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Gibbs free energy of transfer of glycine and diglycine from water to alkylurea solutions

Measured vs. calculated values

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Ternary systems comprising water (1), glycine (diglycine) (2) and alkylurea (3) have been investigated using vapor pressure osmometry. Equations were obtained in terms of the molalities of the solutes for the activity coefficients of glycine and diglycine in these systems. The alkylureas used were methyl-, ethyl- and N,N'-dimethylurea. Using the activity coefficients the Gibbs free energy of transfer at infinite dilution of component 2 from water to alkylurea solutions was determined. Since the enthalpies of transfer are known, the corresponding entropies could also be obtained. Calculation of the Gibbs free energy of transfer at infinite dilution of component 2 rests on the assumption that it can be divided into two parts: the difference between the Gibbs free energy of cavity formation and that of interaction in the alkylurea solution and water, respectively. The first part was calculated by scaled particle theory using experimental density and surface tension data. The second part was taken to be due mainly to the change in dipole-dipole interactions.

1. Introduction

Alkylureas are interesting to compare with urea in order to help understand its denaturant action which is by no means clear. It is thought that urea interacts with both peptide groups and apolar side chains through direct or indirect interactions [1]. There is thermodynamic evidence, however, that urea interacts directly with peptide groups, competing with water [2,3]; its favorable interaction with apolar side chains probably reflects a decrease in the hydrophobic interaction between the latter [3–8]. In contrast, for alkylureas, as alkyl substitution increases, the interaction with peptide groups is less favorable [1], but they may interact strongly with hydrophobic side chains [6].

In this study ternary systems consisting of water (1), (di)glycine (2), and alkylurea (3) were investigated using vapor pressure osmometry. Thermodynamic analysis of the data yields $\Delta G^{*\mathrm{tr}}$, the Gibbs free energy of transfer at infinite dilution of solute 2 in ternary systems. The complication of self-association is therefore avoided, the interaction being only of the solute-solvent type.

$\Delta G^{*\mathrm{tr}}$ was also calculated. In the calculation a general scheme of dividing the Gibbs free energy into cavity and interaction terms was adopted [7–9]. The Gibbs free energy of cavity formation in solution is directly proportional to the surface tension of the solvent and the surface area of the solute. Sinanoglu [10] introduced a correction for cavities of microscopic dimensions. As this correc-

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tion factor for calculation of the Gibbs free energy is not available for binary solvents, however, we used instead the scaled particle theory (SPT). SPT was originally developed for hard-sphere solutes [11,12]. It is a semi-empirical theory based on geometry. Later, it was successfully applied to nonpolar solutes in nonaqueous and aqueous solutions [13,14] as well as to polar (ionic) solutes [15]. For aqueous solutions a modification of SPT was developed by Stillinger [16]. Density and surface tension introduce the concept of solvent 'structure' into the theory. In our case the measured surface tension [17] was used to obtain an estimate of the average diameter of the binary solvent unit at a given density. For the Gibbs free energy of cavity formation in water alone, both methods gave the same result.

A dipolar solute like glycine undergoes numerous interactions with water and (alkyl)urea molecules. There are strong hydrogen bonds in the first solvation shell and dipole-dipole interactions are probably quite important. Further out, solute may be taken to interact with bulk solvent, not distinguishing between different molecules. As for nonpolar solutes, when another molecule approaches very closely, there are repulsive and dispersive forces. The Gibbs free energy of transfer from water to alkylurea solution involves differences in various interactions between ternary and binary systems, and where no change on transfer occurs the contribution of that interaction is zero. The number and energy of hydrogen bonds in the first solvation shell are assumed to remain unchanged. The calculation has shown that the competitive interaction of the dipoles of water and alkylurea molecules with that of glycine or diglycine can account for the (positive) change in the Gibbs free energy of interaction.

2. Experimental

Glycine, diglycine and urea were supplied by Sigma (St. Louis, MO). The alkylureas were purchased from Fluka (Buchs, Switzerland). Before use, they were recrystallized from hot methanol.

Osmotic coefficients were determined by vapor pressure osmometry which is an alternative method to isopiestic measurements. It has the advantage of speed but the disadvantage of lower accuracy.

The Knauer (Berlin) vapor pressure osmometer was used. The principles of its operation have been described [18]. All measurements were made at 25 °C.

