Heterotactic Enthalpic Interactions of L-Arginine and L-Proline with 1,3-Butanediol and 2,3-Butanediol in Aqueous Solutions

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The enthalpies of the mixture of (*S*)-2-amino-5-guanidinopentanoic acid and (2*S*)-pyrrolidine-2-carboxylic acid with 1,3-butanediol, 2,3-butanediol, and their respective enthalpies of dilution in aqueous solutions at 310.15 K were determined as a function of the mole fraction by flow microcalorimetric measurements. These experimental results have been analyzed to obtain the heterotactic enthalpic interaction coefficients (h_{xy}) according to the McMillan−Mayer theory. It has been found that the h_{xy} coefficients between (*S*)-2-amino-5-guanidinopentanoic acid and (2*S*)-pyrrolidine-2-carboxylic acid with butanediol molecules in aqueous solutions at 310.15 K are all positive. The results are discussed in terms of solute−solute and solute−solvent interactions.

Introduction

Many studies have been done on the effects of polyols in proteins, and it was found that polyols help in stabilizing the native conformations of globular proteins.[1−3] Some authors correlated the stabilizing effect of sugars with the number and position of hydroxyl groups. However, our understanding of the stabilization mechanism of proteins is still incomplete. To understand the nature of interactions between sugars and proteins in aqueous solutions, it is necessary to study biochemical model compounds owing to the complex structure of the biological macromolecules. Amino acids are basic components of protein molecules and are considered to be the model compounds. The native structure of proteins is governed by weak, nonbonding interactions between the amino acid residues or between these residues and the aqueous environment.[4]

Although the polyols under investigation are not found in cellular or extracellular fluids of living organisms, they find wide applications in pharmacology and the cosmetics industry. When introduced into a living organism as vehicles for pharmaceuticals or cosmetics, they affect the components of cellular fluids. This has been confirmed by numerous biochemical studies devoted to the interactions between polyols and components of biological cells.[5]

In our previous studies, the enthalpies of mixing of amino acids with butanol,[6] 1,2-ethanediols,[7] and 2-chlorethanol[8] as well as *N*,*N*-dimethylformamide with polyalcohols (glycol, 1,2-propanediol, 1,3-propanediol, glycerol)[9] in aqueous solution were measured by the method of microcalorimetry. As a continuation of the work, this present work reports the enthalpic interaction coefficients between (*S*)-2-amino-5-guanidinopentanoic acid (L-arginine) and (2*S*)-pyrrolidine-2-carboxylic acid (L-proline) with 1,3-butanediol and 2,3-butanediol in aqueous solution at 310.15 K according to the McMillan−Mayer theory.[10] These coefficients reflect the sum of the enthalpic effects of interactions between the components in aqueous solutions.

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Materials and Methods

L-Arginine and L-proline were obtained from Shanghai Chemical Co., China, and used after recrystallization from a water−methanol mixture and dried in vacuum desiccators until their weights became constant. Analytical reagent grade 1,3-butanediol and 2,3-butanediol were used without further purification. The water used in the experiments was deionized, distilled, and degassed. Both the aqueous L-arginine and L-proline solutions as well as the aqueous 1,3-butanediol and 2,3-butanediol solutions were prepared by mass using a Mettler AE 200 balance with a precision of 0.0001 g. All of the solutions were degassed and used within 12 h after preparation to avoid possible bacterial contamination. The measurements of enthalpies of mixing and dilution were carried out with a flow microcalorimeter (2277 thermal activity monitor, made in Sweden) at 310.15 K. The calorimeter has a high temperature control accuracy (0.001 K). The baseline stability (over a period of 24 h) is 0.2 μW. The solutions were pumped through the mixing-flow vessel of the calorimeter using a pair of LKB-2132 microperpex peristaltic pumps. The flow rates were determined from the mass of the samples delivered in 8 min. The variation of flow rates was less than 0.1 % both before and after a complete experiment. The relative mean deviation of the thermal power determined was 0.3 %, and that of the enthalpies of mixing and dilution was less than 1 %. The apparatus and the procedure used were the same as those described in earlier work.[6−8]

The enthalpies of mixing and dilution can be treated to determine the enthalpic interaction coefficients based on the McMillan−Mayer theory.[10] The excess enthalpy $H^E(m_x, m_y)$ of a solution containing two solute species *x* and *y* can be expressed as a virial expansion of solute molalities using the following equation.

