

A combined QM/MM study of the nucleophilic addition reaction of methanethiolate and N-methylacetamide

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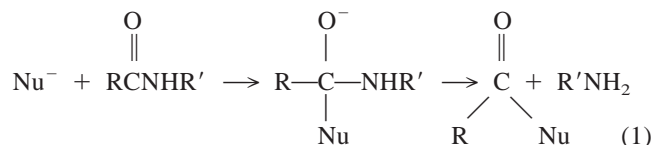
A combined quantum mechanical (QM) and molecular mechanical (MM) method was used to study the nucleophilic addition reaction of methanethiolate to N-methylacetamide (NMA) in the gas phase and aqueous solution. At the B3LYP/aug-cc-pVDZ//HF/6-31+G(d) level, the ion-dipole complex was found to be the global minimum on the potential energy surface in the gas phase with a binding energy of 21.2 kcal/mol. The complex has a C-S distance of 4.33 Å, and no stabilized tetrahedral intermediate was located. The computed potential of mean force in water shows that solvent effects stabilize the reactants over the tetrahedral adduct by 36.5 kcal/mol, and that the tetrahedral intermediate does not exist for the present reaction in water. The present study provides an initial step for modeling the cysteine protease hydrolysis reactions in enzymes. © 2000 by Elsevier Science Inc.

Keywords: nucleophilic addition reaction, solvent effects, combined QM/MM simulations, Monte Carlo simulation, potential of mean force

INTRODUCTION

Nucleophilic addition-elimination reactions at the carbonyl center have been extensively studied in organic chemistry and biochemistry. In general, the reaction mechanism is described in terms of addition-elimination processes through a tetrahedral intermediate by an initial nucleophilic attack of the carbonyl group, followed by collapsing to the final products (Equation

1).¹ Guthrie's analyses of ester and amide hydrolysis reactions revealed that the formation of the tetrahedral intermediate is normally the rate-determining step.^{2,3}



Experimental investigations have been carried in the gas phase by several groups.⁴ It was found that esters generally undergo competitive proton transfer, elimination, and S_N2 reactions in the absence of solvent. Furthermore, Asubiojo and Brauman⁵ concluded, based on studies of halide addition reactions to acyl halides, that the gas-phase mechanism involves a tetrahedral transition state, not a tetrahedral intermediate. Consequently, the reaction in the gas-phase features a double-well potential energy surface with the tetrahedral species as the transition state and the ion-dipole complex as a minimum.⁵

On the computational side, a number of studies of nucleophilic attack involving simple anions to formaldehyde and other carbonyl substrates indicate that there is no barrier to the addition reaction. Yamabe and Minato⁶ and Blake and Jorgensen⁷ found that the nucleophilic addition of halide ions to acyl chlorides can be described by a double-well potential with a tetrahedral transition state at the Hartree-Fock level. Solvent effects on these reactions also were investigated by Weiner et al.⁸ and Madura and Jorgensen.⁹ Employing a combined quantum mechanical and molecular mechanical (QM/MM) approach, Weiner et al.⁸ showed that there is no barrier to the formation of the tetrahedral adduct in the gas phase. Madura and Jorgensen⁹ found that the most stable entity is the tetrahedral adduct in the gas phase for the reaction of hydroxide with formaldehyde, although a small barrier exists between the tetrahedral intermediate and the ion-dipole complex. Monte Carlo simulations by Madura and Jorgensen⁹ using an empir-

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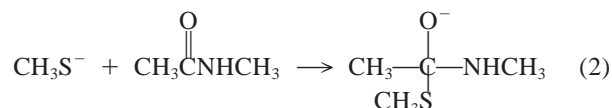
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ical potential function give qualitatively similar results as those obtained from the hydroxide-formamide computation by Weiner et al.⁸ and others.^{9,10}

Howard and Kollman¹¹ further investigated the gas and solution phase reaction of thiolate nucleophilic displacement on formamide. The gas-phase energy surface from that study is unusual in that it is distinguished by the absence of a stable tetrahedral complex,¹¹ in contrast to the hydroxide-formamide reaction.⁸ The geometry for the ion-molecule complex has a sulfur-carbonyl carbon distance of 3.87 Å, which is 23.3 kcal/mol more stable than the separated species.¹¹ Molecular mechanic calculations revealed that the reactant is more stabilized by solvation effects than the tetrahedral adduct in the thiolate-formamide system, but not as effectively as in the hydroxide-formamide reaction. This is because hydrogen bonding interactions and the free energy of hydration are greater for the hydroxide anion than thiolate ion in water. To extend this study, we investigated the nucleophilic addition reaction of methanethiolate (CH₃S[−]) to N-methylacetamide (NMA). In addition to its intrinsic importance for the understanding of addition-elimination reactions in water, this system also serves as a model for the cysteine protease hydrolysis process in papain.^{12,13} The enzyme papain is a target for development of inhibitors and pharmaceutical drugs against tumors, inflammatory diseases, bacterial infections, and pathological processes such as degradation of bone or muscle.^{14,15}