3. Theoretical interpretation

3.1. Coefficients a_{ij} and Gibbs free energy of transfer

The activity of the solvent, i.e., water, is by definition

$$\ln a_1 = -M_1 m \phi \tag{1}$$

where m is the molality of the solute, M_1 the molecular weight of water, and ϕ the molal osmotic coefficient.

Also, from vapor pressure osmometry [18],

$$\ln a_1 = K'\Delta R; \quad \Delta R/K = m\phi \tag{2}$$

where ΔR is the resistance change on the Wheatstone bridge whose two arms are thermistor beads; on one of them is the solvent drop, on the other the solution drop. K is an instrument calibration constant which is determined from measurements of ΔR for standard solutions of known ϕ.

The usual way of analyzing data in ternary systems (1, water; 2, glycine or diglycine; 3, alkylurea), is in terms of a function Δ [19–21], where

$$\Delta = \Delta R/K - m_2{}^{\circ}\phi_2 - m_3{}^{\circ}\phi_3 \tag{3}$$

where ${}^{\circ}\phi_2$ is the osmotic coefficient in the binary system ($m_3 \to 0$) and ${}^{\circ}\phi_3$ that in the binary system ($m_2 \to 0$).

Osmotic coefficients in the binary systems glycine-water and diglycine-water were taken from the literature [22]. Also, ${}^{\circ}\phi_3$ is available [23], but we preferred the values we measured. First, the binary solvent was studied (ΔR°), followed by the

Table 1

Coefficients a_{ij} for representation of $\Delta/(m_2 m_3)$ in ternary systems at 25 °C

Component 2	Concentration range (mol/kg)	Component 3	Concentration range (mol/kg)	a_{11}	a_{21}	a_{31}	a_{41}	a_{12}	a_{13}	a_{14}	a_{22}	$10^2\sigma$
Glycine	0.198–0.434	urea [a]	0.021–16.40	−0.0398	0.0115	−0.0009	–	0.0059	−0.0002	–	−0.0007	
	0.130–2.150	methyl-urea	0.486– 5.80	0.4682	−0.0080	−0.0625	0.0282	−0.3986	0.1209	−0.0114	–	2.9
	0.162–1.22	ethyl-urea	1.27 – 6.10	0.4213	−0.2719	0.0572	–	−0.1372	0.0117	–	0.0576	3.8
	0.113–1.55	dimethyl-urea	0.480– 4.89	0.6755	−0.8496	0.9608	−0.3248	−0.3521	0.1296	−0.0144	–	2.2
	0.158–1.538	urea [a]	0.412– 6.328	−0.0995	0.0999	−0.0473	0.0076	0.0097	0.0030	−0.0004	−0.0173	
Diglycine	0.330–1.15	methyl-urea	1.31 – 6.98	0.1246	0.0313	−0.0015	–	−0.0025	–	–	−0.0139	1.5
	0.173–1.04	ethyl-urea	0.631– 6.07	0.2596	−0.2639	0.1289	–	−0.0330	–	–	0.0222	1.5
	0.211–0.891	dimethyl-urea	0.794– 5.18	0.6193	−0.6206	1.6297	−1.2171	−0.3838	0.1678	−0.0213	–	1.9

[a] From refs. 29 and 30.

ternary solution (ΔR). For this case, the function Δ can be written

$$\Delta = \Delta R/K - \Delta R^{\circ}/K' - m_2{}^{\circ}\phi_2 \qquad (4)$$

The function Δ/m_2m_3 was analyzed as a power series in coefficients a_{ij} and molalities

$$\Delta/m_2m_3 = \sum_{i=1}\sum_{j=1} a_{ij} m_2^{i-1} m_3^{j-1} \qquad (5)$$

by a multiple regression program. No restrictions on possible coefficients were applied. The criterion for decision between alternative, compatible polynomials was that the standard deviation and the number of coefficients should be minimal.

The activity coefficients of components 2 and 3 in ternary solutions, γ_2 and γ_3, are related to Δ/m_2m_3 by the following equations [19]

$$\Delta/m_2m_3 = \left[\frac{\delta \ln \gamma_2}{\delta m_3}\right]_{m_2} = \left[\frac{\delta \ln \gamma_3}{\delta m_2}\right]_{m_3} \qquad (6)$$

Integration of eq. 6 then leads to polynomials representing the dependence of ln γ_2 and ln γ_3 on m_2 and m_3, respectively.

