On the Role of Water in Amide Hydrolysis

Dirk Zahn[a]

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The mechanism of the water-assisted amide hydrolysis of *N*-methylacetamide has been elucidated by Car-Parrinello simulation and related to prior investigations of the acid- and the base-catalyzed pathways. Each process was investigated in aqueous solution, and the water molecules were found to play a crucial role in the amide hydrolysis, with several reaction steps involving proton-transfer events in which the water molecules serve as donors or acceptors. This calls for a quantum treatment of all electronic degrees of freedom, including those of the solvent molecules. A detailed analysis of the mechanisms of the amide hydrolysis pathways obtained from our simulations is given.

Introduction

The hydrolysis of amides is of fundamental importance to biochemistry and has been intensively studied both experimentally[1−4] and in theory.[5−11] Depending on the pH, the reaction in aqueous solution may follow several pathways: at high pH the system follows the so-called "base-catalyzed" route, in which a hydroxide ion performs a nucleophilic attack on the carbonyl carbon atom next to an amide bond, while at low pH protonation of the carbonyl oxygen atom followed by nucleophilic attack carried out by a water molecule is known as the "acid-catalyzed" pathway. In neutral water, both H^+ and OH^- concentrations are low and the hydrolysis preferentially follows a third route.[12] In this case, the nucleophilic attack is also carried out by a water molecule, but it is not facilitated by protonation of the amide carbonyl group.

The hydrolysis of small amides had been the subject of theoretical studies prior to the simulations presented in this work.[5−8] There the mechanistic analysis was biased by the limitation of considering no, or at most one, solvent water molecule as a potential reactant. Recently, amide hydrolysis was also investigated in Car-Parinello simulation studies,[9−13] in which the water molecules are regarded as potential reactants and so are described in quantum mechanical fashion. Accordingly, they are allowed to act as proton donors or acceptors, instead of simply being passive solvent molecules.

In this work, amide hydrolysis at pH = 7 is investigated by Car-Parrinello molecular dynamics (CPMD)[14] simulation, *N*-methylacetamide having been taken as a model to describe the amide group of a peptide. The simulation system has been chosen in analogy to our previous studies,[9−11] and provides a comprehensive picture of the H^+-, OH^--, and water-assisted pathways.

[a] Max-Planck-Institut für Chemische Physik fester Stoffe, Nöthnitzerstr. 40, 01087 Dresden, Germany
Fax: (internat) +49-(0)351-46464002
E-mail: zahn@cpfs.mpg.de

Results and Discussion

Each pathway of amide hydrolysis presented here was investigated in constraint CPMD simulations. The process is divided into several sub-steps, each elucidated as a function of a reaction coordinate, typically chosen as the distance between the atoms in bonds being formed or dissociated.

The Base-Catalyzed Pathway

Base-catalyzed amide hydrolysis is initiated by nucleophilic attack of a hydroxide ion on the carbon atom of the amide group (B0 → B1 in Scheme 1); this is also the rate-determining step.[11] After the addition of the OH^-, the intermediate B1 is negatively charged and exhibits basic character. It may induce dissociation of a solvent water molecule, which was found to occur through protonation of the nitrogen atom, yielding the intermediate B2 and a solvated OH^- ion. In principle, breaking of the C−N bond in B2 is possible, but dissociation through a further sub-step, in which the OH group of B2 is deprotonated by a hydroxide ion, was found to be strongly favored. This reaction yields the intermediate B3, which allows the opening of the C−N bond at much lower energetic costs. The barriers for the cleavage of the C−N bond in B2 and B3 were found to be 72.2 ± 12 kJ/mol and 42.5 ± 12 kJ/mol, respectively.[11] Accordingly, the final step of amide hydrolysis would be expected to be the dissociation of B3 into the amide B4 and the carboxylic acid B5. Carloni and co-workers studied the hydroxy addition step in formamide hydrolysis by a steered CPMD approach,[13] and found the related barrier to be 63 kJ/mol (error margins were not given).

