Activity Coefficients for the System Glycylglycine–Urea–Water

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Activity coefficients for the ternary system glycylglycine–urea–water were measured by the isopiestic vapor pressure method at 25°C. The activity coefficients of glycylglycine were lowered by urea. The lowering increased with increasing concentration of urea and with decreasing concentration of the amino acid. It was dependent on amino acid concentration to a greater extent than in the system α-amino-*n*-butyric acid–urea–water. The activity coefficients of urea were also decreased by glycylglycine. The free energy of the transfer of glycylglycine from water to urea solutions in various concentrations was calculated. The values obtained were combined with enthalpy data to yield −3.9 e. u. as the entropy value for the transfer from water to 6 M urea solution. Results have been interpreted to support the existence of interaction between urea and glycylglycine.

The interaction of urea on amino acids or oligopeptides in aqueous solutions has been investigated for the purpose of elucidating the denaturing action of urea on globular protein.

The solubility of amino acids and peptides in water and aqueous urea solutions was measured by Whitney and Tanford,[1] and Nozaki and Tanford.[2] They showed that aqueous urea solutions are able to accomodate nonpolar amino acid side chains better than water and that urea also interacts favorably with peptide groups, so that urea stabilizes both nonpolar groups and peptide groups.

Robinson and Jencks[3] examined the effect of a series of denaturing agents on the solubility of acetyltetraglycine ethyl ester and three carbobenzoxyglycine derivatives. They suggested that a nonhydrophobic effect of urea makes a major contribution to its denaturing action on some proteins.

Heat of transfer of amino acids from water to 6 M urea solution was measured by Kresheck and Benjamin.[4] It was shown that the ordering of water molecules around a solute molecule is increased due to the presence of the nonpolar part of the solute molecules and that part of this ordering is removed by urea. It was also shown that there is evidence for the existence of specific interactions between urea and polar parts of the solute molecules including the peptide bond.

The change in interaction as a continuous function of urea and amino acid concentrations can be obtained from the activity coefficients measured by the isopiestic vapor pressure method. Cussler[5] measured activity coefficients for the system α-amino-*n*-butyric acid–urea–water by the isopiestic method. In this system, the activity coefficient of the amino acid decreased with increasing urea concentration and increased with amino acid concentration.

The activity coefficients for the system glycylglycine—urea—water were measured by the isopiestic method to examine their dependence on concentrations of both amino acid and urea. Hydrophobic interaction need not be taken into consideration, as the peptide has no hydrophobic side chain.

Experimental

Materials. Glycylglycine (G. R.) was decolorized and recrystallized twice from a water–ethanol solution. Urea (G. R.) was recrystallized twice from water, without being heated above 60°C. The peptide and urea were dried *in vacuo* over phosphorus pentoxide at room temperature. Potassium chloride of analytical grade (Merck Co.) was not purified further and was dried in vacuo over phosphorus pentoxide at 110°C. Solutions were prepared with deionized water freed of air by boiling.

Measurements and calculation of the activity coefficients for the ternary solutions. Osmotic and activity coefficients were determined with an apparatus[6] similar to that used by Robinson and Stokes.[7] Four or five ternary peptide-urea solutions with varying compositions were put in silver dishes. Reference potassium cholride solutions were present in triplicate or quadruplicate. The dishes were placed on a flat copper block in a glass vacuum desiccator set in a thermostat bath at 25°C and controlled within 0.01°C. All the solutions were prepared afresh for each measurement. The intial concentrations in the solutions were adjusted to be sufficiently close to their equilibrium concentrations by the method reported previously.[8] The time required for attainment of equilibrium was 4—7 days. Equilibrium concentration was measured by weighing, all weights being corrected to those in vacuum.

An experimental quantity Δ is defined[7] by

$$\Delta = 2m_R\phi_R - m_1\phi_1 - m_2\phi_2 \quad (1)$$

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

The value of Δ is thus obtained experimentally from the concentrations m_R, m_1, and m_2, provided that the osmotic coefficients of reference solution and binary solutions of glycylglycine and urea are known.

