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Enthalpic interactions of L-alanine and L-serine in aqueous urea solutions

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Abstract

The enthalpies of dilution of L-alanine and L-serine in various aqueous urea solutions have been determined by flow microcalorimetry at 298.15 K. The homogeneous enthalpic interaction coefficients over the whole range of aqueous urea solutions have been calculated according to the excess enthalpy concept. The results were interpreted from the point of view of solute–solute interactions moderated by solvent effects. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Enthalpy of dilution; Amino acids; Aqueous urea solutions

1. Introduction

Thermodynamic properties of aqueous solutions of amino acids are of current interest as a means of increasing our understanding of complex biochemical systems [1–3]. Amino acids and urea are attractive model compounds for studies on multicomponent liquid mixtures that mimic naturally occurring biological systems. It is well known that urea is a strong denaturant of globular proteins [4–6] and can affect the structure of water [7]. The interactions of sparingly soluble solutes in aqueous solutions of urea are of contemporary interest at both the theoretical and practical level [8]. There has been significant interest in the investigation of interactions between zwitterionic α-amino acids and different molecules of biological importance, based on the McMillan–Mayer approach [9–11]. Recently, the thermodynamic properties of biologically important compounds such as small peptides [12], amines [13], and guanidinium hydrochloride [14,15] in aqueous urea solutions have been reported. The amino acid–urea interactions are of great importance in biophysical chemistry, since they concern denaturation processes, and are often used in perturbative approaches to gain insight into the stability of proteins.

Continuing our investigations on the enthalpic interactions of some typical amino acids in aqueous binary solutions [16–19], we turn our attention to aqueous urea solutions. L-alanine and L-serine were chosen as representative amino acids with non-polar and polar side-groups. The enthalpies of dilution of the two amino acids and the calculated homogeneous enthalpic interaction coefficients in various aqueous urea solutions are reported. The results are interpreted from the point of view of solute–solute and solute–solvent interactions.

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2. Experimental

2.1. Reagents

Biochemical reagent grade L-alanine and L-serine were used after recrystallization from methanol–water mixtures and drying in vacuum over P_2O_5 at room temperature for at least 72 h. Analytical reagent grade urea was recrystallized from ethanol and dried under vacuum for 72 h at 330 K. The water used for the preparation of solutions was deionized and distilled using a quartz sub-boiling purifier. Both the aqueous urea solutions (water+urea) which were used as solvents or diluents and the amino acid solutions (amino acid+water+urea) were prepared by mass.

2.2. Calorimetric procedure

The heat of dilution was measured with a mixing-flow microcalorimeter (LKB-2277 BioActivity Monitor). All measurements were carried out at 298.15 K. The solutions were pumped through the mixing-cell at constant rates by a pair of microperpex peristaltic pumps (LKB-2132). The flow rates were determined by weighing the masses of the liquids through each pump within 3 min. The liquids passing through pumps A and B were changed in the following sequence:

1. A(aqueous urea solution)+B(aqueous urea solution) — baseline determined
2. A(aqueous urea solution)+B(amino acid solution) — dilution thermal power determined
3. A(aqueous urea solution)+B(aqueous urea solution) — baseline re-established

The enthalpies of dilution $\Delta_{\mathrm{dil}}H_{\mathrm{m}}$ were calculated from the equation

$$\Delta_{\mathrm{dil}}H_{\mathrm{m}} = \frac{P(1 + m_{\mathrm{i}}M)}{m_{\mathrm{i}}f_2} \tag{1}$$

in which P is the dilution thermal power (μW), M the molar mass of amino acid (kg mol^{-1}) and f_2 is the flow rate of amino acid solution (mg s^{-1}). The final molality m_{f} was calculated from the equation

$$m_{\mathrm{f}} = \frac{m_{\mathrm{i}}f_2}{[f_1(m_{\mathrm{i}}M + 1) + f_2]} \tag{2}$$

in which f_1 is the flow rate of diluent (aqueous urea solution).

