Interactions of Some Glycyl Dipeptides with Sodium Butyrate in Aqueous Solutions at 298.15 K: A Volumetric and Conductometric Study

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Densities and conductivity data for the sodium butyrate−glycyl dipeptide (glycylglycine, glycyl-L-valine, glycyl-L-leucine)−water systems were determined at 298.15 K. These data have been used to calculate apparent molar volumes of the dipeptides and limiting molar conductivity (Λ_o) of sodium butyrate. The standard partial molar volumes ($V^o_{2,\phi}$), standard partial molar volumes of transfer for dipeptide from water to aqueous sodium butyrate solutions ($\Delta_t V^o$), the hydration number, and volumetric interaction coefficients have been calculated. An increase in the transfer volumes and a decrease in hydration number of the dipeptide with increasing sodium butyrate concentrations have been explained due to stronger interactions of sodium butyrate with the charged center and glycyl unit of the peptide compared to the sodium butyrate−nonpolar group in peptide interactions. Using the $V^o_{2,\phi}$ values of the amino acids and an additivity relationship, the standard partial molar volumes of the peptides were estimated. The decrease in Λ_o with an increase in dipeptide concentration is attributed to the interaction of sodium butyrate with the dipeptides and the friction resistance of the solvent medium.

Introduction

The study of the interactions between proteins and salt solutions provides an insight into the conformational stability and unfolding behavior of the biopolymers. Salt solutions have large effects on the structure and properties of proteins including their solubility, denaturation, dissociation into subunits, and the activity of enzymes.[1,2] However, for their complete understanding, we need a proper idea for the state and the behavior of the protein in the medium.[3] Because of the structural complexities of proteins and the nonfeasibility of direct thermodynamic studies, amino acids and small peptides are often used as model compounds because they are building blocks of proteins. Small peptides, which contain more complex structure and more components of proteins than amino acids, have attracted wide interest recently. There have been some investigations on thermodynamic properties of some peptides in aqueous solutions[4–9] and aqueous simple salt solutions.[10–20] However, there are only a few studies about properties of peptides in aqueous organic salt solutions,[13,14,21,22] probably due to the complex nature of their interactions.

An organic salt sodium butyrate (NaC_4) has hydrophilic and lipophilic properties. It has many biological characteristics. For example, sodium butyrate can promote the growth of beneficial bacteria and inhibit the growth of harmful bacteria in the gastrointestinal tract. It is an energy source of cell differentiation, which can ensure the repair of injured chorion and accelerate proliferation and maturation of gastrointestinal cells, etc. Sodium butyrate is known to influence the dissociation of proteins in solution[23] and cause a salting-out of nonelectrolytes.[24] On the other hand, the peptides are important molecules due to their wide application in drug production and their role as signal transmitters in cell communications.[2] The systematic study of peptides can provide valuable information about their behavior in solutions and insight into the conformational stability of proteins. For this purpose, in the present paper, we report the studies on the interaction of sodium butyrate with glycyl dipeptides (glycylglycine, glycyl-L-valine, glycyl-L-leucine) by densimetry and conductometry at 298.15 K. The results are discussed in terms of the structural interaction and electrostatic interaction and the hydration model of glycyl dipeptide molecules.

Experimental Section

Chemicals. Glycylglycine (99.0 %, Sigma), glycyl-L-valine (99.0 %, Sigma), and glycyl-L-leucine (99.0 %, Sigma) were twice recrystallized from aqueous ethanol solutions and dried for 24 h under vacuum at room temperature. They were then stored over P_2O_5 in a desiccator before use. Analytical reagent grade sodium butyrate (> 99.0 %, Shanghai Chem. Co.) was twice recrystallized from aqueous ethanol solutions and dried under vacuum at 383 K for 2 days before use. Potassium chloride (99.999 %, Aldrich Chem. Co.) was dried for 2 days at 373 K and was used to determine the conductance cell constant. Water with a conductivity of < 1.0 $\mu\Omega^{-1}\cdot cm^{-1}$ was obtained by distilling deionized water from alkaline $KMnO_4$ to remove any organic matter. All solutions were prepared freshly by weighing on the molality scale.

Apparatus and Procedures. Solution densities were measured to $\pm 3\cdot10^{-6}$ g·cm^{-3} with an Anton Paar DMA 60/602 vibrating-tube digital densimeter that was calibrated daily at 298.15 K using dry air and water with a conductivity of less than 1.0 $\mu\Omega^{-1}\cdot cm^{-1}$. The densimeter was thermostatted using Schott thermostat units, which have a thermal stability of ± 0.005 K.

