the constraint imposed on the computation of the potential of the mean force. Such computations predicted a rather high energy for this intermediate (44 kcal mol^{-1}) with a small barrier to dissociation (2 kcal mol^{-1}).

The reaction through the amino-*gem*-diol intermediate INT is related to the stepwise mechanism previously described in the literature;[41] the carbonyl double bond is hydrated (TS1) prior to the breaking of the NC amide bond (TS2). The concerted mechanism previously described leads directly from the reactants to the products through TS3. In our scheme, a zwitterionic intermediate ZW is also found, which may be formed directly from the reactants through TS4 (see below), but interconversion with the intermediate INT (TS6) is possible. As mentioned above, this zwitterion has been described by us[51] and other authors[50] in connection with the ester aminolysis reaction. It is stabilized by hydrogen bonds formed with surrounding water molecules and at least four explicit water molecules must be considered in the calculation.[50]

The transition structure TS4 deserves a few comments. In spite of several trials, it was not located in our calculations using Model 1, which systematically converges to TS3. In fact, if it exists, TS4 should be very similar to TS3. Moreover, TS3 and TS4 could formally represent the same structure if the potential-energy surface exhibits a bifurcation in the path towards the products. From our calculations, we cannot make any definitive conclusions concerning this point but the hypotheses above are supported by molecular dynamics simulations using combined DFT/MM potentials.[51] A number of reactive trajectories going through transition structures of the concerted (non-assisted) type were simulated and their fate was found to depend on the initial conditions in a very subtle way. After crossing the barrier, some trajectories proceeded directly to the products whereas others proceeded to the intermediate ZW.

Geometry of relevant structures: The geometries of the reactants, products, reaction intermediates, and transition structures are summarized in Figure 1. The stable structures (energy minima) display typical hydrogen-bond networks with six to seven hydrogen bonds. In the reactant structure, the carbonyl oxygen atom and the hydrogen atoms of the amino unit form hydrogen bonds with water molecules. In contrast, no hydrogen bonds are formed with the nitrogen atom. This picture is consistent with the results of molecular dynamics simulations of formamide in water. For instance, in ref. [52] (see also refs. [1]–[6] and [24] in that paper), the N(formamide)−H(water) radial distribution function exhibits a very small feature at 2.33 Å indicating a negligible interaction. In addition, each hydrogen atom on the amino unit was shown to form one hydrogen bond with water whereas the carbonyl oxygen atom was shown to form two to three hydrogen bonds. In the case of the TSs, the presence of several water molecules interacting with the solute favors charge separation and tends to increase the asynchronous character of proton transfer. Actually, by looking at the transition structures, one may identify some hydroxy or hydronium-like units interacting with the rest of the system. For instance, in TS1 (assisted or non-assisted), proton transfer from water to the formamide oxygen atom is almost achieved whereas the forming CO bond distance is still large (a little shorter in the assisted process).

To obtain a deeper insight into the structure of the reaction intermediates in water, we carried out molecular dynamics simulations using a combined DFT/MM potential for the systems in a box of water molecules (Model 3). The formamide molecule has already been studied using a similar model.[52] In addition, Bakowies and Kollman[16] have carried out classical Monte Carlo simulations for a structure similar to INT in water [H_2N–C(OH)O^-, that is, the adduct formed by the reaction of formamide + OH^-].

Intra- and intermolecular parameters obtained in these simulations are collected in Table 2. Radial distribution functions (RDFs) for the intermolecular interactions of INT and ZW intermediates are presented in Figure 2 and Figure 3, respectively. In Table 2, the bond lengths obtained with Model 3 are compared with those obtained with Model 1 above. Note that both models predict a long CN bond length for ZW, around 1.62–1.63 Å. In addition, Model 3 predicts large fluctuations of this bond, as expected. A histogram for the CN bond length is given in Figure 4. However, the system does not dissociate and no proton transfer is observed between the subunits during the 30 ps of the simulation suggesting a non-negligible stability of the