3. Results and discussion

The thermodynamics formalism used to treat the enthalpies of dilution is based on the excess enthalpy concept. The excess enthalpy h^{E} of a binary solution is defined by

$$h^{\mathrm{E}} = h - h_{\mathrm{w}}^{*} - mH_2 \tag{3}$$

where h is the enthalpy of a solution of molality m, h_{w}^{*} the enthalpy of water, and H_2 is the partial molar enthalpy of the solution at infinite dilution. The excess enthalpy can be expressed as a power series in the solution molality.

$$h^{\mathrm{E}} = h_2m^2 + h_3m^3 + h_4m^4 + \cdots \tag{4}$$

where h_2 is the enthalpic interaction coefficient which represents the interaction between pairs of solvated solute molecules, h_3 relates to triplet interactions, and h_4 relates to quartet interactions. For the dilution of a solution of initial molality m_{i} to give a solution of final molality m_{f}, the molar enthalpy of dilution $\Delta_{\mathrm{dil}}H_{\mathrm{m}}$ is given by

$$\Delta_{\mathrm{dil}}H_{\mathrm{m}} = \frac{h^{\mathrm{E}}(m_{\mathrm{f}})}{m_{\mathrm{f}}} - \frac{h^{\mathrm{E}}(m_{\mathrm{i}})}{m_{\mathrm{i}}} \tag{5}$$

where $h^{\mathrm{E}}(m_{\mathrm{f}})$ is the excess enthalpy for the solution of molality m_{f} and $h^{\mathrm{E}}(m_{\mathrm{i}})$ is that for the solution of molality m_{i}. From Eqs. (4) and (5), it follows that

$$\Delta_{\mathrm{dil}}H_{\mathrm{m}} = h_2(m_{\mathrm{f}} \boxminus m_{\mathrm{i}}) \boxplus h_3(m_{\mathrm{f}}^2 \boxminus m_{\mathrm{i}}^2) \boxplus h_4(m_{\mathrm{f}}^3 \boxminus m_{\mathrm{i}}^3) + \cdots \tag{6}$$

or

$$\frac{\Delta_{\mathrm{dil}}H_{\mathrm{m}}}{(m_{\mathrm{f}} \boxminus m_{\mathrm{i}})} = h_2 \boxplus h_3(m_{\mathrm{f}} + m_{\mathrm{i}}) \boxplus h_4(m_{\mathrm{f}}^2 + m_{\mathrm{i}}m_{\mathrm{f}} + m_{\mathrm{i}}^2) + \cdots \tag{7}$$

In the present work, urea–water mixtures are treated wholly as solvents. The experimental values $\Delta_{\mathrm{dil,exp}}H_{\mathrm{m}}$ of alanine and serine together with the initial and final molality in various aqueous urea solutions are given in Tables 1 and 2, respectively. The results were fitted to Eq. (7) using a least-squares procedure and the enthalpic interaction coefficients are listed in Tables 3 and 4. The calculated values $\Delta_{\mathrm{dil,calc}}H_{\mathrm{m}}$ according to Eq. (6) are also listed in Tables 1 and 2. The variations in h_2 coefficients for the two amino acids with the mass fractions of urea in solutions are

Table 1
Enthalpies of dilution of L-alanine in aqueous urea solutions at 298.15 K

