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Effect of temperature on viscosity properties of some α-amino acids in aqueous urea solutions

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Abstract

Viscosities for solutions of glycine, DL-α-alanine, DL-α-amino-*n*-butyric acid, DL-valine, DL-leucine and L-serine in 5 mol kg^{-1} aqueous urea have been determined at 278.15, 288.15, 298.15 and 308.15 K. The viscosity *B*-coefficients for the amino acids in the aqueous urea solution have been calculated at different temperatures. The effect of temperature on the *B*-coefficients is discussed on the basis of the Feakins equation. The contribution of solute to the activation parameters ($\Delta\mu_2^{0\neq}$, $\Delta H_2^{0\neq}$, $\Delta S_2^{0\neq}$) for viscous flow of the solution have been calculated, together with the Gibbs energy, enthalpy and entropy of transfer for the amino acids from the ground-state solvent to the hypothetical viscous transition state solvent. The contributions of the charged end group (NH_3^+, COO^-) and CH_2 groups of the amino acids to *B*-coefficient and $\Delta\mu_2^{0\neq}$ have been also estimated using the linear correlations between *B*-coefficient or $\Delta\mu_2^{0\neq}$ and the number of carbon atoms in the alkyl chains of the amino acids. All the activation parameters are discussed in terms of the solute–solvent interactions in the ground and transition states.

Keywords: α-Amino acids; Viscosity *B*-coefficient; Activation parameters for viscous flow; Aqueous urea solution

1. Introduction

Knowledge of the individual contributions of all the protein constituents is needed for a proper understanding of the significance of thermodynamic parameters for the denaturation of proteins in denaturant solutions. Urea is a strong denaturant of proteins. The precise role of urea in the denaturation of protein systems remains unclear in spite of extensive investigations [1–3]. In order to more effectively predict the actions of urea on secondary and tertiary protein conformations, an understanding of the interactions of urea with less complicated molecules that model protein side chains, e.g. amino acids or simple oligopeptides must first be developed.

There have been many studies on the behavior of the model compounds of proteins in aqueous

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urea solutions. Standard Gibbs energies, enthalpies and entropies of transfer for some amino acids and/or peptides from water to urea solutions have been investigated by Nozaki and Tanford [4], Abu-Hamdlyyah and Shehabuddin [5], and Lapanje et al. [6]. Volumes and heat capacities of amino acids in urea solutions have also been studied by some authors [3,7–11]. Recently, extensive work has been reported by Hakin et al. [12] on thermodynamic properties of transfer of glycine and some small peptides from water to urea–water solutions. However, most of these studies are restricted to the standard temperature of 298.15 K and no viscosity investigations have been reported for amino acids + urea + water systems, although viscosity has been proven to be a sensitive and accurate probe for solution studies. To understand the behavior of amino acids and their groups in aqueous urea solutions at different temperatures and to further investigate this ongoing puzzle of urea-based protein denaturation, we have studied the viscosity properties of glycine (Gly), DL-α-alanine (Ala), DL-α-amino-*n*-butyric acid (Abu), DL-valine (Val), DL-leucine (Leu) and L-serine (Ser) in 5 mol kg^{-1} aqueous urea solutions at 278.15, 288.15, 298.15 and 308.15 K. The use of the first five of these amino acids permits individual estimations of *B*-coefficient for the charged end group (NH_3^+, COO^-) and the CH_2 group. L-Serine was chosen to study the effect of OH group in the alkyl chain on the viscosity *B*-coefficients.

In addition, the transition state theory has been applied to calculate the activation parameters for the viscous flow of the amino acids and their groups in aqueous urea solutions. The solute–solvent and solvent–solvent interactions in ground and transition states are discussed.