The coefficients from analysis of the function Δ/m_2m_3 are given in table 1. The significance of individual coefficients a_{ij} is diminished because of the relatively large experimental error of vapor pressure osmometry. The error of function Δ was estimated to be around 15%. From the coefficients a_{ij}, ΔG^{tr} can be obtained. For the transfer at infinite dilution of component 2, the following relation applies

$$\Delta G^{\circ tr} = RT \ln {}^{\circ}\gamma_{2u} \left({}^{\circ}\gamma_{2w} = 1\right) \qquad (7)$$

where ${}^{\circ}\gamma_{2u}$ is the activity coefficient of component 2 at infinite dilution in a ternary system and ${}^{\circ}\gamma_{2w}$ in a binary system. In terms of coefficients a_{ij}

$$\Delta G^{\circ tr} = RT \sum_{k=1} a_{1k}/k m_3^k \qquad (8)$$

The difference between the molal fraction and molality basis at infinite dilution, ΔG^{+tr}, is constant

$$\Delta G^{+tr} - \Delta G^{\circ tr} = RT \ln(1 + m_3 M_1) \qquad (9)$$

The difference from the molarity basis is similarly constant

$$\Delta G^{*tr} - \Delta G^{\circ tr} = RT \ln(1 + m_3 v_3 \rho_1) \qquad (10)$$

where m_3 is the molality and v_3 the apparent specific volume of alkylurea, and M_1 and ρ_1 the

Table 2

Gibbs free energy of transfer at infinite dilution (ΔG^{*tr}, in J/mol) of glycine, diglycine and the peptide group from water to aqueous urea and alkylurea solutions at 25 °C

Solvent	Concentration (M)	ΔG^{*tr} [a] (J/mol)		
		Glycine	Diglycine	Peptide group
Urea-water	2	40 (230) [b]	−220 (310) [b]	−260 (80) [b]
	4	120	−300	−420
	6	225 (790)	−280 (570)	−505 (−220)
	8	350 (1160)	−290 (730)	−640 (−430)
Methylurea-water	2	1280	840	−440
	4	1970 (1715)	1740 (1660)	−230 (−50)
	6	2910	2450	−460
	8	2530 (3795)	2850 (3900)	320 (105)
Ethylurea-water	2	1850	1580	−270
	4	2750 (2350)	2600 (2440)	−150 (110)
	6	3180 (3850)	3340 (4100)	160 (250)
N,*N*′-Dimethylurea-water	2	2980	2430	−550
	4	4960 (3105)	4700 (3420)	−260 (315)
	6	6830	5850	980

[a] Estimated relative errors are about 15%.

[b] Values in parentheses were obtained from solubility studies and the activity coefficient data from the literature (ref. 1).