m_i (mol kg^{-1})	m_f (mol kg^{-1})	$\Delta_{dil,exp}H_m$ (J mol^{-1})	$\Delta_{dil,calc}H_m$ (J mol^{-1})	m_i (mol kg^{-1})	m_f (mol kg^{-1})	$\Delta_{dil,exp}H_m$ (J mol^{-1})	$\Delta_{dil,calc}H_m$ (J mol^{-1})
wt=2.5%							
0.2022	0.1005	−25.32	−25.59	0.6024	0.2941	−76.61	−77.37
0.2022	0.1344	−16.56	−16.68	0.6024	0.3959	−51.60	−51.22
0.4009	0.1307	−68.50	−68.93	0.8066	0.2569	−137.44	−137.40
0.4009	0.1975	−51.61	−51.49	0.8066	0.3904	−102.59	−103.03
0.4009	0.2650	−34.07	−33.95	0.8066	0.5270	−68.08	−68.34
0.5021	0.1627	−86.01	−86.28	1.0022	0.3157	−169.77	−169.19
0.5021	0.2462	−64.80	−64.54	1.0022	0.4810	−125.70	−126.92
0.5021	0.3309	−43.10	−42.66	1.0022	0.6511	−84.90	−84.23
0.6024	0.1941	−103.81	−103.31				
wt=5.0%							
0.2019	0.0694	−47.23	−47.11	0.5973	0.2966	−97.05	−96.90
0.2019	0.1017	−35.58	−35.43	0.5973	0.3901	−65.63	−65.80
0.2019	0.1332	−24.22	−24.15	0.8109	0.2767	−167.60	−167.44
0.3918	0.1357	−87.11	−87.37	0.8109	0.4018	−126.00	−125.76
0.3918	0.1964	−66.25	−66.01	0.8109	0.5274	−85.27	−85.54
0.3918	0.2562	−45.41	−45.37	0.9566	0.3241	−193.00	−192.89
0.5973	0.2052	−127.90	−128.21	0.9566	0.6200	−98.13	−98.26
wt=7.5%							
0.2018	0.0647	−44.10	−43.59	0.6029	0.1889	−128.33	−127.67
0.2018	0.0987	−32.31	−32.71	0.6029	0.2897	−95.13	−95.98
0.2018	0.1332	−21.54	−21.73	0.6029	0.3933	−63.53	−63.85
0.3992	0.1265	−86.41	−85.41	0.8061	0.2496	−170.78	−168.85
0.3992	0.1936	−63.56	−64.16	0.8061	0.3840	−126.21	−127.01
0.3992	0.2620	−42.27	−42.65	0.8061	0.5227	−84.18	−84.53
0.4999	0.1575	−108.47	−106.42	1.0081	0.3085	−211.52	−208.75
0.4999	0.2413	−79.83	−79.98	1.0081	0.4760	−157.81	−157.08
0.4999	0.3271	−53.08	−53.19	1.0081	0.6498	−103.59	−104.56
wt=10.0%							
0.2090	0.0735	−40.45	−40.68	0.5894	0.3856	−66.32	−66.78
0.2090	0.1061	−31.01	−30.82	0.8024	0.3995	−132.60	−132.39
0.2090	0.1386	−20.88	−20.88	0.8024	0.5198	−92.38	−92.33
0.4066	0.2049	−63.93	−64.40	1.0263	0.3443	−220.17	−220.23
0.4066	0.2678	−44.62	−44.31	1.0263	0.5087	−165.08	−165.66
0.5894	0.2024	−126.63	−126.15	1.0263	0.6683	−113.02	−112.43
0.5894	0.2919	−97.44	−97.47				
wt=15.0%							
0.2004	0.0993	−34.38	−34.86	0.6126	0.3013	−113.94	−113.88
0.2004	0.1326	−23.50	−23.53	0.6126	0.4032	−76.75	−76.93
0.2975	0.0972	−70.29	−70.04	0.8010	0.2585	−197.58	−197.52
0.2975	0.1468	−53.38	−53.14	0.8010	0.3908	−148.01	−149.76
0.2975	0.1962	−36.38	−35.95	0.8010	0.5243	−101.02	−100.78
0.4007	0.1302	−97.14	−96.25	1.0126	0.3230	−247.66	−247.39
0.4007	0.1968	−72.49	−73.18	1.0126	0.4897	−186.13	−186.51
0.4007	0.2635	−49.36	−49.60	1.0126	0.6588	−125.37	−124.38
0.6126	0.1998	−152.48	−149.95				
wt=20.0%							
0.2041	0.0696	−51.02	−51.53	0.6174	0.4120	−83.10	−82.98
0.2041	0.1042	−38.95	−38.50	0.8119	0.2715	−220.09	−216.50
0.2041	0.1374	−25.80	−25.81	0.8119	0.4045	−160.81	−163.27