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$$H^{E}(m_x, m_y)/w_1 = H(m_x, m_y)/w_1 - h_w^* - m_x H_{x,m}^{\infty} - m_y H_{y,m}^{\infty} = h_{x,x} m_x^2 + 2h_{xy} m_x m_y + h_{yy} m_y^2 + h_{xxx} m_x^3 + 3h_{xxy} m_x^2 m_y + 3h_{xyy} m_x m_y^2 + h_{yyy} m_y^3 + \ldots \quad (1)$$

where $H^{E}(m_x, m_y)$ and $H(m_x, m_y)$ represent the excess enthalpy and the total enthalpy of a solution containing m_x of x and m_y of y species in w_1 of water, respectively; h_w* is the standard enthalpy of 1 kg of pure water; $H_{x,m}^{\infty}$ and $H_{y,m}^{\infty}$ are the limiting partial molar enthalpies of solutes x and y, respectively; and the various h_{ij} and h_{iij} are the interaction coefficients that represent the contribution of solute−solute interactions between pairs, triplets, and higher-order interactions of solvated solute molecules in a binary solution.

The dilution enthalpies $\Delta_{dil}H$ ($J \cdot kg^{-1}$) are determined by measuring thermal power P (μW) and flow rates of solution and solvent (f_A and f_B, $mg \cdot s^{-1}$)

$$\Delta_{dil}H = P/(f_A + f_B - m_{x,i} M_x f_A) \quad (2)$$

where M_x is the molar mass of solute ($kg \cdot mol^{-1}$) and $m_{x,i}$ is initial molality ($mol \cdot kg^{-1}$).

The final molality m_x ($mol \cdot kg^{-1}$) may be calculated from the equation

$$m_x = m_{x,i} f_A / [f_B (m_{x,i} M_x + 1) + f_A] \quad (3)$$

The mixing enthalpy $\Delta_{mix}H$ ($J \cdot kg^{-1}$) of aqueous x solution and aqueous y solution is calculated from the equation

$$\Delta_{mix}H = P^*/(f_x + f_y - m_{x,i} M_x f_x - m_{y,i} M_y f_y) \quad (4)$$

where P* is the mixing thermal power (μW); f_x and f_y are the flow rates of solutions x and y; and $m_{x,I}$ and $m_{y,I}$ are the initial molalities of solutions x and y before mixing, respectively.

To facilitate the calculation, an auxiliary function ΔH* is introduced

$$\Delta H^* = \Delta H_{mix} - \Delta H_{dil}(x) - \Delta H_{dil}(y) = H^{E}(m_x, m_y) - H^{E}(m_x) - H^{E}(m_y) \quad (5)$$

Therefore the equation for the heterotactic interaction coefficients can be evaluated for the combination of eqs 1 and 5

$$\Delta H^*/w_1 = 2h_{xy} m_x m_y + 3h_{xxy} m_x^2 m_y + 3h_{xyy} m_x m_y^2 + \ldots \quad (6)$$

If the mixing experiments are carried out at different values of m_x and m_y, then the pairwise and triplet enthalpic interaction coefficients can be evaluated.

