Activity Coefficients of Glycylglycine and α-Aminobutyric Acid in Aqueous Sucrose Solutions

Hatsuho UEDAIRA
Research Institute for Polymers and Textiles, Sawatari 4, Kanagawa-ku, Yokohama 221

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The activity coefficients of glycylglycine and α-aminobutyric acid in aqueous sucrose solutions have been measured by the isopiestic vapor pressure method at 25 °C. α-Aminobutyric acid is salted-out by sucrose, the effect increasing with an increase in the concentration of sucrose and a decrease in the concentration of amino acid. However, in the glycylglycine–sucrose–water system at lower concentrations of solutes salting-in of glycylglycine by sucrose takes place and then salting-out predominates at concentrations higher than 1 mol kg^{-1} glycylglycine and 2 mol kg^{-1} sucrose. The pairwise interactions and the free energy of the transfer of amino acids from water to various concentractions of sucrose solutions are calculated. The results account for the stabilizing effect of sucrose on the higher structure of globular proteins.

Although sugars have long been observed to affect the stability and activity of proteins, the mechanism of the effect remains unclear. Beilinson1) found that sucrose and glycerol inhibit the heat coagulation of ovalbumin. Ball *et al.*2) reported that various sugars prevent heat coagulation of serum albumin. Gersma and Stuur3) found that polyvalent alcohols show protective effects on the reversible thermal denaturation of lysozyme, ribonuclease, and chymotrypsinogen A.

Shimpson and Kauzman4) found that sucrose and glycerol inhibit the denaturation of ovalbumin by urea. According to Shikama,5) the bovin serum albumin molecule denaturated by urea has different conformations after the renaturation in 1 mol kg^{-1} sucrose solutions. Metral and Yon6) showed that the inhibition caused by sucrose on triptic hydrolysis of β-lactoglobulin A is due to the fixation of sucrose on this protein and to the existence of strong stabilizing interactions. Furthermore, at low concentration the sugars accelerate the rate of subunit dissociation of the olygomeric enzyme L-asparaginase with urea, and at higher concentrations, sugars decrease the rate at which the enzyme dissociates in the presence of urea.7)

In order to clarify the fundemantal mechanisms involved in these phenomena, a thermodynamic study with model compounds is one of the best approaches. Lakshimi and Nandi8) obtained the activity coefficients of aromatic amino acids and their *N*-acetyl ethyl esters in sucrose and glucose solutions by means of solubility measurements.

In the present work, the activity coefficients of glycylglycine (glygly) and α-aminobutyric acid (αABA) in sucrose solutions were determined by the isopiestic vapor pressure methods. These amino acids were selected as models of peptide unit and amino acids having alkyl side chains. The isopiestic method is superior to the solubility method in that the change in interactions with both concentrations of amino acid and sucrose can be obtained.

Experimental

Materials. Glygly (G. R.) was decolorized and recrystallized twice from a water–ethanol solution. Sucrose and αABA were recrystallized from a water ethanol solution. The samples were dried *in vacuo* over phosphorus pentaoxide at room temperature. Potassium chloride (analytical grade, Merck Co.) was dried *in vacuo* over phosphorus pentaoxide at 110 °C. Solutions were prepared with deionized water freed of air by boiling.

Measurements and Calculation of the Activity Coefficients. Osmotic and activity coefficients were determined with the same apparatus as previously reported.9) Several ternary solutions of amino acid and sucrose with varing compositions and reference potassium chloride solutions were put in silver dishes. The dishes were placed on a flat copper block in a glass vacuum dessicator set in a thermostat bath at 25 °C.9) The initial concentrations in the ternary solutions were adjusted to be sufficiently close to their equilibrium concentrations.10) The time required for attainment of equilibrium was 4—14 days. Equilibrium concentration was measured by weighing, all the weights being corrected to those in vacuum.