Table 1 (*Continued*)

m_i (mol kg^{-1})	m_f (mol kg^{-1})	$\Delta_{dil,exp}H_m$ (J mol^{-1})	$\Delta_{dil,calc}H_m$ (J mol^{-1})	m_i (mol kg^{-1})	m_f (mol kg^{-1})	$\Delta_{dil,exp}H_m$ (J mol^{-1})	$\Delta_{dil,calc}H_m$ (J mol^{-1})
0.4076	0.1383	−107.40	−106.47	0.8119	0.5390	−110.21	−108.91
0.4076	0.2055	−79.24	−80.41	1.0072	0.3323	−265.32	−266.22
0.4076	0.2727	−53.76	−53.97	1.0072	0.4986	−198.30	−199.20
0.6174	0.2078	−166.81	−164.31	1.0072	0.6628	−133.08	−132.96
0.6174	0.3104	−122.11	−123.76				
wt=25.0%							
0.2022	0.0999	−41.97	−41.74	0.6041	0.1937	−178.00	−179.44
0.2022	0.1337	−28.28	−28.22	0.8062	0.3880	−178.93	−181.96
0.2022	0.0664	−54.15	−54.89	0.8062	0.5236	−124.10	−121.86
0.4042	0.1980	−89.08	−89.14	0.8062	0.2556	−236.06	−239.60
0.4042	0.2656	−61.18	−60.50	1.0006	0.4776	−214.73	−217.21
0.4042	0.1311	−118.18	−116.62	1.0006	0.6461	−144.31	−142.82
0.6041	0.2933	−135.58	−137.10	1.0006	0.3137	−289.15	−289.45
0.6041	0.3946	−94.25	−92.76				
wt=30.0%							
0.2053	0.0683	−59.63	−58.70	0.6135	0.4043	−98.83	−98.48
0.2053	0.1027	−44.49	−44.41	0.8166	0.2625	−258.36	−256.23
0.2053	0.1369	−29.43	−29.89	0.8166	0.3979	−190.71	−193.57
0.4033	0.1327	−122.89	−122.55	0.8166	0.5350	−126.98	−128.98
0.4033	0.2000	−92.47	−93.21	1.0030	0.3190	−309.62	−306.87
0.4033	0.2674	−62.95	−62.97	1.0030	0.4849	−229.30	−229.17
0.6135	0.1995	−194.15	−192.39	1.0030	0.6536	−150.49	−150.04
0.6135	0.3016	−147.49	−146.26				
wt=35.0%							
0.2009	0.0991	−46.40	−47.05	0.6038	0.1920	−195.67	−192.18
0.2009	0.1305	−33.03	−32.62	0.8015	0.3838	−188.47	−191.63
0.2009	0.0654	−62.13	−62.51	0.8015	0.5201	−126.75	−127.95
0.4042	0.1977	−95.35	−96.64	0.8015	0.2519	−256.31	−253.41
0.4042	0.2610	−67.20	−67.12	1.0078	0.4784	−234.10	−235.01
0.4042	0.1299	−129.79	−128.02	1.0078	0.6500	−156.20	−155.64
0.6038	0.2917	−144.72	−145.64	1.0078	0.3131	−316.36	−312.42
0.6038	0.3941	−97.39	−97.64				
wt=40.0%							
0.2023	0.0680	−68.59	−69.38	0.7993	0.2596	−268.49	−265.64
0.2023	0.1018	−51.48	−52.03	0.7993	0.3919	−197.31	−197.25
0.2023	0.1356	−35.26	−34.57	0.7993	0.5267	−129.24	−128.92
0.4000	0.1329	−138.38	−138.29	1.0012	0.3216	−313.82	−316.24
0.4000	0.1995	−105.40	−103.79	1.0012	0.4867	−229.07	−231.70
0.6044	0.1985	−203.27	−206.74	1.0012	0.6560	−150.33	−148.56
0.6044	0.4005	−101.70	−102.18				

illustrated in Fig. 1. The values of h_2 for L-alanine and L-serine in pure water are 209.6 and −740.3 J kg mol^{-2}, respectively, which are in good agreement with those of other workers [20]. As there are some difficulties in the interpretation of the higher h coefficients, only the pairwise coefficients h_2 are considered here.

