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Enthalpic Interaction Coefficients of *N*-(*N*- Glycylglycyl)glycine in Aqueous Sodium Chloride and Potassium Chloride Solutions at 298.15 K[†]

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The enthalpies of dilution have been measured for *N*-(*N*-glycylglycyl)glycine in aqueous sodium chloride and potassium chloride solutions at 298.15 K using a mixing-flow microcalorimeter. The enthalpic interaction coefficients h_2, h_3, and h_4 in the studied aqueous solutions have been calculated according to the McMillan−Mayer model. The results show that the enthalpic pairwise interaction coefficients h_2 of *N*-(*N*-glycylglycyl)glycine in both electrolyte solutions are negative and become less negative as the molality of sodium chloride or potassium chloride increases. The value of h_2 in aqueous sodium chloride solutions is less than that in aqueous potassium chloride solutions of the same molality. The effects of the two salts on h_2 are discussed from the point of view of electrostatic and structural interactions.

Introduction

It has been known for a very long time that different salts have different effects on the stability of globular protein structures.[1] However, globular proteins are extremely complicated molecules, and their interactions with salts in aqueous solutions must necessarily be multifarious and also in a general sense are environmentally mediated. Because of the complex conformational and configurational three-dimensional structures of proteins, small solutes that incorporate some of the structural features found in proteins, such as amino acids, oligopeptides, and acylamides, have been used as model compounds for specific aspects of proteins in aqueous solution.[2−5] Most previous studies on oligopeptides have been restricted to pure water.[6−9] However, biological fluids are not pure water, and the properties of proteins, such as their structure, solubility, denaturation, activity of enzymes, etc., are greatly influenced by electrolytes.[10−13] As an extension of our earlier work,[14] this paper presents experimental information on the interactions of *N*-(*N*-glycylglycyl)glycine (hereafter called tripeptide) with sodium chloride and potassium chloride in aqueous solutions at 298.15 K. The main objective of the present work was to see whether there is significant energetic discrimination between tripeptide and *N*-glycylglycine (dipeptide) for the two salts. Protein is a kind of bioactive macromolecular compound composed of many peptide-bonded amino acids. Therefore, the number of peptide bonds should have an important influence on the interactions between protein molecules. The conclusions obtained from this paper will aid in the understanding of the stabilization mechanism of proteins in body fluids.

Experimental Section

Reagents. Tripeptide was purchased from Tokyo Chemical Industry Co., Ltd. (Japan), and its purity was better than 99 %. It was used without further purification. Sodium chloride and potassium chloride were analytical reagents with mass fraction > 99 % obtained from Tianjin Kermel Chemical Reagent Co., Ltd. They were recrystallized from distilled water and dried under reduced pressure at 393 K. All of the reagents were stored over P_2O_5 in a vacuum desiccator for 72 h at room temperature prior to use.

Twice-distilled water was deionized by passage through a quartz sub-boiling purifier before use in the preparation of solutions. All of the solutions were prepared by mass using a Mettler AG 135 balance with precision to ± 0.00001 g. The molality ranges of the aqueous solutions of sodium chloride and potassium chloride were (0 to 0.60) $\text{mol}\cdot\text{kg}^{-1}$. The molality range of the aqueous tripeptide solutions was (0.08 to 0.35) $\text{mol}\cdot\text{kg}^{-1}$. All of the solutions were degassed with ultrasonic waves and used within 12 h of preparation.

Calorimetric Measurements. The enthalpies of dilution for tripeptide in aqueous salt solutions were determined with a 2277-204 measuring cylinder and a Thermometric 2277 thermal activity monitor (Thermometric, Sweden) at 298.15 K. The solutions of tripeptide and mixed solvents were pumped through the mixing-flow vessel of the calorimeter using VS2-10R MIDI dual-channel pumps. The flow rates were determined from the mass of the samples delivered in 6 min. The variation in the flow rates was less than 0.1 % both before and after a complete dilution experiment. Details of this apparatus, associated equipment, and the experimental procedure have been reported elsewhere.[15−17]