In the analysis of the results we use the function Δ, defined by11)

$$\Delta = 2m_R\phi_R - m_1\phi_1 - m_2\phi_2 , \tag{1}$$

where m_1 and m_2 are the molalities of solute 1 (glygly or αABA) and solute 2 (sucrose), respectively, in aqueous ternary solutions; ϕ_1 and ϕ_2 are the osmotic coefficients of binary aqueous solutions of solutes 1 and 2 at a molality of m_1 and m_2, respectively; m_R and ϕ_R are the molality and osmotic coefficient, respectively, of the reference potassium chloride solution which is in vapor pressure equilibrium with a ternary solution containing solutes 1 and 2 at molalities m_1 and m_2.

The value of Δ/m_1m_2 is given by the equations

$$\Delta/m_1m_2 = \sum_{i=0}^{n}\sum_{j=0}^{n} A_{ij}m_1{}^i m_2{}^j \qquad (n=i+j=2 \text{ or } 3) \tag{2}$$

From Eqs. 1 and 2, the activity coefficient for solute 1 in the ternary solution is given11,12) by

$$\begin{aligned}\ln \gamma_1 = \ln \gamma_{10} &+ A_{00}m_2 + A_{10}m_1m_2 + \frac{1}{2}A_{01}m_2{}^2 + A_{20}m_1{}^2m_2 \\ &+ \frac{2}{3}A_{11}m_1m_2{}^2 + \frac{1}{3}A_{02}m_2{}^3 + A_{30}m_1{}^3m_2 + \frac{3}{4}A_{21}m_1{}^2m_2{}^2 \\ &+ \frac{1}{2}A_{12}m_1m_2{}^3 + \frac{1}{4}A_{03}m_2{}^4 ,\end{aligned} \tag{3}$$

and that of solute 2 by

$$\begin{aligned}\ln \gamma_2 = \ln \gamma_{20} &+ A_{00}m_1 + \frac{1}{2}A_{10}m_1{}^2 + A_{01}m_1m_2 + \frac{1}{3}A_{20}m_1{}^3 \\ &+ \frac{2}{3}A_{11}m_1{}^2m_2 + A_{02}m_1m_2{}^2 + \frac{1}{4}A_{30}m_1{}^4 + \frac{1}{2}A_{21}m_1{}^3m_2 \\ &+ \frac{3}{4}A_{12}m_1{}^2m_2{}^2 + A_{03}m_1m_2{}^3 ,\end{aligned} \tag{4}$$

where γ_1 and γ_2 are the molal activity coefficients of solutes

TABLE 1. TERNARY ISOPIESTIC DATA AT 25 °C FOR THE SYSTEMS GLYCYLGLYCINE-SUCROSE-WATER AND α-AMINOBUTYRIC ACID-SUCROSE-WATER