It is generally accepted that the h coefficients are attributable to the interactions between two solvated solute molecules and very sensitive to solvent variation. Two effects may give contributions to the enthalpic interaction coefficients: one results from the electrostatic interaction and the other from the structural interaction. For the pairwise interaction of two

Table 2
Enthalpies of dilution of L-serine in aqueous urea solutions at 298.15 K

m_i (mol kg^{-1})	m_f (mol kg^{-1})	$\Delta_{dil,exp}H_m$ (J mol^{-1})	$\Delta_{dil,calc}H_m$ (J mol^{-1})	m_i (mol kg^{-1})	m_f (mol kg^{-1})	$\Delta_{dil,exp}H_m$ (J mol^{-1})	$\Delta_{dil,calc}H_m$ (J mol^{-1})
wt=5.0%							
0.1996	0.0985	59.20	58.60	0.6027	0.1914	210.47	208.59
0.1996	0.1318	38.40	38.89	0.8045	0.3849	196.58	194.67
0.1996	0.0652	80.04	78.74	0.8045	0.5203	130.05	128.17
0.4019	0.1963	107.86	109.39	0.8045	0.2521	266.18	264.57
0.4019	0.2636	71.18	72.21	1.0061	0.4766	232.38	234.61
0.4019	0.1294	145.89	147.85	1.0061	0.6463	154.06	155.18
0.6027	0.2914	154.45	153.70	1.0061	0.3110	317.69	318.31
0.6027	0.3925	101.50	101.16				
wt=10.0%							
0.2123	0.0725	68.37	68.20	0.6067	0.4058	68.95	67.72
0.2123	0.1075	49.57	49.97	0.7688	0.2549	177.22	177.66
0.2123	0.1427	33.10	32.46	0.7688	0.3859	127.39	125.81
0.4072	0.1377	112.38	113.13	0.7688	0.5115	80.94	81.75
0.4072	0.2059	79.75	81.13	0.9355	0.3050	211.63	210.77
0.4072	0.2733	51.42	51.93	0.9355	0.4671	150.36	150.60
0.6067	0.2036	148.67	149.35	0.9355	0.6211	99.48	99.99
0.6067	0.3051	107.15	106.13				
wt=15.0%							
0.1993	0.0654	50.51	49.83	0.6010	0.2912	70.54	71.42
0.1993	0.0986	36.28	36.38	0.6010	0.3917	44.44	45.09
0.1993	0.1317	23.49	23.71	0.8064	0.2581	121.19	120.95
0.4002	0.1295	80.07	81.06	0.8064	0.3910	86.20	85.14
0.4002	0.1959	56.94	57.66	1.0128	0.3198	150.52	149.35
0.4002	0.2626	37.12	36.69	1.0128	0.4861	110.62	109.37
0.5022	0.1614	93.09	92.44	1.0128	0.6556	74.072	75.25
0.5022	0.2446	66.04	65.08				
wt=20.0%							
0.2045	0.1042	27.16	26.82	0.6016	0.3970	30.21	29.78
0.2074	0.1064	26.89	26.89	0.8035	0.2781	77.50	78.18
0.4058	0.1426	55.48	56.19	0.8035	0.4038	55.47	54.79
0.4058	0.2066	39.14	39.75	0.8035	0.5285	36.49	35.96
0.4058	0.2686	25.72	25.72	1.0008	0.3456	94.79	95.42
0.6016	0.2102	66.46	67.21	1.0008	0.4997	69.84	70.51
0.6016	0.3037	47.63	46.82				
wt=25.0%							
0.2027	0.0676	17.54	17.51	0.6062	0.3994	3.88	3.81
0.2027	0.1016	12.23	12.29	0.8103	0.2594	13.01	13.20
0.2027	0.1354	7.75	7.66	0.8103	0.3938	5.70	5.64
0.4043	0.1330	20.64	20.92	0.8103	0.5302	2.39	2.36
0.4043	0.2006	13.04	13.17	1.0023	0.3169	15.89	15.66
0.4043	0.2682	7.35	7.28	1.0023	0.4825	9.61	9.50
0.6062	0.1967	16.98	17.19	1.0023	0.6516	7.57	7.71
0.6062	0.2977	9.03	8.87				
wt=30.0%							
0.2009	0.0667	11.15	11.07	0.6099	0.3989	−2.63	−2.60
0.2009	0.1001	8.58	8.72	0.8036	0.2545	−11.42	−11.54
0.3054	0.1000	10.67	10.60	0.8036	0.3865	−11.56	−11.75
0.3054	0.1507	7.80	7.77	0.8036	0.5221	−8.59	−8.47
0.4535	0.1470	6.24	6.34	1.0232	0.3213	−20.94	−20.78