2. Materials and experimental techniques

Glycine (Shanghai Chem. Co.), DL-α-alanine (Shanghai Chem. Co.), DL-α-amino-*n*-butyric acid (Shanghai Chem. Co.), DL-valine (Fluka), DL-leucine (Baker) and L-serine (Beijing Chem. Co.) were twice recrystalized from aqueous ethanol solutions and dried under vacuum at 348 K for 6 h. Then they were stored over P_2O_5 in a desiccator before use. Analytical reagent grade urea (Beijing Chem. Co.) was used after drying under vacuum at 328 K. Water with a conductivity of 1.2 $\mu\Omega^{-1}$ cm^{-1} was obtained by distilling the deionized water from alkaline $KMnO_4$ to remove any organic matter. All solutions were prepared freshly by weighing on the molality concentration scale.

Solution viscosities were measured with a suspended level Ubbelohde viscometer, which was placed in a water thermostat (Schott, Germany) and has a flow time of approximately 200 s for water at 298.15 K. The temperature of the water thermostat was controlled by a CT 1450 temperature controller and a CK-100 ultracryostat to ± 0.005 K. Flow time measurements were performed with a SCHOTT AVS 310 photoelectric time unit with a resolution of 0.01 s. The procedure details are given elsewhere [13]. The density data reported in a previous paper [11] were used in the present work.

3. Results

The viscosity *B*-coefficient for the amino acids in aqueous urea solutions was obtained from the following equation [14] using the measured viscosity data for amino acid + urea + water systems:

$$\eta_r = \eta/\eta_0 = 1 + Bc \quad (1)$$

where η and η_0 are viscosities of the solutions (amino acid + urea + water) and the solvent (urea + water), respectively. η_r is the relative viscosity, and c the molarity of amino acid in solution. Viscosity *B*-coefficients were obtained by the least-squares method and are given in Table 1 together with their standard deviations. To the

Table 1
Viscosity B-coefficients for α-amino acids in 5 mol kg^{-1} aqueous urea solution at different temperatures

Amino acid	B (dm^3 mol^{-1})			
	278.15 K	288.15 K	298.15 K	308.15 K
Gly	0.195 ± 0.001	0.190 ± 0.003	0.187 ± 0.003	0.1843 ± 0.0006
DL-Ala	0.336 ± 0.004	0.310 ± 0.003	0.288 ± 0.006	0.275 ± 0.007
DL-Abu	0.457 ± 0.004	0.416 ± 0.005	0.387 ± 0.005	0.369 ± 0.003
DL-Val	0.550 ± 0.006	0.509 ± 0.005	0.471 ± 0.005	0.433 ± 0.004
DL-Leu	0.610 ± 0.004	0.564 ± 0.005	0.519 ± 0.004	0.472 ± 0.002
L-Ser	0.319 ± 0.007	0.311 ± 0.004	0.299 ± 0.003	0.285 ± 0.004

best of our knowledge, these data are being reported for the first time.

In the mechanism of viscous flow, the displacement of a liquid layer with respect to the adjacent one can be described as the movement of the molecules of the layer from their equilibrium positions to the adjacent ones within the same layer. Eying [15] considered that the jump of the molecule from its equilibrium position to an adjacent one can be regarded as a process accompanied by overcoming a potential energy barrier. That is to say, the process is similar to that acting in chemical reactions. Then the molecules in their equilibrium position are at the ground states and the molecules in the position of the top of the barrier are at the transition states. Based on the Eying transition state theory, the thermodynamic activation parameters (Gibbs energy, entropy and enthalpy) of viscous flow for the amino acids were evaluated by using Feakins et al. [16] extension of Eying transition state theory:

$$B = \left(V^{\circ}_{1,\phi} - V^{\circ}_{2,\phi}\right)/1000 + \left(V^{\circ}_{1,\phi}/1000\right)\left(\Delta\mu_2^{0\neq} - \Delta\mu_1^{0\neq}\right)/RT \quad (2)$$