A conductivity meter (model 145A+, Thermo Orion) with a precision of ± 0.5 % and a conductivity cell (model 011510, Thermo Orion) were used for the measurement of conductivity of each sample. The conductance cell was equipped with a water circulating jacket, and the temperature was controlled within

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Table 1. Solution Densities (ρ) and Apparent Molar Volumes ($V_{2,\phi}$) for the Glycyl Dipeptides in Aqueous Solution as a Function of the Molality of the Glycyl Dipeptides (m_p) at $T = 298.15$ K

Glycylglycine			Glycyl-L-valine			Glycyl-L-leucine		
m_p / mol·kg^{-1}	ρ / g·cm^{-3}	$V_{2,\phi}$ / cm^3·mol^{-1}	m_p / mol·kg^{-1}	ρ / g·cm^{-3}	$V_{2,\phi}$ / cm^3·mol^{-1}	m_p / mol·kg^{-1}	ρ / g·cm^{-3}	$V_{2,\phi}$ / cm^3·mol^{-1}
0.0000	0.999705	-----	0.0000	0.999705	-----	0.0000	0.999705	-----
0.06033	1.003053	76.38	0.02261	1.000877	122.25	0.01944	1.000645	139.75
0.08146	1.004212	76.45	0.03982	1.001763	122.29	0.02958	1.001134	139.75
0.1018	1.005326	76.48	0.06223	1.002900	122.48	0.03950	1.001609	139.78
0.1481	1.007842	76.56	0.07858	1.003731	122.49	0.04928	1.002078	139.78
0.2504	1.013330	76.67	0.1017	1.004894	122.56	0.05700	1.002444	139.83
0.2965	1.015779	76.68	0.1513	1.007378	122.57	0.07128	1.003122	139.84
0.3421	1.018080	77.00	0.1799	1.008775	122.69	0.07696	1.003390	139.86
						0.09125	1.004060	139.92

Table 2. Solution Densities (ρ) and Apparent Molar Volumes ($V_{2,\phi}$) for the Glycyl Dipeptides in Aqueous Sodium Butyrate Solutions as a Function of the Molality of the Glycyl Dipeptides (m_p) and Sodium Butyrate (m_{NaC4}) at $T = 298.15$ K

$m_{NaC4} = 0.5$ mol·kg^{-1}			$m_{NaC4} = 1.0$ mol·kg^{-1}			$m_{NaC4} = 1.5$ mol·kg^{-1}			$m_{NaC4} = 2.0$ mol·kg^{-1}		
m_p / mol·kg^{-1}	ρ / g·cm^{-3}	$V_{2,\phi}$ / cm^3·mol^{-1}	m_p / mol·kg^{-1}	ρ / g·cm^{-3}	$V_{2,\phi}$ / cm^3·mol^{-1}	m_p / mol·kg^{-1}	ρ / g·cm^{-3}	$V_{2,\phi}$ / cm^3·mol^{-1}	m_p / mol·kg^{-1}	ρ / g·cm^{-3}	$V_{2,\phi}$ / cm^3·mol^{-1}
Glycylglycine											
0.0000	1.016306	-----	0.0000	1.033731	-----	0.0000	1.049358	-----	0.0000	1.064029	-----
0.04371	1.018589	79.26	0.02997	1.035230	80.89	0.03309	1.050936	82.47	0.03356	1.065557	83.83
0.06080	1.019479	79.23	0.04358	1.035914	80.76	0.04353	1.051434	82.43	0.04442	1.066052	83.78
0.08197	1.020576	79.23	0.05946	1.036711	80.68	0.05905	1.052175	82.36	0.06026	1.066775	83.70
0.09915	1.021467	79.20	0.08059	1.037761	80.70	0.07914	1.053136	82.26	0.08199	1.067760	83.68
0.1977	1.026527	79.15	0.1018	1.038820	80.63	0.09991	1.054130	82.16	0.1025	1.068679	82.73
0.2515	1.029258	79.13	0.1509	1.041255	80.56	0.1509	1.056553	82.04	0.1511	1.070900	83.47
0.3142	1.032398	79.16	0.2028	1.043807	80.53	0.2019	1.058949	82.02	0.2016	1.073180	83.36
Glycyl-L-valine											
0.0000	1.016313	-----	0.0000	1.033731	-------	0.0000	1.049358	-----	0.0000	1.064029	-----
0.02131	1.017330	125.07	0.02070	1.034684	125.32	0.01980	1.050197	127.42	0.01923	1.064796	128.40
0.04013	1.018233	124.85	0.03876	1.035505	125.47	0.03992	1.051048	127.36	0.04040	1.065636	128.39
0.05917	1.019151	124.62	0.05930	1.036437	125.48	0.06225	1.051996	127.20	0.06126	1.066470	128.23
0.08053	1.020178	124.46	0.08059	1.037411	125.34	0.08085	1.052785	127.10	0.08011	1.067225	128.09
0.1011	1.021168	124.32	0.09878	1.038212	125.52	0.1005	1.053644	126.76	0.1016	1.068075	128.06
0.1543	1.023679	124.29	0.1528	1.040614	125.53	0.1515	1.055788	126.69	0.1500	1.069973	128.00
0.1987	1.025781	124.12	0.1767	1.041644	125.65	0.1946	1.057585	126.62	0.1937	1.071667	127.97
			0.2019	1.042756	125.59						
Glycyl-L-leucine											
0.0000	1.016306	------	0.0000	1.033796	-------	0.0000	1.049358	-------	0.0000	1.064029	-------
0.01941	1.017169	142.04	0.01958	1.034608	143.16	0.02000	1.050126	144.40	0.03010	1.065100	145.33
0.04004	1.018082	142.02	0.02968	1.035024	143.19	0.03023	1.050521	144.28	0.04026	1.065463	145.25
0.06057	1.018989	142.95	0.04027	1.035465	143.07	0.04059	1.050920	144.21	0.05051	1.065832	145.13
0.07994	1.019844	141.87	0.05083	1.035901	143.04	0.05032	1.051294	144.17	0.06052	1.066186	145.13
0.1002	1.020738	141.77	0.06061	1.036313	142.87	0.06082	1.051696	144.15	0.07058	1.066541	145.12
0.1310	1.022079	141.74	0.07067	1.036727	142.86	0.07047	1.052067	144.09	0.08018	1.066877	145.14
0.1609	1.023385	141.63	0.08075	1.037159	142.64	0.07865	1.052382	144.04	0.08993	1.067215	145.17
			0.1043	1.038124	142.65	0.08773	1.052724	144.07			