Table 2 (*Continued*)

m_i (mol kg^{-1})	m_f (mol kg^{-1})	$\Delta_{dil,exp}H_m$ (J mol^{-1})	$\Delta_{dil,calc}H_m$ (J mol^{-1})	m_i (mol kg^{-1})	m_f (mol kg^{-1})	$\Delta_{dil,exp}H_m$ (J mol^{-1})	$\Delta_{dil,calc}H_m$ (J mol^{-1})
0.4535	0.2221	3.56	3.55	1.0232	0.4887	−18.13	−18.17
0.6099	0.1957	−1.07	−1.07	1.0232	0.6616	−11.29	−11.43
0.6099	0.2962	−3.10	−3.07				
wt=35.0%							
0.3059	0.0986	−8.10	−8.16	0.5998	0.3903	−16.23	−16.37
0.3059	0.1493	−6.83	−6.94	0.8059	0.2512	−42.84	−42.00
0.3059	0.2011	−5.20	−5.19	0.8059	0.3835	−34.08	−34.16
0.4051	0.1297	−14.01	−13.91	0.8059	0.5207	−23.47	−23.85
0.4051	0.1968	−11.64	−11.76	1.0024	0.3084	−54.16	−53.20
0.4051	0.2654	−8.80	−8.64	1.0024	0.4723	−41.47	−41.94
0.5998	0.1896	−27.85	−27.37	1.0024	0.6433	−28.08	−27.91
0.5998	0.2884	−22.50	−22.74				
wt=40.0%							
0.2018	0.0711	−9.38	−9.47	0.6134	0.4150	−26.90	−26.75
0.2018	0.1051	−7.43	−7.48	0.8108	0.2745	−70.80	−69.58
0.3048	0.1579	−14.00	−14.15	0.8108	0.4098	−52.80	−53.67
0.3048	0.2083	−9.91	−9.86	0.8108	0.5451	−35.20	−35.67
0.4001	0.1391	−27.40	−26.96	1.0177	0.3400	−85.00	−84.25
0.4001	0.2063	−21.60	−21.41	1.0177	0.5094	−61.80	−62.56
0.6134	0.2103	−49.80	−49.68	1.0177	0.6798	−39.70	−39.17
0.6134	0.3130	−38.70	−39.09				

Table 3
Enthalpic pair interaction coefficients of L-alanine in aqueous urea solutions at 298.15 K

Mass fraction of urea (%)	h_2 (J kg mol^{-2})	h_3 (J kg^2 mol^{-3})	h_4 (J kg^3 mol^{-4})	m_i (mol kg^{-1})
2.5	264.3	−9.0	−3.2	0.20∼1.00
5.0	368.6	−148.9	78.1	0.20∼1.00
7.5	322.7	−17.6	−0.8	0.20∼1.00
10.0	271.3	104.5	−60.0	0.20∼1.00
15.0	323.1	84.6	−53.0	0.20∼1.00
20.0	364.8	78.9	−52.1	0.20∼1.00
25.0	368.3	157.1	−108.5	0.20∼1.00
30.0	389.5	168.2	−114.3	0.20∼1.00
35.0	450.0	52.7	−48.9	0.20∼1.00
40.0	505.7	58.8	−82.3	0.20∼1.00

Table 4
Enthalpic pair interaction coefficients of L-serine in aqueous urea solutions at 298.15 K

Mass fraction of urea (%)	h_2 (J kg mol^{-2})	h_3 (J kg^2 mol^{-3})	h_4 (J kg^3 mol^{-4})	m_i (mol kg^{-1})
5.0	−635.8	202.7	−62.7	0.20∼1.00
10.0	−584.4	380.2	−176.7	0.20∼1.00
15.0	−466.9	399.8	−193.8	0.20∼1.00
20.0	−361.4	344.7	−19.3	0.20∼1.00
25.0	−200.3	293.9	−147.8	0.20∼1.00
30.0	−101.6	199.4	−88.8	0.20∼1.00
35.0	−7.5	138.8	−69.4	0.20∼1.00
40.0	28.7	182.7	−101.8	0.20∼1.00