m_R	m_1	m_2	Δ/m_1m_2		Diff. %[b]
			Exptl	Calcd[a]	
		Glycylglycine-Sucrose-Water			
0.41292	0.17518	0.55396	-0.021_{54}	-0.023_{21}	−0.02
	0.26074	0.48299	-0.026_{12}	-0.024_{97}	0.02
	0.42168	0.34959	-0.023_{01}	-0.026_{94}	−0.08
	0.55101	0.24348	-0.026_{43}	-0.027_{52}	−0.02
	0.73539	0.091227	-0.037_{60}	-0.027_{70}	0.09
0.59975	0.25219	0.78754	-0.003_{77}	-0.005_{91}	−0.04
	0.44359	0.63745	-0.004_{98}	-0.006_{91}	−0.05
	0.65823	0.46967	-0.002_{82}	-0.006_{15}	−0.10
	0.86390	0.30841	-0.002_{42}	-0.004_{94}	−0.06
	1.0524	0.15741	0.000_{29}	-0.004_{75}	−0.08
0.81372	0.58512	0.86910	-0.002_{63}	-0.011_{47}	0.49
	0.88214	0.64024	0.017_{32}	0.014_{28}	−0.12
	1.1583	0.43184	0.016_{74}	0.015_{29}	−0.05
	1.3936	0.24999	0.008_{29}	0.012_{10}	0.09
1.1610	0.27866	1.5876	0.018_{51}	0.026_{77}	0.18
	0.57402	1.3735	0.032_{71}	0.029_{18}	−0.13
	0.87125	1.1631	0.037_{45}	0.033_{68}	−0.18
	1.2258	0.91511	0.035_{03}	0.036_{07}	0.06
	1.4723	0.73519	0.035_{02}	0.032_{15}	−0.15
1.5188	0.30159	2.0461	0.038_{00}	0.035_{47}	−0.06
	0.52929	1.8921	0.038_{31}	0.038_{13}	−0.01
	0.80052	1.7067	0.044_{58}	0.042_{97}	−0.08
	1.0730	1.5256	0.044_{76}	0.046_{84}	0.12
	1.3232	1.3569	0.044_{37}	0.046_{75}	0.16
2.1221	0.28058	2.8240	0.041_{21}	0.038_{25}	−0.06
	0.60993	2.6101	0.046_{47}	0.043_{59}	−0.12
	0.92873	2.4068	0.050_{01}	0.050_{06}	0.00
	1.2170	2.2253	0.050_{62}	0.052_{80}	0.15
	1.4622	2.0690	0.050_{56}	0.049_{96}	−0.05
3.0536	0.25757	3.9720	0.028_{15}	0.030_{84}	0.05
	0.83440	3.6171	0.043_{89}	0.043_{45}	−0.02
	1.1102	3.4518	0.046_{36}	0.048_{11}	0.12
	1.4335	3.2582	0.047_{59}	0.046_{61}	−0.08
3.8765	0.24079	4.9649	0.029_{35}	0.029_{11}	0.00
	0.51140	4.7984	0.037_{50}	0.035_{35}	−0.07
	0.82222	4.6149	0.040_{67}	0.044_{09}	0.17
	1.1378	4.4055	0.051_{77}	0.049_{87}	−0.13
		α-Aminobutyric Acid-Sucrose-Water			
0.41893	0.15289	0.56465	0.132_{34}	0.098_{44}	−0.39
	0.27378	0.45279	0.083_{34}	0.083_{99}	0.01
	0.36844	0.36689	0.040_{44}	0.075_{27}	0.62
	0.45887	0.27343	0.087_{31}	0.068_{99}	−0.30
	0.61716	0.12142	0.052_{02}	0.062_{11}	0.10
0.60585	0.20515	0.81427	0.072_{69}	0.089_{71}	0.26
	0.37736	0.65626	0.061_{24}	0.072_{97}	0.27
	0.57167	0.47473	0.051_{24}	0.061_{99}	0.27
	0.74552	0.30529	0.055_{71}	0.058_{15}	0.05
	0.91333	0.13887	0.059_{70}	0.058_{81}	−0.01
0.83636	0.19248	1.1680	0.092_{16}	0.089_{43}	−0.04
	0.48244	0.91170	0.067_{75}	0.065_{11}	−0.08
	0.72733	0.68513	0.067_{53}	0.057_{27}	−0.34
	1.0272	0.40075	0.063_{39}	0.058_{95}	−0.12
	1.2812	0.14770	0.079_{81}	0.066_{51}	−0.17

TABLE 1. (Continued)