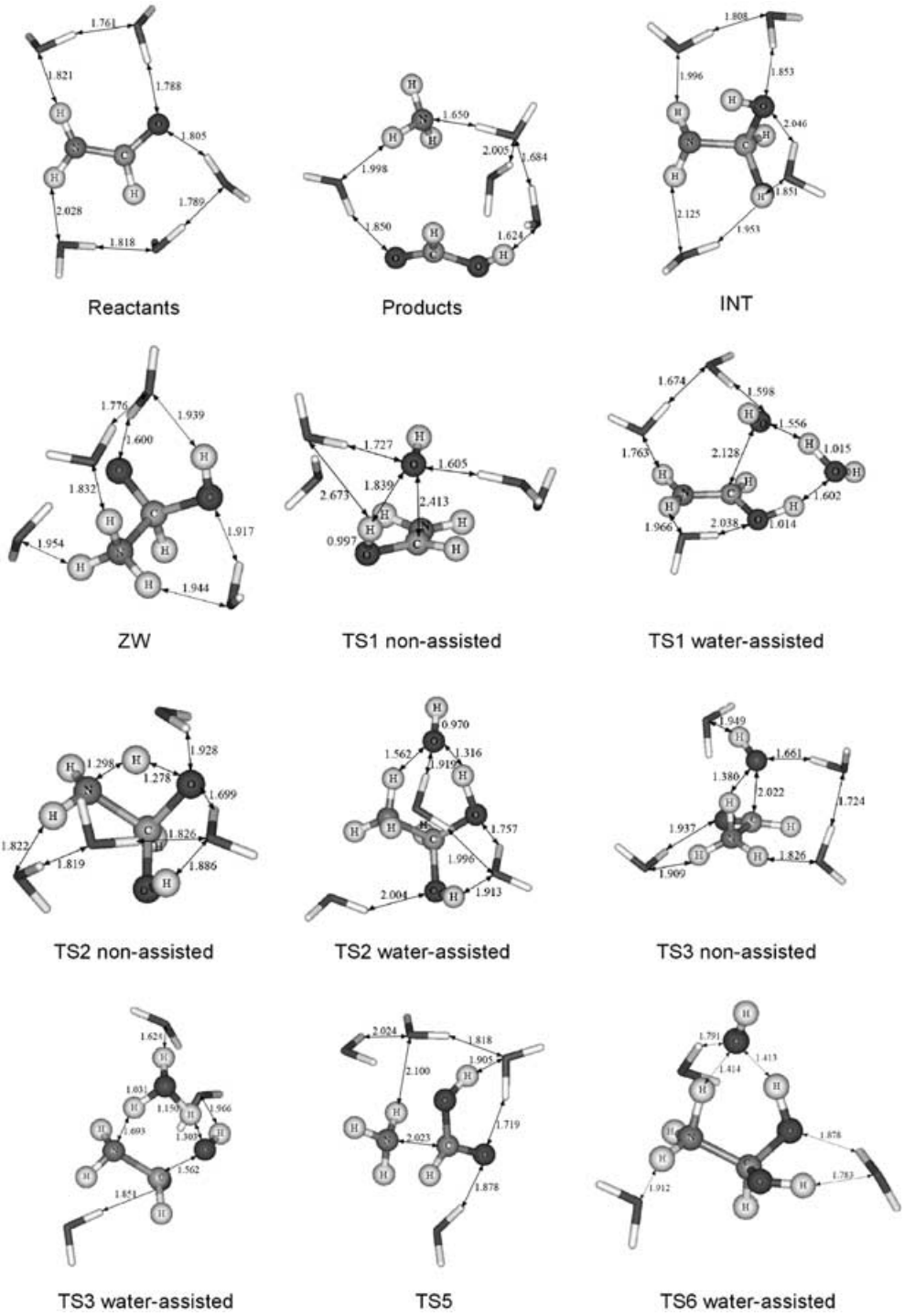


Figure 1. Optimized structures at the B3LYP/6-31G* level of theory for the neutral hydrolysis of formamide. TS4 is assumed to be equal to TS3 (see main text).

intermediate. Energy considerations (see below) will confirm this point.