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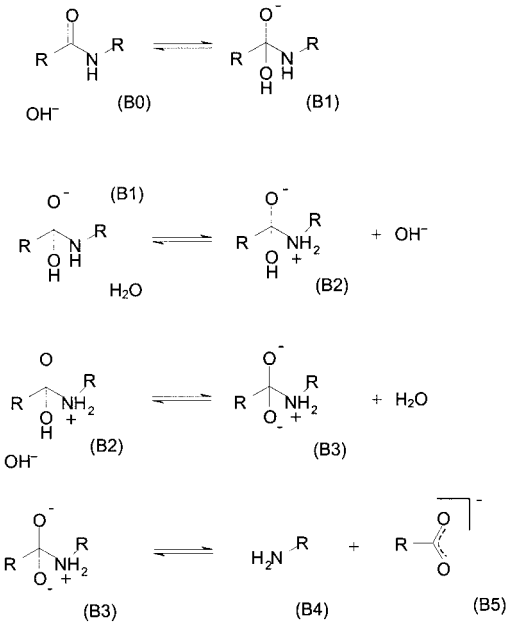
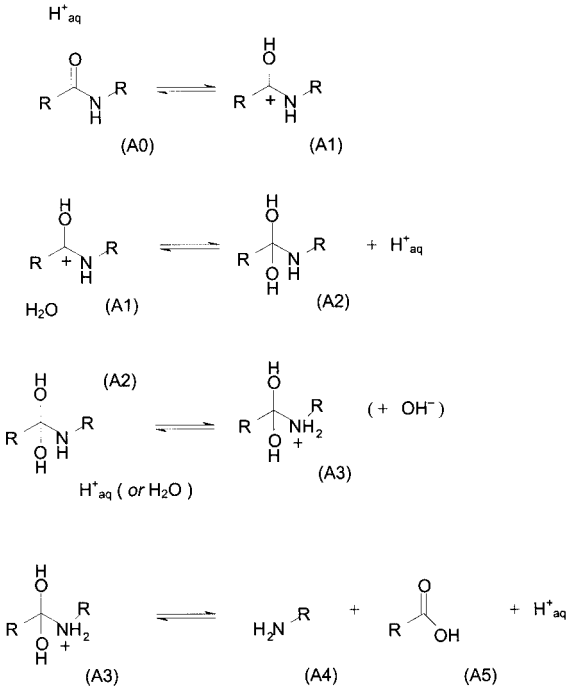


Scheme 1. Base-catalyzed amide hydrolysis



Scheme 2. Acid-catalyzed amide hydrolysis

The Acid-Catalyzed Pathway

Acid-catalyzed amide hydrolysis starts with the addition of a proton to the amide. The protonation is commonly believed to occur at the oxygen atom of the carbonyl group (A0 → A1 in Scheme 2).[4,15−18] In the next step, the protonated amide A1 is the subject of a nucleophilic attack by the oxygen atom of an adjacent water molecule. In the course of the addition of an OH group to A1, this water molecule is dissociated into OH^- and H^+.[9,10] The excess proton migrates to the solvent in a series of proton-transfer reactions, as described by the Grotthus mechanism.[19−23] Explicit quantum treatment of the solvent molecules is crucial for this observation, and so it was elusive in earlier studies that did not involve the solvent in the quantum picture.[5,6] The reaction A1 → A2 is the rate-determining step and has also been intensively studied experimentally.[4] On this basis, the mechanism, as illustrated in Scheme 2, was proposed more than a decade ago. In the following reaction step, the nitrogen atom of the intermediate A2 is protonated. This may be carried out either by excess protons from the water phase or by dissociation of a water molecule. While both processes are possible, the first route is believed to be more likely.[9,10] In the preceding reaction step an excess proton is produced. Though it was always transferred to the solvent, path-sampling molecular dynamics simulations revealed trajectories in which the proton diffused in proximity of A2 and finally protonated the nitrogen atom, yielding the intermediate A3.[10] In the last step, the C−N bond of A3 is broken. This is accompanied by the deprotonation of one of the OH groups, resulting in the carboxylic acid A5 and the amine A4.