Cysteine proteases have been widely studied and shown to play important roles in degradation of muscular protein^{16–18} and in immunopharmacological response.^{19,20} However, the mechanism for cysteine protease hydrolysis is still poorly understood.^{12,13,21} Computational insight into the reaction mechanism of methanethiolate and NMA will pave out the necessary step toward an understanding of the enzymatic process.



In this article, we present results from a combined QM/MM study of the nucleophilic attack of methanethiolate ion on NMA (Equation 2). Here, we focus on the reaction path and energetics in the gas phase, and solvation effects along this path. Our approach for these computations features *ab initio* quantum mechanical calculations to characterize the gas-phase reaction path, and Monte Carlo statistical mechanical simulations to determine the free energy change for the reaction in water. In the following, computational details are given, followed by results and discussion.

COMPUTATIONAL DETAILS

Ab Initio Calculations of the Gas-Phase Reaction

QM calculations for the gas-phase reaction have been carried out using the Gaussian 92 program.²² The gas-phase reaction profile is determined using *ab initio* Hartree-Fock theory with the 6-31+G(d) basis set.^{23,24} In comparison with results obtained at the HF/6-31G(d) level, it was clearly that diffuse functions are essential for these systems.²⁵ In the geometry optimization, all degrees of freedom are relaxed except the distance between the sulfur atom of methanethiolate and the carbonyl carbon in NMA.

Monte Carlo Simulations

A series of Monte-Carlo simulations using a combined QM/MM AM1/TIP3P potential have been carried out using the free energy perturbation method to obtain the potential of mean force in aqueous solution.^{26–28} A total of 400 water molecules plus the reactant species are included in a rectangular box in the NPT ensemble at 25°C and 1 atm. In these calculations, the reactant is treated quantum mechanically by the semiempirical AM1 model,²⁹ whereas the solvent molecules are modeled by the TIP3P potential for water.³⁰ These calculations for the solution simulations were carried out using the MCQUB program.³¹

The molecular complex was transformed from the reactant region ($R_c = 7.0$ Å) to the final tetrahedral complex ($R_c = 1.8$ Å) using “double-wide” sampling¹⁰ at intervals of ± 0.1 Å for the sulfur-carbon (S-C) distance. Each simulation was performed for a reference state ($RC0 = 0.5$) and two perturbed states ($RC1 = 0.0$, $RC2 = 1.0$), using the ZMATRIX = 2 option in the MCQUB program.³¹ The free energy calculations were performed using Zwanzig’s free energy perturbation method^{32,33}:

$$\Delta G = kT \ln \langle \exp(-\Delta U/kT) \rangle_0 \quad (3)$$

where $\Delta U = U(RC1, \text{ or } RC2) - U(RC0)$, and $U(R_c)$ is the total solute-solvent (QM/MM) interaction energy corresponding to the solute reaction coordinate R_c . Each simulation consists of an equilibration phase of 5×10^5 configurations, and an additional 1.5×10^6 configurations for averaging. Standard Metropolis sampling is used along with preferential sampling, in which the probability of attempted is made proportional to $1/(r^2 + c)$, where c is fixed at 150 Å^2 . This allows solvent molecules in close proximity of the QM region to be selected more frequently than distant water molecules by approximately a factor of 3. A change in volume is tried on every 500 attempted moves.

Solute-solvent interactions are determined by a combined AM1/TIP3P potential,^{34–39} where the necessary Lennard-Jones parameters for C, O, and H are taken from previous publications.³⁴ The OPLS (optimized potential for liquid simulations) parameters are used for sulfur without further modification. One complication exists in the present study is that, accompanying the chemical reaction, the Lennard-Jones parameters for oxygen must be gradually changed from the corresponding sp^2 value for the carbonyl group to the sp^3 value for the tetrahedral anion. For simplicity, these parameters for oxygen are linearly scaled to its corresponding values from 1.8 Å (sp^3) to 7 Å (sp^2). In all calculations, a spherical cutoff switching scheme between 9.0 and 9.5 Å was used to evaluate all intermolecular interactions. Combined QM/MM simulations were performed using fixed solute geometries obtained at the HF/6-31+G(d) level and water at the experimental values.

