Computational Modeling of the Enolization in a Direct Mechanism of Racemization of the Aspartic Acid Residue

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The rapid racemization of aspartic acid (Asp) residues in peptides and proteins is due mainly to the succinimide intermediate. However, there should be another mechanism for Asp racemization without intermediacy of the succinimide. The direct H-atom abstraction from the C(α)-atom that leads to the enol form of the Asp residue is one possibility. In another study, we have computationally predicted that the corresponding enolization in the succinimide intermediate occurs by assistance of two H2O molecules. In the present study, we, therefore, investigated the similar two-H2O-assisted enolization for an Asp-containing model compound by the same computational method as before (B3LYP/6-31 + G**). Rather surprisingly, the activation barrier for the two-H2O-assisted enolization of the Asp residue (protonated form) was calculated to be almost equal to that for the corresponding succinimide. Therefore, an Asp residue is expected to be prone to enolization to almost the same degree as the corresponding succinimide form, and the ‘direct’ (i.e., non-succinimide-mediated) mechanism of Asp racemization may compete with the succinimide-mediated mechanism.

Introduction. – Aspartic acid (Asp) residues are among the most racemization-prone amino acid residues in peptides and proteins. Recently, the racemization of Asp residues has been attracting considerable attention, due to its relevance to aging and age-related diseases [1]. The rapid racemization of Asp residues is attributed to the succinimide intermediate formed by intramolecular cyclization [2–4]. In this mechanism, the species that actually undergoes racemization is the succinimide intermediate. The intermediacy of the succinimide also explains the simultaneously occurring isomerization to β-Asp. The contribution by Geiger and Clarke [2a] is most often cited for the succinimide-mediated mechanism. It should be noted here that the succinimide-mediated mechanism does not account for 100% of the total racemization of Asp residues. The work of Geiger and Clarke shows that ‘greater than two-thirds of the total racemization can be attributed to the succinimide’, and another mechanism has to be invoked for more than 20% of the observed racemization.

In the succinimide-mediated mechanism, the enolization involving the H(α)-atom and the CO(α) group occurs in the succinimide intermediate [4]. We have computationally shown that this enolization most probably proceeds by a two-H2O-assisted mechanism, as shown in Fig. 1 [4]. This mechanism, however, could be possible also for the Asp residue itself. Indeed, Geiger and Clarke [2a] mentioned the possibility of the direct H-atom abstraction from the C(α)-atom of the Asp residue as a racemization mechanism.
In the present study, we investigated the two-\(H_2O\)-assisted enolization in the Asp residue itself. In the study of the succinimide enolization \[4\], we used 1 as a model compound. Therefore, compound 2 was used in the present study as an Asp-containing model compound (compound 3 is its enol form). The side-chain COOH group was taken as protonated, since the deprotonated form (i.e., the anion) would disfavor the enolization, in the course of which negative charge accumulates on the C(\(\alpha\))-atom; indeed, attempts to locate a transition state for the anionic form were not successful.

As in the previous study of the succinimide 1 \[4\], we used the density-functional theory (DFT) method with the B3LYP functional and the 6-31+G** basis set (B3LYP/6-31+G**) to optimize the geometries of energy minima and the transition state. All geometry optimizations were followed by vibrational-frequency calculations to confirm the nature of the stationary points. All calculations were performed using Spartan’04 \[5\].

Results and Discussion. – Fig. 2 shows the calculated energy profile for the two-\(H_2O\)-assisted enolization of 2. The optimized geometries of the reactant complex (RC, 2·2\(H_2O\)), the transition state (TS), and the product complex (PC, 3·2\(H_2O\)) are shown in Fig. 3. The RC and PC are dihydrates of 2 and its enol form 3, respectively. The energy of the RC is lower by 16.9 kcal mol\(^{-1}\) than that of the separated reactants (SR), i.e., the sum of the energies of the three molecules optimized separately (the optimized geometry for the isolated 2 is not shown). The PC is higher in energy than the RC by 16.1 kcal mol\(^{-1}\) and lower than the separated products (SP) by 23.0 kcal mol\(^{-1}\) (the optimized geometry for the isolated 3 is not shown).

The activation barrier calculated as the energy difference between the RC and the TS is 31.1 kcal mol\(^{-1}\). Rather surprisingly, this value is ‘equal’ to the corresponding value for the succinimide analogue 1 \[4\]. When corrected for the zero-point energy, the barrier is lowered to 27.3 kcal mol\(^{-1}\), which is 0.8 kcal mol\(^{-1}\) lower than for 1. Therefore, the enolization of an Asp residue in its protonated form can occur as easily as that of the corresponding succinimide form.
As in the case of 1 [4], a C–H· · ·O interaction (2.197 Å) involving the C–H bond to be cleaved (C1–H1) and one of the H2O molecules is observed in the RC. Moreover, in all of the RC, TS, and PC, there is a H-bond between the CO O-atom of the side chain (O2) and the N–H bond of the neighboring residue on the C terminal (N1–H2).

It is interesting to note that, in the TS (Fig. 3), the side-chain C–C bond (C3–C4) is antiperiplanar to the breaking C–H bond (C1–H1), the H1–C1–C3–C4 dihedral angle being ~175°. This is also the case in the RC, where the H1–C1–C3–C4 dihedral angle is ~180°. Moreover, on going from the RC to the TS, the C1–C3 bond distance becomes shorter (from 1.551 to 1.534 Å) and the C3–C4 distance becomes slightly longer (from 1.510 to 1.511 Å). These results are similar to the case of a serine residue reported in a separated paper of this issue [6] and may indicate that the C3–C4 bond stabilizes the TS by negative hyperconjugation (i.e., the negative charge that accumulates on C1 as the C1–H1 bond is cleaved delocalizes to the antibonding σ* orbital of the C3–C4 bond). A more detailed analysis of such hyperconjugation involving a COO C-atom is now in progress.
In conclusion, our results show that the Asp residue in its protonated form is prone to enolization to almost the same degree as the corresponding succinimide form. Therefore, the ‘direct’ mechanism of Asp racemization may compete with the succinimide-mediated mechanism, in agreement with the experiment on a synthetic peptide [2a].

REFERENCES