Where $V^{\circ}_{1,\phi}$ and $V^{\circ}_{2,\phi}$ are the partial molar volumes of the solvent and solute at infinite dilution, respectively. $\Delta\mu_1^{0\neq}$ is the Gibbs energy of activation per mole of pure solvent, and is given by:

$$\Delta\mu_1^{0\neq} = RT\ln\left(\eta_0 V^{\circ}_{1,\phi}/hN_A\right) \quad (3)$$

where h is Planck's constant and N_A is Avogadro's number. $\Delta\mu_2^{0\neq}$ is the contribution per mole of solute to the Gibbs energy of activation for viscous flow of the solution. Hence, Eq. (2) can be rearranged as follows:

$$\Delta\mu_2^{0\neq} = \Delta\mu_1^{0\neq} + \left(RT/V^{\circ}_{1,\phi}\right) \times \left[1000B - \left(V^{\circ}_{1,\phi} - V^{\circ}_{2,\phi}\right)\right] \quad (4)$$

Thus obtained $\Delta\mu_2^{0\neq}$ values at different temperatures are recorded in Table 2.

Over the temperature range concerned, the entropy and enthalpy of activation for viscous flow of the amino acids in 5 mol kg^{-1} aqueous urea solution were calculated with Eqs. (5) and (6). The results are given in Table 3.

$$\Delta S_2^{0\neq} = -d\left(\Delta\mu_2^{0\neq}\right)/dT \quad (5)$$

Table 2
Activation Gibbs energies $\Delta\mu_2^{0\neq}$ for viscous flow of amino acids in 5 mol kg^{-1} aqueous urea solution at different temperatures

Amino acid	$\Delta\mu_2^{0\neq}$ (kJ mol^{-1})			
	278.15 K	288.15 K	298.15 K	308.15 K
Gly	35.6 ± 0.1	35.9 ± 0.3	36.0 ± 0.4	36.3 ± 0.1
DL-Ala	53.8 ± 0.5	51.8 ± 0.4	50.4 ± 0.7	48.7 ± 0.8
DL-Abu	69.3 ± 0.5	66.3 ± 0.6	64.3 ± 0.6	63.4 ± 0.4
DL-Val	81.8 ± 0.7	79.1 ± 0.6	76.4 ± 0.6	73.4 ± 0.5
DL-Leu	90.5 ± 0.5	87.5 ± 0.6	84.2 ± 0.5	80.4 ± 0.3
L-Ser	51.9 ± 0.8	52.1 ± 0.5	51.8 ± 0.4	51.1 ± 0.5

Table 3
Activation enthalpy $\Delta H_2^{0\neq}$ and entropy $\Delta S_2^{0\neq}$ for viscous flow of amino acids in 5 mol kg^{-1} aqueous urea solution

Amino acid	$\Delta H_2^{0\neq}$ (kJ mol^{-1})	$\Delta S_2^{0\neq}$ (kJ^{-1} mol^{-1})
Gly	30.2 ± 0.8	−20 ± 3
DL-Ala	91.7 ± 2.3	137 ± 8
DL-Abu	123.7 ± 9.7	197 ± 33
DL-Val	159.4 ± 1.5	279 ± 5
DL-Leu	184.3 ± 3.7	338 ± 13
L-Ser	58.8 ± 4.2	24 ± 14

$$\Delta H_2^{0\neq} = \Delta\mu_2^{0\neq} + T\Delta S_2^{0\neq} \quad (6)$$

For a solution in thermodynamic equilibrium, a chosen solute molecule interacts with every solvent molecule; the sum of these interactions over all solute and solvent molecules is the familiar solute–solvent interaction. There are two contributions to $\Delta\mu_2^{0\neq}$ [17]. The first one is thought of as the Gibbs energy of interaction of the solute with a hypothetical transition-state solvent. The effect of the solute on the Gibbs energy of activation of the solvent molecule is, in fact, the difference between the solvation energies of the solute in the ground-state solvent and in the transition-state solvent, or Gibbs energy of transfer, $\Delta G_2^{\circ}(1{-}1')$, between them. The second contribution to $\Delta\mu_2^{0\neq}$ comes from the movement of the solute through its own viscous transition state, $\Delta G_2^{\circ}(2{-}2')$. Thus,

$$\Delta\mu_2^{0\neq} = \Delta G_2^{\circ}(1{-}1') + \Delta G_2^{\circ}(2{-}2') \quad (7)$$

We are interested in the solute–solvent interactions. Therefore, it is necessary to estimate $\Delta G_2^{\circ}(2{-}2')$ first, and then to obtain $\Delta G_2^{\circ}(1{-}1')$ from the measured $\Delta\mu_2^{0\neq}$ values. $\Delta\mu_1^{0\neq}$ can be used as a common value for $\Delta G_2^{\circ}(2{-}2')$ [17]. The other activation parameters of transfer $\Delta H_2^{\circ}(1{-}1')$ and $\Delta S_2^{\circ}(1{-}1')$ can be obtained by equations similar to Eqs. (5) and (6). All the results have been collected in Table 4.