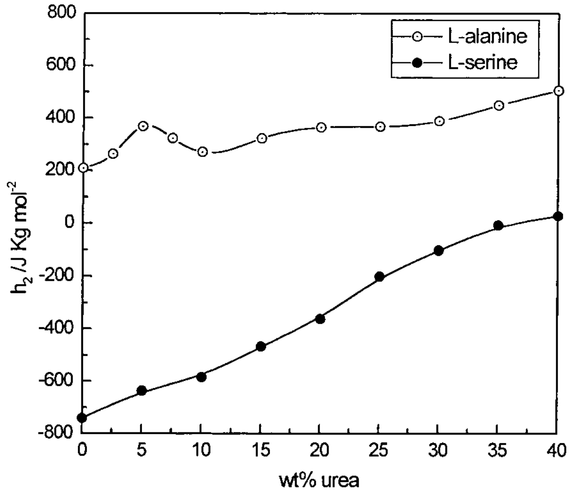

Fig. 1. Variation of enthalpic pair interaction coefficients of L-alanine and L-serine in aqueous urea solutions at 298.15 K.

hydrated amino acid molecules, the most probable configuration is one where the positively charged amino group of one zwitterion interacts with the negatively charged carboxyl group of the second molecule in a side-by-side manner. Associated with this electrostatic interaction is a contribution arising from the perturbation of the solvent cospheres [3]. In the process of pairwise interaction of amino acids, there will be a coalescence of the hydration cospheres of the charged groups, with a subsequent relaxation of water to the bulk solvent. If these effects dominate the pairwise interaction a negative h coefficient should be observed. The structural interaction, arising from the side-group on the α-carbon, will also contribute to the pairwise interactions. The exact contribution will depend on the nature of the side-groups involved [17].

The results shown in Fig. 1 support the above views. The h_2 coefficients for alanine are positive while those for serine are negative at lower mass fractions of urea, and the h_2 coefficients for serine are more negative than that for alanine.

For the two amino acids, the h_2 coefficients generally increase with the increasing mass fraction of urea (Fig. 1), which can be attributed to the structure alteration of the binary solutions (urea+water). Urea partakes in the formation of urea–water clusters that are responsible for the formation of interstices in the solution which accommodate the non-polar moieties of amino acids [21]. The effect of urea on the strength of the hydrophobic bond is to weaken the hydrophobic bonding in all cases except for CH_4 and C_2H_6 molecules [22]. The geometric sizes of hydrophobic moieties of serine and alanine ($-CH_2-$ and $-CH_2CH_3$) are similar to those of CH_4 and C_2H_6 molecules and hence the exacting geometric requirements for the formation of the interstices that accommodate these moieties can also be satisfied. This effect strengthens the hydrophobic bonding for the non-polar moieties of alanine and serine upon increasing addition of urea. On the other hand, the addition of urea to water raises the dielectric constant [21] and results in weakened electrostatic interaction. These two effects lead to the trends of h_2 coefficients of the two amino acids (Fig. 1).

For alanine in aqueous urea solutions, the maximum value of h_2 is at about 5% urea (ca. 0.9 mol kg^{-1}). Frank and Franks [23] have proposed that the effect of urea on the water structure is rather subtle in the sense that it acts as a statistical structure breaker, unlike the structure-breaking action by ions of common electrolytes. In the water-rich region (<2 mol kg^{-1}), urea molecules do not enhance the formation of 'bulky' ice-like structures in water, but possibly occupy interstitial spaces forming a regular solution. The non-polar moieties of amino acids may also enter these interstices and enhance the hydrophobic interactions. Beyond the maximum point, urea–water and urea–urea clusters begin to form [24], leading to the weakening of hydrophobic interactions.

At higher mass fractions of urea (>40%, ~6.7 mol kg^{-1}), the h_2 coefficients for serine become positive, which also serves as evidence for the structure-breaking effect on water of urea and the prevailing of hydrophobic hydration over the electrostatic interaction. Far away from the region rich in water, the structure-breaking effect of urea increases appreciably with increasing urea concentration until almost reaching the saturation limit around 7 mol kg^{-1} [25] where the ratio of water to urea is about 6:1. At urea concentrations above 7 mol kg^{-1}, the urea-water clusters undergo disruption possibly owing to increasing packing imbalance [26] and can result in the collapse of protein quaternary structure [27]. Solutions of about 7 mol kg^{-1} of urea are often used as a denaturation reagent of proteins.

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