Table 3. Standard Partial Molar Volumes, $V^o_{2,\phi}$ (cm^3·mol^{-1}), for the Glycyl Dipeptides in Aqueous Sodium Butyrate Solutions at $T = 298.15$ K

m_{NaC4}	0 mol·kg^{-1}	0.5 mol·kg^{-1}	1.0 mol·kg^{-1}	1.5 mol·kg^{-1}	2.0 mol·kg^{-1}
glycylglycine	76.29 ± 0.07 76.23 ± 0.03[a]	79.25 ± 0.02	80.79 ± 0.04	82.45 ± 0.05	83.86 ± 0.04
glycyl-L-valine	122.25 ± 0.05 122.25 ± 0.05[b] 121.99 ± 0.02[b]	124.99 ± 0.10	125.35 ± 0.05	127.49 ± 0.08	128.41 ± 0.06
glycyl-L-leucine	139.69 ± 0.10 139.70 ± 0.07[a]	142.12 ± 0.02	143.35 ± 0.06	144.44 ± 0.03	145.33 ± 0.07

[a] Ref 25. [b] Ref 5.

± 0.02 K with a low temperature thermostat (model DC-2006, Shanghai Hengping Instrument Factory). The cell constant is 1.092 cm^{-1}, which was calculated by repeated measurements of KCl solutions. All data were corrected at 298.15 K with specific conductivity of the solvent.

Results and Discussion

The density data measured for the glycyl dipeptide + water binary systems and glycyl dipeptide + sodium butyrate + water ternary systems at 298.15 K are given in Table 1 and Table 2, respectively, as a function of the molality of the glycyl dipeptides (m_p) and sodium butyrate (m_{NaC4}). Apparent molar volumes, $V_{2,\phi}$, of the glycyl dipeptides in water and in (0.5, 1.0, 1.5, and 2.0) mol·kg^{-1} aqueous sodium butyrate solutions were calculated from the solution densities, ρ, using the equation

$$V_{2,\phi} = M/\rho - 10^3(\rho - \rho_o)/m_p\rho\rho_o \quad (1)$$

where M is the molar mass of the glycyl dipeptides and ρ_o is the density of solvent. Calculated apparent molar volumes for