4. Discussion

The B values of the amino acids reflect the net structural effect of the charged groups and the hydrophobic alkyl groups on the amino acid. These two effects can be separated by noting that the B-coefficients of amino acid are linear in n_c, the number of carbon atoms in alkyl chains, i.e.:

$$B = B(NH_3^+, COO^-) + n_c B(CH_2) \quad (8)$$

The regression parameters, $B(NH_3^+, COO^-)$, the zwitterionic group contribution and $B(CH_2)$,

Table 4
Thermodynamic activation parameters of transfer for the amino acids from ground-state to transition-state in 5 mol kg^{-1} aqueous urea solution

Amino acid	$\Delta G_2^{\circ}(1{-}1')$ (kJ mol^{-1})				$\Delta H_2^{\circ}(1{-}1')$ (kJ mol^{-1})	$\Delta S_2^{\circ}(1{-}1')$ (kJ mol^{-1})
	278.15 K	288.15 K	298.15 K	308.15 K		
Gly	25.2 ± 0.1	25.7 ± 0.3	26.0 ± 0.4	26.5 ± 0.1	13.5 ± 0.8	−42 ± 3
DL-Ala	43.4 ± 0.5	41.6 ± 0.4	40.4 ± 0.7	38.9 ± 0.8	84.2 ± 2.3	147 ± 8
DL-Abu	58.9 ± 0.5	56.1 ± 0.6	54.3 ± 0.6	53.6 ± 0.4	107.6 ± 9.7	177 ± 33
DL-Val	71.4 ± 0.7	68.9 ± 0.6	66.4 ± 0.6	63.6 ± 0.5	143.5 ± 1.5	259 ± 5
DL-Leu	80.1 ± 0.6	77.3 ± 0.6	74.2 ± 0.5	70.6 ± 0.4	168.2 ± 3.7	316 ± 13
L-Ser	41.5 ± 0.8	41.9 ± 0.5	41.8 ± 0.4	41.3 ± 0.5	43.7 ± 4.2	7 ± 14

Table 5
Contribution of (NH_3^+,COO^-) and CH_2 groups to viscosity B-coefficient of the amino acids at different temperatures

Group	B ($dm^3\ mol^{-1}$)			
	278.15 K	288.15 K	298.15 K	308.15 K
(NH_3^+,COO^-)	0.114 ± 0.031	0.117 ± 0.024	0.122 ± 0.021	0.131 ± 0.022
CH_2	0.105 ± 0.009	0.094 ± 0.007	0.084 ± 0.006	0.072 ± 0.007

the methylene group contribution to B-values of amino acid, are listed in Table 5. A similar linear correlation has been reported by Wadi and Goyal [18] for some amino acids (glycine, DL-alanine, L-proline, L-threonine, β-alanine, γ-aminobutyric acid and ε-aminocaproic acid) in aqueous potassium thiocyanate solutions. Our recent viscosity data for the α-amino acids in aqueous guanidine hydrochloride (GuHCl) solutions also suggested the same linear relationship [13]. However, it should be pointed out that $B(CH_2)$ obtained here characterizes the mean contribution of CH and CH_3 groups to B-coefficients of the amino acids.

The dB/dT values of the groups can provide direct evidence regarding their structural effects in solutions. The results given in Table 5 clearly reveal the structural features of these groups. The dB/dT values are positive for the structure-breaking zwitterion group, i.e. as the water structure decreases with increasing temperature, this group become a less effective structure breaker and its B-coefficient increases. However, the hydrophobic structure-making CH_2 groups show negative dB/dT as required. As seen from Table 1, the α-amino acids studied have negative dB/dT values. This suggests that the effect of the nonpolar part of the amino acids on solvent structure overcomes that of the charged end group in aqueous urea solutions.