The enthalpies of dilution $\Delta_{\text{dil}}H_{\text{m}}$ were calculated from the equation[18]

$$\Delta_{\text{dil}}H_{\text{m}} = -P\frac{(1 + m_i M)}{m_i f_2} \qquad (1)$$

in which P is the dilution thermal power of the solute, m_{i} and M are the initial molality and the molar mass of tripeptide, respectively, and f_2 is the flow rate of the tripeptide solution. The uncertainties in P, m_{i}, and f_2 were $\pm$ 0.2 μW, $\pm$ 0.0001 $\text{mol}\cdot\text{kg}^{-1}$, and $\pm$ 0.002 $\text{mg}\cdot\text{s}^{-1}$, respectively. The relative mean deviations of all of the $\Delta_{\text{dil}}H_{\text{m}}$ values for duplicate runs at each initial molality were within 1 %.

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Table 1. Molar Enthalpies of Dilution ($\Delta_{\mathrm{dil}}H_{\mathrm{m}}$) of Tripeptide in Water and Aqueous Sodium Chloride Solutions at 298.15 K[a]

$\frac{m_{\mathrm{i}}}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m_{\mathrm{f}}}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{\Delta_{\mathrm{dil}}H_{\mathrm{m}}}{\mathrm{J \cdot mol^{-1}}}$	$\frac{\delta}{\mathrm{J \cdot mol^{-1}}}$	$\frac{m_{\mathrm{i}}}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m_{\mathrm{f}}}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{\Delta_{\mathrm{dil}}H_{\mathrm{m}}}{\mathrm{J \cdot mol^{-1}}}$	$\frac{\delta}{\mathrm{J \cdot mol^{-1}}}$
$m(\mathrm{NaCl}) = 0.0000\ \mathrm{mol \cdot kg^{-1}}$							
0.0800	0.0394	41.76 ± 0.38	0.02	0.2200	0.1076	153.15 ± 1.38	−0.03
0.1000	0.0491	58.68 ± 0.53	−0.04	0.2500	0.1216	176.48 ± 1.59	−0.06
0.1200	0.0589	75.09 ± 0.68	−0.03	0.2800	0.1361	198.89 ± 1.79	−0.08
0.1500	0.0735	99.21 ± 0.89	−0.03	0.3000	0.1454	214.42 ± 1.93	−0.11
0.1800	0.0879	123.02 ± 1.11	−0.02	0.3200	0.1550	229.45 ± 2.07	−0.15
0.2000	0.0975	138.51 ± 1.25	−0.03	0.3500	0.1695	252.34 ± 2.27	0.02
$m(\mathrm{NaCl}) = 0.1000\ \mathrm{mol \cdot kg^{-1}}$							
0.0800	0.0392	42.92 ± 0.39	−0.03	0.2200	0.1071	146.04 ± 1.31	−0.05
0.1000	0.0491	58.64 ± 0.53	−0.03	0.2500	0.1212	167.08 ± 1.50	−0.03
0.1200	0.0587	74.20 ± 0.67	−0.02	0.2800	0.1360	186.75 ± 1.68	−0.02
0.1500	0.0733	96.63 ± 0.87	−0.03	0.3000	0.1452	200.90 ± 1.81	−0.01
0.1800	0.0879	118.04 ± 1.06	−0.04	0.3200	0.1547	214.67 ± 1.93	0.01
0.2000	0.0975	132.21 ± 1.19	−0.03	0.3500	0.1686	236.06 ± 2.12	0.05
$m(\mathrm{NaCl}) = 0.2000\ \mathrm{mol \cdot kg^{-1}}$							
0.0800	0.0403	42.53 ± 0.38	−0.19	0.2200	0.1102	140.35 ± 1.26	−0.07
0.1000	0.0505	57.37 ± 0.52	−0.06	0.2500	0.1251	159.42 ± 1.43	−0.14