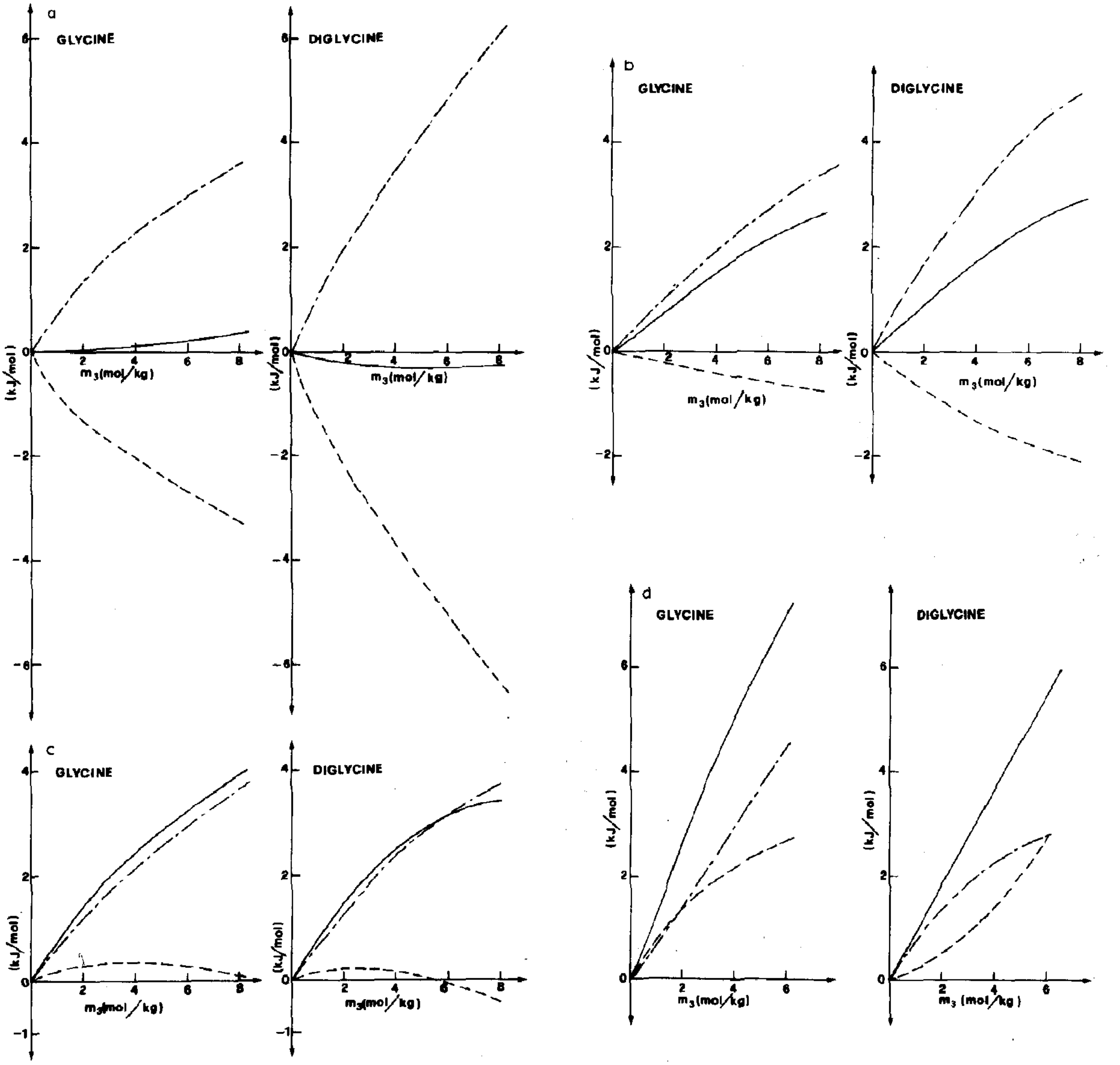

Fig. 1. Plots of thermodynamic functions of transfer of glycine and diglycine at infinite dilution from water to aqueous solutions of alkylureas vs. alkylurea molality, m_3. (– – – –) $-T\Delta S^{tr}$, (———) ΔG^{tr}, (------) ΔH^{tr}. (a) Urea, (b) methylurea, (c) ethylurea, (d) N,N'-dimethylurea.

molecular weight and density of water, respectively. Molality and molarity in the above expressions are 1/1000 of the nominal values.

We were interested mainly in ΔG^{*tr}, the Gibbs free energy of transfer at infinite dilution of component 2 on a molarity basis, as recommended by Ben-Naim [24]. The Gibbs free energies of transfer ΔG^{*tr} are given in table 2.

Entropies of transfer were calculated from values of ΔG^{tr} and published results of the enthalpy of transfer [1]. From the curve of ΔH^{tr} vs molality the ΔH^{tr} values at required molalities were

Table 3

Gibbs free energy of cavity formation, ΔG_{cav}, in alkylurea solutions, calculated by scaled particle theory (SPT), and the difference for the transfer from water to alkylurea solutions, ΔG_{cav}^{tr}, in J/mol at 25 °C [a]