Both systems form well-defined hydrogen bonds with water. In the diol intermediate INT, the N−O(w) RDF exhibits almost three hydrogen bonds (see coordination numbers in Table 2), suggesting that the nitrogen atom forms one direct bond with water, in contrast to formamide.[52] We have analyzed the MD trajectories in order to check for the formation of hydrogen bonds with water molecules that could form bridged structures between acceptor and donor groups in the solute. This analysis was based on X-(solute)···H(solvent) and H(solute)···O(solvent) distances for the whole system throughout the simulation. It shows that 1) water molecules that accept a hydrogen bond from the

Table 2. Structural properties obtained for solvated reaction intermediates using Model 1 and Model 3.[a]

	INT		ZW	
Internal geometry	Model 1	Model 3	Model 1	Model 3
d_{CN} [Å]	1.431	1.455	1.629	1.620
d_{CO-} [Å]	–	–	1.302	1.317
$d_{CO(H)}$ [Å]	1.402, 1.455	1.432	1.419	1.452
d_{NH} [Å]	1.022, 1.028	1.046	1.045, 1.032, 1.033	1.063
hydrogen bonds[b]				
$d_{NO(w)}$ (N) [Å]		2.76 (2.8)		2.65 (3.0)
$d_{O-O(w)}$ (N) [Å]		–		2.72 (3.8)
$d_{O(H)O(w)}$ (N) [Å]		2.79 (2.9)		2.79 (3.0)
$d_{H(O)O(w)}$ (N) [Å]		1.59 (1.0)		1.68 (1.0)
dipole moment [debye]		4.18		10.13

[a] Average values are given for Model 3. [b] Position of first maximum in RDFs (integrated number of neighbors).

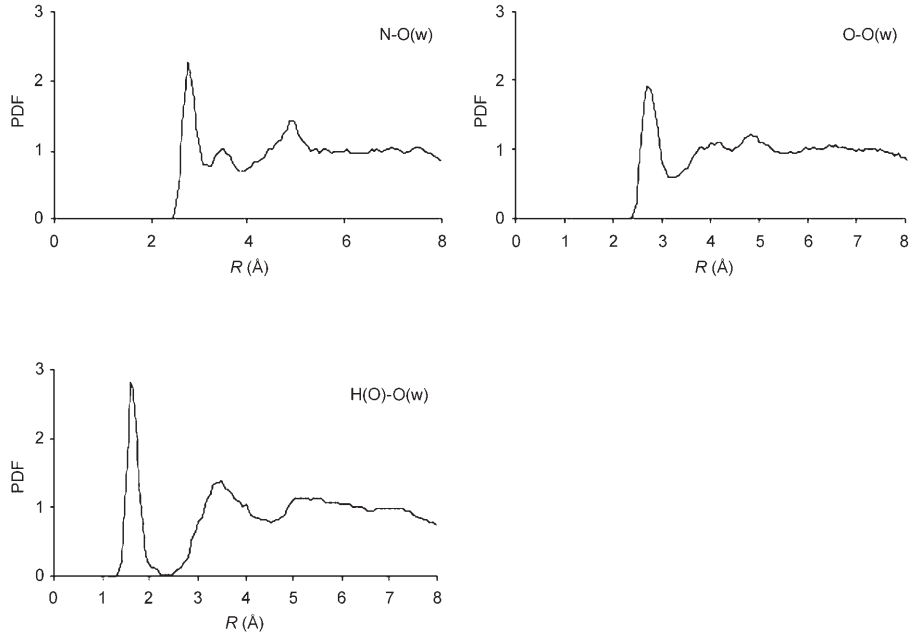


Figure 2. Radial distribution functions for the amino-*gem*-diol intermediate INT in aqueous solution as obtained in a DFT/MM molecular dynamics simulation.

hydrogen atom of a hydroxy group often act as a hydrogen-bond donor with respect to the nitrogen atom and 2) hydrogen bonds formed by hydroxy groups as donors involve different water molecules although these water molecules may interact with each other.