0.1200	0.0606	71.95 ± 0.65	0.02	0.2800	0.1400	178.22 ± 1.60	−0.18
0.1500	0.0755	93.39 ± 0.84	0.05	0.3000	0.1495	191.48 ± 1.72	−0.14
0.1800	0.0905	113.75 ± 1.02	0.03	0.3200	0.1594	203.98 ± 1.84	−0.09
0.2000	0.1002	127.37 ± 1.15	−0.02	0.3500	0.1737	223.54 ± 2.01	0.11
$m(\mathrm{NaCl}) = 0.3000\ \mathrm{mol \cdot kg^{-1}}$							
0.0800	0.0397	43.94 ± 0.39	−0.07	0.2200	0.1081	142.62 ± 1.28	−0.18
0.1000	0.0496	58.73 ± 0.53	−0.08	0.2500	0.1225	162.58 ± 1.46	−0.20
0.1200	0.0594	73.34 ± 0.66	−0.09	0.2800	0.1371	181.91 ± 1.64	−0.18
0.1500	0.0739	94.97 ± 0.85	−0.12	0.3000	0.1469	194.59 ± 1.75	−0.15
0.1800	0.0889	115.25 ± 1.04	−0.15	0.3200	0.1562	207.83 ± 1.87	−0.10
0.2000	0.0986	128.92 ± 1.16	−0.17	0.3500	0.1704	227.08 ± 2.04	0.02
$m(\mathrm{NaCl}) = 0.4000\ \mathrm{mol \cdot kg^{-1}}$							
0.0800	0.0393	44.56 ± 0.40	0.53	0.2200	0.1070	138.33 ± 1.24	0.12
0.1000	0.0491	57.64 ± 0.52	−0.44	0.2500	0.1213	157.78 ± 1.42	0.12
0.1200	0.0588	71.68 ± 0.65	−0.29	0.2800	0.1358	176.56 ± 1.59	0.07
0.1500	0.0733	92.29 ± 0.83	−0.09	0.3000	0.1452	189.51 ± 1.71	0.02
0.1800	0.0878	112.30 ± 1.01	0.05	0.3200	0.1554	201.27 ± 1.81	−0.05
0.2000	0.0974	125.39 ± 1.13	0.09	0.3500	0.1695	221.08 ± 1.99	0.23
$m(\mathrm{NaCl}) = 0.5000\ \mathrm{mol \cdot kg^{-1}}$							
0.0800	0.0379	37.99 ± 0.34	−0.03	0.2200	0.1035	134.62 ± 1.21	0.08
0.1000	0.0474	52.42 ± 0.47	0.00	0.2500	0.1173	154.42 ± 1.39	0.07
0.1200	0.0568	66.69 ± 0.60	0.03	0.2800	0.1315	173.40 ± 1.56	0.06
0.1500	0.0710	87.37 ± 0.79	0.06	0.3000	0.1406	186.33 ± 1.68	0.04
0.1800	0.0850	107.85 ± 0.97	0.08	0.3200	0.1497	199.20 ± 1.79	0.04
0.2000	0.0941	121.49 ± 1.09	0.08	0.3500	0.1633	218.64 ± 1.97	0.09
$m(\mathrm{NaCl}) = 0.6000\ \mathrm{mol \cdot kg^{-1}}$							
0.0800	0.0400	22.96 ± 0.21	−0.08	0.2200	0.1090	110.23 ± 0.99	−0.52
0.1000	0.0498	36.43 ± 0.33	0.21	0.2500	0.1236	128.96 ± 1.16	0.41
0.1200	0.0560	53.98 ± 0.49	−0.29	0.2800	0.1384	145.93 ± 1.31	0.10
0.1500	0.0747	67.87 ± 0.61	−0.02	0.3000	0.1478	157.57 ± 1.42	−0.23
0.1800	0.0894	86.81 ± 0.78	0.32	0.3200	0.1576	168.99 ± 1.52	−0.15
0.2000	0.0992	99.16 ± 0.89	0.45	0.3500	0.1719	186.67 ± 1.68	0.25

[a] The symbols m_{i} and m_{f} represent the initial and final molalities of tripeptide, respectively; the symbol δ is defined as $\delta = \Delta_{\mathrm{dil}}H_{\mathrm{m}} - \Delta_{\mathrm{dil}}H_{\mathrm{m}}(\mathrm{calcd})$, where $\Delta_{\mathrm{dil}}H_{\mathrm{m}}(\mathrm{calcd})$ was calculated using eq 5 with coefficients obtained by fitting the data at the corresponding $m(\mathrm{NaCl})$.