Serine can be regarded as a derivative of alanine in which −H is replaced by an −OH group. Therefore, the B-coefficient of serine should increase by virtue of its increased size. But L-serine behaves as a better structure-breaker than alanine because −OH is a hydrophilic group. The increase in B-coefficients due to an increase in size is offset by a decrease in B values due to the structure-breaking property of the −OH group in serine. Thus, the B-coefficient of serine is generally comparable with that of alanine.

A comparison with our recent results [13] for these α-amino acids in guanidine hydrochloride solutions reveals that the B-coefficients in GuHCl solution are larger than the corresponding values in aqueous urea. From Table 5 and results in the literature [13], it is found that the $B(CH_2)$ values are almost the same in both urea and guanidine hydrochloride solutions. In other words, the main difference between B(urea) and B(GuHCl) comes from the difference in interactions between charged end groups of amino acids and the denaturants. Guanidine hydrochloride can interact with the zwitterion of amino acids by ion–ion interactions and hydrogen bonding [19], but only hydrogen bonding can be expected with urea. This is believed to be the molecular configuration reasons.

Moreover, it is observed that B values show a linear correlation with the standard partial molar volumes $V^{\circ}_{2,\phi}$ reported previously [11] for the α-amino acids in aqueous urea solutions. The coefficients a_1 and a_2 of the equation:

$$B = a_1 + a_2 V^{\circ}_{2,\phi} \tag{9}$$

are given in Table 6 together with their standard deviations and correlation coefficients. The a_2 value reflects the size and shape of the solute [20]. It has been reported [21] that the a_2 value lies between 0 and 2.5 for unsolvated spherical species. It can be noted from Table 6 that this value is greater than 2.5. Therefore, the amino acid is strongly solvated in aqueous urea solutions.

Using the linear correlation between enthalpy of solution at infinite dilution, ΔH_s°, of the amino

Table 6
Coefficients a_1 and a_2 of Eq. (9) for α-amino acids in 5 mol kg^{-1} aqueous urea solution

T (K)	a_1 (dm^3 mol^{-1})	a_2	R[a]
278.15	-0.084 ± 0.044	6.8 ± 0.6	0.990
288.15	-0.070 ± 0.036	6.1 ± 0.5	0.992
298.15	-0.051 ± 0.033	5.5 ± 0.4	0.991
308.15	-0.021 ± 0.036	4.7 ± 0.5	0.987

[a] R is the correlation coefficient.

acids in aqueous urea solutions and urea concentration [5], the values of ΔH_s° at 298.15 K for glycine, alanine and leucine in 5 mol kg^{-1} aqueous urea solution have been calculated. If the B-coefficients of these amino acids (glycine, alanine and leucine) are plotted as a function of their ΔH_s° a reasonable linear relation (correlation coefficient $R = 0.934$) is observed:

$$B = 0.5826 - 2 \times 10^{-4} \Delta H_s^\circ \quad (10)$$

This correlation is unexpected, taking into account that enthalpy and viscosity reflect different features of solutions. It can be only supposed that the influence of urea on various properties of the amino acids is similar.

According to Feakins et al. [16], the chemical potential change of solute ($\Delta\mu_2^{0\neq}$) is greater than that of pure solvent ($\Delta\mu_1^{0\neq}$) for net structure-makers. The values of $\Delta\mu_1^{0\neq}$ for 5 mol kg^{-1} urea solution are 10.4, 10.2, 9.97 and 9.80 kJ mol^{-1} at 278.15, 288.15, 298.15 and 308.15 K, respectively. From the data in Table 2, one can conclude that these amino acids are acting as structure-makers. This is consistent with the conclusion obtained from B-coefficients. Except for glycine the values of $\Delta S_2^{0\neq}$ and $\Delta H_2^{0\neq}$ in Table 3 are positive, indicating that the formation of the activated complex is associated with bond breaking and a decrease in order. This suggests that the ground-states are in the ordered region.