RESULTS AND DISCUSSION

At the HF/6-31+G(d), the most stable structure corresponds to the ion-dipole complex with an S-C distance of 4.33 Å (Figure 1). The ion-dipole complex was predicted to be -18.0 kcal/mol lower in energy than the separated methanethiolate and NMA species. When the HF/3-21G method is used, the global minimum is located at an S-C distance of 4.20 Å and the complexion energy is -21.8 kcal/mol. Switching to a larger basis

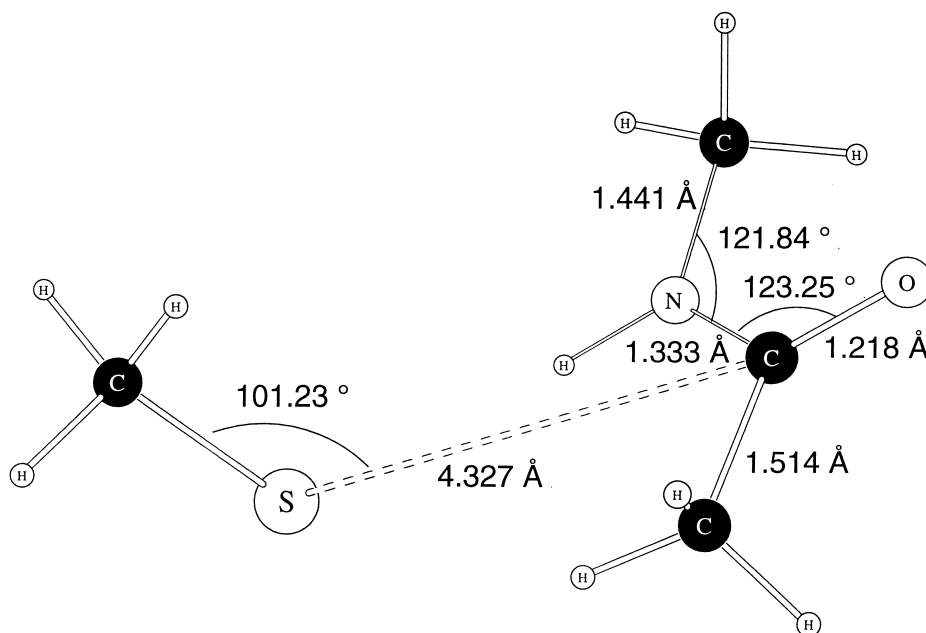


Figure 1. Optimized geometry of the ion-dipole complex of methanethiolate and NMA at the HF/6-31+(d) level. The S-C distance is 4.33 Å and the relative energy to separated reactants is -18.0 kcal/mol.

function plus electron correlation contributions at the density functional level, B3LYP/aug-cc-pVDZ, the binding energy for the ion-dipole complex is lowered to -21.2 kcal/mol. Comparison between HF/6-31+G(d) and HF/3-21G results indicates that diffuse functions provide more stabilization to the separated species than the ion-dipole complex, making this complex 3.8 kcal/mol less attractive than that predicted at the

HF/3-21G level. On the other hand, inclusion of electron correlation effects favors the ion-dipole complex. Thus, the results at the B3LYP/aug-cc-pVDZ level turn out to be fortuitously similar to the HF/3-21G values.

The free energy reaction profile for the nucleophilic addition reaction, computed at the B3LYP/aug-cc-pVDZ//HF/6-31+G(d) level, is depicted in Figure 2. In the region between