The final molality m_{f} after dilution was calculated from the equation

$$m_{\mathrm{f}} = \frac{m_i f_2}{f_1(1 + m_i M) + f_2} \qquad (2)$$

in which f_1 is the flow rate of diluent (water or aqueous solution of sodium chloride or potassium chloride).

Results and Discussion

For a solution with molality m, the molar excess enthalpy $H_{\mathrm{m}}^{\mathrm{E}}$ can be written as a power series in m with coefficients h_n:[19]

$$H_{\mathrm{m}}^{\mathrm{E}} = L_{2\phi} = h_2 m + h_3 m^2 + h_4 m^3 + \cdots \qquad (3)$$

where$L_{2\phi}$ is the relative apparent molar enthalpy. The enthalpic interaction coefficients h_n, which are related to the McMillan−Mayer coefficients,[20−22] measure the enthalpic interactions mediated by the solvent occurring between pairs, triplets, and higher-order multiplets of solute species. According to the relationship $L_2 = L_{2\phi} + m(\partial L_{2\phi}/\partial m)_{T,P,n_1}$, we can obtain the following expression for the relative partial molar enthalpy, L_2:

$$L_2 = 2h_2 m + 3h_3 m^2 + 4h_4 m^3 + \cdots \qquad (4)$$

Therefore, $\Delta_{\mathrm{dil}}H_{\mathrm{m}}$, the molar enthalpy of dilution of the solution from an initial molality m_{i} to a final molality m_{f}, is given by:

$$\begin{aligned}\Delta_{\mathrm{dil}}H_{\mathrm{m}} &= H_{\mathrm{m}}^{\mathrm{E}}(m_{\mathrm{f}}) - H_{\mathrm{m}}^{\mathrm{E}}(m_{\mathrm{i}}) \\ &= h_2(m_{\mathrm{f}} - m_{\mathrm{i}}) + h_3(m_{\mathrm{f}}^2 - m_{\mathrm{i}}^2) + \\ &\quad h_4(m_{\mathrm{f}}^3 - m_{\mathrm{i}}^3) + \cdots \end{aligned} \qquad (5)$$

in which $H_{\mathrm{m}}^{\mathrm{E}}(m_{\mathrm{i}})$ and $H_{\mathrm{m}}^{\mathrm{E}}(m_{\mathrm{f}})$ are the molar excess enthalpies of the solute in the solutions before and after dilution, respectively.

Table 2. Molar Enthalpies of Dilution $\Delta_{dil}H_m$ of Tripeptide in Aqueous Potassium Chloride Solutions at 298.15 K[a]

