

Just three water molecules can trigger the undesired nonenzymatic reactions of aspartic acid residues: new insight from a quantum-chemical study

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Abstract. Aspartic acid (Asp) residues in peptides and proteins (L-Asp) can undergo spontaneous, nonenzymatic reactions under physiological conditions by which abnormal L- β -Asp, D-Asp, and/or D- β -Asp residues are formed. These altered Asp residues may affect the three-dimensional structures of the peptides and proteins and hence their properties and functions. In fact, the altered Asp residues are relevant to age-related diseases such as cataract and Alzheimer's disease. Most of the above reactions of the L-Asp residue proceed via a cyclic succinimide intermediate. In this paper, I propose a detailed mechanism of cyclization of an Asp residue (forming a precursor of the succinimide) by the B3LYP/6-31+G(d,p) density functional theory calculations carried out for a small Asp-containing model compound complexed with three water molecules which act as general acid–base catalysts in proton transfers. In the proposed mechanism, the amide group on the C-terminal side of the Asp residue is first converted to the tautomeric iminol form. Then, successive reorientation of a water molecule and conformational change occur followed by the nucleophilic attack of the iminol nitrogen atom on the carboxyl carbon atom of the Asp side chain to form a five-membered ring. A satisfactory agreement was obtained between the calculated and experimental energetics.

1. Introduction

The aspartic acid (Asp) residues (L-Asp residues) in peptides and proteins tend to undergo nonenzymatic alteration to β -Asp, D-Asp, and/or D- β -Asp residues [1–3]. These reactions which occur under physiological conditions can affect the three-dimensional structures of the peptides and proteins and hence their properties and functions. In fact, the altered Asp residues are relevant to age-related diseases such as cataract and Alzheimer's disease [3].

Most of the above “undesired” reactions of the L-Asp residue are believed to proceed via a cyclic succinimide intermediate as shown in figure 1 [1–4]. However, there have been no quantum-chemical calculations reported which reasonably explain the experimental findings about the formation of the succinimide intermediate, especially from a viewpoint of energetics. In this paper, I present a plausible computational model of the cyclization of an Asp residue to a *gem*-diol species (a possible precursor of the succinimide). In the proposed model, three water molecules play important roles as general acid–base catalysts in proton transfers. The results strongly suggest that water molecules are crucial for the succinimide formation in peptides and proteins.



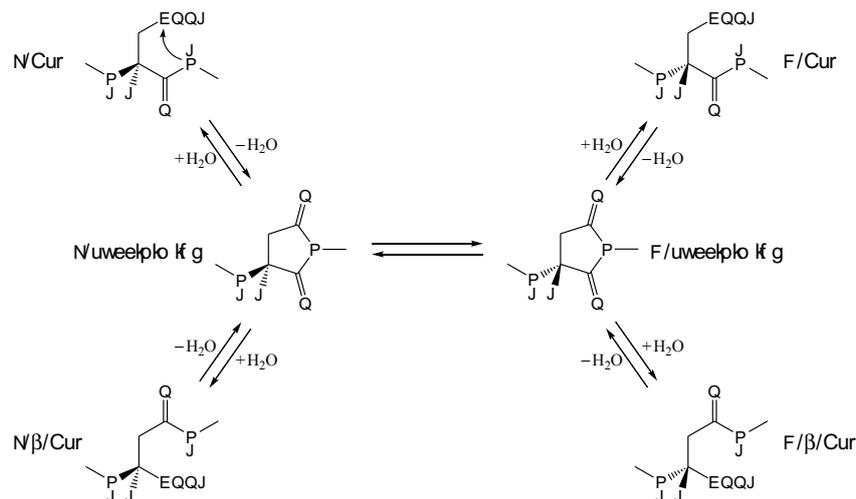


Figure 1. Succinimide-mediated reactions of an Asp residue.

2. Method

All calculations were performed using Spartan '08 (Wavefunction, Inc., Irvine, CA, USA). Figure 2 shows the model compound used in the present study. The side-chain carboxyl group was taken as protonated, since only the protonated form is considered to undergo the nucleophilic attack by the nitrogen atom of the following residue [2,4]. Three water molecules were placed to form the reactant complex so that they make a bridge between the oxygen and NH hydrogen atoms of the amide group on the C-terminal side of the Asp residue.

Energy-minimum and transition state (TS) geometries were located by the density functional theory (DFT) calculation with the B3LYP functional and the 6-31+G(d,p) basis set. The relative energies were corrected for the zero-point energy (ZPE).

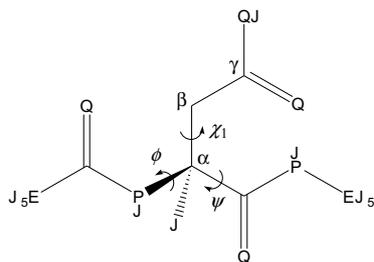


Figure 2. Model compound (Ace-Asp-Nme) used in the present study. Dihedral angles ϕ , ψ , and χ_1 characterize the main-chain and side-chain conformations.