In the case of the ZW intermediate, the oxygen atom carrying the negative charge forms almost four hydrogen bonds. This result is consistent with the experimental coordination number of the oxygen atom of the hydroxide anion in water[82] and with the theoretical results of Bakowies and Kollman[16] for a related system, $H_2N{-}C(OH)O^-$.

One may wonder whether intramolecular hydrogen bonds are formed or not. Although some stabilizing interactions of this type can be found during the simulation, one may describe the hydroxy groups in INT and the O^- and hydroxy groups in ZW as relatively independent. The water solvation patterns described above for these groups are consistent with this statement. Indeed, the average intramolecular O···H distances are rather large (2.75 Å in INT, 2.47 Å in ZW).

The dipole moments of both intermediates are also given in Table 2. Not surprisingly, the magnitude of this property is very large for ZW but is also quite substantial for the diol intermediate. The dipole moment of this intermediate is much smaller (2.54 D) in the gas phase showing that the system is highly polarized in aqueous solution.

Reaction energies: Data from the energy analysis using Models 1 and 2 are given in Table 3. Note that the complex formed by the interaction of the formamide molecule with five water molecules is the reference system. We include in Table 3 the result of computations at the B3LYP/6-31G* and MP2/6-311+G** levels of theory. Energy calculations at the MP2 level were carried out using the optimized geometries, zero-point energies, and thermal contributions to the free energy determined at the B3LYP level, as well as the solvation energy corrections due to interactions with the continuum. As shown, the MP2 values are a little larger than the B3LYP values but there is a good qualitative agreement between both methods. According to the predicted free energies shown in Table 3, the hydrolysis reaction should proceed through a water-assisted stepwise mechanism. In the first stage of the process, the amino-*gem*-diol intermediate should always be formed. The corresponding activation free energy is 34.9 kcal mol^{-1} at the MP2 level, which is much less than the value for the assisted concerted process (42.4 kcal mol^{-1}). Then, the amino-*gem*-diol intermediate should evolve directly to the product through TS2 with an activation energy of about 11.2 kcal mol^{-1}. However, the formation of the zwitterionic intermediate through TS6 cannot be completely excluded although, if formed, ZW will easily decompose into the products through TS5 (3.6 kcal mol^{-1} above ZW).

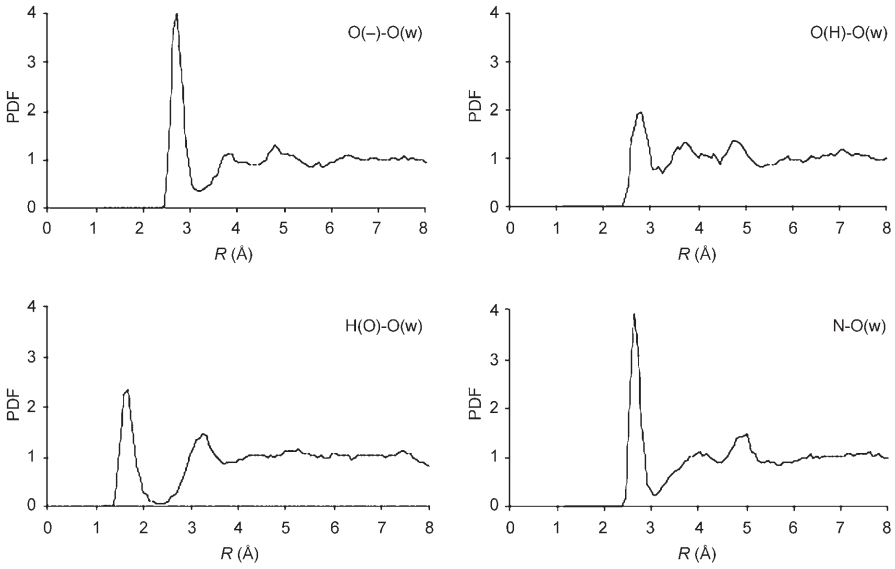


Figure 3. Radial distribution functions for the ZW intermediate in aqueous solution as obtained in a DFT/MM molecular dynamics simulation.