The value of Δ/m_1m_2 is given by the equation

$$\Delta/m_1m_2 = A + Bm_1 + Cm_2 + Dm_1^2 + Em_1m_2 + Fm_1m_2^2 + Gm_1^3 + Hm_1^2m_2 + Im_1m_2^2 + Jm_2^3 \quad (2)$$

1) P. L. Whitney and C. Tanford, *J. Biol. Chem.*, **237**, PC1735 (1962).
2) Y. Nozaki and C. Tanford, *ibid.*, **238**, 4074 (1963).
3) D. R. Robinson and W. P. Jencks, *J. Amer. Chem. Soc.*, **87**, 2462 (1965).
4) G. C. Kresheck and L. Benjamin, *J. Phys. Chem.*, **68**, 2476 (1964).
5) E. L. Cussler, Jr., *ibid.*, **71**, 901 (1967).
6) H. Uedaira and H. Uedaira, This Bulletin, **42**, 2137 (1969).
7) R. A. Robinson and R. H. Stokes, *J. Amer. Chem. Soc.*, **65**, 1954 (1961).
8) H. Uedaira and H. Uedaira, *J. Phys. Chem.*, **74**, 1931 (1970).

TABLE 1. TERNARY ISOPIESTIC DATA AT 25°C FOR THE SYSTEM GLYCYLGLYCINE-UREA-WATER

m_R	m_1	m_2	Δ/m_1m_2		
			Exptl	Calcd	Diff. %
0.59422	0.30393	0.82598	-0.070_{52}	-0.067_{25}	0.08
	0.45138	0.69074	-0.062_{61}	-0.059_{86}	0.08
	0.61061	0.54705	-0.055_{52}	-0.052_{89}	0.08
0.81451	0.63366	0.95362	-0.045_{67}	-0.049_{78}	−0.17
	1.0306	0.59809	-0.035_{41}	-0.038_{99}	−0.15
	1.2386	0.41251	-0.031_{94}	-0.035_{03}	−0.11
0.95786	1.5529	0.41159	-0.035_{18}	-0.031_{58}	0.13
1.0731	0.39843	1.6926	-0.052_{29}	-0.053_{27}	−0.03
	0.71011	1.3975	-0.036_{03}	-0.044_{12}	−0.42
	0.92038	1.2157	-0.038_{57}	-0.039_{57}	−0.06
	1.2219	0.93663	-0.030_{43}	-0.035_{07}	−0.28
1.2522	0.30117	2.1531	-0.048_{38}	-0.052_{22}	−0.11
	0.61438	1.8642	-0.041_{74}	-0.043_{24}	−0.08
	0.91965	1.5867	-0.037_{06}	-0.037_{45}	−0.03
	1.2086	1.3210	-0.033_{01}	-0.034_{07}	−0.08
	1.5161	1.0381	-0.032_{68}	-0.031_{96}	0.05
1.4119	0.93465	1.9066	-0.032_{77}	-0.034_{96}	−0.15
	1.2077	1.6855	-0.043_{12}	-0.032_{34}	0.86
1.4549	1.3471	1.6164	-0.029_{23}	-0.031_{30}	−0.17
1.4964	0.85004	2.2067	-0.051_{71}	-0.034_{22}	1.21
	0.99471	2.0532	-0.044_{21}	-0.032_{79}	0.87
	1.1709	1.8832	-0.035_{23}	-0.031_{61}	0.30
1.7651	0.49254	3.0884	-0.033_{78}	-0.034_{10}	−0.02
	0.72814	2.8648	-0.029_{06}	-0.030_{73}	−0.11
	0.95557	2.6500	-0.025_{75}	-0.028_{72}	−0.23
	1.1841	2.4365	-0.024_{37}	-0.027_{63}	−0.29
1.9521	0.21485	3.7775	-0.033_{38}	-0.035_{10}	−0.04
	0.67882	3.3300	-0.025_{58}	-0.027_{32}	−0.11
	0.93178	3.0880	-0.022_{23}	-0.025_{34}	−0.25
	1.1929	2.8392	-0.020_{33}	-0.024_{44}	−0.39
2.0182	1.5386	2.6560	-0.018_{73}	-0.023_{13}	−0.49
2.1249	0.15800	4.2375	-0.031_{28}	-0.032_{12}	−0.01
	0.64568	3.7717	-0.027_{33}	-0.024_{00}	0.21
	0.90599	3.5115	-0.021_{08}	-0.022_{16}	−0.09
	1.1643	3.2603	-0.018_{57}	-0.021_{36}	−0.27
2.4102	0.47371	4.6065	-0.022_{35}	-0.021_{06}	0.06
	0.65589	4.4279	-0.020_{35}	-0.019_{01}	0.09
	0.83587	4.2494	-0.018_{00}	-0.017_{66}	0.03
	1.0141	4.0763	-0.016_{86}	-0.016_{78}	0.01
	1.2086	3.8855	-0.015_{85}	-0.016_{23}	−0.04
2.6062	0.31875	5.2391	-0.019_{89}	-0.021_{30}	−0.05
	0.62787	4.9292	-0.017_{19}	-0.017_{00}	0.01
	0.95470	4.6020	-0.014_{23}	-0.014_{41}	−0.02
	1.2895	4.2645	-0.012_{11}	-0.012_{96}	−0.10
	1.6230	3.9288	-0.011_{42}	-0.011_{81}	−0.05
2.6519	0.30391	5.3890	-0.029_{48}	-0.021_{26}	0.27
	0.52501	5.1472	-0.017_{25}	-0.017_{86}	−0.03
	0.75794	4.9153	-0.013_{27}	-0.015_{35}	−0.16
	0.98926	4.6885	-0.015_{06}	-0.013_{67}	0.13
	1.2086	4.4617	-0.012_{73}	-0.012_{60}	0.01
2.8825	0.30271	5.9615	-0.018_{87}	-0.021_{59}	−0.09
	0.52619	5.7316	-0.016_{20}	-0.017_{59}	−0.08
	0.75920	5.4942	-0.013_{92}	-0.014_{45}	−0.04
	0.99162	5.2676	-0.013_{54}	-0.012_{04}	0.15
	1.2107	5.0338	-0.010_{98}	-0.010_{18}	0.09
3.0133	0.29538	6.3277	-0.025_{10}	-0.023_{44}	0.06
	0.61972	5.9654	-0.012_{22}	-0.017_{07}	−0.32
	0.92474	5.6789	-0.014_{18}	-0.012_{85}	0.12
	1.2332	5.3342	-0.008_{86}	-0.009_{29}	−0.05
	1.5479	5.0076	-0.008_{24}	-0.005_{80}	0.33

