Two-water-assisted racemization of the succinimide intermediate formed in proteins. A computational model study

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ABSTRACT

Racemization of aspartic acid (Asp) residues in proteins plays an important role in the molecular biology of aging. In the widely accepted mechanism of the Asp racemization, a succinimide (SI) intermediate is the species which actually undergo the direct racemization. In the present study, a two-water-assisted mechanism of the SI racemization was computationally investigated using a model compound in which an aminosuccinyl (Asu) residue is capped with acetyl and NMe groups on the N- and C-termini, respectively. The two water molecules catalyze the enolization of the $H_\alpha-C_\alpha-C=O$ portion in the Asu residue by mediating proton relay from the α-carbon atom to the carboxyl oxygen atom. After the enolization, migration of the water molecules and conformational change lead to the mirror image of the initially formed enol two-water complex, and the racemization is completed by the following ketonization. The overall activation barrier (28.2 kcal·mol⁻¹) corresponds to the enolization and ketonization steps, and falls within the available experimental activation energies (21.4 - 29.0 kcal·mol⁻¹). Therefore, the two-water-assisted mechanism investigated here is plausible for the *in vivo* and *in vitro* racemization reactions of the SI intermediates formed in peptides and proteins.

Keywords: Aspartic Acid Residue; Racemization; Nonenzymatic Reaction; Succinimide; Water Catalysis; Enolization; Computational Chemistry; Density Functional Theory

1. INTRODUCTION

Aspartic acid (Asp) residues (L-Asp residues) in pep-

tides and proteins are prone to nonenzymatic racemization [1-3]. This reaction of Asp residues in long-lived proteins is a spontaneous, inevitable process during their natural aging and plays an important role in the molecular biology of aging [3-5]. The Asp racemization has also relevance to many age-related diseases such as cataract and Alzheimer's disease [3,4].

It has been widely accepted that a large part of the Asp racemization proceeds via the succinimide (SI) intermediate [1,2,6,7]. As shown in **Figure 1**, the SI intermediate, which is formed by intramolecular cyclization from the Asp residue (with release of a water molecule), is the actual species which undergo the direct racemization. Also, the structural isomerization to the β-form can accompany the racemization, since the SI has two carbonyl groups at which the reaction with water can occur.

At the physiological pH of 7.4, it is plausible that the racemization of the SI intermediate occurs via an enol species as shown in **Figure 1** [7-9]. Note that, in the enol form, the α-carbon atom has a planar configuration. Recently, the mechanism of the SI intermediate racemization via the enol form was computationally studied using a model compound in which an aminosuccinyl (Asu) residue is capped with a formyl (HCO) group and an NMe group (Nme) on the C- and N-termini, respectively, and it was shown that two water molecules effectively catalyze the enolization process by mediating proton relay from the α-carbon atom to the carboxyl oxygen atom [7]. In the present study, similar calculations were performed using a more suitable model compound shown in **Figure 2**. In this compound, the N- and C-termini of an Asu residue are capped with acetyl (Ace) and Nme groups, respectively.

2. METHOD

All calculations were performed using Spartan '08 (Wavefunction, Inc., Irvine, CA, USA). Two water mole-
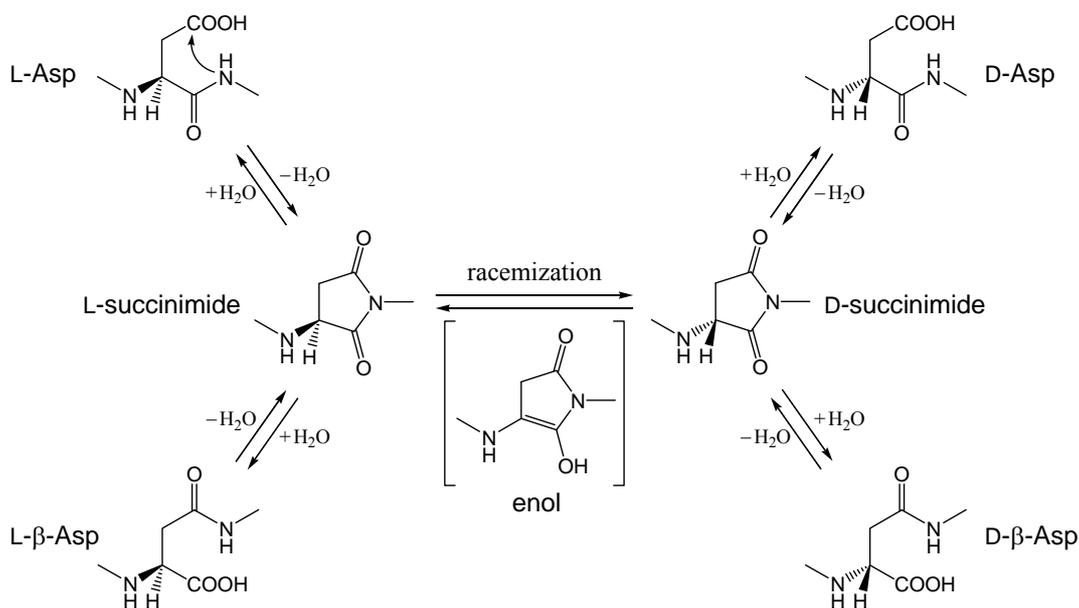
Figure 1. Succinimide-mediated reactions of an L-Asp residue.
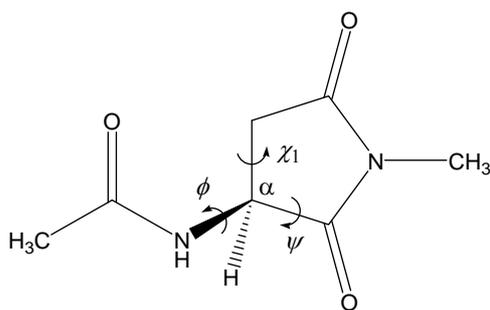
Figure 2. Model compound used in the present study (Ace-Asu-Nme). The dihedral angles ϕ (C-N-C_α-C) and ψ (N-C_α-C-N) characterize the main-chain conformation.
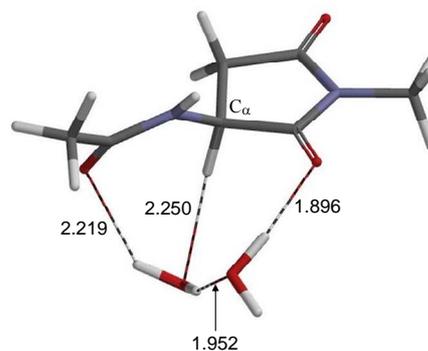
Figure 3. The geometry of the reactant complex L-**SI** ($\phi = -144°$, $\psi = -139°$). Selected interatomic distances are shown in Å.