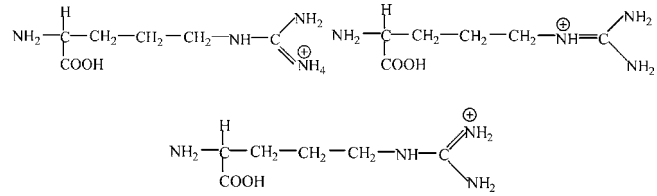
Results and Discussion

Values of ΔH* calculated from experimental data using eq 6 by the least-squares procedure, together with the experimental $\Delta_{mix}H$, $\Delta_{dil}H(x)$, and $\Delta_{dil}H(y)$, are given in Table 1. The data were fitted to eq 6 to obtain the heterotactic enthalpic interaction coefficients (Table 2). The enthalpic pairwise interaction coefficients are regarded as a measure of the enthalpy effect (i.e., the enthalpy of interaction) when two solute particles approach each other. The physical meaning of the pair interaction coefficients of an excess property is linked to the variation of the thermodynamic property when two hydrated molecules are brought from an infinite distance, where solute−solvent interactions prevail, to a finite distance where solute−solute, water-mediated interactions are operating.[11] Since it is difficult to interpret the higher h_{xy} coefficients, only the pairwise coefficients h_{xy} are considered here.

The enthalpic interaction coefficients represent a measure of interactions between two hydrated solutes and depend on the interactions between the solute molecules and the solvent water. On the whole, the global effects between amino acids (L-proline and L-arginine) and butanediol molecules in the aqueous solutions reflect three superimposed processes. The first is the partial dehydration of the hydration shell of the amino acid zwitterions (endothermic process). The second is the partial dehydration of the hydration shell of butanol (endothermic process), and the third is the direct interaction between the molecules of amino acids and butanol, which plays the dominant role in the overall interaction process.

Since both of the amino acids (L-proline and L-arginine) and butanediol molecules have hydrophobic and hydrophilic groups, the direct interaction between them can be summarized as: (a) the hydrophobic−hydrophobic interaction (endothermic process, making positive contributions to h_{xy}); (b) the hydrophobic−hydrophilic interaction (endothermic process, making positive contributions to h_{xy}); (c) the hydrophilic−hydrophilic interaction (exothermic process, making negative contributions to h_{xy}); and (d) hydrogen-bond interactions (exothermic process, making negative contributions to h_{xy}).

For the ternary interaction systems, the heterotactic enthalpic interaction coefficients and the correlation coefficients between L-arginine and L-proline with 1,3-butanediol and 2,3-butanediol were achieved by multiple linear regression analysis with eq 6 from the experimental data in Table 1 and shown in Table 2. From Table 2 it can be seen that the values of h_{xy} have large uncertainty intervals but the correlations are near 1, which may result from the theoretical defects of eq 6. However, they can provide us with useful information. h_{xy} are all positive. This shows that the interaction of arginine and proline with the same kind of butanediol isomers is an endothermic process (h_{xy} is positive), and the value of h_{xy} is determined by the structural difference between L-arginine and L-proline. L-Arginine is an amphoteric amino acid. It not only has a longer hydrophobic alkyl side chain but also has a hydrophilic guanidino group at the end of the side chain. In neutral, acidic, or alkaline environments it bears a positive charge. Because of the conjugated electronic system between its double bond and nitrogen isolated electron pair, its positive electrode delocalizes. The resonance structures are depicted as follows:



Both a guanidine fragment[12,13] and a butanediol can be involved in hydrogen bonding. L-Proline is a natural amino acid that has one pyrrole ring. A comparison of the molecular structure of the two amino acids shows that they have the same long alkyl side chain, but L-arginine has a guanidino group at the end of its alkyl side chain, while L-proline has a pyrrole ring. From the experimental data in Table 2 it can be seen that h_{xy} (arginine) $\ll h_{xy}$ (proline) in aqueous solution at 310.15 K. This mainly results from three reasons: first, the cyclic structure in L-proline results in relatively larger hydrophobicity,[7,14] while the alkyl side chain of L-arginine embedded between hydrophilic groups reduces the hydrophobicity, so the force of (a) and (b) predominates in L-proline; second, because of the existence of

