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Staggered side-chain conformers of aspartyl residues prerequisite to transformation from L-α- to D-β-aspartate 58 in human-lens αA-crystallin fragment

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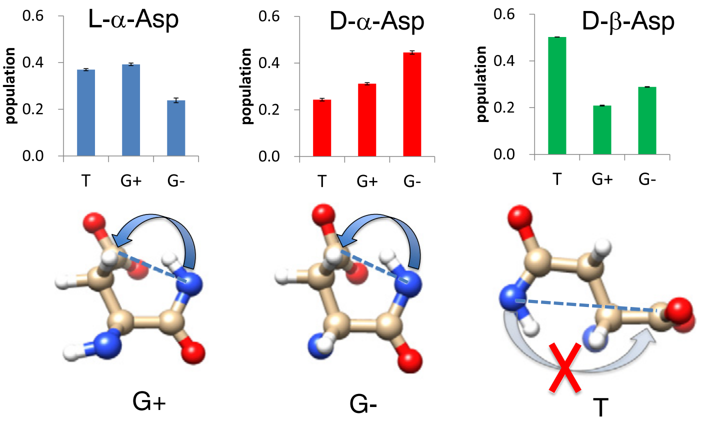
HIGHLIGHTS

- Staggered side-chain conformers of Lα-, Dα-, and Dβ-Asp58 isomers in αA-crystallin
- $^1H-^1H$ coupling constants of Asp isomers quantified by high-resolution NMR
- Gauche conformers in Lα- and Dα-Asp promote the transformation to uncommon Dβ-Asp.
- Uncommon Dβ-Asp is stabilized by the side-chain trans conformer in aged proteins.
- Change from gauche to trans conformer is enthalpy-driven and favorable in D-β-Asp.

GRAPHICAL ABSTRACT



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ABSTRACT

D-β-aspartyl (Asp) residues are found in aged human-lens αA-crystallin. To explore why the uncommon D-β-Asp is accumulated, the stability of L-α-, D-α-, and D-β-Asp residues is compared in view of the staggered side-chain conformers. By using αA-crystallin fragment, $T^{55}VLD^{58}SGISEVR^{65}$, composed of Asp58 isomers, the vicinal spin–spin coupling constants of Asp58 H_{α}-$H_{\beta1}$ and H_{α}-$H_{\beta2}$ are quantified by high-resolution solution NMR. The trans conformer is most preferred in the D-β-Asp side-chain, whereas gauche+ and gauche− are abundant in L-α- and D-α-Asp. The close distance between Asp58 carboxylate carbon ($\mathbf{C}_{COO-}$) and Ser59 nitrogen (**N**) in gauche+ and gauche− is advantageous to the intramolecular cyclization to form succinimide intermediate, followed by the transformation from α- to β-Asp. The cyclization is not allowed in the trans conformer because of the long distance between $\mathbf{C}_{COO-}$ and **N**, to keep D-β-Asp stable. The change from gauche to trans conformer in D-β-Asp is exothermic and enthalpy-driven.

1. Introduction

It has been thought that D-amino acid does not exist in living tissues because proteins in natural tissues consist of L-amino acid. However, D-aspartyl (Asp) residues have been found in aged lens [1–4], tooth [5], aorta [6], brain [7,8], bone [9,10], and ligament [11]. D-Asp is produced

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by the racemization of natural L-Asp residues in the protein. The racemization is accompanied by the isomerization from the natural α-Asp to the uncommon β-Asp form via a succinimide intermediate [12]. L (or D)-succinimide is formed by the intramolecular cyclization, converted to D (or L)-succinimide, and finally, the D- and L-succinimides are hydrolyzed at either side of their two carbonyl groups, yielding both α- and β-Asp residues, respectively [13]. Therefore, 4 isomers, L-α-Asp, L-β-Asp, D-α-Asp and D-β-Asp, are formed in the protein. These isomers are responsible for the structural change of proteins or peptides, because D-isomers with different side-chain orientation and β-isomers which prolong main peptide bond can induce uncommon main chain structures, to trigger the abnormal unfolding or aggregation leading to a disease. For the racemization, the formation of succinimide is thought to be the rate-determining step [13,14]. Previous studies have suggested that β-Asp is more stable than α-Asp in human-lens αA-crystallin peptides [14]. It remains unsolved, however, why the β-Asp is stable as compared to α-Asp in such peptides.