By means of Eqs. (1) and (2), the activity coefficient for glycylglycine in the ternary solution is given[9,10] by

$$\ln \gamma_1 = \ln \gamma_{10} + Am_2 + Bm_1m_2 + \frac{C}{2}m_2^2 + Dm_1^2m_2 + \frac{2E}{3}m_1m_2^2 + \frac{F}{3}m_2^3 + Gm_1^3m_2 + \frac{3H}{4}m_1^2m_2^2 + \frac{I}{2}m_1m_2^3 + \frac{J}{4}m_2^4 \quad (3)$$

and that of urea by

$$\ln \gamma_2 = \ln \gamma_{20} + Am_1 + \frac{B}{2}m_1^2 + Cm_1m_2 + \frac{D}{3}m_1^3 + \frac{2E}{3}m_1^2m_2 + Fm_1m_2^2 + \frac{G}{4}m_1^4 + \frac{H}{2}m_1^3m_2 + \frac{3I}{4}m_1^2m_2^2 + Jm_1m_2^3 \quad (4)$$

where γ_1, and γ_2 are the molal activity coefficients of solutes 1 and 2 in a ternary solution containing solute 1 and 2 with molalities m_1 and m_2, respectively. γ_{10} and γ_{20} are the molal activity coefficients, respectivley, 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.[11] The values of osmotic and activity coefficients for glycylglycine solutions were taken from the data of Ellerton *et al.*,[12] and the values for urea solutions, from Ellerton and Dunlop.[10]