Table 4. Contributions of the Glycyl Group (CH_2CONH), (CONH), and the Alkyl Side Chain to the Standard Partial Molar Volumes, $V^o_{2,\phi}$ ($cm^3 \cdot mol^{-1}$), for the Glycyl Dipeptides in Aqueous and Aqueous Sodium Butyrate Solutions at $T = 298.15$ K

m_{NaC4}	0 $mol \cdot kg^{-1}$[a]	0.5 $mol \cdot kg^{-1}$	1.0 $mol \cdot kg^{-1}$	1.5 $mol \cdot kg^{-1}$	2.0 $mol \cdot kg^{-1}$	method
CH_2CONH	33.04 ± 0.04	34.67 ± 0.04	34.83 ± 0.05	35.54 ± 0.07	35.69 ± 0.06	Glygly-glycine
	31.34 ± 0.04	33.53 ± 0.10	33.84 ± 0.07	35.68 ± 0.09	35.80 ± 0.07	Glyval-valine
	31.87 ± 0.08	34.50 ± 0.04	35.49 ± 0.06	36.23 ± 0.05	36.86 ± 0.08	Glyleu-leucine
CONH	15.81 ± 0.04	17.76 ± 0.03	18.35 ± 0.05	19.52 ± 0.05	20.08 ± 0.05	Glygly-alanine
$CH(CH_3)_2$	45.96 ± 0.09	45.74 ± 0.10	44.56 ± 0.06	45.04 ± 0.09	44.55 ± 0.07	Glyval-glygly
$CH_2CH(CH_3)_2$	63.40 ± 0.12	62.87 ± 0.03	62.56 ± 0.07	61.99 ± 0.06	61.47 ± 0.08	Glyleu-glygly

[a] Ref 25.

Table 5. Standard Partial Molar Volumes, $V^o_{2,\phi}$ ($cm^3 \cdot mol^{-1}$), for the Dipeptides in 1.0 $mol \cdot kg^{-1}$ Salt Solutions at $T = 298.15$ K

salt	glycylglycine	glycylleucine
CH_3COONa	80.65[a]	142.90[a]
	80.05[b]	
NaCl	78.74[c]	
NaSCN	77.62[b]	

[a] Ref 22. [b] Ref 13. [c] Ref 19.

Table 6. Standard Partial Molar Volumes, $V^o_{2,\phi}$ ($cm^3 \cdot mol^{-1}$), for the Glycyl Dipeptides in Aqueous Sodium Butyrate Solutions Calculated by Using the Standard Partial Molar Volumes of the Amino Acids

m_{NaC4}	0.5 $mol \cdot kg^{-1}$	1.0 $mol \cdot kg^{-1}$	1.5 $mol \cdot kg^{-1}$	2.0 $mol \cdot kg^{-1}$
glycylglycine	78.26 ± 0.06	81.02 ± 0.04	82.92 ± 0.07	85.44 ± 0.06
glycyl-L-valine	125.14 ± 0.06	126.57 ± 0.06	127.84 ± 0.06	129.08 ± 0.06
glycyl-L-leucine	141.30 ± 0.06	142.92 ± 0.04	144.22 ± 0.06	145.74 ± 0.06

Table 7. Standard Volumes of Transfer, $\Delta_t V^o$ ($cm^3 \cdot mol^{-1}$), for the Glycyl Dipeptides from Water to Aqueous Sodium Butyrate Solutions at $T = 298.15$ K

m_{NaC4}	0.5 $mol \cdot kg^{-1}$	1.0 $mol \cdot kg^{-1}$	1.5 $mol \cdot kg^{-1}$	2.0 $mol \cdot kg^{-1}$
glycylglycine	2.96 ± 0.07	4.50 ± 0.08	6.16 ± 0.09	7.57 ± 0.08
glycyl-L-valine	2.74 ± 0.11	3.10 ± 0.07	5.24 ± 0.09	6.16 ± 0.08
glycyl-L-leucine	2.43 ± 0.10	3.66 ± 0.12	4.75 ± 0.10	5.64 ± 0.12

the glycyl dipeptides are also listed in Table 1 and Table 2. The reported apparent molar volume data for the peptides were found to be adequately presented by the linear equation

$$V_{2,\phi} = V^o_{2,\phi} + S_v m_p \quad (2)$$

where $V^o_{2,\phi}$ is the infinite dilution apparent molar volume that equals the standard partial molar volume and S_v is an experimentally determined parameter. Values of $V^o_{2,\phi}$ have been evaluated by weighted least-squares regression analysis. The standard partial molar volumes for the glycyl dipeptides in water and in aqueous solutions of sodium butyrate are represented in Table 3 along with their standard deviations. It can be seen that the $V^o_{2,\phi}$ values for the studied peptides in water agree well with literature values.[5,25] The $V^o_{2,\phi}$ values for the dipeptides are higher in aqueous sodium butyrate solutions than in water, and these values increase with the concentration of the cosolute. Further, the $V^o_{2,\phi}$ values increase from glycylglycine to glycylvaline to glycylleucine. The increase in the $V^o_{2,\phi}$ values is due to the increase in the size of the alkyl side chain of amino acids constituting the dipeptides.