In this study, the stability between α- and β-Asp forms in the peptide is discussed in relation to the population of staggered side-chain conformers (trans (T), gauche+ (G^+) and gauche− (G^-)) of Asp isomers. By using human-lens αA-crystallin fragment, T6 ($T^{55}VLD^{58}SGISEVR^{65}$) composed of L-$\alpha$-, D-$\alpha$-, and D-$\beta$-Asp 58 residues, the vicinal spin–spin coupling constants (J) of Asp 58 H_α-$H_{\beta 1}$ ($J_{\alpha\beta 1}$) and Asp 58 H_α-$H_{\beta 2}$ ($J_{\alpha\beta 2}$) are quantified and compared by high-resolution solution NMR at 10–60 °C. We adopt T6 sequence because Asp 58 and Asp 151 in αA-crystallin are highly converted from L- to D-isomer in human lens [2]. From the respective coupling constants, the population of staggered side-chain conformers of L-α-, D-α-, and D-β-Asp 58 are determined independently. The staggered side-chain conformers in the Asp 58 isomers are shown in Fig. 1. Here, the difference in the population of side chain conformers between α- and β-Asp is interpreted by how easily the succinimide is formed by the intramolecular cyclization. Moreover, the temperature dependences of the conformer populations are investigated to compare the thermodynamic stability between the respective conformers T, G^+, and G^- in each Asp isomer. Changes in Gibbs free energy (ΔG), the enthalpy (ΔH), and the entropy (ΔS) accompanied by the conformational variations give insight into how the transformation of side chain conformers is induced in relation to the stability of α- and β-Asp isomers in T6.

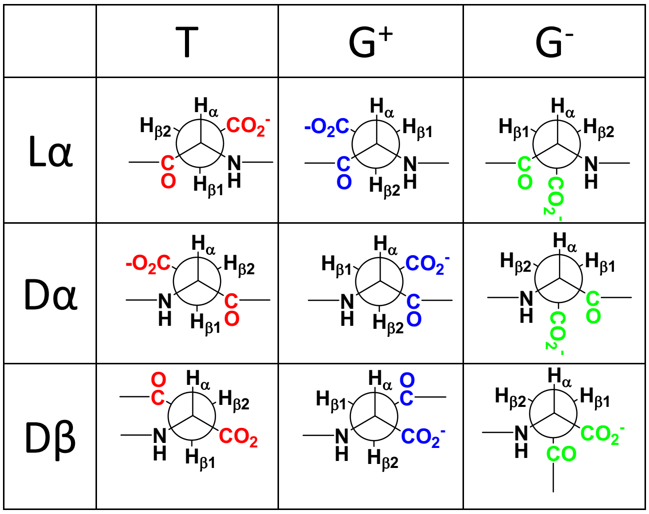

Fig. 1. Newman projections of staggered side-chain conformers of L-α-, D-α-, and D-β-Asp residues in the peptide with respect to the dihedral angles. Here we define T as the conformer where β carboxylate carbon is located in the trans side against the α carbonyl carbon (left column), G^+ as the conformer where $H_{\beta 2}$ is located in the trans side against α proton (center column), and G^- as the conformer where $H_{\beta 1}$ is located in the trans side against the α amide nitrogen (right column).

2. Materials and methods

2.1. Materials

Fmoc (9-fluorenylmethoxycarbonyl) -amino acids were purchased from Watanabe Chemical Industry (Hiroshima, Japan). Solvents used in the peptide synthesis were of special reagent grade. HPLC grade solvent was used to confirm the purity of the peptide. All other reagents were of special reagent grade and used without further purification. Solvent heavy water (D_2O; 99.9% atom D) was from ISOTEC (USA).