m_R	m_1	m_2	Δ/m_1m_2 Exptl	Δ/m_1m_2 Calcd[a]	Diff. %[b]
0.87464	0.20041	1.2161	0.093_{17}	0.088_{27}	−0.08
	0.50305	0.95060	0.067_{91}	0.063_{97}	−0.12
	0.75888	0.71485	0.066_{60}	0.056_{85}	−0.34
	1.0716	0.41810	0.063_{99}	0.059_{69}	−0.12
1.1436	0.39163	1.4369	0.066_{35}	0.070_{08}	0.10
	0.68999	1.1789	0.061_{96}	0.057_{75}	−0.17
	0.97043	0.92902	0.057_{86}	0.057_{08}	−0.03
	1.3165	0.60988	0.049_{78}	0.064_{11}	0.56
	1.6161	0.31341	0.073_{10}	0.071_{53}	−0.04
1.5601	0.32964	2.0478	0.071_{68}	0.073_{67}	0.05
	0.63128	1.8023	0.066_{54}	0.059_{82}	−0.27
	1.1290	1.3856	0.059_{23}	0.059_{44}	0.01
	1.5484	1.0166	0.053_{21}	0.064_{68}	0.64
	1.8648	0.70963	0.061_{28}	0.062_{73}	0.07
2.0610	0.32614	2.6944	0.059_{28}	0.071_{88}	0.29
	0.64124	2.4470	0.063_{35}	0.060_{35}	−0.13
	1.0351	2.1369	0.060_{04}	0.059_{63}	−0.02
	1.3484	1.8829	0.057_{79}	0.061_{90}	0.28
	1.7479	1.5432	0.057_{04}	0.057_{53}	0.03
2.5831	0.19494	3.4234	0.072_{38}	0.074_{88}	0.03
	0.54857	3.1652	0.060_{54}	0.060_{55}	0.00
	0.80056	2.9752	0.060_{33}	0.058_{88}	−0.07
	1.1392	2.7143	0.060_{40}	0.060_{92}	0.03
	1.3856	2.5261	0.057_{78}	0.061_{16}	0.25
	1.6907	2.2794	0.058_{50}	0.054_{73}	−0.30
2.7287	0.20449	3.5911	0.070_{49}	0.071_{82}	0.02
	0.57545	3.3203	0.061_{14}	0.059_{16}	−0.07
	0.84047	3.1235	0.059_{53}	0.058_{60}	−0.05
	1.1971	2.8522	0.058_{90}	0.061_{00}	0.14
	1.4500	2.6435	0.063_{69}	0.060_{08}	−0.27
	1.7794	2.3989	0.056_{34}	0.049_{06}	−0.61
2.9161	0.35899	3.7005	0.067_{04}	0.061_{60}	−0.13
	0.66476	3.4811	0.059_{48}	0.057_{09}	−0.10
	0.94791	3.2676	0.060_{52}	0.058_{64}	−0.11
	1.2875	3.0172	0.056_{93}	0.060_{50}	0.25
	1.6002	2.7794	0.055_{31}	0.055_{38}	0.01
3.0421	0.37288	3.8437	0.062_{53}	0.059_{04}	−0.09
	0.69071	3.6170	0.056_{55}	0.055_{92}	−0.03
	0.98479	3.3948	0.058_{94}	0.058_{30}	−0.04
	1.6642	2.8906	0.053_{55}	0.051_{67}	−0.16
3.3771	0.35322	4.2703	0.046_{28}	0.052_{05}	0.14
	1.0433	3.7607	0.055_{42}	0.056_{08}	0.04
	1.3038	3.5743	0.052_{85}	0.057_{17}	0.31
3.6267	0.37633	4.5497	0.048_{34}	0.044_{86}	−0.09
	1.1130	4.0121	0.053_{65}	0.053_{64}	−0.00
	1.3920	3.8161	0.050_{45}	0.053_{23}	0.21

a) Calculated by the least-squares treatment of Eq. 20 and Table 2. b) Percentage error defined by Kelly *et al.*[16]

1 and 2 in a ternary solution containing solutes 1 and 2 with molalities m_1 and m_2, respectively; γ_{10} and γ_{20} are the molal activity coefficients, respectively, of binary solutions containing only solute 1 at molality m_1 or solute 2 at molality m_2. The values of osmotic and activity coefficients for reference potassium chloride solutions were taken from the values given by Robinson and Stokes.[13] The values of osmotic and activity coefficients for glygly and αABA solutions were taken from the data of Ellerton *et al.*,[14] and the values for sucrose solutions, from Robinson and Stokes.[15]

Results

The equilibrium molalities of solute 1 and 2 in ternary solutions and of potassium chloride in reference solutions for the systems, glygly–sucrose–water

Table 2. Coefficients in Eq. 2 for the ternary solutions glycylglycine-sucrose-water (I) and α-aminobutyric acid-sucrose-water(II) at 25 °C