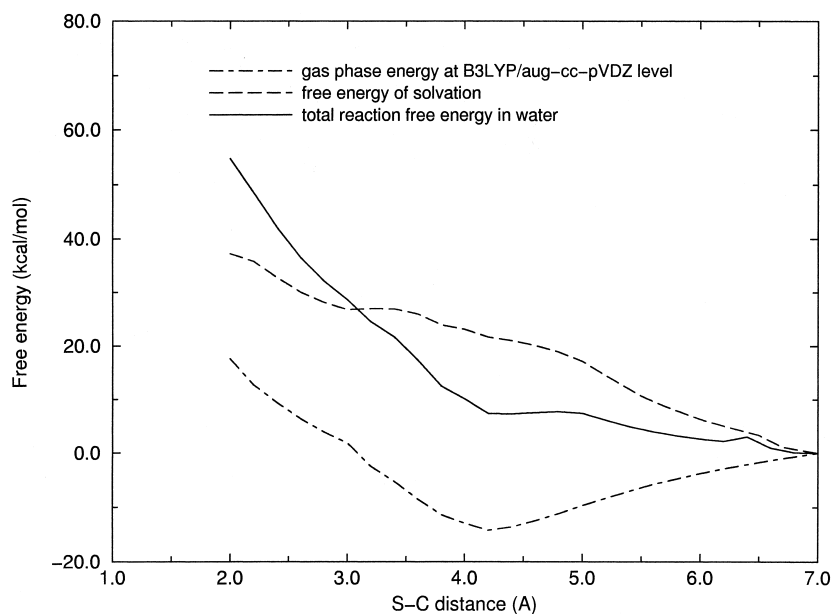


Figure 2. Computed potential of mean force for the reaction of CH_3S^- and NMA in the gas phase using the B3LYP/aug-cc-pVDZ//HF/6-31+G(d) method (dot-dash curve), and in water from combined QM-AM1/MM simulations (solid curve). The solvation free energy profile along the reaction path, which is defined as the distance between the sulfur and carbonyl carbon atom, is shown in the long-dash curve.

1.8 and 2.0 Å, which corresponds to a distance for a typical S-C bond, we did not locate an energy minimum for the tetrahedral intermediate. At 2.0 Å, the "tetrahedral adduct" is estimated to be 17.6 kcal/mol higher in energy than the species with an S-C distance of 7.0 Å. The energy profile shows a gradual decrease in free energy as the sulfur-carbon distance shortens to 4.33 Å at which the binding energy is -21.2 kcal/mol. Then, the energy rapidly increases as the sulfur-carbon distance is further reduced. Examination of the geometrical trend in Table 1 reveals the change in hybridization of the amide group, which changes from the planar sp^2 to the tetrahedral sp^3 hybridization about the nitrogen atom. The carbon-nitrogen interatomic distance of the amide group concomitantly increases as a result of losing the electronic resonance effect in the amide bond. For comparison, Howard and Kollman¹¹ found that the S-C bond length for the reaction of the O-protonated formamide with HS^- is 1.96 Å. In this case, the resulting neutral adduct is stabilized by charge annihilation, giving rise to a stable tetrahedral intermediate. Howard and Kollman¹¹ also studied the reaction of HS^- with formamide ($HS^- + HCONH_2$). It was found that the most stable species is the ion-dipole complex with a S-C distance of 3.87 Å and a binding energy of -28.9 kcal/mol relative to the initial reactants at RHF/4-31G//RHF/4-31G level.¹¹

The potential of mean force in aqueous solution is also shown in Figure 2, along with the curve illustrating the net solvation effect from combined QM/MM simulations. As the molecular complex approaches the tetrahedral intermediate (~1.9 to 2.0 Å), the free energy continuously increases. Overall, solvation effects destabilize the tetrahedral adduct by about 36.5 kcal/mol relative to the species at an S-C distance of 7.0 Å. Evidently, the solvation effect, which is destabilizing along the entire reaction path, is more than enough to offset the binding energy of the ion-dipole complex. Although the free energy profile may be further reduced by 5 to 10 kcal/mol if the simulation was extended beyond the present 7 Å range, the overall energetic trend for the reaction is clear. There is no stable tetrahedral intermediate both in the gas phase and in aqueous solution for the nucleophilic addition step of the reaction between methanethiolate and NMA. Solvent effects strongly stabilize the separated species in water.

It often has been postulated that a tetrahedral intermediate in nucleophilic addition reaction usually is produced before elimination takes place, which can be studied by spectroscopic

methods in solution.^{40,41} The presence of the tetrahedral intermediate in the gas phase is more equivocal. Madura and Jorgensen,⁹ who studied the addition reaction of hydroxide and formaldehyde, found that there are two minima along the reaction path, located at O-C distances of 2.74 Å and at 1.47 Å. The first energy minimum is the ion-dipole complex, whereas the second is the tetrahedral intermediate. At $R(O-C) = 2.39$ Å, there is a negligibly small barrier from the ion-dipole complex to the tetrahedral adduct. In contrast, in aqueous solution, the reaction is dictated by a solvation-induced barrier of 28 kcal/mol and a tetrahedral intermediate that is 14 kcal/mol above the separated species.⁹ Howard and Kollman¹¹ studied the reaction of hydrogenthiolate and formamide, a system similar to the present reaction. A single energy minimum was located, corresponding to the ion-dipole complex at a S-C separation of 3.87 Å, similar to the reaction profile shown in Figure 2. In aqueous solution, the solvation energy difference between the tetrahedral intermediate and the separated reactants was 19.0 kcal/mol in favor of the reactants, compared to the present result of 36.5 kcal/mol for $CH_3S^- + NMA$.