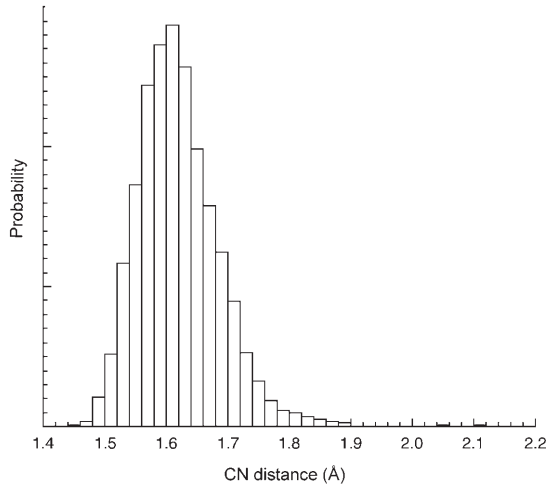


Figure 4. Histogram of CN bond length as obtained in the DFT/MM molecular dynamics simulation of intermediate ZW in water.

Our calculations clearly suggest that 1) non-assisted processes in water solution are very unlikely to occur, 2) in contrast to assisted processes, for the non-assisted ones, the concerted mechanism is slightly favored with respect to the stepwise one, and 3) water catalysis is particularly important for TS2 but has a modest effect on TS3. It is also interesting that the results from Models 1 and 2 are quite similar. In other words, bulk solvent effects seems to be rather small, the largest changes in ΔG being less than 2 kcal mol^{-1}. This is in agreement with the previous suggestion made by Antonczak et al.[12]

Further calculations have been performed to check the validity of our results. Specifically, calculations at the MP4/6-311+G** level of theory were carried out for the assisted processes by using the geometries optimized at the B3LYP/6-31G* level. The activation energies for TS1, TS2, and TS3 are 33.9, 30.8, and 40.4 kcal mol^{-1}, respectively, which are very close to the MP2 values reported in Table 3. This suggests that the analysis presented above is not expected to change by increasing the computational level.

Comparison with previous theoretical studies: It is interesting to compare our results with previous computations in the literature for the reaction in solution. The concerted water-assisted process was considered by Antonczak et al.[12] They evaluated the corresponding free energy of activation to be 49.57 kcal mol^{-1}, with respect to separated molecules, and 39.4 kcal mol^{-1}, with respect to the pre-reactant complex formed by formamide and two water molecules (MP2/6-31G*//HF/3-21G level of theory, see Table 6 of that paper; the effect of the dielectric continuum is not included in this value but the authors noted that it should be small). The latter value may be compared with our MP2 result for TS3, which is not very different (42.4 kcal mol^{-1}). Kallies and Mitzner,[43] performed calculations on a system immersed in a dielectric continuum and with three water molecules interacting with formamide (B3LYP/6-31G* level). Unfortunately, their activation energies cannot be compared directly with our results since they considered a different reference system (the separated molecules at infinity). However, one can make two remarks. First, the ΔG value for the concerted water-assisted reaction (49.78 kcal mol^{-1}) is very similar to that reported by Antonczak et al.[12] who used an equivalent reference system (49.57 kcal mol^{-1}). Second, their results suggest a preference for the stepwise mechanism, in agreement with our calculations (although this preference is larger in our case).