Results and Discussion

The isopiestic data for the system glycylglycine—urea—water are given in Table 1. The values of Δ/m_1m_2 (calcd) were calculated by the least-squares treatment of Eq. (2) and Table 2. The percentage

TABLE 2. COEFFICIENTS IN EQ. (2) FOR TERNARY SOLUTIONS GLYCYLGLYCINE-UREA-WATER AT 25°C

Coefficients		Coefficients	
A	−0.099457	F	0.002963
B	0.099905	G	0.007619
C	0.009731	H	0.004752
D	−0.047333	I	0.000847
E	−0.017318	J	−0.000434

error in the molality of the reference solution is defined by[7,13]

$$\text{diff. } \% = \frac{m_R(\text{calcd}) - m_R(\text{exptl})}{m_R(\text{exptl})} \times 100 \quad (5)$$

where

$$m_R(\text{calcd}) = \frac{m_1\phi_1 + m_2\phi_2 + \Delta(\text{calcd})}{2\phi_R} \quad (6)$$

The coefficients of Eq. (2) were calculated from the experimental values of Δ/m_1m_2 by the method of least-squares with a HITAC 8400 computer, and are given in Table 2.

Substituting the numerical values into Eqs. (3) and (4), we have for glycylglycine

$$\ln \gamma_1 = \ln \gamma_{10} - 0.099457m_2 + 0.099905m_1m_2 + 0.004865m_2^2 - 0.047333m_1^2m_2 - 0.011546m_1m_2^2 - 0.000988m_2^3 + 0.007619m_1^3m_2 + 0.003564m_1^2m_2^2 + 0.000424m_1m_2^3 - 0.000109m_2^4 \quad (7)$$

and for urea

$$\ln \gamma_2 = \ln \gamma_{20} - 0.099457m_1 + 0.049952m_1^2 + 0.009731m_1m_2 - 0.015778m_1^3 - 0.011546m_1^2m_2 + 0.002963m_1m_2^2 + 0.001905m_1^4 + 0.002376m_1^3m_2 + 0.000636m_1^2m_2^2 - 0.000434m_1m_2^3 \quad (8)$$

The activity coefficients of glycylglycine at various concentrations of urea in ternary solutions are shown in Fig. 1. The solid line shows the activity coefficients in binary solutions. The activity coefficients of glycylglycine decreased with the increase in concentration of urea. The lowering effect of urea was more marked at smaller concentrations of glycylglycine.

The activity coefficients of urea at various concentrations of glycylglycine are shown in Fig. 2. The activity coefficients of urea also decreased with the increase in concentration of glycylglycine.

Unlike the activity coefficients of glycylglycine, those of α-amino-*n*-butyric acid in aqueous solution are greater than unity and increase with concentration.[5] Addition of urea to aqueous solution of α-amino-*n*-butyric acid, however, also decreases the activity coefficients of the amino acid.[5] Urea decreases hydrophobic bonding of amino acids having larger hydrocarbon residues, resulting in an increase in

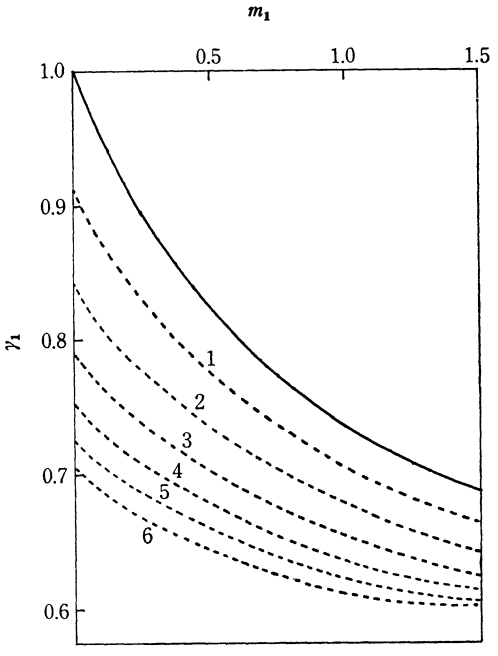

Fig. 1. Activity coefficients of glycylglycine in urea solutions: ——, in water; ----, in urea solution of various concentrations. The concentrations of urea: 1 1.0 m; 2, 2.0 m; 3, 3.0 m; 4, 4.0 m; 5, 5.0 m; 6, 6.0 m.