It can be seen from Table 4 that values of $\Delta G_2^\circ(1-1')$ are positive and increase from glycine to leucine at given temperatures. Thus, their transfer from the ground-state solvent to the transition-state solvent is unfavored and becomes more difficult for the amino acids which have longer alkyl chains. The interactions of amino acids with the ground-state solvent are much stronger. Accordingly, more solute–solvent bonds must be broken to form the transition state and more energy is needed. Therefore $\Delta\mu_2^{0\neq}$ should increase gradually from glycine to leucine. From the $\Delta\mu_2^{0\neq}$ data in Table 2, the same conclusion can be obtained. This trend can be also explained from the solute–solvent interactions. As these amino acids have the same charged end groups, the difference between them is the length of alkyl chain. It can be deduced that the increasing $\Delta\mu_2^{0\neq}$ comes from the difference in interactions of the alkyl groups of the amino acids with urea and those with water molecules in solvent mixtures. According to Frank and Wen [22], the hydrophobic effect of a molecule with non-polar and polar parts on water increases with increasing length of non-polar parts. However, the study on the effect of urea on the hydrophobic chain of the protein [23] pointed out that this effect is directly proportional to the length of hydrophobic chain of the protein. Therefore, the interactions of the alkyl groups with urea and water molecules increase with increasing alkyl chains length of the amino acids and $\Delta\mu_2^{0\neq}$ increases gradually from glycine to leucine.

As for the standard partial molar volumes [11] and the B-coefficient of α-amino acids, $\Delta\mu_2^{0\neq}$

Table 7
Contribution of (NH_3^+,COO^-) and CH_2 groups to $\Delta\mu_2^{0\neq}$, $\Delta H_2^{0\neq}$ and $\Delta S_2^{0\neq}$ in 5 mol kg^{-1} aqueous urea solution

Group	$\Delta H_2^{0\neq}$ (kJ mol^{-1})	$\Delta S_2^{0\neq}$ (J K^{-1} mol^{-1})	$\Delta\mu_2^{0\neq}$ (kJ mol^{-1})			
			278.15 K	288.15 K	298.15 K	308.15 K
(NH_3^+,COO^-)	5.1 ± 6.8	-70 ± 23	24.9 ± 3.6	25.1 ± 2.8	25.5 ± 2.5	27.1 ± 2.8
CH_2	37.6 ± 1.0	85 ± 3	13.8 ± 1.1	13.0 ± 0.8	12.2 ± 0.8	11.2 ± 0.8

also varies linearly with n_c. The regression of $\Delta\mu_2^{0\neq}$ against n_c using Eq. (11):

$$\Delta\mu_2^{0\neq} = \Delta\mu_2^{0\neq}(NH_3^+,COO^-) + n_c\Delta\mu_2^{0\neq}(CH_2) \quad (11)$$

gives $\Delta\mu_2^{0\neq}(NH_3^+,COO^-)$ and $\Delta\mu_2^{0\neq}(CH_2)$ as the respective contributions of (NH_3^+,COO^-) and the CH_2 groups. Using a similar method as in Eqs. (5) and (6), $\Delta H_2^{0\neq}$ and $\Delta S_2^{0\neq}$ values for zwitterion (NH_3^+,COO^-) and CH_2 groups can be obtained. These results are presented in Table 7.

It can be seen from Table 7 that $\Delta\mu_2^{0\neq}(CH_2)$ decreases while $\Delta\mu_2^{0\neq}(NH_3^+,COO^-)$ increases from 278.15 to 308.15 K, giving a positive $\Delta S_2^{0\neq}$ for the CH_2 group and a negative $\Delta S_2^{0\neq}$ for the zwitterion, respectively. Similar trends are observed in aqueous guanidine hydrochloride [13]. Activation enthalpy and entropy data suggested that interaction of solvents with (NH_3^+,COO^-) are not very strong in the ground state, significant solute–solvent bonds will be made in the transition state, while the reverse is true for the CH_2 group.

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