Very recently, the neutral hydrolysis of *N*-methylacetamide was simulated by Zahn[45] using DFT Car–Parrinello molecular dynamics (CPMD). The transition-state picture of the nucleophilic attack was nearly identical to that reported by Antonczak et al[12] for the assisted concerted process. The author reported an activation free energy of 35.2 ± 3 kcal mol^{-1}. This value is in excellent agreement with the value (37.1 kcal mol^{-}1) we obtained by DFT for the same mechanism. The computational levels used in both approaches are very similar so that one may conclude that the simple solvation models employed in our work are a very good approximation. In particular, the lack of first-solvation-shell configurational averages seems to have a limited influence on the free-energy computations. Indeed, the error made may be

Table 3. Thermodynamic properties at the B3LYP/6-31G* and MP2/6-311+G** levels of theory for stationary points at $T=298$ K.[a]

	B3LYP/6-31G*					MP2/6-311+G**	
	ΔE	ΔZPE	$-T\Delta S$	ΔG	ΔG_s	ΔE	ΔG_s
FOR	0.0	0.0	0.0	0.0	0.0	0.0	0.0
INT	23.2	1.4	1.4	25.5	23.5	22.9	23.2
ZW	14.3	2.5	4.3	19.5	18.9	19.2	23.8
PROD	1.1	0.0	1.0	1.8	3.5	4.2	6.6
non-assisted stepwise reaction							
TS1	39.0	−1.0	3.5	40.4	41.0	40.8	42.8
TS2	43.1	−1.0	3.8	44.7	45.0	46.4	48.3
assisted stepwise reaction							
TS1	29.3	−1.2	4.1	30.7	30.4	33.8	34.9
TS2	26.3	0.4	6.6	30.3	30.3	30.4	34.4
non-assisted concerted reaction							
TS3	43.8	−1.8	3.0	44.0	43.9	47.5	47.6
assisted concerted reaction							
TS3	34.8	−0.6	5.1	37.3	37.1	40.1	42.4
other transition structures							
TS5 non-assisted	15.4	0.8	3.4	21.0	22.0	20.8	27.4
TS6 assisted	27.9	0.0	5.2	33.4	32.6	31.1	35.8

[a] Values in kcal mol^{-1} relative to the initial complex of formamide + five water molecules. ΔG_s includes corrections from bulk solvent effects in aqueous solution (TS4 may be assumed to be equal or very close to TS3, see text).

roughly estimated as the difference between the activation free energies, that is, 2 kcal mol^{-1}.

As mentioned above, similar CPMD simulations have been carried out by Cascella et al.,[17] who described the formation of the intermediate shown in Scheme 3. The corresponding free energy of activation (44 kcal mol^{-1}), is significantly larger than that obtained by Zahn for the concerted hydrolysis. Differences between the two CPMD simulations have been commented on in more detail by Zahn.[45]

Before ending this section it is worth commenting on the reverse reaction ($NH_3+HCOOH \rightarrow NH_2COH+H_2O$). As for the forward reaction, assisted and stepwise mechanisms are preferred. However, in this case, the zwitterionic intermediate ZW is much more likely to be formed initially than the amino-*gem*-diol intermediate INT. This intermediate must be formed for the products to be obtained and the transition structure for its direct formation from NH_3 and HCOOH has a slightly lower energy than the transition structure for its formation from ZW (compare TS2 and TS6).