In another related study involving the hydroxide anion-formamide ($HO^- + HCONH_2$) reaction in water,⁸ solvent effects bring about 42 kcal/mol destabilization of the tetrahedral adduct, compared to 19 kcal/mol for the HS^- counterpart.¹¹ The difference between the sulfur and oxygen nucleophiles may be rationalized by the difference in solvation free energy between the two nucleophiles. Hydroxide ion has a much greater solvation free energy (~106 kcal/mol) than that of a thiolate anion (76 kcal/mol) in water. Consequently, there is a greater penalty to remove the solvent molecules from the first solution shell of OH^- than from SH^- in order for the reactions to occur.

Our present results are similar to the findings by Howard and Kollman,¹¹ especially for the gas-phase reaction. In aqueous solution, the reaction of $CH_3S^- + NMA$ does not form a stable intermediate under uncatalyzed conditions in going from the reactants to the tetrahedral adduct (~2.0 Å). The present results are qualitatively in accord with findings by Howard and Kollman¹¹ in that the tetrahedral species was not found to be a viable reaction intermediate in water. This is in contrast to the hydroxide-formaldehyde reaction in water investigated by Madura and Jorgensen,⁹ which exhibits a minimum for the tetrahedral intermediate.

The computational uncertainties in the present study include

Table 1. Key geometrical variables for the reaction of $CH_3S^- + NMA$ along the reaction path defined by the distance between sulfur and the carbonyl carbon atom

R(S-C)	R(C-N)	R(C-O)	A(CCO)	A(CSC)	A(SCO)
8.0	1.345	1.208	121.13	115.72	149.35
6.0	1.341	1.211	120.83	107.59	144.91
5.0	1.338	1.214	120.91	105.62	154.80
4.4	1.334	1.217	120.92	101.46	163.08
4.0	1.330	1.221	120.28	88.79	167.38
3.6	1.346	1.216	120.40	71.80	141.17
3.0	1.377	1.206	121.21	97.10	108.40
2.6	1.395	1.212	120.84	98.25	104.52
2.0	1.469	1.272	115.71	101.38	110.72

Bond distances are given angstroms and bond angles in degrees.

the use a semiempirical method (AM1) for the treatment of solute-solvent interactions in hybrid QM/MM simulations, and fixed reactant geometries obtained from gas-phase reaction path calculations. However, because the nucleophilic attack of a methanethiolate of the amide carbonyl group is strongly endothermic in the gas phase, which is further exacerbated by solvation effect, these computational uncertainties are unlikely to alter the qualitative picture of the reaction. The present study suggests that it is of interest to examine other alternative pathways in aqueous solution, including a generalized acid catalysis mechanism for the amide bond cleavage.^{11,42} In a recent QM/MM study of the amide bond hydrolysis by the enzyme papain, Harrison et al.²¹ found that the reaction of Cys-thiolate anion and NMA in the enzyme active site proceeds via a transition state without the intervention of a tetrahedral intermediate. In the enzymatic reaction, His159 donates a proton to the amino group, leading to a concerted C-N bond cleavage and the formation of the acyl enzyme complex. The computed activation energy decreases from 39.2 kcal/mol in the gas phase to 20.1 kcal/mol in the enzyme.²¹

CONCLUSIONS

The nucleophilic addition reaction of methanethiolate with NMA has been investigated in the gas phase and aqueous solution using *ab initio* molecular orbital and combined QM/MM simulation methods. In the gas phase, the ion-dipole complex (4.328 Å between sulfur and carbon) was found to be the global energy minimum, which is 18.0 kcal/mol more stable than that of the separated reactants at the HF/6-31+G(d) level. As the S-C distance is reduced to form the tetrahedral intermediate, there is a continuous increase in energy in the gas phase. In aqueous solution, the ion-dipole minimum disappears, and the free energy increase is further escalated by solvent effects. The results demonstrate that the tetrahedral species represents a transition structure for the reaction of CH₃S⁻ and NMA in water. Alternatively, a likely mechanism in aqueous hydrolysis is through general acid catalysis. In the region between S-C separations of 4.0 to 7.0 Å, the change in the free energy profile is minimal as a result of compensating effects between desolvation and ion-dipole attraction. Then, there is a rapid rise in free energy. Overall, solvation effects stabilize the reactants over the tetrahedral adduct by 36.5 kcal/mol.

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