Coefficients	I	II
$A_{00}\times 10$	−0.77385	1.3170
$A_{10}\times 10$	0.34859	−2.0566
$A_{01}\times 10$	1.0532	−0.15888
$A_{20}\times 10$	0.50339	1.8176
$A_{11}\times 10^2$	−3.2769	2.1159
$A_{02}\times 10^2$	−3.0133	0.4932
$A_{30}\times 10^2$	−2.6230	−4.6886
$A_{21}\times 10^2$	0.1266	−1.3365
$A_{12}\times 10^3$	5.098	0.668
$A_{03}\times 10^3$	2.647	1.012

and αABA-sucrose-water, are given in Table 1, together with the values of experimental quantities Δ/m_1m_2 defined by Eq. 1. In order to fit the experimental values of Δ/m_1m_2 to a power series in m_1 and m_2 with the form of Eq. 2, two polynomials involving terms up to squares or cubes in m_1 and m_2 were examined with a HITAC 8450 computer. In both systems, the better polynomials for representing the data are equations containing terms up to cubic. Their coefficients are given in Table 2.

From Eqs. 3 and 4 and Table 2, we obtain

$$\begin{aligned}\ln\gamma_1 = \ln\gamma_{10} &- 0.077385m_2 + 0.034859m_1m_2 \\ &+ 0.052662m_2^2 + 0.050339m_1^2m_2 - 0.021846m_1m_2^2 \\ &- 0.010044m_2^3 - 0.026230m_1^3m_2 + 0.000949m_1^2m_2^2 \\ &+ 0.002549m_1m_2^3 + 0.000662m_2^4, \end{aligned} \tag{5}$$

$$\begin{aligned}\ln\gamma_2 = \ln\gamma_{20} &- 0.077385m_1 + 0.017429m_1^2 \\ &+ 0.10532m_1m_2 + 0.016780m_1^3 - 0.021846m_1^2m_2 \\ &- 0.030133m_1m_2^2 - 0.006557m_1^4 + 0.000633m_1^3m_2 \\ &+ 0.003824m_1^2m_2^2 + 0.002647m_1m_2^3, \end{aligned} \tag{6}$$

for the system glygly–sucrose–water, and

$$\begin{aligned}\ln\gamma_1 = \ln\gamma_{10} &+ 0.13170m_2 - 0.20566m_1m_2 \\ &- 0.007944m_2^2 + 0.18176m_1^2m_2 + 0.014106m_1m_2^2 \\ &+ 0.0016439m_2^3 - 0.046886m_1^3m_2 - 0.010024m_1^2m_2^2 \\ &+ 0.000334m_1m_2^3 - 0.000253m_2^4, \end{aligned} \tag{7}$$

$$\begin{aligned}\ln\gamma_2 = \ln\gamma_{20} &+ 0.13170m_1 - 0.10283m_1^2 - 0.015888m_1m_2 \\ &+ 0.060588m_1^3 + 0.014106m_1^2m_2 + 0.004932m_1m_2^2 \\ &- 0.011721m_1^4 - 0.006683m_1^3m_2 + 0.000501m_1^2m_2^2 \\ &- 0.001012m_1m_2^3, \end{aligned} \tag{8}$$

for the system αABA–sucrose–water.

The activity coefficients of glygly in binary solution and in ternary solutions containing several concentrations of sucrose are given in Fig. 1 and the activity coefficients of αABA in binary and in ternary solutions in Fig. 2. The activity coefficients of glygly and αABA increase with the concentration of sucrose. At lower concentrations, however, sucrose has a different effect on the activity coefficients of glygly and αABA. As shown in Fig. 1, when sucrose concentration is lower than 2 mol kg⁻¹ glygly is salted-in by sucrose, but at higher concentrations of both glygly (>1 mol kg⁻¹) and sucrose (>2 mol kg⁻¹) sating-out predominates. The activity coefficient of αABA increases for all the concentrations studied, the increasing effect being greater at lower concentration of αABA.