2.2. Peptide synthesis

In this study, Leu 57 in T6 is substituted by 4-trifluoromethyl-phenylalanine (CF_3-Phe) to use ring proton NMR signal of Phe as a reference. Here we call the sequence ($T^{55}V$ CF_3-Phe $D^{58}SGISEVR^{65}$) T6f. The isomers of T6f in which L-α-Asp was replaced with D-α- and D-β-Asp at position 58 were synthesized by Fmoc solid-phase chemistry using an automated solid-phase peptide synthesizer (PSSM-8; Shimadzu, Kyoto, Japan). The three isomers are called T6fLα, T6fDα, and T6fDβ, respectively. The purity of each peptide was confirmed to be 95% by reversed phase-high performance liquid chromatography and electrospray ionization-mass spectrometry. For the ^{1}H-NMR signal assignment of the three T6f isomers, the 6 positions of T6fLα amino acid residues were replaced with alanine (Ala) one by one, and obtained by Fmoc solid-phase synthesis (called T6fA). The sequences of the T6fA peptides are listed in Table 1.

2.3. ^{1}H-NMR measurement

Proton-NMR measurements were carried out on 400 MHz spectrometer (JEOL ECA400) equipped with a super conducting magnet of 9.4 T. A high sensitivity probe (JEOL, NM40T10A/AT) for 10 mm o.d. sample tube was used to obtain good S/N ratio. About 2 mg of T6f and T6fA peptides was dissolved in 4 ml PBS/D_2O (pD 7.6) and subject to NMR measurement at 37 °C. Free-induction decays (FIDs) were accumulated 256 times. The digital frequency resolution was set to 0.3 Hz, unless otherwise stated. Although D_2O was selected as a solvent, the DANTE presaturation pulse sequence was applied to avoid the signal overlapping of impurity light water (HDO) with the target ^{1}H NMR peak. Chemical shifts were obtained by referring to the absorption frequency of the solvent deuteron monitored as the lock signal.

To assign each ^{1}H NMR signal of T6f, the two-dimensional (2D) ^{1}H–^{1}H COSY measurement was also performed for T6f isomers and 6 kinds of T6fA peptides at 37 °C. FID signals were accumulated 64 times with the typical 90° width of 31 μs.

To evaluate vicinal ^{1}H–^{1}H coupling constants (J), the high resolution ^{1}H-NMR measurements were carried out at 10–60 °C. The digital resolution was as high as 0.02 Hz to obtain the coupling constants with high accuracy. FID signals were accumulated 2048 times.

Table 1
Amino acid sequences of T6f (top) and 6 kinds of T6fA used in this study. The position shown by the red character is replaced by Ala in each T6fA.

Sequence
Thr-Val-CF_3Phe-Asp-Ser-Gly-Ile-Ser-Glu-Val-Arg
Thr-Ala-CF_3Phe-Asp-Ser-Gly-Ile-Ser-Glu-Val-Arg
Thr-Val-CF_3Phe-Asp-Ala-Gly-Ile-Ser-Glu-Val-Arg
Thr-Val-CF_3Phe-Asp-Ser-Gly-Ala-Ser-Glu-Val-Arg
Thr-Val-CF_3Phe-Asp-Ser-Gly-Ile-Ala-Glu-Val-Arg
Thr-Val-CF_3Phe-Asp-Ser-Gly-Ile-Ser-Ala-Val-Arg
Thr-Val-CF_3Phe-Asp-Ser-Gly-Ile-Ser-Glu-Ala-Arg

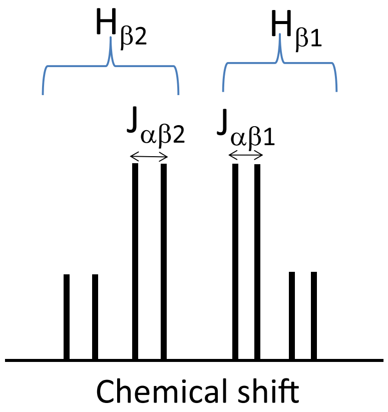

Fig. 2. Schematic view of how $J_{\alpha\beta1}$ and $J_{\alpha\beta2}$ are determined from the ^{1}H NMR spectra (see text).

2.4. Evaluation of ^{1}H–^{1}H coupling constant

The coupling constants of Asp H_α–$H_{\beta1}$ ($J_{\alpha\beta1}$) and H_α–$H_{\beta2}$ ($J_{\alpha\beta2}$) were evaluated by using Asp 58 H_β at 2.5–2.9 ppm. Fig. 2 schematically illustrates how $J_{\alpha\beta1}$ and $J_{\alpha\beta2}$ were determined from the spectra. Vertical bars represent the signals of Asp H_β which has ABX-type spin system. According to the previous report [15], we assigned the Asp H_β in the high field as $H_{\beta1}$, and that in the low field as $H_{\beta2}$, and evaluated $J_{\alpha\beta1}$ and $J_{\alpha\beta2}$ as shown in Fig. 2.