3. Results and discussion

As shown in figure 3, a four-step reaction pathway was found starting from the reactant complex **AM** (amide form). The geometry of **AM** is shown in figure 4.

The first step is iminization (i.e., conversion to the tautomeric iminol form) of the amide group on the C-terminal side to form **IM1**. This step may be schematically designated as in figure 5; namely, the three water molecules enable a quadruple proton transfer by which the conversion of the amide group to the iminol form occurs. By iminization, the nucleophilicity of the nitrogen atom is expected to be greatly reinforced [4]. The TS for the first step is denoted **TS_{AI}**. The corresponding activation barrier was calculated to be 21.2 kcal mol⁻¹, which is possible for a biological reaction. **IM1** is 16.2 kcal mol⁻¹ higher in energy than **AM**. The geometries of **TS_{AI}** and **IM1** are shown in figure 6. The iminol OH group in **IM1** is *cis* with respect to the C=N bond.

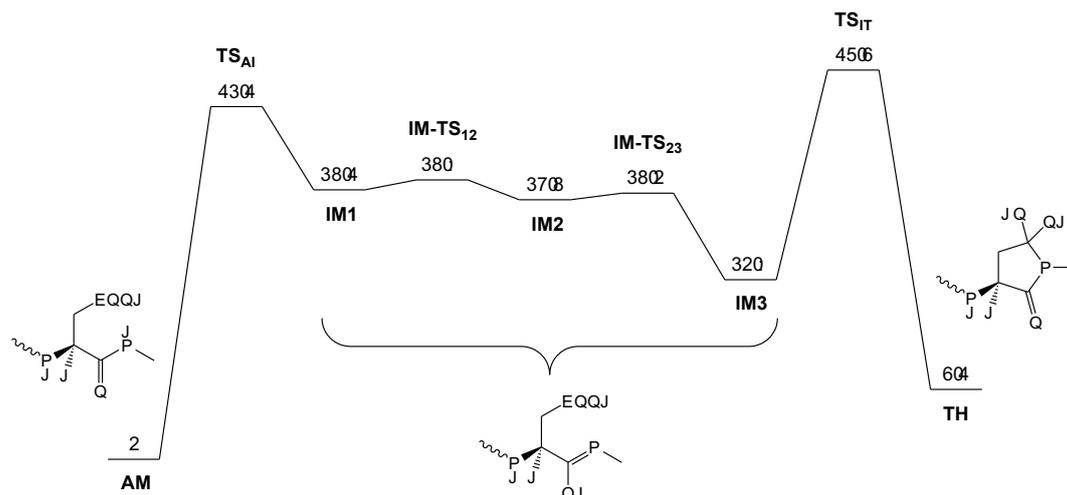


Figure 3. Energy diagram. ZPE-corrected energies relative to the reactant complex **AM** are shown in kcal mol⁻¹.

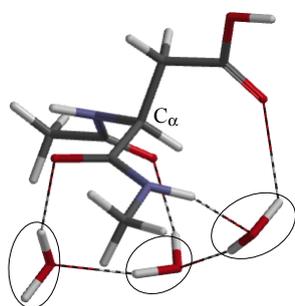


Figure 4. The geometry of **AM** ($\phi = -120^\circ$, $\psi = 158^\circ$, $\chi_1 = -158^\circ$). The three catalytic water molecules are circled.

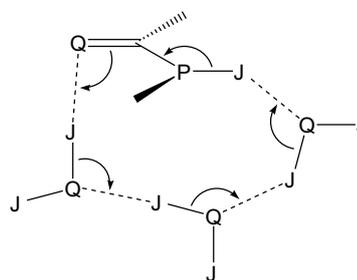


Figure 5. A schematic representation of the three-water-mediated iminization of the amide group.

The second step is reorientation of a water molecule leading to **IM2** (figure 6) The corresponding TS is **IM-TS₁₂** (geometry not shown). More specifically, the hydrogen bond involving the iminol nitrogen in **IM1** is broken and a new hydrogen-bond bridge is formed between the iminol OH group and the side-chain C=O group. This step can occur with an extreme ease, the energy barrier being only 0.6 kcal mol⁻¹. **IM2** is lower in energy than **IM1** by 0.6 kcal mol⁻¹.

The third step is a conformational change to **IM3** (figure 6) through the transition state **IM-TS₂₃** (geometry not shown) by which the iminol OH group becomes *trans*. The barrier for this step is also very small (0.4 kcal mol⁻¹), and the energy of **IM3** is lower than that of **IM2** by 4.8 kcal mol⁻¹.