9) V. E. Bower and R. A. Robinson, *J. Phys. Chem.*, **67**, 1524 (1963).
10) H. D. Ellerton and P. J. Dunlop, *ibid.*, **70**, 1831 (1966).
11) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," 2nd ed., Butterworths, London (1959).
12) H. D. Ellerton, G. Reinfelds, D. E. Mulcahy, and P. J. Dunlop, *J. Phys. Chem.*, **68**, 398 (1964).
13) F. J. Kelly, R. A. Robinson, and R. H. Stokes, *ibid.*, **65**, 1958 (1961).

TABLE 3. FREE ENERGY OF TRANSFER (in cal/mol) OF GLYCYLGLYCINE FROM WATER TO UREA SOLUTIONS

m_1	m_2					
	1.0	2.0	3.0	4.0	5.0	6.0
0	−55.5	−103	−140	−169	−190	−207
0.5	−36.0	− 68.4	− 95.2	−116	−133	−147
1.0	−25.3	− 49.8	− 70.8	− 87.5	− 99.7	−109
1.5	−20.4	− 40.7	− 57.8	− 68.5	− 72.7	− 70.4

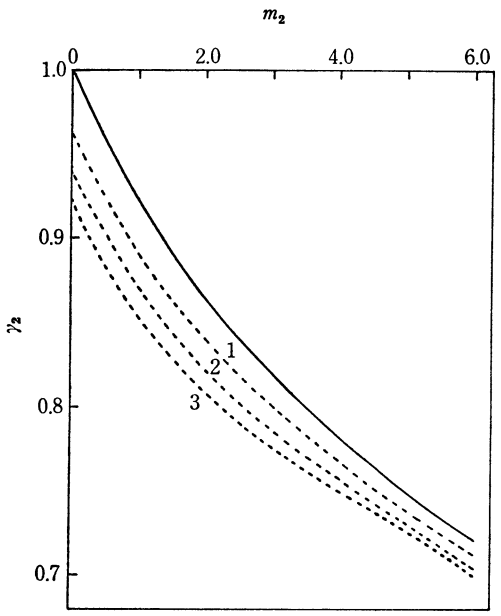

Fig. 2. Activity coefficients of urea in glycylglycine solutions: ——, in water; ----, in glycylglycine solutions at several concentrations of glycylglycine. The concentrations of glycylglycine: 1, 0.5 m; 2, 1.0 m; 3, 1.5 m.

solubility.[2] The decrease in activity coefficient of α-amino-*n*-butyric acid is caused by the decrease in hydrophobic bonding.[5]

On the contraty, as there is no hydrophobic part in glycylglycine, the decrease in activity coefficient is due to some factor other than hydrophobic interaction.

Robinson and Jencks[3] showed that the increase in solubility of acetyltetraglycine ethyl ester by urea is not caused by hydrophobic interaction.

At lower concentrations of amino acids, urea decreases the activity coefficient of glycylglycine to a greater extent than that of α-amino-*n*-butyric acid, the degree of decreases being reversed at relatively high concentrations ($\gtrsim 1.2\ m$). The decrease in activity coefficients of glycylglycine in urea solutions is due to the factor which is more dependent on the concentration of amino acid than hydrophobic interaction.

The activity coefficients of solute i in urea solutions are all relative to unity in the standard state in water and do not refer to the standard state in a water–urea solvent,[14] because of the method of calculation adopted in isopiestic measurements.[9,10] Thus, the free energy of transfer ΔG_t of solute i at constant concentration of the solute, from water to urea solution, is given by an equation containing only the term of the change in activity coefficients. When urea is treated as a component of solvent, the appropriate concentration scale for solute i is the mole fraction. The free energy of transfer ΔG_t for solute i is given by