2.5. Population of staggered side-chain conformers

Asp has 3 staggered side-chain conformers (trans (T), gauche+ (G^+) and gauche− (G^-)) which have different dihedral angles. As shown in Fig. 1, we define T as the conformer where β carboxylate carbon is located in the trans side against the α carbonyl carbon (left column), G^+ as the conformers where $H_{\beta2}$ is located in the trans side against α proton (center column), and G^- as the conformers where $H_{\beta1}$ is located in the trans side against the α amide nitrogen (right column). Using $J_{\alpha\beta1}$ and $J_{\alpha\beta2}$ obtained by the high-resolution ^{1}H NMR measurement, the population of staggered side-chain conformers of Asp in T6f isomers is calculated by the following equations [16],

$$\begin{aligned}J_{\alpha\beta1} &= P(T)J_t + P\left(G^+\right)J_g + P(G^-)J_g\\ J_{\alpha\beta2} &= P(T)J_g + P\left(G^+\right)J_t + P(G^-)J_g\\ P(T) &+ P\left(G^+\right) + P(G^-) = 1\end{aligned} \tag{1}$$

Here P(T), P(G^+), and P(G^-) are the probabilities for conformers T, G^+, and G^- defined in Fig. 1, and J_t and J_g are the vicinal spin–spin coupling constants between the α- and β-protons in the trans and the gauche conformers, respectively. In this study, J_t (13.56 Hz) and J_g (2.60 Hz) were referred to Pachler's parameter set [17].

2.6. Thermodynamic parameters

From the population of Asp 58 side-chain conformers, the equilibrium constants ($K_{G+/T}$ and $K_{G-/T}$) between conformers T and G^+, and between T and G^- in T6f were quantified. The equilibrium constants are given by

$$K_{G^+/T} = \frac{P(G^+)}{P(T)} \tag{2}$$

$$K_{G^-/T} = \frac{P(G^-)}{P(T)} \tag{3}$$

By using $K_{G+/T}$ and $K_{G-/T}$, the standard Gibbs energy change ΔG^o were obtained as

$$\Delta G^0\left(T\rightarrow G^+\right) = -RT\ \ln K_{G^+/T} \tag{4}$$

$$\Delta G^0(T\rightarrow G^-) = -RT\ \ln K_{G^-/T} \tag{5}$$

where T and R are the absolute temperature and the gas constant, respectively. The change in the standard enthalpy ΔH^o was obtained by the van't Hoff plot. The entropy term $T\Delta S^o$ was obtained by $\Delta G^o - \Delta H^o$.