m_i / mol·kg^{-1}	m_f / mol·kg^{-1}	$\Delta_{dil}H_m$ / J·mol^{-1}	δ / J·mol^{-1}	m_i / mol·kg^{-1}	m_f / mol·kg^{-1}	$\Delta_{dil}H_m$ / J·mol^{-1}	δ / J·mol^{-1}
m(KCl) = 0.1000 mol·kg^{-1}							
0.0800	0.0382	44.52 ± 0.40	0.05	0.2200	0.1031	155.12 ± 1.40	−0.04
0.1000	0.0476	60.83 ± 0.55	−0.07	0.2500	0.1170	177.68 ± 1.60	0.07
0.1200	0.0568	77.45 ± 0.70	−0.01	0.2800	0.1311	199.76 ± 1.80	0.10
0.1500	0.0708	101.21 ± 0.91	0.04	0.3000	0.1400	215.15 ± 1.94	0.04
0.1800	0.0848	124.34 ± 1.12	−0.03	0.3200	0.1488	230.81 ± 2.08	0.05
0.2000	0.0945	139.02 ± 1.25	0.04	0.3500	0.1624	253.67 ± 2.28	−0.05
m(KCl) = 0.2000 mol·kg^{-1}							
0.0800	0.0383	43.24 ± 0.39	0.00	0.2200	0.1043	146.70 ± 1.32	−0.02
0.1000	0.0480	58.64 ± 0.53	0.02	0.2500	0.1185	167.28 ± 1.51	−0.03
0.1200	0.0574	74.01 ± 0.67	0.02	0.2800	0.1326	187.65 ± 1.69	−0.03
0.1500	0.0718	96.11 ± 0.86	0.02	0.3000	0.1421	201.02 ± 1.81	−0.02
0.1800	0.0858	118.01 ± 1.06	0.00	0.3200	0.1509	215.21 ± 1.94	−0.02
0.2000	0.0954	131.94 ± 1.19	−0.01	0.3500	0.1645	236.17 ± 2.13	0.03
m(KCl) = 0.3000 mol·kg^{-1}							
0.0800	0.0373	39.88 ± 0.36	−0.02	0.2200	0.0991	148.99 ± 1.34	0.00
0.1000	0.0468	55.21 ± 0.50	0.00	0.2500	0.1118	171.81 ± 1.55	0.01
0.1200	0.0563	70.35 ± 0.63	−0.04	0.2800	0.1259	191.88 ± 1.73	0.02
0.1500	0.0696	93.90 ± 0.85	−0.03	0.3000	0.1374	202.01 ± 1.82	0.02
0.1800	0.0828	117.05 ± 1.05	−0.02	0.3200	0.1460	216.22 ± 1.95	0.01
0.2000	0.0916	132.24 ± 1.19	−0.05	0.3500	0.1594	236.49 ± 2.13	0.00
m(KCl) = 0.4000 mol·kg^{-1}							
0.0800	0.0371	41.28 ± 0.37	0.09	0.2200	0.1012	142.13 ± 1.28	0.01
0.1000	0.0462	56.33 ± 0.51	0.01	0.2500	0.1143	163.39 ± 1.47	−0.01
0.1200	0.0556	70.76 ± 0.64	−0.05	0.2800	0.1280	183.78 ± 1.65	0.03
0.1500	0.0694	92.54 ± 0.83	−0.06	0.3000	0.1371	197.23 ± 1.78	0.03
0.1800	0.0832	113.78 ± 1.02	−0.05	0.3200	0.1462	210.68 ± 1.90	0.01
0.2000	0.0918	128.58 ± 1.16	−0.05	0.3500	0.1595	231.26 ± 2.08	−0.05
m(KCl) = 0.5000 mol·kg^{-1}							
0.0800	0.0362	40.41 ± 0.36	−0.06	0.2200	0.0989	136.83 ± 1.23	0.07
0.1000	0.0452	54.76 ± 0.49	0.00	0.2500	0.1122	156.69 ± 1.41	0.06
0.1200	0.0543	68.91 ± 0.62	0.05	0.2800	0.1254	176.43 ± 1.59	0.04
0.1500	0.0676	89.84 ± 0.81	0.08	0.3000	0.1344	189.33 ± 1.70	0.03
0.1800	0.0810	110.23 ± 0.99	0.07	0.3200	0.1430	202.70 ± 1.82	0.02
0.2000	0.0898	123.79 ± 1.11	0.06	0.3500	0.1562	222.48 ± 2.00	0.03
m(KCl) = 0.6000 mol·kg^{-1}							
0.0800	0.0397	28.02 ± 0.25	0.03	0.2200	0.1083	119.14 ± 1.07	0.03
0.1000	0.0495	41.30 ± 0.37	0.05	0.2500	0.1226	138.82 ± 1.25	0.01
0.1200	0.0595	54.25 ± 0.49	0.05	0.2800	0.1373	157.87 ± 1.42	0.00
0.1500	0.0741	73.95 ± 0.67	0.05	0.3000	0.1470	170.86 ± 1.54	0.00
0.1800	0.0891	92.96 ± 0.84	0.05	0.3200	0.1563	184.33 ± 1.66	−0.01
0.2000	0.0986	106.19 ± 0.96	0.04	0.3500	0.1707	204.01 ± 1.84	0.01

[a] The symbols m_i and m_f represent the initial and final molalities of tripeptide, respectively; the symbol δ is defined as $\delta = \Delta_{dil}H_m - \Delta_{dil}H_m$(calcd), where $\Delta_{dil}H_m$(calcd) was calculated using eq 5 with coefficients obtained by fitting the data at the corresponding m(KCl).