The Water-Assisted Pathway

The first step of the water-assisted amide hydrolysis is the addition of an OH group to the amide. As in the acid-catalyzed pathway, this occurs through nucleophilic attack by a neighboring water molecule. One might thus expect a similar reaction mechanism, but this is not the case. In analogy with our previous study of acid-catalyzed hydrolysis,[9] we investigated the nucleophilic attack at neutral pH by use of constraint CPMD simulations in which the distance between the carbonyl carbon atom in A1 and the oxygen atom of an adjacent water molecule is taken as the reaction coordinate. In a series of 5 ps simulation runs, the O−C distance was fixed to r_{O-C} = 3.8, 3.4, 3.0, 2.6, 2.3, 2.0, 1.8, 1.6, 1.5, and 1.4 Å. We calculated the average constraint force for each constraint situation and derived the potential of mean force from integration. From this, the activation energy of the water-assisted hydrolysis of *N*-methylacetamide was found to be 147 ± 12 kJ/mol. The barriers of the H^+- and OH^--assisted hydrolysis obtained by the same computational approach were reported to be 78 ± 12 and 66 ± 12 kJ/mol.[9,11] Hansen and co-workers investigated the rate of a peptide bond hydrolysis as a function of the pH,[12] and in their experiments the water-assisted mechanism was found to be dominant at neutral pH. Accordingly, at pH = 7 the preference of the H^+/OH^--catalyzed pathways over the water-assisted route caused by lower activation energies is overcompensated by the differences in concentration of H^+/OH^- and water.

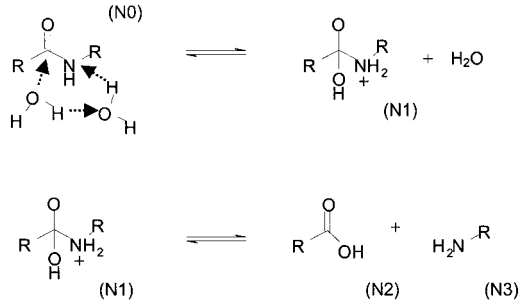
In the acid-catalyzed route, the addition of the OH group to A1 causes the nitrogen atom of the intermediate A2 to

become basic, which produces A3 in a subsequent reaction step.[9,10] In the water-assisted route, the carbonyl oxygen atom of the amide is not protonated prior to the nucleophilic attack (compare A1 in Scheme 2 and N0 in Scheme 3), so the nitrogen atom in the corresponding analogue of A2 should surely be even more susceptible to protonation. Actually, though, no such intermediate could be observed from our simulations. Instead, the addition of the OH group and the protonation of the nitrogen atom of N0 was found to occur as a concerted process (N0 → N1) involving the dissociation of a proton from the water molecule performing the nucleophilic attack. This proton binds to an adjacent water molecule, which − in a Grotthuss type of "proton hopping" mechanism − transfers one of its other protons to the nitrogen atom of N1. In the final step, the C−N bond of the intermediate N1 is dissociated, yielding the amine N2 and the carboxylic acid N3.

Carloni and co-workers investigated the water addition step in formamide hydrolysis.[13] Their study is based on steered CPMD, in which the system was moved along the presumed reaction coordinate (the O−C distance, analogously to this work) at a velocity of 1.1 Å·ps^{-1}. These authors were unable to observe a simultaneous series of proton hopping events (N0 → N1), but instead obtained a $RCOOH_2NHR$ (R = H for formamide) intermediate. This discrepancy could originate from mechanistic differences in formamide and *N*-methylacetamide hydrolysis. The two amides are fairly similar, however, and one should also consider the possibility of methodological problems. In the constrained CPMD simulations described in this work the reaction coordinate is scanned in intervals of 0.1 to 0.4 Å, each of these steps being separated from the preceding one by 5 ps. This corresponds to a maximum "scanning velocity" of 0.08 Å·ps^{-1}, over ten times slower than the velocity used in the steered CPMD by Carloni and co-workers. The faster the system is moved along the reaction coordinate, the higher the risk of skipping configurations to which the system cannot relax in a sufficiently short time. The concerted proton transfer we observed for the acid-catalyzed and the water-assisted hydrolysis of *N*-methylacetamide requires a favorable arrangement of the hydrogen-bonded network, which typically occurs on a picosecond scale. It would be interesting to see if concerted proton transfers could be observed in a steered CPMD simulation using lower scanning velocities. While the barrier observed for hydroxy addition to formamide[13] is somewhat lower than that of *N*-methylacetamide,[11] the barrier for water addition to formamide reported by Carloni and co-workers is about 40 kJ/mol larger than the one we obtained for *N*-methylacetamide from constraint CPMD. This indicates that the non-concerted reaction involves a high-energy intermediate and is disfavored once the system is given enough time to establish a hydrogen-bonded network allowing for concerted proton transfer reaction.