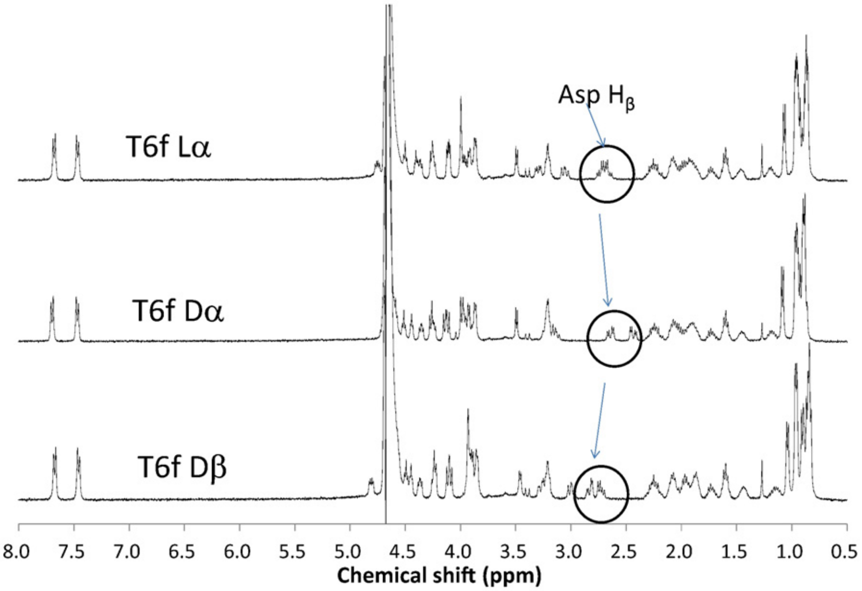

Fig. 3. ^{1}H NMR spectra of T6f composed of L-α-, D-α-, and D-β-Asp 58 isomers in PBS at 37 °C. The Asp H_β in each isomer is surrounded by the circle.

Table 2
Assignment of the ^1H NMR signals of T6fLα in PBS/D_2O at 37 °C.

Residue	Chemical shift (ppm) Hα	Hβ	Hγ	Hδ
Thr	CH 3.48, 3.50	CH 3.95, 3.96, 3.98[a]	Me 1.06, 1.08	
Val	CH 4.10, 4.11	CH 1.83–2.11[b]	Me 0.85, 0.86 Me 0.87, 0.88	
CF_3Phe	CH 4.74, 4.75, 4.76, 4.77	CH(β1) 3.03, 3.05, 3.06, 3.09 CH(β2) 3.27, 3.29, 3.31, 3,32	phe (2, 6) 7.46, 7.48 phe (3, 5) 7.67, 7.69	
Asp	N.D.	CH(β1) 2.63, 2.65, 2.67, 2.69 CH(β2) 2.71, 2,73, 2.75, 2.77		
Ser	CH 4.39, 4.41, 4.42	CH_2 3.91–3.93		
Gly	CH_2 4.00			
Ile	CH 4.25–4.27	CH 1.83–2.11[b]	Me 0.89 0.91, 0.93, 0.94[c] CH_2 1.16–1.23, 1.44–1.51	Me 0.89 0.91, 0.93, 0.94[c]
Ser	CH 4.49, 4.50, 4.52	CH_2 3.86–3.87		
Glu	CH 4.35, 4.36, 4.37, 4.39	CH_2 1.83–2.11[b]	CH_2 2.18–2.33	
Val	CH 4.114[b], 4.126	CH 1.83–2.11[b]	Me 0.95, 0.96 Me 0.97, 0.98	
Arg	CH 4.23, 4.24, 4.25, 4.27	CH_2 1.68–1.77[b]	CH_2 1.56–1.64	CH_2 3.21

[a] Thr Hβ overlaps with Ser Hβ.

[b] Val, Ile, Glu, and part of Arg Hβ overlap with each other.

[c] Ile γ and δ Me are partly overlapped and also partly superposed by Val Me.

3. Result and discussion

3.1. ^1H-NMR spectra of T6f and signal assignment

To examine whether or not the side-chain conformers of Asp 58 isomers are different in αA-crystallin fragment, we first observe the ^1H NMR spectra of T6f composed of L-α-, D-α-, and D-β-Asp 58 in PBS/D_2O at 37 °C. The spectra are shown in Fig. 3. To assign each ^1H NMR signal, 6 amino acid residues in T6fLα are replaced with alanine one by one. Such derivatives, called as T6fA, are listed in Table 1, together with the amino acid sequences. The signal assignment is performed by comparing the respective 1D ^1H NMR and ^1H–^1H COSY signals of each T6fA with those of the original T6f. For example, when Val 56 of T6f is replaced with Ala, the signals disappearing in T6fA can be assigned to Val 56. Almost all ^1H-NMR signals are successfully assigned in this way and the assignment is summarized in Table 2. In this study, Asp H_β, indicated by circles in Fig. 3, is used to quantify the spin–spin coupling constants.