No direct cyclization pathways from **IM1** and **IM2** leading to a five-membered ring could be found. Instead, a cyclization pathway from **IM3** was successfully found (the fourth step). The *gem*-diol product of this step, denoted **TH**, is an example of the tetrahedral intermediates of the nucleophilic substitution reactions at carbonyl carbon atoms. The energy of **TH** relative to **AM** is 4.2 kcal mol⁻¹. The TS connecting **IM3** and **TH** is denoted **TS_{IT}**. The geometries of **TS_{IT}** and **TH** are also shown in figure 6. It should be noted that the three water molecules again act as catalysts in this step, mediating the proton transfer from the iminol OH group to the side-chain C=O group. The energy of **TS_{IT}** is 23.4 kcal mol⁻¹ with respect to **AM**, and the cyclization step is rate-determining in the overall reaction shown in figure 3. Interestingly, this value is close to the experimental activation energy (21.7 kcal mol⁻¹) estimated for the succinimide formation from the Asp residue in the hexapeptide Val-Tyr-Pro-Asp-Gly-Ala [1]. The succinimide is formed by dehydration of **TH**. This process has not been examined in the present study, because other water molecules (near the *gem*-diol group) should

catalyze it. Nevertheless, the cyclization step may be rate-determining in the succinimide formation from the Asp residue.

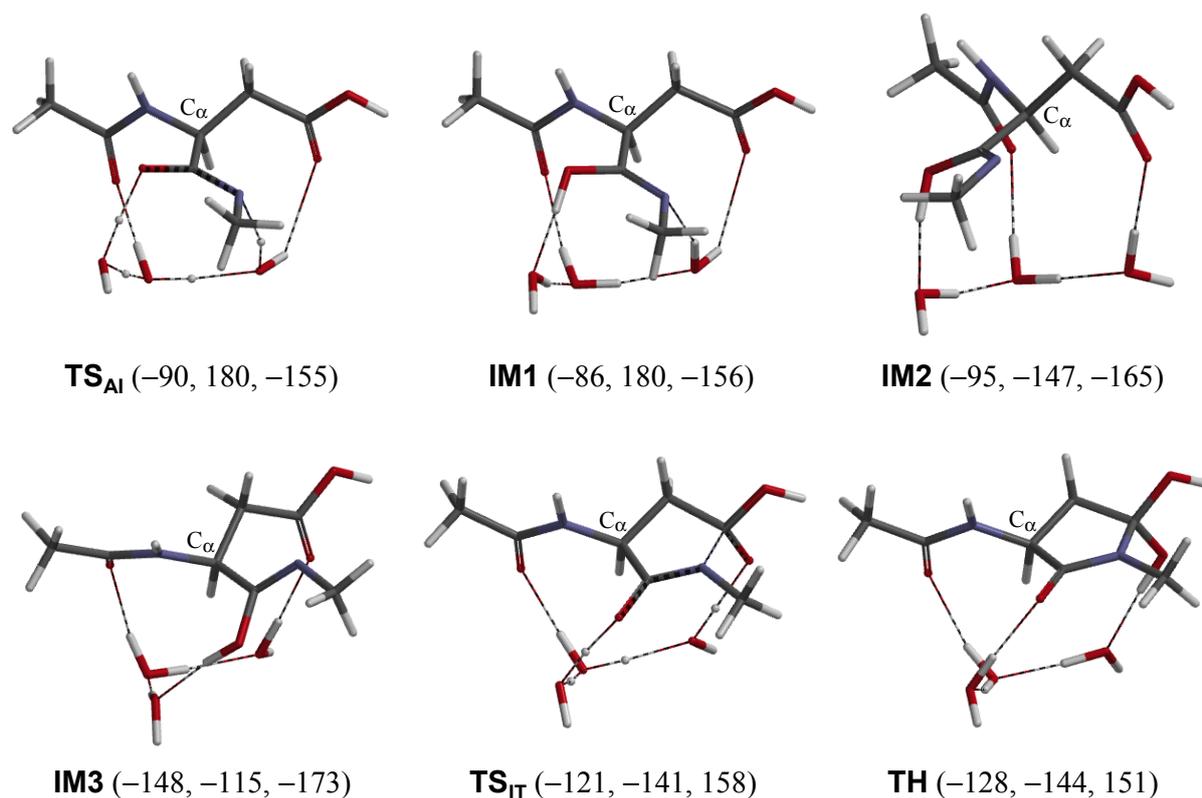


Figure 6. The geometries of **TS_{AI}**, **IM1**, **IM2**, **IM3**, **TS_{IT}**, and **TH**. For each geometry, the ϕ , ψ , and χ_1 values (in degrees) are given in parentheses in this order.

4. Conclusion

A reaction model has been proposed for the cyclization reaction of Asp residues leading to the tetrahedral intermediate in the succinimide formation. Key steps are (1) iminolization of the amide group on the C-terminal side, and (2) cyclization from the iminol intermediate. Both steps are assisted by three water molecules, which act as general acid–base catalysts in proton transfers. The “undesired” reactions of the Asp residues in proteins and peptides may be triggered by just three water molecules.

References

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