To illuminate the contribution of the groups of the peptides to $V^o_{2,\phi}$, the $V^o_{2,\phi}$ values of the groups have been calculated. From the difference between $V^o_{2,\phi}$ values of the studied dipeptides and the corresponding amino acids,[26] the $V^o_{2,\phi}$ values of the peptide backbone unit (CH_2CONH) and the group (CONH) have been calculated and are reported in Table 4. The alkyl side chain of glycylvaline and glycylleucine contributions to the $V^o_{2,\phi}$ values given in Table 4 have been calculated from the difference in $V^o_{2,\phi}$ values of homologous glycyl peptides. The $V^o_{2,\phi}$ values of the groups in aqueous solution are also listed in Table 4. It is seen from this table that $V^o_{2,\phi}(CH_2CONH)$ and $V^o_{2,\phi}(CONH)$ are larger than those in water, whereas the alkyl side chain contributions to $V^o_{2,\phi}$ of the peptides are smaller than those in water. These results indicate that these groups have a different hydration effect in mixed solutions. The interaction of ions of NaC_4 with the hydrophilic peptide backbone unit of peptides through electrostatic interactions leads to a reduction in their electrostriction of the solvent and an increase in $V^o_{2,\phi}(CH_2CONH)$ and $V^o_{2,\phi}(CONH)$. This interaction results in a positive volume change. On the other hand, the sodium butyrate-side chain group interactions give a negative volume effect.

In previous studies, the volume properties of some dipeptides (especially glycylglycine) in NaCl,[19] NaSCN,[13] and CH_3COONa[22] solutions have been reported. The values of $V^o_{2,\phi}$ for glycylglycine and glycyl-L-leucine in aqueous solutions of 1.0 $mol \cdot kg^{-1}$ NaCl, NaSCN, and CH_3COONa are given in Table 5. It is found that the trend of $V^o_{2,\phi}$ for the peptides at the same salt concentration follows the order

$$CH_3(CH_2)_2COO^- > CH_3COO^- > Cl^- > SCN^-$$

It suggests that the order for the interactions between the anion and the peptides follows the above order. This order is consistent with that of the hydration ability of these anions.[27,28] Robinson and Jencks[24] studied the effect of a number of salt solutions on the activity coefficient of acetyltetraglycine ethyl ester (ATGEE), which is a model compound for peptides and proteins. From their salting-out constants, the order of the salting-out effect $CH_3COO^- > Cl^- > SCN^-$ was obtained. This indicated that the dehydration effect of ions on dipeptides is one of the important reasons for the salting-out of dipeptides.

Mishra and Ahluwalia[25] proposed an additivity relationship in which the $V^o_{2,\phi}$ values of a dipeptide were made up of the sum of the $V^o_{2,\phi}$ values of the respective amino acids, with additional terms to allow for the loss of groups and changes in electrostriction. The relationship is $V^o_{2,\phi}$(dipeptide) = $V^o_{2,\phi}$(first amino acid) + $V^o_{2,\phi}$(second amino acid) + 10.5 (for a decrease in electrostriction due to loss of CO_2^- and NH_3^+ groups) − 18 (for removal of 1 mol of H_2O) − 3.4 (for the increase in electrostriction due to separation of charged centers). Using the $V^o_{2,\phi}$ values of glycine, valine, and leucine in aqueous NaC_4 solutions[26] and the above equation, the $V^o_{2,\phi}$ values of glycyl dipeptides studied in this work can be calculated. The results are given in Table 6. It is found that excellent agreement

Table 8. Values of Hydration Number, n_H, for the Glycyl Dipeptides in Aqueous Sodium Butyrate Solutions at $T = 298.15$ K

m_{NaC4}	0 $mol \cdot kg^{-1}$	0.5 $mol \cdot kg^{-1}$	1.0 $mol \cdot kg^{-1}$	1.5 $mol \cdot kg^{-1}$	2.0 $mol \cdot kg^{-1}$
glycylglycine	5.69	4.80	4.33	3.83	3.40
glycyl-L-valine	9.54	8.71	8.60	7.95	7.68
glycyl-L-leucine	11.22	10.48	1.11	9.78	9.51

Table 9. Pair, V_{xy}, and Triplet, V_{xyy}, Interaction Coefficients for Glycyl Dipeptides in Aqueous Sodium Butyrate Solution at T = 298.15 K

	V_{xy}	V_{xyy}
peptides	$m^3 \cdot mol^{-2} \cdot kg$	$m^3 \cdot mol^{-3} \cdot kg^2$
glycylglycine	2.8111 ± 0.2463	−0.3148 ± 0.0956
glycyl-L-valine	2.2036 ± 0.5127	−0.2241 ± 0.1990
glycyl-L-leucine	2.3810 ± 0.1949	−0.3316 ± 0.0757

between the calculated values and the experimental values of the peptides is observed.