3.2. Evaluation of spin–spin coupling constants

To evaluate spin–spin coupling constants (J) of Asp H_α–$H_{\beta 1}$ ($J_{\alpha\beta 1}$) and H_α–$H_{\beta 2}$ ($J_{\alpha\beta 2}$), the high-resolution ^1H-NMR measurement is performed at 10–60 °C. In this experiment, the digital resolution is 0.02 Hz, which is high enough to determine $J_{\alpha\beta 1}$ and $J_{\alpha\beta 2}$ with high accuracy. The obtained results of $J_{\alpha\beta 1}$ and $J_{\alpha\beta 2}$ in the three isomers are summarized in Table 3. For T6fLα, it is found that $J_{\alpha\beta 1}$ is smaller by 0.1–0.3 Hz in comparison with $J_{\alpha\beta 2}$ at the respective temperature. Similarly, for T6fDα, the value of $J_{\alpha\beta 1}$ is 0.4–0.9 Hz as small as $J_{\alpha\beta 2}$. For T6Dβ, however, $J_{\alpha\beta 1}$ is found to be 2.7–3.9 Hz larger than $J_{\alpha\beta 2}$ at all temperatures examined. Such phenomena should be due to the differences in the population of side-chain conformers (T, G^+, G^-), the detail of which will be described in the next section.

3.3. Population of staggered side-chain conformers

Using $J_{\alpha\beta 1}$ and $J_{\alpha\beta 2}$, the population of T, G^+ and G^- conformers of L-α-, D-α-, and D-β-Asp side chains is evaluated in T6fLα, T6fDα, and T6fDβ at 10–60 °C. Eq. (1) is used to analyze each conformer population. Fig. 4 shows the typical result of Asp side-chain conformation in T6fLα (left), T6fDα (center), and T6fDβ (right) at 37 °C. It is found that the population of the preferred conformer depends on which of the Asp isomer is contained in the peptide. In T6fLα, the population of G^+ is 0.39, the highest in the side-chain conformers of Asp. In T6fDα, the population of G^- is most preferred, whereas the T conformer is the least. In contrast, the population of T conformer is most preferable in T6fDβ; more than half of the Asp side-chain is in the T state. Such preferences are the case at all the temperatures examined.

The present result shows that the population of the gauche conformers, G^+ and G^- is relatively high in α-Asp containing T6fLα and T6fDα, as compared to the T conformer. The gauche conformers are considered to be advantageous to the racemization and isomerization of Asp via a succinimide intermediate by the following reason. The racemization and isomerization proceed as: (i) when the carboxylate carbon ($\mathbf{C}_{COO-}$) of the Asp 58 side chain is attacked by the nitrogen (**N**) of Ser 59, L(or D)-succinimide is formed by the intramolecular cyclization; (ii) L(or D)-succinimide is converted to D (or L)-succinimide through an intermediate that has the prochiral α-carbon in the plane of the ring; and (iii) the D- and L-succinimide are hydrolyzed at either side of their two carbonyl groups, yielding both α- and β-Asp residues, respectively [13]. The close distance between Asp 58 $\mathbf{C}_{COO-}$ and Ser 59 **N** should be advantageous to this reaction. For G^+ and G^- conformers, the distance between Asp $\mathbf{C}_{COO-}$ and Ser **N** is, actually, 3.5 ± 0.7 Å, smaller than the distance 4.5 ± 0.5 Å for the T conformer; see the broken lines of the 3D model in Fig. 4. Therefore, Asp $\mathbf{C}_{COO-}$ in G^+ and G^- conformers are easily attacked by Ser **N** for the isomerization of Asp 58 as shown in the 3D model of G^+ and G^-. In contrast, T6fDβ has the highest population of the T conformer of Asp 58 side-chain. In such case, Ser 59 **N** in T6fDβ is hard to attack Asp 58 $\mathbf{C}_{COO-}$ to form succinimide due to rather long distance between Asp $\mathbf{C}_{COO-}$ and Ser **N**, as illustrated by the 3D model of T. In this sense, the Dβ form is thought to be the most stable in the 3 isomers of Asp 58, the result consistent with the previous kinetics studies [14].

Table 3
Spin–spin coupling constants, $J_{\alpha\beta 1}$ and $J_{\alpha\beta 2}$ (Hz) of Asp 58 in the three isomers at 10, 20, 30, 37, 45, and 60 °C. The experimental errors are ±0.06 Hz.