Kinetic constants, comparison with experiment: Experimentally,[10] the pseudo-first-order kinetic constant for the neutral hydrolysis of formamide has been determined at 56 °C ($3.6\pm0.1\times10^{-9}$ s^{-1}) and 120 °C ($1.09\pm0.29\times10^{-6}$ s^{-1}) from which the authors estimated a value of $k=1.1\times10^{-10}$ s^{-1} at 25 °C using a two-point Arrhenius plot. This value is close to those reported by Radzicka and Wolfenden[7] for the hydrolysis of the amide bond in small peptides at 25 °C ($k\approx10^{-11}$ s^{-1}). In a later study, a free energy of activation of 31.71 kcal mol^{-1} was estimated for the hydrolysis of *N*-acetylglycyl-glycine.[83] Other experimental estimations for the neutral hydrolysis of amides and references may be found in the paper of Slebocka-Tilk et al.[10]

To compare the results of our calculations with experimental data, one should note that the values reported by Slebocka-Tilk et al. correspond to pseudo-first-order kinetic constants. If the reaction is *n*th order with respect to water, the computed kinetic constants should be multiplied by $[H_2O]^n$ before comparison with experiment is made (here, *n* will be assumed to be the number of water molecules participating in the reaction coordinate).

Computed kinetic constants at 25 °C obtained using transition-state theory are summarized in Table 4. As shown, the pseudo-first-order kinetic constant for the first step of the stepwise water-assisted process (3.9×10^{-10} s^{-1}), which is the rate-limiting step, is very close to the experimental estimate for formamide hydrolysis (1.1×10^{-10} s^{-1}). Computed kinetic constants for the other reaction mechanisms are at variance with experimental measurements, differences in k_1 being 5–11 orders of magnitude.

Table 4. Kinetic (k) and pseudo-first-order kinetic (k') constants at 25 °C computed using the MP2/6-311+G** activation free energies given in Table 3 and transition state theory.

Reaction mechanism[a]	ΔG [kcal mol^{-1}]	k [s^{-1}]	n	$k'=k(H_2O)^n$ [s^{-1}]
concerted, non-assisted				
k_3	47.6	5.7×10^{-23}	1	3.2×10^{-21}
concerted, assisted				
k_3	42.4	3.8×10^{-19}	2	1.2×10^{-15}
stepwise, non-assisted				
k_1	42.8	1.9×10^{-19}	1	1.1×10^{-17}
k_{-1}	19.6	2.3×10^{-2}	0	2.3×10^{-2}
k_2	25.1	2.1×10^{-6}	1	1.1×10^{-4}
stepwise, assisted				
k_1	34.9	1.3×10^{-13}	2	3.9×10^{-10}
k_{-1}	11.7	1.5×10^{4}	1	8.4×10^{5}
k_2	11.2	3.5×10^{4}	1	2.0×10^{6}

[a] k_i and k_{-i} stand respectively for forward and backward processes going through TS*i*.

Based on the absence of ^{18}O=C exchange accompanying the hydrolysis, Slebocka-Tilk et al.[10] have suggested that if the diol intermediate exists, it should break down to products faster than it reforms to reactants, that is, k_2 should be larger than k_{-1}. The theoretical data in Table 4 confirms this hypothesis although the differences between the kinetic constants are not very large.

Conclusions

There has been a lengthy discussion in the literature concerning the uncatalyzed hydrolysis of formamide in water. Most previous theoretical studies essentially focused on gas-phase processes. Some did consider solvent effects but inadequate models were used or were limited to particular reaction steps such that the results were in strong disagreement with the available experimental data. The calculations presented in this paper strongly support the uncatalyzed, non-concerted mechanism for the hydrolysis of formamide in water in which the first step is the rate-limiting process. Its free energy of activation is 34.9 kcal mol^{-1} (MP2/6-311+G**//B3LYP/6-31G*) and has a pseudo-first-order kinetic constant (3.9×10^{-10} s^{-1}) that is in excellent agreement with very recent experimental data (1.1×10^{-10} s^{-1}). Clearly, our calculations show that 1) the hydrolysis reaction is water-assisted and 2) concerted hydrolysis is unlikely to occur. This work also provides information on the water-assisted reaction coordinates which can be used to obtain more accurate free-energy profiles by computing the potential of the mean force through Car–Parrinello or combined QM/MM molecular dynamics simulations. Though such a study was beyond the scope of this project it is envisaged that it will be a focus of forthcoming investigations.

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