The standard volumes of transfer for the glycyl dipeptides from water to aqueous solutions of sodium butyrate are listed in Table 7. They were calculated from the relation

$$\Delta_t V^o = V^o_{2,\phi}\,(\text{in aqueous } NaC_4) - V^o_{2,\phi}\,(\text{in water}) \quad (3)$$

where $V^o_{2,\phi}$ (in water) is the infinite dilution apparent molar volume for the glycyl dipeptides in water. It is evident that the $\Delta_t V^o$ values are positive and increase with concentration of NaC_4. The results can be explained by the cosphere overlap model, as developed by Gurney[28] and Frank and Evans.[29]

Table 10. Molar Conductivity of Sodium Butyrate in Aqueous Peptide Solution at T = 298.15 K

m_p = 0.05 mol·kg^{-1}		m_p = 0.1 mol·kg^{-1}		m_p = 0.15 mol·kg^{-1}		m_p = 0.2 mol·kg^{-1}	
c	Λ	c	Λ	c	Λ	c	Λ
mol·dm^{-3}	S·cm^2·mol^{-1}	mol·dm^{-3}	S·cm^2·mol^{-1}	mol·dm^{-3}	S·cm^2·mol^{-1}	mol·dm^{-3}	S·cm^2·mol^{-1}
			Water + Glycylglycine				
0.0032954	72.32	0.0028475	71.35	0.0032098	68.74	0.0031081	67.97
0.0049942	72.00	0.0043307	70.88	0.0046585	68.70	0.0043911	67.76
0.0063605	71.82	0.0055069	70.62	0.0062672	68.32	0.0057945	67.56
0.0077472	71.38	0.0070397	70.29	0.0078544	68.15	0.0072033	67.39
0.0091976	71.05	0.0084149	69.97	0.0091706	67.90	0.0091189	67.14
0.010131	70.87	0.0097071	69.78	0.010623	67.56	0.010558	66.99
0.011499	70.60	0.011128	69.61	0.011922	67.44	0.011921	66.75
0.012837	70.40	0.012446	69.35	0.013222	67.18	0.013404	66.46
0.014154	70.10	0.013754	69.19	0.014491	67.02	0.014710	66.28
0.015655	69.80	0.015063	68.98	0.015731	66.82	0.015889	66.18
0.016762	69.63	0.016399	68.69	0.016994	66.61	0.017361	65.92
0.018171	69.52	0.017716	68.64	0.018295	66.59	0.018640	65.91
0.019364	69.36	0.018967	68.44	0.019761	66.41	0.020143	65.71
0.021081	69.10	0.020163	68.28	0.020829	66.31	0.021677	65.50
0.022281	68.86	0.021248	68.14	0.022110	66.13		
0.023696	68.63	0.022900	67.81	0.023311	66.00		
0.024982	68.47			0.025103	65.78		
			Water + Glycylvaline				
0.0043247	70.77	0.0024978	69.09	0.0028719	67.58	0.0028792	65.25
0.0060325	70.29	0.0040032	68.76	0.0045667	67.14	0.0045573	64.72
0.0075595	69.82	0.0056393	68.38	0.0061642	66.76	0.0062826	64.16
0.0090231	69.40	0.0071912	67.90	0.0077154	66.36	0.0079668	63.62
0.010600	68.97	0.0087958	67.56	0.0090626	66.02	0.0094385	63.43
0.012175	68.75	0.010419	67.21	0.010615	65.73	0.011070	63.06
0.013866	68.57	0.011750	66.85	0.011938	65.41	0.012638	62.76
0.015404	68.25	0.013225	66.58	0.013311	65.14	0.014197	62.49
0.016850	67.97	0.014603	66.29	0.014550	65.01	0.015809	62.20
0.018124	67.90	0.016074	66.00	0.015854	64.69	0.017368	61.97
0.019468	67.64	0.017515	65.75	0.017172	64.44	0.018883	61.86
0.020870	67.39	0.018819	65.73	0.018517	64.42	0.020365	61.65
0.022167	67.15	0.020246	65.47	0.019657	64.18	0.021858	61.44
0.023299	66.98	0.021697	65.22	0.021022	63.97	0.023245	61.21
0.024367	66.83	0.023041	65.02	0.022258	63.81	0.024624	61.06
0.025741	66.62	0.024509	64.78	0.023509	63.62	0.025948	60.90
0.026606	66.43	0.025874	64.54	0.024687	63.46	0.026404	60.84
				0.025309	63.37		
			Water + Glycylleucine				
0.0040919	70.63	0.0042841	68.89	0.0039682	65.98	0.0059562	62.08
0.0054830	70.25	0.0060025	68.09	0.0055520	65.65	0.0074347	61.93
0.0071202	69.75	0.0076373	67.68	0.0070102	65.24	0.0088917	61.61
0.0086843	69.39	0.0093034	67.30	0.0084356	64.84	0.010423	61.47
0.010054	68.96	0.010849	66.98	0.0098562	64.47	0.011870	61.25
0.011622	68.58	0.012440	66.67	0.011325	64.12	0.013300	60.99
0.013062	68.22	0.014109	66.30	0.012810	63.85	0.014731	60.85
0.014504	67.92	0.015573	65.96	0.014250	63.61	0.016290	60.60
0.015899	67.59	0.017008	65.73	0.015754	63.30	0.017608	60.41
0.017329	67.31	0.018399	65.63	0.017154	63.03	0.019058	60.22
0.018719	67.16	0.019788	65.39	0.018523	62.80	0.020385	60.17
0.020117	66.95	0.021289	65.14	0.019923	62.73	0.021704	59.98
0.021438	66.70	0.022582	64.95	0.021285	62.46	0.023034	59.80
0.022780	66.46	0.023834	64.74	0.022637	62.26	0.024534	59.62
0.024065	66.24	0.025295	64.51	0.023886	62.07	0.025855	59.44
0.025211	66.04			0.025036	61.88	0.026479	59.37
0.026434	65.84			0.025866	61.75		
			Pure Water				
0.00032342	79.62	0.0020761	78.68	0.011243	73.74	0.020910	71.69
0.0011161	79.50	0.0024303	78.42	0.013530	73.15	0.022843	71.37
0.0012729	79.36	0.0031665	76.56	0.015498	72.68	0.024810	71.00
0.0014883	79.15	0.0062982	75.26	0.017206	72.39	0.026472	70.72
0.0018798	78.83	0.0088494	74.43	0.019159	72.03		