Isomers		Temperature (°C) 10	20	30	37	45	60
T6fLα	$J_{\alpha\beta 1}$	6.78	6.71	–	6.65	6.67	6.61
	$J_{\alpha\beta 2}$	6.91	6.97	–	6.90	6.97	6.82
T6fDα	$J_{\alpha\beta 1}$	5.02	5.27	5.28	5.27	–	5.81
	$J_{\alpha\beta 2}$	5.99	6.07	6.09	6.01	–	6.25
T6fDβ	$J_{\alpha\beta 1}$	8.39	8.24	–	8.11	7.91	7.82
	$J_{\alpha\beta 2}$	4.45	4.84	–	4.89	4.94	5.15

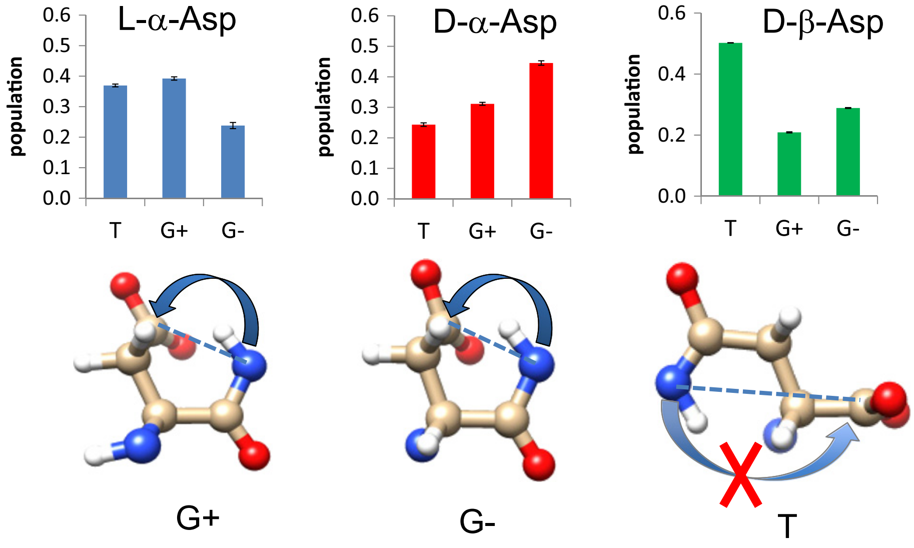

Fig. 4. Population of the side-chain conformers T, G$^+$, and G$^-$ in L-α- (left), D-α- (center), and D-β-Asp 58 (right) at 37 °C. The major conformers in each Asp 58 isomer, G$^+$ for L-α-Asp, G$^-$ for D-α-Asp, and T for D-β-Asp, are also illustrated by the 3D model in the bottom, where carbon, hydrogen, nitrogen, and oxygen atoms are shown in light brown, white, blue, and red colors, respectively. The distance between Asp 58 carboxylate carbon and Ser 59 nitrogen in each conformer is indicated by the broken line. Notice that the intramolecular cyclization (shown by arrows) is allowed in G$^+$ and G$^-$ conformers, whereas not allowed in T conformer (see text).

3.4. Thermodynamic parameters accompanied by trans–gauche change of Asp side-chain

To compare the thermodynamic stability between the respective conformers T, G$^+$, and G$^-$ in L-α-, D-α-, and D-β-Asp isomers, we evaluate the thermodynamic parameters (ΔG, ΔH, and $T\Delta S$) accompanied by trans–gauche change of Asp side-chain conformers. From the population of each conformer T, G$^+$, and G$^-$, the equilibrium constants $K_{G+/T}$ and $K_{G-/T}$ are obtained by Eqs. (2) and (3) at 10–60 °C. $K_{G+/T}$ and $K_{G-/T}$ give the standard Gibbs energy change ΔG^o of the respective conformer transformation by Eqs. (4) and (5). The standard enthalpy change, ΔH^o, is obtained as the slope of the van't Hoff plot at 10–60 °C. The entropy term $T\Delta S^o$ is calculated as $\Delta G^o - \Delta H^o$. In Table 4 is summarized the result of ΔG^o, ΔH^o, and $-T\Delta S^o$ at 37 °C. It is found that ΔG^o (T → G$^+$) and ΔG^o (T → G$^-$) are −0.6 and −1.6 kJ/mol, respectively, both negative for T6fDα. The result means that gauche conformers are rather stable in comparison to the trans conformer in T6fDα. In contrast, ΔG^o (T → G$^+$) and ΔG^o (T → G$^-$) for T6fDβ are 2.3 and 1.4 kJ/mol, respectively. In T6fDβ, the trans conformer is most stable and energetically favored in 3 conformers. In T6fLα, ΔG^o (T → G$^+$) is slightly negative (−0.2 kJ/mol), whereas ΔG^o (T → G$^-$) is positive (1.1 kJ/mol). Although the values of ΔG^o (T → G$^+$) and ΔG^o (T → G$^-$) are found to be within the magnitude of thermal fluctuation, it is nonetheless predicted that the deflection of conformers may influence the succinimide formation to transform from L-α- or D-α- to D-β-Asp for the human life span.