cules were placed to the model compound to form the reactant complex such that they make a bridge between the C_α-H_α bond and the α-carbonyl group of the Asu residue. Hereafter, the reactant complex is denoted as L-**SI** (and its mirror image as D-**SI**). As in our previous studies [7-10], 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).

3. RESULTS AND DISCUSSION

Overall, the present results are very similar to those obtained previously using a more simple model compound [7].

The geometry of the reactant complex L-**SI** is shown in **Figure 3**. The two water molecules make a bridge between the C_α-H_α bond and the α-carbonyl group of the

Asu residue by a C-H···O interaction (2.250 Å) and hydrogen bonds to form a eight-membered cyclic structure between the model compound and the two water molecules. In addition, there is a weak hydrogen bond (2.219 Å) between the oxygen atom of the Ace group ("neighboring residue") and the water molecule interacting with the C_α-H_α bond.

The entire energy diagram for the conversion of L-**SI** to its mirror image D-**SI** is shown in **Figure 4**.

In the first step, a concerted bond reorganization involving a triple proton transfer occurs in the above-mentioned eight-membered cycle of L-**SI** via the transition state **TS1** (**Figure 5**) to give an enol two-water complex **EN1** (**Figure 6**). The activation barrier for this step was calculated to be 28.2 kcal·mol^{-1}. The hydrogen bond between the oxygen atom of the Ace group and one of the water molecules becomes considerably shorter in **TS1** (1.706 Å), implying that this hydrogen bond has an im-
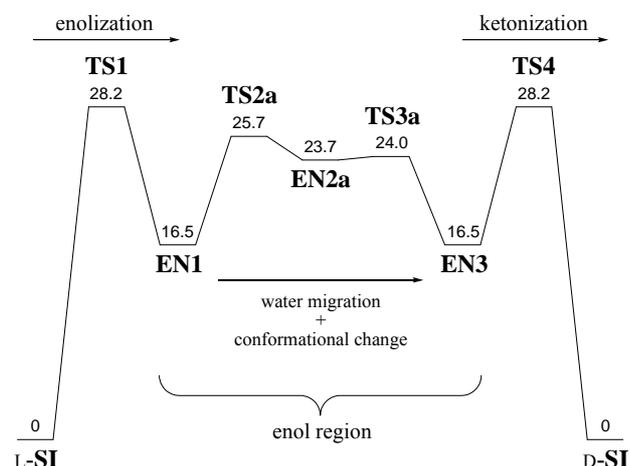
Figure 4. Energy diagram. ZPE-corrected energies relative to L-**SI** are shown in kcal·mol^{-1}.
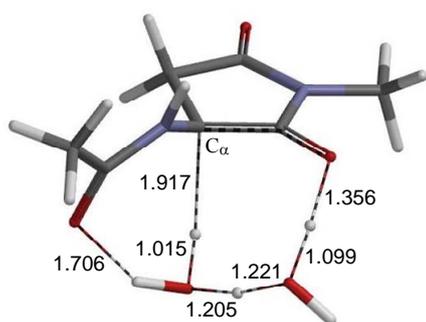
Figure 5. The geometry of **TS1** (ϕ = −135˚, ψ = −150˚). Selected interatomic distances are shown in Å.
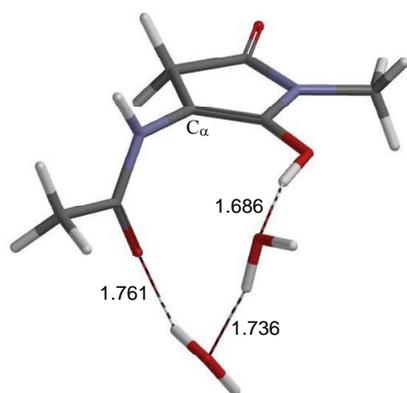
Figure 6. The geometry of **EN1** (ϕ = −76˚, ψ = 179˚). Hydrogen bond distances are shown in Å.