$$\Delta G_t = RT \ln(f_i/f_{i0}) \tag{9}$$

where f_{i0} and f_i are the activity coefficients in mole fraction scale of solute in binary and ternary solutions, respectively, both at mole fraction N_{i0}. The activity coefficient in the molality scale was calculated at a certain molality in a binary solution from the data referred to before. The activity coefficients of the solute were calculated at the same concentration in mole fraction scale in a ternary solution, by Eq. (7). The activity coefficients in the molality scale were then converted into the rational activity coefficients by the equation[11]

$$f = \gamma(1 + 0.001 m_i W_A) \tag{10}$$

where W_A is the molecular weight of solvent. In a binary solution, W_A is equal to the molecular weight of water. In a ternary solution, W_A is the molecular weight of mixed solvent (water–urea) defined by

$$W_A = \frac{55.51 W_{A0} + W_u m_u}{55.51 + m_u} \tag{11}$$

where W_{A0} is the molecular weight of water, and W_u and m_u are the molecular weight and molality of urea, respectively, in the mixed solvent.

The free energy of transfer of glycylglycine from water to urea solutions at various concentrations is shown in Table 3. At comparatively small concentrations of glycylglycine, the free energy of transfer becomes more negative with increasing concentration of urea, and at higher concentrations, the value changes a little with urea concentration and rather tends to approach zero at higher concentrations of urea. In addition, at the same concentration of urea, the values of ΔG_t approach zero with increasing concentrations of glycylglycine. The values of ΔG_t for glycylglycine (Table 3) have neither maximum not minimum as observed by Nozaki and Tanford[2] from measurement of solubility.[15]

The values of ΔG_t at $m_1=0$ are the free energy change when a mole of glycylglycine is transferred from water with a given mole fraction to the mixture urea–water of the same mole fraction in the limit $m_1=0$. Consequently, they can be compared with the enthalpy of transfer of the solute from water to aqueous urea at infinite dilution ΔH_t which is the difference between partial molal enthalpy of the solute at infinite dilution

14) H. S. Harned and R. A. Robinson, "Multicomponent Electrolyte Solutions," Pergamon Press Inc., London (1968), p. 94.

15) The values of transfer free energy for glycylglycine from water to various urea solution were: −5, to 2 M urea; −12, to 4 M urea; −1, to 6 M urea; and −4 (cal/mol), to 8 M urea.[2]

in ternary and binary solutions.

The enthalpy of transfer of the solute from water to aqueous 6 M (molarity scale) urea solution at infinite dilution was obtained by Kresheck and Benjamin.[4] The value obtained was −1425 cal/mol. Concentration of urea in the molality scale was converted into that in the molality scale by means of density of solution,[16] *i. e.* 6 molarity urea solution is 8.2402 molality. The corresponding values of ΔG_t of glycylglycine at infinite dilution is obtained by extrapolating Eq. (7). The extrapolated value for activity coefficient gives $\Delta G_t = -259$ cal/mol.[17] The entropy of transfer ΔS_t was thus calculated to be −3.9 e. u. Kresheck and Benjamin[4] calculated it with the ΔG_t of Nozaki and Tanford,[2] giving −4.8 e. u. Though the two values are not the same, their signs are negative in both calculations. The transfer of glycylglycine from water to urea solution is accompanied by negative free energy change, negative enthalpy change and negative entropy change. The negative value of free energy of transfer is caused by the large negative value of enthalpy of transfer which surpasses the unfavorable entropy decrease.

It is known that urea destroys the ordered structure of water in aqueous solution.[18] The self diffusion coefficients of water in aqueous glycylglycine solution were measured at 25—50°C by Altunina *et al.*[19] Glycylglycine decreases the activation energy of self diffusion of water. The peptide molecule shifts the equilibrium between ordered and free structures of water to the disordered structure by decreasing activation energy of self diffusion of water. Glycylglycine is ranked thus as a sturcture breaker (negative hydration), that is, water molecules move more easily in glycylglycine solution than in pure water.