Regarding the standard enthalpy, ΔH^o (T → G$^+$) and ΔH^o (T → G$^-$) in T6fLα and T6fDβ are positive. Therefore, the changes from trans to gauche conformers in these peptides are found to be endothermic. This is remarkable in T6fDβ, showing that the transformation from trans to gauche conformers is not energetically favored because the trans form is stable in D-β-Asp. In contrast, ΔH^o (T → G$^+$) and ΔH^o (T → G$^-$) in T6fDα are negative. The transformation from trans to gauche conformers is exothermic in T6fDα. The transformation from trans to gauche conformers is energetically favorable in T6fDα, indicating the stability of gauche conformers in D-α-Asp. Because the absolute values of ΔH^o are larger than $T\Delta S^o$ except the case of T → G$^+$ in T6fLα, the transformation from trans to gauche conformers in T6fDα and gauche to trans conformer in T6fDβ is considered to be a enthalpy-driven process.

4. Conclusion

In this study, we quantify the population of staggered side-chain conformers (trans (T), gauche+ (G$^+$) and gauche− (G$^-$)) in αA-crystallin mimic peptide, T6f (T^{55}V-CF$_3$Phe-D^{58}SGISEVR^{65}) composed of L-α-, D-α-, and D-β-Asp 58 isomers, to gain insight into why the natural L-α-Asp is converted to uncommon D-β-Asp in the aged protein. The vicinal spin–spin coupling constants of Asp 58 H_α–$H_{\beta1}$ ($J_{\alpha\beta1}$) and H_α–$H_{\beta2}$ ($J_{\alpha\beta2}$) are determined for this purpose, by using high-resolution solution NMR at 10–60 °C. The T conformer is most preferred in the D-β-Asp side-chain, whereas G$^+$ and G$^-$ are favored in L-α- and D-α-Asp. In G$^+$ and G$^-$ conformers, the close distance between carboxylate carbon ($\mathbf{C}_{COO-}$) of Asp 58 side-chain and Ser 59 nitrogen (**N**) is advantageous to the intramolecular cyclization to form succinimide intermediate, followed by the transformation from α- to β-Asp. In contrast, the T conformer in the D-β-Asp does not allow the intramolecular cyclization because Asp 58 $\mathbf{C}_{COO-}$ and Ser 59 **N** is far from

Table 4
Change in the standard Gibbs energy ΔG^o, the enthalpy ΔH^o, and the entropy $-T\Delta S^o$ (kJ/mol) accompanied by the transformation of Asp side chain conformers at 37 °C. The experimental errors are given in brackets.

	T → G$^+$			T → G$^-$		
	ΔG^o	ΔH^o	$-T\Delta S^o$	ΔG^o	ΔH^o	$-T\Delta S^o$
T6fLα	−0.2 (±0.0)	0.3 (±0.0)	−0.4 (±0.0)	1.1 (±0.2)	1.9 (±0.1)	−0.8 (±0.0)
T6fDα	−0.6 (±0.0)	−3.0 (±0.1)	2.4 (±0.1)	−1.6 (±0.1)	−7.2 (±0.1)	5.6 (±0.3)
T6fDβ	2.3 (±0.0)	5.9 (±0.0)	−3.6 (±0.0)	1.4 (±0.0)	1.6 (±0.0)	−0.2 (±0.0)

each other to keep the D-β-Asp form stable. This is the first molecular view to answer the question why the D-β-Asp is gradually accumulated in aged αA-crystallin for the human life span. From the thermodynamic analysis, it is also found that the transformation from gauche to trans conformer in D-β-Asp is enthalpy-driven and energetically favorable.

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