This is also supported by the findings of Antonczak et al. who investigated formamide hydrolysis by ab initio geometry optimizations[6] in which two water molecules were treated explicitly, while the solvent effect on the reaction was modeled by an electric continuum. In the course of the nucleophilic attack, Antonczak et al. observed a concerted proton transfer via the two explicitly considered water molecules. In our CPMD simulations the number of quantum mechanically described water molecules could be increased to 27. While this is much closer to − but still far from − a proper aqueous solution, we nevertheless expect that addition of more water molecules to the simulation model should not favor mechanistic routes in which fewer water molecules are involved.

Our picture of the transition state of the nucleophilic attack is nearly identical to that in the work by Antonczak et al.[6] The O−C distance between the carbonyl carbon atom in A1 and the oxygen atom of the attacking water molecule was found to be 1.674 Å in ref.[6] and 1.65 Å in our CPMD simulations. Two protons are roughly equally shared in their respective hydrogen bonds. One proton of the water molecule performing the nucleophilic attack is donated to an adjacent water molecule, which acts both as an acceptor for the proton released in the course of the O−C bond formation and as a donor for the protonation of the nitrogen atom of A1.



Scheme 3. Water-assisted amide hydrolysis

Summary and Conclusion

We present a theoretical study of amide hydrolysis in aqueous solution. The process was investigated in its H^+-, OH^--, and water-assisted mechanisms through the use of comparable simulation setups.

The rate-determining step common to all the pathways is a nucleophilic attack resulting in the addition of an OH group to the amide. The related barriers were found to be 66 ± 12, 78 ± 12, and 147 ± 12 kJ/mol for the OH^--, H^+-, and the H_2O-assisted hydrolysis, respectively. From experiment, the barriers were observed as 59 kJ/mol[24] and 90 kJ/mol[25,26] for the base- and the acid-catalyzed hydrolysis of *N*-methylacetamide, respectively. No experimentally derived value for the barrier of the water-assisted process could be found in the literature.

Each pathway was explored, including all of its sub-steps. The mechanistic analysis revealed that the inclusion of all (or at least a sufficiently large number) of the water molecules in the explicit quantum calculations is essential.

Theoretical investigations prior to this work considered no or at most one solvent water molecule as potential reactants.[5−8] As a consequence, some of the mechanistic routes described in this work eluded those studies.

Simulation Details

The simulation system consists of one molecule of *N*-methylacetamide (R = CH_3 in Schemes 1−3) and 27 water molecules. An orthorhombic simulation box of dimensions 12.27 Å × 8 Å × 8 Å was used in combination with periodic boundary conditions to mimic an aqueous solution. The temperature was kept constant at 300 K. In order to be able to use a relatively large electronic mass parameter of 700 au, all hydrogen atoms were replaced by deuterium. Since only static properties are investigated, the simulation results are not affected by this numerical trick. The setup of the constraint CPMD simulations and the calculation of the potential of mean force profiles, including their error margins, are described in ref.[9]. The quantum approach is identical to our previous simulations of acid- and the base-catalyzed hydrolysis.[9−11] Through this analogy, effects related to the simulation approach are diminished, providing good comparability of the studies of the different pathways of the hydrolysis of *N*-methylacetamide.

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