Table 11. Values of Limiting Molar Conductivity Λ_0 ($S \cdot cm^2 \cdot mol^{-1}$) for Sodium Butyrate in Water and Water−Dipeptide Mixtures at $T = 298.15$ K

m_p/mol·kg^{-1}	Λ_0/S·cm^2·mol^{-1}
Water	
0.0000	82.24 ± 0.59
	82.66[a]
Water + Glycylglycine	
0.05000	74.22 ± 0.17
0.1000	72.92 ± 0.14
0.1500	70.26 ± 0.21
0.2000	68.98 ± 0.14
Water + Glycylvaline	
0.05000	73.49 ± 0.32
0.1000	71.57 ± 0.27
0.1500	69.69 ± 0.15
0.2000	68.14 ± 0.29
Water + Glycylleucine	
0.05000	73.74 ± 0.20
0.1000	71.57 ± 0.27
0.1500	68.82 ± 0.21
0.2000	63.90 ± 0.16

[a] Ref 38.

Properties of the water molecules in the hydration cosphere depend on the nature of the solute species.[30] The following type of interactions can occur in the ternary system of peptide−NaC_4−water: (a) ion−ion interactions between the Na^+ ion and the COO^- group in the peptide and between the $CH_3CH_2CH_2COO^-$ group and the NH_3^+ group in the peptide; (b) ion−peptide group interactions between ions of sodium butyrate (Na^+, $CH_3CH_2CH_2COO^-$) and the peptide backbone unit (−CH_2CONH) of dipeptides; (c) ion−nonpolar (hydrophobic) group interactions between ions of sodium butyrate and nonpolar groups of the dipeptides. If the cosphere overlap model is followed, ion−ion interactions and ion−peptide group interactions would lead to a positive $\Delta_t V^o$. On the other hand, ion−nonpolar group interactions would lead to a negative $\Delta_t V^o$. Since we have observed positive $\Delta_t V^o$ for all the dipeptides, we conclude that ion−ion interactions and ion−peptide group interactions are stronger than ion−nonpolar group interactions. This conclusion supports our results of the standard partial molar volumes. With increasing NaC_4 concentration, this interaction will become stronger, and therefore $\Delta_t V^o$ increases. Since there are larger nonpolar side chains of the valine (leucine) unit in glycyl-L-valine (glycyl-L-leucine) than diglycine, more positive $\Delta_t V^o$ values for diglycine in comparison to glycyl-L-valine and glycyl-L-leucine were observed.