Component 3	m_3	a	y	K_2	K_1	K_0	Component 2			
							Glycine		Diglycine	
							ΔG_{cav}	ΔG_{cav}^{tr}	ΔG_{cav}	ΔG_{cav}^{tr}
Urea	0.5	2.971	0.4318	5477	−12470	17838	51594	594	81801	801
	2.0	3.160	0.4531	5532	−13586	9149	52354	1350	82915	1920
	3.0	3.344	0.4731	5604	−14748	10612	52322	2300	84374	3370
	6.0	3.479	0.4872	5660	−15633	11720	53883	2880	85207	4200
	8.0	3.591	0.5008	5693	−16650	12940	55164	4160	87220	6220
	12.0	3.728	0.5108	5733	−17209	13929	54809		86576	5570
Methylurea	0.5	3.016	0.4334	5368	−12419	7929	50717	−283	80357	−643
	1.0	3.122	0.4421	5292	−12748	8450	50216	−800	79485	−1510
	2.0	3.282	0.4537	5148	−13133	9187	49181	−1820	77723	−3300
	4.0	3.542	0.4708	4922	−13698	10400	47536	−3500	74933	−6100
	8.0	3.842	0.4883	4675	−14268	11817	45712	−5300	71854	−9150
	12.0	4.021	0.4986	4556	−14644	12736	44852		70392	
Ethylurea	0.5	3.053	0.4291	5099	−11908	7685	48441		76660	
	1.0	3.186	0.4335	4816	−11771	7940	46157	−4840	72900	−8100
	2.0	3.400	0.4411	4433	−11621	8386	43075	−7930	67823	−13180
	4.0	3.700	0.4522	4013	−11531	9090	39702	−11300	62260	−18740
	6.0	3.889	0.4550	3697	−11186	9276	37072	−13930	57962	−23040
N,*N*′-Dimethylurea	0.5	3.074	0.4374	5298	−12526	8162	50195	−805	79478	−1520
	1.0	3.223	0.4481	5155	−12869	8825	49166	−1830	77732	−3300
	2.0	3.461	0.4645	4954	−13419	9935	47719	−2380	75269	−5730
	4.0	3.788	0.4845	4693	−14088	11490	45811	−5200	72039	−8960
	6.0	4.007	0.4971	4543	−14537	12593	44721	−6300	70188	−10810
	8.0	4.160	0.5039	4404	−14689	13239	43239	−7360	68384	−12620

[a] The value of ΔG_{cav} in water is 51000 J/mol for glycine and 81000 J/mol for diglycine. K_2, K_1, K_0 and y are parameters of the SPT; a is the diameter of the solvent sphere.

interpolated and $-T\Delta S^{tr}$ calculated. The three thermodynamic functions for the systems in question are shown in figs. 1a–d.

3.2. Calculation of Gibbs free energy of cavity formation by SPT [25]

According to SPT, ΔG_{cav}^{tr} is

$$\Delta G_{cav} = K_0 + K_1 r + K_2 r^2 + K_3 r^3 \tag{11}$$

$$K_0 = RT\left[-\ln(1-y) + 9/2(y/(1-y))^2\right] - \pi P a^3/6 \tag{11a}$$

$$K_1 = -RT/a\left[6y/(1-y) + 18(y/(1-y))^2\right] + \pi P a^2 \tag{11b}$$

$$K_2 = RT/a^2\left[12y/(1-y) + 18(y/(1-y))^2\right] - 2\pi P a \tag{11c}$$

$$K_3 = 4/3\pi P \tag{11d}$$

$r = (a_1 + a)/2$, where a_1 is the diameter of the solute and a that of the solvent spheres, $y = \pi/6a^3\rho$ the volume fraction of the solvent, ρ the number density of the pure solvent and P the pressure in the system. The terms containing P are negligible for small cavities and atmospheric pressure.

The mean diameter of the binary solvent alkylurea-water was estimated from the measured surface tension at various molalities as follows. The macroscopic surface tension γ_∞ is related to the K_2 parameter of SPT by the relation [25]

$$K_2 = \gamma_\infty 4\pi N_A \qquad (12)$$

where N_A is Avogadro's number. From eq. 12 an estimate of K_2 was obtained and then using eq. 11c a was determined. The parameters of SPT and resultant ΔG_{cav} and ΔG_{cav}^{tr} are listed in table 3.

3.3. Calculation of the Gibbs free energy of interaction upon transfer

The total value of ΔG_{int} on dissolving glycine or diglycine in the binary solvent alkylurea-water is composed of $\Delta G_{int,np}$, $\Delta G_{int,hb}$, $\Delta G_{int,dd}$ and $\Delta G_{int,bl}$. np denotes the nonpolar repulsive and dispersive interactions at short distances, hb the hydrogen bonds, dd the interaction between dipoles (both glycine and urea possess high dipole moments in solution) and bl the interaction with bulk solvent beyond the third solvation shell.