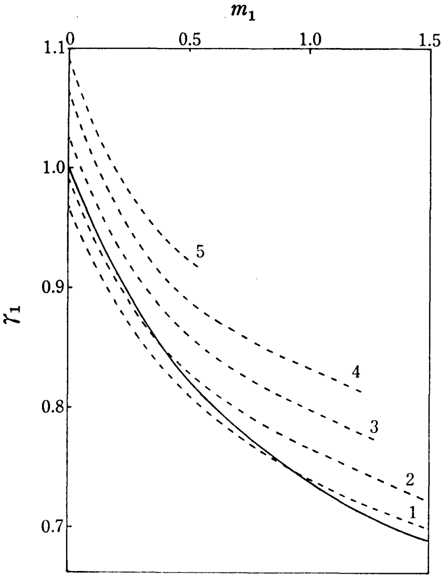

Fig. 1. Activity coefficients of glycylglycine in sucrose solutions: ——, in water; ----, in sucrose solutions at several concentrations of sucrose. The concentrations of sucrose (mol kg⁻¹): 1, 1.0; 2, 2.0; 3, 3.0; 4, 4.0; 5, 5.0.

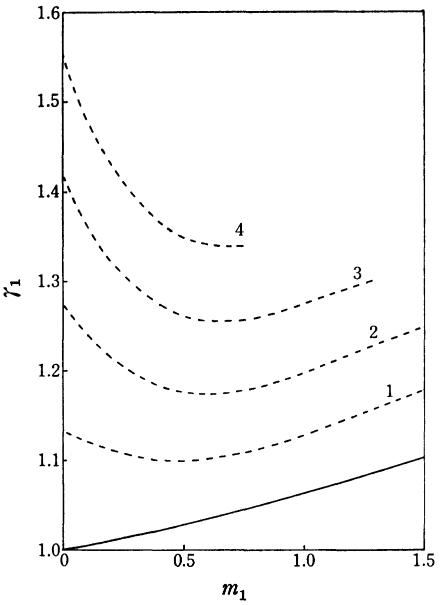

Fig. 2. Activity coefficients of α-aminobutyric acid in sucroes solutions: ——, in water; ----, in sucrose solutions at several concentrations of sucrose. The concentrations of sucrose (mol kg⁻¹): 1, 1.0; 2, 2.0; 3, 3.0; 4, 4.0.

Discussion

From the isopiestic data, the excess free energy of mixing per kilogram of solvent, $\Delta_m G_w^{ex}$, can be calculated. $\Delta_m G_w^{ex}$ for a mixture of two solutions of nonelectrolytes A (concentration m_1) and B (m_2) is given

TABLE 3. FREE ENERGY EFFECTS OF PAIRWISE AND TRIPLET INTERACTIONS

A	B	$\{AB\}_g$[a)] J mol^{-2} kg	$\{AAB\}_g$ J mol^{-3} kg^2	$\{ABB\}_g$ J mol^{-3} kg^2
α-Aminobutyric acid	Sucrose	160	−85	−6.6
Glycylglycine	Sucrose	−96	14	44
α-Aminobutyric acid	Urea	−54[b)]	0.13	2.1
Glycylglycine	Urea	−123[b)]	41	4.0
Sucrose	Urea	−155	7.5	6.2

a) $\{AB\}_g$ is $(2.303RT)$ times the "limiting interaction coefficients" which is defined by Schrier and Robinson[19)] as a measure of the mutual interaction of the solutes (and the solvent) free of the contribution from concentration dependent terms. b) Lilley and Scott[20)] obtained the values, −35 and −119 (J mol^{-2} kg), for αABA-urea and glygly-urea, respectively.

by Cassel and Wood[17)] as follows:

$$\begin{aligned}\Delta_m G_w^{ex}/RT &= (m_1+m_2)\phi_{mix} - m_1\phi_1 - m_2\phi_2 \\ &= [2\{AB\}_g m_1 m_2 + 6\{AAB\}_g m_1^2 m_2 \\ &\quad + 6\{ABB\}_g m_1 m_2^2 + \cdots]/RT, \qquad (9)\end{aligned}$$

where ϕ_{mix} is the osmotic coefficient of a ternary solution, the species in brackets denoting the particles interacting, and subscript g the free energy of interaction. The values of the pairwise and triplet interactions calculated by Eq. 9 are given in Table 3, together with values for the systems αABA–urea–water,[18)] glygly–urea–water,[9)] and sucrose–urea–water.[12)] Using an expression[17)] similar to Eq. 9 and the data for binary solutions, the pairwise interactions for binary solutions of αABA,[14)] glygly,[14)] sucrose[15)] and urea[12)] are calculated to be 62, −660, 180 and −110 (J mol^{-2}kg), respectively.