portant role in stabilizing the transition state. **EN1** is higher in energy than L-**SI** by 16.5 kcal·mol^{-1}. The dihedral angle ϕ changes largely from −144˚ to −76˚ by this enolization step.

From **EN1**, there exist two energetically equivalent routes, Route A and Route B, which are related by re-

flection, to reach its mirror image **EN3** (ϕ = 76˚, ψ = −179˚). In Route A (**Figure 4**), migration of the two water molecules and a conformational change from ϕ = −76˚ to ϕ = −150˚ first occurs though the transition state **TS2a** (**Figure 7**) leading to **EN2a** (**Figure 8**), followed by a conformational change to ϕ = 76˚ through the transition state **TS3a** (**Figure 9**) leading to **EN3**. In Route B,
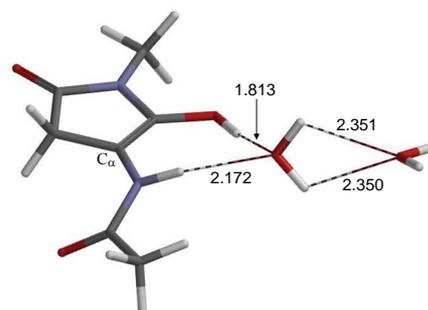
Figure 7. The geometry of **TS2a** (ϕ = −147˚, ψ = −176˚). Selected interatomic distances are shown in Å.
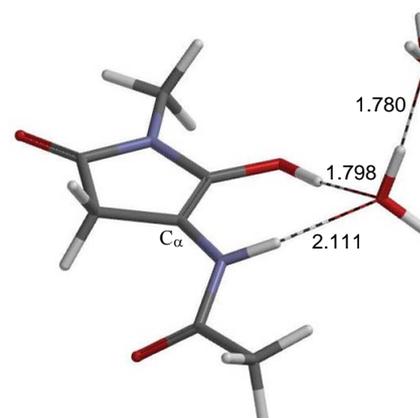
Figure 8. The geometry of **EN2a** (ϕ = −150˚, ψ = −176˚). Hydrogen bond distances are shown in Å.
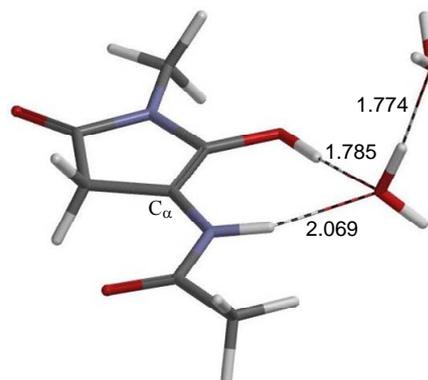
Figure 9. The geometry of **TS3a** (ϕ = −175˚, ψ = −179˚). Hydrogen bond distances are shown in Å.

a conformational change to $\phi = 150°$ occurs first, and the water migration and conformational change leading to **EN3** follow. The stereoinversion of L-**SI** is completed by the ketonization (*i.e.*, the reverse reaction of enolization) of **EN3** catalyzed by the two water molecules to give D-**SI** ($\phi = 144°$, $\psi = 139°$). The transition state of this final step is denoted as **TS4** in **Figure 4**. It should be noted that, in **EN3**, the two water molecules reside on the opposite side of the five-membered ring compared to **EN1**.

The overall activation barrier is 28.2 kcal·mol^{-1}, corresponding to the initial enolization and the final ketonization steps. This value falls within the experimental activation energies (21.4 - 29.0 kcal·mol^{-1}) determined by Arrhenius plots for systhetic peptides corresponding to fragments of αA-crystalline and elastin [11,12]. This implies that the two-water-assisted racemization mechanism investigated here may be one of those which actually occur *in vivo* and *in vitro* for peptides and proteins.

4. CONCLUSION

A two-water-assisted mechanism of the SI racemization in the formation of D-Asp residues was computationally investigated using Ace-Asu-Nme as a model compound. The energetic results were very similar to those of the previous study [7] using a more simple model compound. The calculated activation barrier fairly agreed with the available experimental results. Therefore, it is highly plausible that the two-water-assisted mechanism of the SI racemization actually occurs in peptides and proteins. Water, while indispensable for life, may also catalyze the biological reaction which is related to aging. Mechanisms where more than two water molecules catalyze the SI racemization can also be conceivable. Such mechanisms will be investigated in the near future.

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