As already mentioned, we were interested only in the change of these contributions to ΔG_{int} upon transfer from water to alkylurea-water solution.

Calculation of the nonspecific part of the electrostatic interactions $\Delta G_{int,bl}$ was performed using the theory of Kirkwood [26]. The very slight increase of the static dielectric constant of alkylurea solutions [17] as compared to water makes the nonspecific part of the electrostatic interactions not significantly different from zero. ($\Delta G_{int,bl}^{tr} = 0$).

Calculation of the hydrogen bonding in these complicated systems was not attempted and the number and energy of the strong hydrogen bonds between glycine and water in the first solvation shell were assumed not to change on transfer ($\Delta G_{int,hb}^{tr} = 0$).

After an approximate calculation of the Gibbs free energy of the nonpolar interactions (20 kJ/mol) and its change upon transfer amounting to 10% of that value [27], only the interactions between permanent dipoles were left for consideration. These were calculated using a potential of the form [28]

$$U(r) = -2/3\mu_1\mu_2/r_{12}^3 \qquad (13)$$

where μ_1 and μ_2 are the dipole moments of components 1 and 2, and r_{12} the distance between the two dipoles. In the following equation, uniformly

Table 4

Gibbs free energy of dipole-dipole interactions in ternary solutions ($\Delta G_{d\text{-}d}$, in kJ/mol) and the difference for the transfer from water to alkylurea solutions ($\Delta G_{d\text{-}d}^{tr}$, in J/mol) at 25 ° C [a]

Urea	Glycine		Diglycine		Methylurea	Glycine		Diglycine	
(m_3)	($\Delta G_{d\text{-}d}$)	($\Delta G_{d\text{-}d}^{tr}$)	($\Delta G_{d\text{-}d}$)	($\Delta G_{d\text{-}d}^{tr}$)	(m_3)	($\Delta G_{d\text{-}d}$)	($\Delta G_{d\text{-}d}^{tr}$)	($\Delta G_{d\text{-}d}$)	($\Delta G_{d\text{-}d}^{tr}$)
1.0	−225.4	−630	−363.5	−1010	1.0	−223.1	1680	359.8	2710
2.0	−226.0	−1170	−364.4	−1890	2.0	−221.6	3187	−357.3	5140
4.0	−226.9	−2065	−365.8	−3330	4.0	−219.1	5675	−353.3	9150
6.0	−227.5	−2720	−366.9	−4390	6.0	−217.0	7750	−349.9	12500
8.0	−228.0	−3200	−367.6	−5170	8.0	−215.3	9510	−347.1	15340
					12.0	−212.5	12310	−342.6	19850
Ethylurea					*N,N′*-Dimethylurea				
1.0	−221.3	3480	−356.9	5610	1.0	−221.9	2890	−357.8	4660
2.0	−218.2	6550	−351.9	10560	2.0	−219.4	5370	−353.8	8665
4.0	−213.1	11690	−343.6	18855	4.0	−215.3	9450	−347.2	15245
6.0	−209.0	15775	−337.0	25440	6.0	−212.1	12660	−342.0	20415
8.0	−205.6	19196	−331.5	30950	8.0	−209.5	15250	−337.9	24590
12.0	−201.7	23110	−325.2	37260	12.0	−205.6	19170	−331.6	30910

[a] Dipole moments (in debye): urea, 6.55; methylurea, 7.5; ethylurea, 8.5; *N,N′*-dimethylurea, 9.0; water, 2.5; glycine, 15.7; diglycine, 27.8. The diameters of glycine and diglycine molecules were taken as 5.4 and 7.0 Å, respectively. The values of $\Delta G_{d\text{-}d}$ for glycine and diglycine in water are −224.8 and −362.5 kJ/mol, respectively.