Pairwise and triplet interactions formally depend only on the solute species, but they are affected by the interactions with the solvent water as Schrier and Robinson[19)] pointed out.

It is interesting to classify the pairwise interactions by the effect of these solutes on water structure. Walrafen[21)] showed from Raman spectral measurements that sucrose has a structure promoting effect on water. Taylor and Rowlinson[22)] measured the heat of dilution of sucrose in water and concluded that strong hydrogen bonding exists between sucrose and surrounding water molecules. The self diffusion coefficients of water in aqueous solutions of glygly and αABA were measured at 25—50 °C by Altunina *et al.*[23)] Glygly and αABA decrease the activation energy of self diffusion of water. On the other hand, the structure-making solutes cause the water near them to be more ice-like than normal; the melting of such a structure requires greater energy with the result that such solutes give rise to positive excess partial molal heat capacities.[24,25)] Spink and Wadsö[26)] measured the heat capacities of solid αABA and aqueous solution of the amino acid at 25 °C and also enthalpies of solution at several temperatures. By both direct and indirect measurements they determined the partial molal heat capacity, $\bar{C}_{p,2}^{\circ}$ and excess partial molal heat capacity, ΔC_p°, of αABA, 222—226 and 76—80 (J k^{-1}mol^{-1}), respectively. The corresponding values for glygly which are determined from the data of heat capacity of solid,[27)] and of aqueous solutions[28)] are 159 and −4.6 (J k^{-1}mol^{-1}) for $\bar{C}_{p,2}^{\circ}$, and ΔC_p°, respectively. The NMR and calorimetric data for αABA are not consistent as to the effect on the water structure, probably because αABA is near the borderline of the structure-making and structure-breaking solutes. We believe, however, that the precise calorimetric results by Spink and Wadsö are more reliable and thus, αABA is considered to be a weak structure maker. Many papers have appeared on the effect of urea on water structure, but it is generally accepted that urea behaves as a structure breaker.[29)]

Thus, αABA and sucrose are ranked as structure makers (positive hydration) and glygly and urea as structure breakers (negative hydration). From Table 3, it is seen that the pairwise interactions between the two structure making solutes are positive (αABA+sucrose, sucrose+sucrose and αABA+αABA), and the interactions between the two structure breaking solutes are negative (glygly+urea, glygly+glygly and urea+urea). For the systems containing structure-making and structure-breaking solutes (sucrose+urea, glygly+sucrose and αABA+urea), the interactions are negative. The triplet interactions have signs opposite to the pairwise interactions between unlike solutes in the same systems.

The nature of the structure-making effect of hydrophylic sucrose on water structure differs from that of αABA having hydrophobic ethyl group, and the structure-breaking effects of glygly and urea are not the same. In spite of these differences, the signs of excess pairwise interactions entirely depend on the above classification by the effect on water structure.

In order to obtain the concentration dependency of the interaction quantitatively, the free energy of the transfer is calculated. The activity coefficients of the amino acids in sucrose solutions measured by the isopiestic method are referred to unity in the standard state in water, and not to the standard state in a water–sucrose solvent.[30)] Thus the free energy of transfer at constant concentration of the solute is calculated from the change in activity coefficients. Consequently, the free energy of transfer, ΔG_t, of solute 1 from water to sucrose solution is given by[31)]

$$\Delta G_t = RT \ln (f_1/f_{10}), \qquad (10)$$

where f_{10} and f_1 are the activity coefficients in mole fraction scale of solute 1 in binary and ternary sucrose solutions, respectively, both at the same mole fraction; f_1 and f_{10} are defined as before.[31)]