In the case of a ternary system containing two sturcture breaking solutes, their activity coefficients are lowered by each other as a result of sturcture salting in,[20,21] occuring in the system, *e. g.*, sodium benzenesulfonate–urea–water.[8] The proportion of free water thus should increase and entropy of transfer should be positive. The data of partial molar heat capacity, obtained with dipeptides by Kresheck and Benjamin,[4] show that the peptide unit has no conspicuous effect on the solvent structure in water, but the negative value of partial molar heat capacities at infinite dilution, associated with transfer of glycylglycine from water to 6 M urea, suggests a peptide-urea interaction resulting in less ordered water structure. The explanations are in line with the lowering of activity coefficients in ternary systems, and with the negative free energy of transfer. However, the entropy of tansfer of glycylglycine from water to urea solutions is negative.

Thus, the interaction between glycylglycine and urea might be the main cause of decreasing activity coefficient and negative free energy of transfer. In order to confirm interaction such as complex formation between glycylglycine and urea, X-ray refraction intensity curves were obtained for the crystalline complex crystallized from aqueous glycylglycine and urea solution (mole ratio, 1 : 4), and soild mixture of glycylglycine and urea of the same mole ratio as crystallized from solution. A difference was found between the curves for the crystalline and solid mixture of glycylglycine and urea.[22] Thus, even in aqueous solution, glycylglycine and urea may form a certain complex aggregation.

Swenson and Koob[23] found by NMR study that rates of exchange for the peptide proton of glycylglycine and triglycine were smaller in aqueous urea than in water. They stated that urea might decrease the base-catalyzed rate by specifically interacting with the peptide hydrogen bond. Their data suggest an interaction between glycylglycine and urea in aqueous solution.

Robinson and Jencks[3] showed that an increase in the number of amide bonds in carbobenzoxyglycine derivatives has no appreciable influence on the effect of urea on the solubility of peptides and that solubility of acetyltetraglycine ethyl ester increases linearly with urea concentration. They pointed out that the evidence is more easily explained in terms of complex formation.

The interaction between urea and glycylglycine increases with urea concentratoin and decreases with glycylglycine concentration. In a relatively small concentration (at about 0.1 M amino acid residue) at which the usual investigations on denaturation of protein by urea are carried out, the decrease in the activity coefficient of glycylglycine is greater than that of α-amino-*n*-butyric acid. These results suggest that urea interacts with polar parts of amino acids, and the effect is important at relatively small concentrations of amino acids. The denaturation of globular protein by urea may thus be caused by the interaction of polar parts of amino acids and urea besides the weakening of hydrophobic bonding between hydrophobic side chains of constituent amino acid residues.

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16) E. P. Eagen, Jr. and B. B. Luff. *J. Chem. Eng. Data*, **11**, 192 (1966).

17) The values of ΔG_t differ from those of Nozaki and Tanford.[2] The results in this paper show that activity coefficients of glycylglycine in urea solutions are a function of the concentrations of both urea and glycylglycine, containing their cross terms. It is therefore thought that the main cause of difference is that Nozaki and Tanford assumed the activity coefficients of glycylglycine in aqueous urea solutions to be dependent on the concentration of glycylglycine only and independent of the concentration of urea.

18) H. S. Frank, *Fed. Proc.*, **S24**, 1 (1965).

19) L. K. Altunina, O. F. Bezrikov, N. A. Smirnova, I. A. Moskbicheva, and V. P. Fokanov, *Struktura i Rol' Vodui v Zhivom Organizme*, **2**, 57 (1968).

20) O. Ya. Samoilov, V. I. Tikhomirov, V. P. Ionov, and A. A. Kuznetzova, *Radiokhimiya*, **1**, 14 (1961); O. Ya. Samoilov, *Stukt. Khim.*, **7**, 15, 175 (1966); O. Ya. Samoilov, "Sostoyanie i Rol' Vodui v Biologicheskiv Obiektov," Nauka, Moskow (1967), p. 31.

21) H. S. Frank, *J. Phys. Chem.*, **67**, 1554 (1963).

22) The samples were rotated vertically to the X-ray beam and the refraction intensity was measured by a Geiger counter in the angle range $2\theta = 10° - 50°$. The crystalline complex showed two new peaks at face distances of 4.85—5.0 Å and 3.25—3.30 Å, and the peak at 5.85—5.90 Å existing in the solid mixture was absent from the complex.

23) C. A. Swenson and L. Koob, *J. Phys. Chem.*, **74**, 3376 (1963).