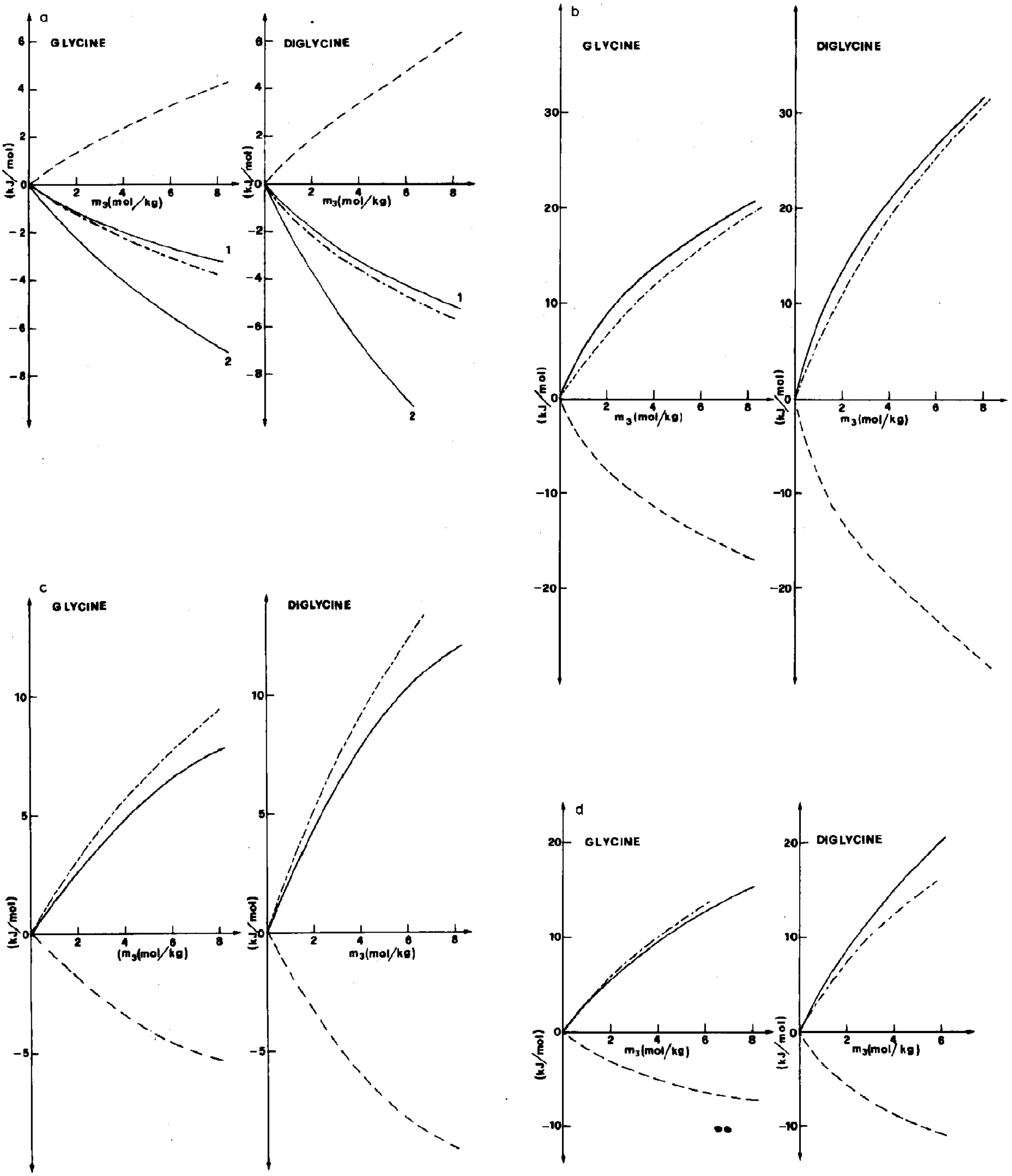

Fig. 2. Plots of the difference between Gibbs free energy of cavity formation of glycine and diglycine in alkylurea solutions and water, $\Delta G^{\mathrm{tr}}_{\mathrm{cav}}$, and the difference between calculated and estimated Gibbs free energy of interaction of glycine and diglycine in alkylurea solutions and water, $\Delta G^{\mathrm{tr}}_{\mathrm{int}}$, vs. alkylurea molality, m_3. (------) $\Delta G^{\mathrm{tr}}_{\mathrm{cav}}$, (———) $\Delta G^{\mathrm{tr}}_{\mathrm{int}}$ calculated, (-—-—-) $\Delta G^{\mathrm{tr}}_{\mathrm{int}}$ estimated. (a) Urea; 1, $\mu_{\mathrm{u}} = 6.55$ debye; 2, $\mu_{\mathrm{u}} = 6.93$ debye; (b) methylurea; (c) ethylurea; (d) N,N'-dimethylurea.