Table 3. Enthalpic Interaction Coefficients of Tripeptide in Water and Aqueous Sodium Chloride Solutions at 298.15 K[a]

m(NaCl) / mol·kg^{-1}	h_2 / J·kg·mol^{-2}	h_3 / J·kg^2·mol^{-3}	h_4 / J·kg^3·mol^{-4}	SD / J·mol^{-1}
0.0000	−1898.6 ± 14.5	1090.7 ± 49.3	−1117.5 ± 65.6	0.09
0.1000	−1855.2 ± 4.6	1340.0 ± 15.7	−1377.5 ± 20.8	0.03
0.2000	−1757.7 ± 58.1	1079.2 ± 195.4	−943.8 ± 258.6	0.35
0.3000	−1637.9 ± 43.9	719.7 ± 148.7	−498.1 ± 197.6	0.27
0.4000	−1561.8 ± 57.3	807.0 ± 194.9	−804.4 ± 260.2	0.35
0.5000	−1504.6 ± 20.3	603.0 ± 69.5	−466.0 ± 93.4	0.13
0.6000	−1425.3 ± 47.7	510.6 ± 159.8	−310.9 ± 212.4	0.34

[a] The h_n values are reported as value ± standard error, the latter of which was given by the computer during the multiple linear regression analysis. SD = standard derivation.

Table 4. Enthalpic Interaction Coefficients of Tripeptide in Aqueous Potassium Chloride Solutions at 298.15 K[a]

m(KCl) / mol·kg^{-1}	h_2 / J·kg·mol^{-2}	h_3 / J·kg^2·mol^{-3}	h_4 / J·kg^3·mol^{-4}	SD / J·mol^{-1}
0.1000	−1751.2 ± 13.4	879.6 ± 46.0	−895.0 ± 61.6	0.08
0.2000	−1697.0 ± 9.0	898.2 ± 30.8	−794.8 ± 41.2	0.06
0.3000	−1591.2 ± 8.0	519.2 ± 28.5	−236.8 ± 38.9	0.05
0.4000	−1521.4 ± 10.4	571.2 ± 35.8	−471.5 ± 48.3	0.06
0.5000	−1451.2 ± 7.5	641.6 ± 26.0	−622.7 ± 35.4	0.05
0.6000	−1381.7 ± 7.7	345.2 ± 25.9	−387.6 ± 34.4	0.05

[a] The h_n values are reported as value ± standard error, the latter of which was given by the computer during the multiple linear regression analysis. SD = standard derivation.

In this work, pure water and the salt solutions are regarded wholly as “solvents”. Tables 1 and 2 give the experimental values of $\Delta_{dil}H_m$, which were fitted to eq 5 by a least-squares procedure using Origin 6.0 software with the intercept locked to 0, together with the differences between the experimental values and the corresponding calculated values as well as the initial and final molalities of tripeptide in various aqueous sodium chloride and potassium chloride solutions, respectively. The enthalpic pairwise interaction coefficients h_2 calculated from eq 5 using the least-squares method for NaCl and KCl solutions are presented in Tables 3 and 4, respectively. The homogeneous enthalpic pairwise parameter for tripeptide in pure water is − 1898.6 J·kg·mol^{-2}, which is in good agreement with that obtained by Lilley et al. (−1899 J·kg·mol^{-2}).[23] Figures 1 and 2 show the relationship between relative partial molar enthalpy