The number of water molecules (n_H) hydrated to the glycyl dipeptides can be estimated from the electrostriction partial molar volume $V^o_{2,\phi}$(elect)[31] by

$$n_H = V^o_{2,\phi}(\text{elect})/(V^o_e - V^o_b) \quad (4)$$

where V_e^o is the molar volume of electrostricted water and V_b^o is the molar volume of bulk water. The value of $(V_e^o - V_b^o)$ is calculated to be −3.3 $cm^3 \cdot mol^{-1}$ at 298.15 K.[31] The $V^o_{2,\phi}$(elect) values can be calculated[32] from the intrinsic partial molar volume of the glycyl dipeptides $V^o_{2,\phi}$(int)[33,34] and the experimentally determined $V^o_{2,\phi}$ values by

$$V^o_{2,\phi}(\text{elect}) = V^o_{2,\phi}(\text{glycyl dipeptides}) - V^o_{2,\phi}(\text{int}) \quad (5)$$

The $V^o_{2,\phi}$(int) is made up of two terms: the van der Waals volume and the volume due to packing effects. The values of $V^o_{2,\phi}$(int) for the glycyl dipeptides were calculated from the crystal molar volume as below[31]

$$V^o_{2,\phi}(\text{int}) = (0.7/0.634)M/\rho_r \quad (6)$$

where ρ_r is the crystal density of the glycyl dipeptide. The values of crystal densities of glycylglycine, glycyl-L-valine, and glycyl-L-leucine determined by single-crystal X-ray diffraction are (1.534, 1.251, and 1.176) g·cm^{-3}, respectively. The obtained n_H values are included in Table 8. The charged end groups of peptides have a strong hydration ability. The peptide bond group, which is a polar group, strengthens the hydration ability of the peptides. The hydration numbers mainly come from the electrostriction effect of the charged end/peptide bond groups of dipeptides on water. It can be seen from Table 8 that n_H values for a given dipeptide are less than those in water and decrease with an increase in the concentration of sodium butyrate. This phenomenon suggests that interactions between ions of sodium butyrate and the charged end/peptide bond groups become stronger with the concentration of cosolutes. The electrostriction of water caused by the charged end/peptide bond groups of dipeptides will be largely reduced, which results in a larger decrease in n_H. This clearly indicates that electrolytes have a dehydration effect on the glycyl dipeptides in aqueous salt solutions. This is consistent with the observed increase in $\Delta_t V^o$ with increasing sodium butyrate concentration. Similar behavior has also been observed in the case of some peptides in KCl,[15] sodium acetate, and magnesium acetate solutions.[22]

Friedman and Kirshnan[35] suggested that the standard thermodynamic properties of transfer can reflect the interaction between solute and solvent. The volume behavior of a solute at infinite dilution is dependent on solute−solvent interactions and thus determined only by the respective intrinsic value and the solute−solvent interactions. The standard transfer volumes can be expressed as

$$\Delta_t V^o = 2V_{xy} m_{NaC_4} + 3V_{xyy} m^2_{NaC_4} \quad (7)$$

where x stands for peptides and y stands for NaC_4, respectively. The V_{xy} and V_{xyy} are the pair and triplet volumetric interaction coefficients, respectively, for the studied peptides. These results are given in Table 9. The V_{xy} and V_{xyy} are positive and negative, respectively, for the studied peptides in the cosolute. The magnitude of V_{xy} is greater than V_{xyy}, which suggests that interactions between peptides and NaC_4 are mainly pairwise and the higher interaction terms are less important.

The conductance data (Λ) of sodium butyrate in aqueous and aqueous (0.05, 0.1, 0.15, and 0.2) mol·kg^{-1} dipeptide solutions are listed in Table 10. Limiting molar conductivity (Λ_0) of NaC_4 was obtained by least-squares fitting the experimental data in Table 10 to the expression[36]

$$\Lambda = \Lambda_0 - \frac{A\sqrt{c}}{1 + B\sqrt{c}} \quad (8)$$

where Λ_0, A, and B are fitting parameters and c is the molarity of NaC_4 in mol·dm^{-3} and was obtained by converting from molality with the density. The resulting values are given in Table 11.

It may be noted from Table 11 that the values of Λ_0 in peptide solutions are very low as compared with that in water. This is expected on account of the highly viscous nature of aqueous peptide solution and also due to a possible stronger ion−solvent interaction.[37] Table 11 shows that the Λ_0 value for NaC_4 decreases with an increasing peptide content in solution. This can be ascribed to the facts that (i) an increase in microscopic viscosity of the medium with an increase in dipeptide concentration increases the frictional coefficient of the medium thereby retarding the mobility of the ions in solution and (ii) an increase

in peptide content of the mixtures causes the attraction between NaC_4 and the peptide to increase, and hence presolvation of ions by peptide molecules, causing an increase of the hydrodynamic radii of ions and a decrease of their mobility.

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