The values of ΔG_t of glygly and αABA from water to various concentrations of sucrose solutions are given

Table 4. FREE ENERGY OF TRANSFER (in J mol) OF GLYCYLGLYCINE AND α-AMINOBUTYRIC ACID FROM WATER TO SUCROSE SOLUTIONS

m_{10}[a)]	m_2 0.5	1.0	2.0	3.0	4.0	5.0
	Glycylglycine					
0	−44.1	−40.3	53.3	190	320	431
0.2	−37.4	−31.3	57.5	183	303	414
0.5	−20.0	−2.6	95.7	223	347	
1.0	13.5	54.4	178	318	462	
1.5	30.2	76.7	188	297		
	α-Aminobutyric acid					
0	181	354	686	992	1260	
0.2	140	275	536	791	1020	
0.5	106	210	418	632	837	
1.0	97.5	194	388	590		
1.5	111	215	402			

a) The molality of solute 1 in a binary solution. The molalities in ternary solutions are slightly greater than m_{10} as the transfer is made at constant mole fraction.[31)]

in Table 4. At higher concentrations of sucrose (>2 *m*), ΔG_t of both glygly and αABA increases with increasing concentration of sucrose, the increasing effect on αABA being much larger than that on glygly. The values of ΔG_t depend differently on the concentration of the amino acids. ΔG_t increases with increasing concentration of glygly and with decreasing concentration of αABA. At lower concentrations ($m_{10}<1.0$ and $m_2<2.0$), the sucrose solution is a more favorable solvent than water for glygly. The results indicate that the sucrose solution environment is much more unforable than water for αABA having an alkyl side chain as compared with glygly which has no side chain. It would require much more work for alkyl groups in the interior of the protein to be exposed in the sucrose solution than in water, they would thus be caused by the sucrose solution to enter into the interior of protein, as Lakshimi and Nandi[8)] reported for amino acids with aromatic side chain.

The denaturing process can be considered to involve a dilution process of local concentrations of amino acid residues in a protein. The dilution process of a solute is accompanied by the decrease of its chemical potential since the term $RT \ln \gamma_1 m_1$ decreases with dilution. While γ_1 of glygly in a sucrose sloution increases with decreasing concentration more solwly than in water, γ_1 of αABA increases with decreasing concentration at a concentration lower than 0.5 mol kg^{-1} even though γ_{10} decreases with decreasing concentration. (Fig. 1 and 2).

Klotz[32)] obtained values for the average molar local concentrations of peptide and low apolar residues (Ala +Val+Ileu+Phe) whihin molecular volume of proteins (insurine, ribonuclease, ovalbumin and bovin serum albumin) to be 12—13 M and 3—4 M, respectively. Though the concentration range of the present work is lower than the values estimated by Klotz, and it is difficult to determine the real local concentration of amino acid residues at the surface of the protein molecules, the results suggest that the decrease in chemical potential with denaturing (dilution) process is less in sucrose solutions than in water for apolar residues. When denaturant molecules cause the loosening of the globular structure of the protein resulting in a contact of the interior hydrophobic side chains to solutions, the decrease in chemical potential with the subsequent extending process would become less in sucrose+denaturant solution than in denaturant solution. This would also result in more stability of a protein molecule in a sucrose solution and would reduce the extent of denaturation of protein molecule induced thermally or by denaturants.

The results with glygly suggest that the interaction of sucrose on peptide group is somewhat complicated. A sucrose solution is a favorable solvent for glygly at low concentration of sucrose, becoming unfavorable at higher concentration. Shifrin and Parrott[7)] have demonstrated that low concentrations of polyhydric alcohols (including glucose and sucrose) accelerate the rate of dissociation of tetrameric L-asparaginase by urea, and higher concentrations of polyols protect the enzyme against the dissociating effect of urea. The change in the effect of polyols on the protein with concentration might be related to the switch from salting-in to salting-out of glygly by sucrose with increase in sucrose concentration.

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