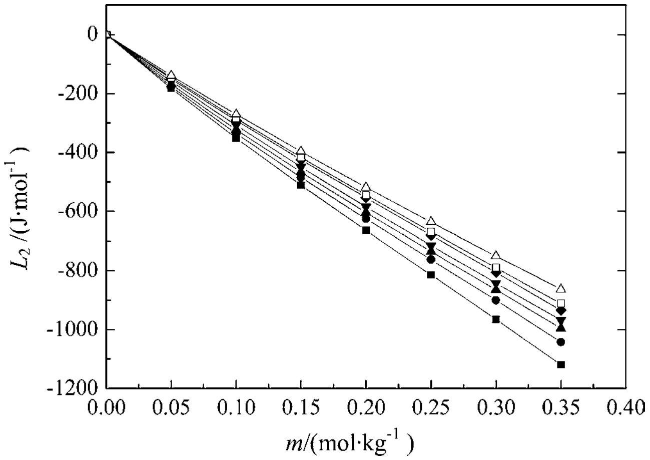

Figure 1. Relative partial molar enthalpy L_2 of tripeptide vs the tripeptide molality m in aqueous sodium chloride solutions at 298.15 K: ■, m(NaCl) = 0.0000 mol·kg^{-1}; ●, m(NaCl) = 0.1000 mol·kg^{-1}; ▲, m(NaCl) = 0.2000 mol·kg^{-1}; ▼, m(NaCl) = 0.3000 mol·kg^{-1}; ◆, m(NaCl) = 0.4000 mol·kg^{-1}; □, m(NaCl) = 0.5000 mol·kg^{-1}; △, m(NaCl) = 0.6000 mol·kg^{-1}.

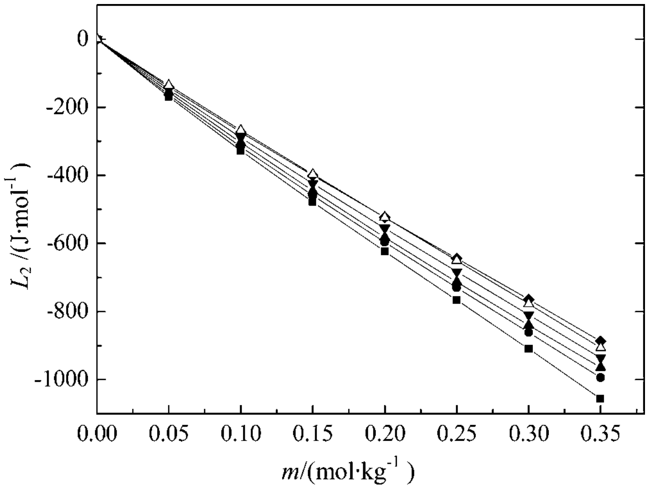

Figure 2. Relative partial molar enthalpy L_2 of tripeptide versus the tripeptide molality m in aqueous potassium chloride solutions at 298.15 K: ■, m(KCl) = 0.1000 mol·kg^{-1}; ●, m(KCl) = 0.2000 mol·kg^{-1}; ▲, m(KCl) = 0.3000 mol·kg^{-1}; ▼, m(KCl) = 0.4000 mol·kg^{-1}; ◆, m(KCl) = 0.5000 mol·kg^{-1}; △, m(KCl) = 0.6000 mol·kg^{-1}.

L_2 and the molality m of tripeptide in aqueous sodium chloride and potassium chloride solutions, respectively. The relationship between L_2 and m is nearly linear in smaller molality ranges, which indicates that the interactions between tripeptide molecules play the dominant role in these solutions.[24] In addition, because of the complexities of the interaction among three or four solute molecules, it is difficult to display their regularities with increasing molality of the aqueous salt solution.[25,26] Thus, only the enthalpic pairwise coefficients h_2 are discussed here.