Table 1. Enthalpies of Dilution and Mixing of Aqueous Solutions L-Arginine and L-Proline with 1,3-Butanediol and 2,3-Butanediol Solutions at Different Temperatures

m_{xi} / mol·kg^{-1}	m_{yi} / mol·kg^{-1}	m_x / mol·kg^{-1}	m_y / mol·kg^{-1}	$H_{dil}(x)$ / J·kg^{-1}	$H_{dil}(y)$ / J·kg^{-1}	H_{mix} / J·kg^{-1}	H^* / J·kg^{-1}
			L-Arginine + 1,3-Butanediol				
0.1000	0.1000	0.0503	0.0491	3.24	−1.60	2.15	0.51
0.1500	0.1500	0.0751	0.0735	6.37	−4.19	5.29	3.10
0.1800	0.1800	0.0898	0.0880	8.67	−6.13	7.53	4.99
0.2000	0.2000	0.0997	0.0977	10.68	−7.25	9.78	6.35
0.2200	0.2200	0.1094	0.1074	12.42	−8.33	11.07	6.98
0.2500	0.2500	0.1241	0.1219	15.91	−10.93	14.15	9.18
0.2800	0.2800	0.1386	0.1363	19.27	−14.77	18.74	14.24
0.3000	0.3000	0.1482	0.1459	21.71	−14.91	22.01	15.21
0.3200	0.3200	0.1579	0.1555	24.27	−16.84	24.85	17.42
0.3500	0.3500	0.1722	0.1699	28.27	−20.74	28.40	20.87
0.3800	0.3800	0.1865	0.1842	32.59	−23.51	33.59	24.51
0.4000	0.4000	0.1960	0.1937	35.90	−26.22	36.32	26.64
0.4200	0.4200	0.2055	0.2032	39.16	−28.87	40.26	29.97
			L-Proline + 1,3-Butanediol				
0.1000	0.1000	0.0504	0.0491	−1.16	−1.60	0.27	3.03
0.1500	0.1500	0.0754	0.0735	−2.58	−4.19	1.70	8.47
0.1800	0.1800	0.0903	0.0880	−3.76	−6.13	2.90	12.80
0.2000	0.2000	0.1002	0.0977	−4.77	−7.25	2.84	14.86
0.2200	0.2200	0.1101	0.1074	−4.64	−8.33	3.74	16.72
0.2500	0.2500	0.1249	0.1219	−6.52	−10.93	5.11	22.56
0.2800	0.2800	0.1397	0.1363	−8.41	−14.77	7.11	30.30
0.3000	0.3000	0.1495	0.1459	−8.53	−14.91	8.14	31.58
0.3200	0.3200	0.1593	0.1555	−9.33	−16.84	9.44	35.61
0.3500	0.3500	0.1739	0.1699	−11.22	−20.74	11.12	43.08
0.3800	0.3800	0.1885	0.1842	−13.01	−23.51	13.51	50.03
0.4000	0.4000	0.1982	0.1937	−14.79	−26.22	14.61	55.61
0.4200	0.4200	0.2079	0.2032	−15.82	−28.87	16.15	60.85
			L-Arginine + 2,3-Butanediol				
0.1000	0.1000	0.0503	0.0491	3.24	−1.76	2.43	0.95
0.1500	0.1500	0.0751	0.0735	6.37	−4.52	5.76	3.91
0.1800	0.1800	0.0898	0.0880	8.67	−6.56	7.96	5.85
0.2000	0.2000	0.0997	0.0977	10.68	−8.00	10.16	7.48
0.2200	0.2200	0.1094	0.1074	12.42	−9.31	11.48	8.37
0.2500	0.2500	0.1241	0.1219	15.91	−12.25	14.82	11.16
0.2800	0.2800	0.1386	0.1363	19.27	−16.16	20.17	17.06
0.3000	0.3000	0.1482	0.1459	21.71	−16.71	22.90	17.90
0.3200	0.3200	0.1579	0.1555	24.27	−18.62	26.10	20.45
0.3500	0.3500	0.1722	0.1699	28.27	−22.55	30.24	24.52
0.3800	0.3800	0.1865	0.1842	32.59	−26.25	35.68	29.34
0.4000	0.4000	0.1960	0.1937	35.90	−29.60	38.59	32.30
0.4200	0.4200	0.2055	0.2032	39.16	−32.85	42.36	36.05
			L-Proline + 2,3-Butanediol				
0.1000	0.1000	0.0504	0.0491	−1.16	−1.76	−0.26	2.66
0.1500	0.1500	0.0754	0.0735	−2.58	−4.52	1.43	8.53
0.1800	0.1800	0.0903	0.0880	−3.76	−6.56	2.22	12.54
0.2000	0.2000	0.1002	0.0977	−4.77	−8.00	3.20	15.98
0.2200	0.2200	0.1101	0.1074	−4.64	−9.31	3.18	17.14
0.2500	0.2500	0.1249	0.1219	−6.52	−12.25	4.05	22.82
0.2800	0.2800	0.1397	0.1363	−8.41	−16.16	5.03	29.60
0.3000	0.3000	0.1495	0.1459	−8.53	−16.71	6.44	31.68
0.3200	0.3200	0.1593	0.1555	−9.33	−18.62	7.31	35.26
0.3500	0.3500	0.1739	0.1699	−11.22	−22.55	8.55	42.32
0.3800	0.3800	0.1885	0.1842	−13.01	−26.25	10.14	49.40
0.4000	0.4000	0.1982	0.1937	−14.79	−29.60	11.14	55.54
0.4200	0.4200	0.2079	0.2032	−15.82	−32.85	12.44	61.10

Table 2. Heterotactic Enthalpic Interaction Coefficients between L-Arginine and L-Proline with 1,3-Butanediol and 2,3-Butanediol Aqueous Solutions at Different Temperatures

solutes $x + y$	h_{xy} / 10^2 J·kg·mol^{-2}	h_{xxy} / 10^4 J·kg^2·mol^{-3}	h_{xyy} / 10^4 J·kg^2·mol^{-3}	R^{2a}	SD^b
L-arginine + 1,3-butanediol	2.95 ± 2.35[c]	2.68 ± 5.37	−2.68 ± 5.36	0.9970	0.61
L-arginine + 2,3-butanediol	3.62 ± 2.65	1.71 ± 6.05	−1.71 ± 6.03	0.9973	0.68
L-proline + 1,3-butanediol	10.89 ± 3.39	−22.82 ± 27.44	23.24 ± 27.97	0.9983	0.89
L-proline + 2,3-butanediol	15.37 ± 2.68	−61.51 ± 21.67	62.68 ± 22.10	0.9989	0.7

[a] Square of correlation coefficient. [b] Standard deviation. [c] The estimated deviation.

the guanidino group, L-arginine has more hydrophilic groups than L-proline, which largely enhances the force of (c) in L-arginine; and third, compared with L-proline, L-arginine has more acceptor and donor sites to form hydrogen bonding, which

enhances the force of (d). To summarize, L-arginine not only has larger hydrophobicity but also a strong hydrogen bonding effect. So, h_{xy} (L-proline) > h_{xy} (L-arginine).

The difference of h_{xy} between butanediol isomers and the same amino acid is mainly determined by the difference between the butanediol isomers studied. The difference between 1,3-butanediol and 2,3-butanediol exists in the different relative position of the two hydroxyl groups. When the two act with arginine and proline, they appear as: h_{xy} (2,3-butanediol) > h_{xy} (1,3-butanediol) if we do not consider the uncertainty intervals. This may mainly result from the following two aspects: the first difference seems to be due to a stronger effect of the hydrophobic hydration of the two methyl groups (CH_3) and the two methylidynes (CH) or the two methylenes (CH_2), which screens the hydroxyl group in 2,3-butanediol, than that of methyl groups (CH_3), the two methylenes (CH_2), and methylidynes (CH). The second is that the two hydroxyl groups of 2,3-butanediol are adjacent. This structure is unstable, and in polar solvent, it is very easy for intramolecular hydrogen bonds to form as a five-membered ring structure. This weakens hydrophilicity−hydrophilic interaction and reduces the formation of intermolecular hydrogen bonds.

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