It is generally accepted that the h_2 coefficients are attributable to the interactions between two solvated solute molecules and very sensitive to solvent variation. The interaction of two solvated species can be represented as the partial dehydration of the solutes and the direct interaction caused by short-range molecular forces.[27]

The types of interaction for the tripeptide−salt−water system discussed here can be classified in detail as follows: (1) partial dehydration of solutes, which leads to a positive contribution to h_2 because of the partial dehydration of the ions' hydration shells and the removal of a number of water molecules from the hydration shell of the polar head of the solute molecules;[28] (2) dipole−dipole interactions between the zwitterionic parts of tripeptide molecules, which lead to a negative contribution to h_2; and (3) electrostatic interactions between the salt ions and the zwitterionic parts of tripeptide molecules, which also lead to a negative contribution to h_2. The resulting sign of h_2 would be a consequence of the competitive equilibrium among the above effects.

First, from Tables 3 and 4 and the corresponding plots in the Supporting Information, it can be seen that the values of the pairwise enthalpic coefficients h_2 for tripeptide in both electrolyte solutions are negative and that the value of h_2 for tripeptide in each aqueous sodium chloride solution is less than that in the aqueous potassium chloride solution of the same molality. The negative values of h_2 testify to the predominance of the dipole−dipole and electrostatic interactions over the partial dehydration of the hydration shell. In addition, Marcus specified that K^+ is a structure-breaking ion while Na^+ is a borderline ion.[29] In other words, the larger size of K^+ makes the interaction between it and water molecules weaker than that between Na^+ and water molecules. Therefore, the dehydration of tripeptide molecules in aqueous potassium chloride solutions is more difficult than that in aqueous sodium chloride solutions, causing the value of h_2 for tripeptide in an aqueous sodium chloride solution to be less than that in an aqueous potassium chloride solution of the same molality.

Second, the enthalpic pairwise interaction coefficient h_2 for tripeptide becomes less negative as the molality of sodium chloride or potassium chloride increases, which can be attributed to the influence of the salt on the enthalpic pairwise interaction. In the tripeptide−salt−water system, hydration sheaths with defined water molecules are formed around dissolved molecules or ions. Mutual interactions between the solute molecules or ions of the electrolyte may cause some water molecules to be squeezed out from the hydration sheaths into the bulk water.[30] Accordingly, the direct interaction between two solute molecules provokes partial dehydration of ions near them, which is an endothermic process (positive contribution to h_2). The higher the molality of sodium chloride or potassium chloride is, the stronger is the associating force of an ion or solute molecule with the water molecules; thus, it is more difficult for water molecules to depart from the ion or solute molecule, and the contribution to the enthalpy is more positive. However, the dominant thermal effect is still the direct interaction between solute molecules. Consequently, the values of h_2 are all negative over the whole investigated molality range of the aqueous salt solutions and become less negative with increasing salt molality. This is different from the change trend of h_2 for dipeptide in aqueous solutions of sodium chloride, which passes through a minimum at about 0.10 mol·kg^{-1} (see the Supporting Information).[14] This occurs mainly because the increase of the electrostatic interactions exceeds that of the dehydration effect for dipeptide when the molality of sodium chloride is less than 0.10 mol·kg^{-1}.

Third, from the variations of the h_2 coefficients for dipeptide[14] and tripeptide with increasing molality of aqueous sodium chloride or potassium chloride (see the Supporting Information), it can be seen that the value of h_2 for tripeptide is more negative than that for dipeptide in aqueous salt solutions of the same molality, which indicates that the mutual approach of tripeptide molecules in water and aqueous salt solutions is more exoergic than that of dipeptide molecules. This is the result of the cooperative effect of two factors: (1) enhancement of the dipole−dipole and electrostatic interactions by the increase in the number of peptide bonds and (2) reorganization of the solvent due to the coalescence of the solvation spheres of the interacting peptide groups.[31,32]

These results provide significant information about the nature of the interactions of the cosolutes with the peptide backbone that will help elucidate the mechanism of interaction of the salts with the proteins, which in turn determines the stability of the proteins.

Supporting Information Available:

Plots of h_2 versus m for the dipeptide and tripeptide in aqueous NaCl and KCl solutions. This material is available free of charge via the Internet at http://pubs.acs.org.

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