distributed solvent molecules are assumed [29]

$$\Delta G_{\mathrm{int}} = N_{\mathrm{A}} \int U(r) 4\pi r^2 \rho g(r)\, \mathrm{d}r \qquad (14)$$

where ρ is the number density and $g(r)$ the radial distribution function. The resultant equation is

$$\Delta G_{\mathrm{int,dd}} = -N_{\mathrm{A}} 8\pi/3\mu_2 \sum_{j=1,3} \rho_j \mu_j \ln(r^{\circ}/r^{\mathrm{in}}) \qquad (15)$$

For transfer from water (W) to alkylurea (AU):

$$\Delta G_{\mathrm{int,dd}} = -N_{\mathrm{A}} 8\pi/3\mu_2 \left[\left(\rho_1^{\mathrm{AU}} - \rho_1^{\mathrm{W}} \right) \mu_1 + \rho_3 \mu_3 \right] \ln(r^{\circ}/r^{\mathrm{in}}) \qquad (16)$$

Boundaries were taken as the second ($r^{\mathrm{in}} = r + 3r_{\mathrm{W}}$) and third ($r^{\circ} = r + 5r_{\mathrm{W}}$) solvation shell around glycine or diglycine. Interactions were calculated between solute and both components of the binary solvent, assuming infinite dilution of the solute. The values of the parameters used in the calculation were taken from the literature. The Gibbs free energies of dipole-dipole interactions and their changes on transfer are collected in table 4. In figs. 2a–d the comparison between calculated (i.e., $\Delta G^{\mathrm{tr}}_{\mathrm{int,d\text{-}d}}$) and estimated $\Delta G^{\mathrm{tr}}_{\mathrm{int}}$ is shown. Estimated values of $\Delta G^{\mathrm{tr}}_{\mathrm{int}}$ are obtained by subtracting $\Delta G^{\mathrm{tr}}_{\mathrm{cav}}$ from $\Delta G^{*\mathrm{tr}}$.

We will start the discussion with the transfer to urea solutions. $\Delta G^{*\mathrm{tr}}$ for glycine is slightly negative and for diglycine slightly positive (cf. fig. 1a). Data were obtained from the literature [30,31]. The small values reflect almost complete compensation between positive ($-T\Delta S^{\mathrm{tr}}$) and negative ΔH^{tr} (fig. 1a) or between positive $\Delta G^{\mathrm{tr}}_{\mathrm{cav}}$ and negative $\Delta G^{\mathrm{tr}}_{\mathrm{int}}$ (fig. 2a). It should be noted though that the compensation in the case of the latter two quantities was achieved using $\mu_{\mathrm{u}} = 6.55$ debye for urea in the calculation of $\Delta G^{\mathrm{tr}}_{\mathrm{int}}$ and not the experimental value 6.93 debye. This discrepancy could reflect neglected interactions, e.g., hydrogen bonding.

The comparison with the previously published results from solubility measurements [1] (cf. table 2) reveals that the values of $\Delta G^{*\mathrm{tr}}$ of glycine and diglycine from water to urea solutions ascertained by osmometry are quite different from those obtained by solubility measurements. For glycine, the values of $\Delta G^{*\mathrm{tr}}$ from osmometry are distinctly lower, although all are positive. For diglycine, on the other hand, the values from osmometry are negative whereas those from solubility measurements are positive. The error involved is estimated at about 15% so that the differences are generally larger than the experimental error. This finding is somewhat surprising since the values of $\Delta G^{*\mathrm{tr}}$ obtained from solubility measurements contain the activity coefficient contribution [1]. The values of activity coefficients were ascertained by applying the experimental equations based on isopiestic measurements [30–32]. They are actually estimates obtained by extrapolation to high urea concentrations where no isopiestic, and osmometric for that matter, measurements can be performed. A previous judgement on the quality of extrapolated data must therefore be considered too optimistic [1].

From the difference of $\Delta G^{*\mathrm{tr}}$ between diglycine and glycine, assuming the additivity of constituent contributions the values of $\Delta G^{*\mathrm{tr}}$ of the peptide group to urea solutions can be calculated (cf. table 2). All the values are negative and range from −260 (2 M urea) to −640 (8 M urea) J/mol and reflect the dominant dipole-dipole interaction (cf. table 4). The values of $\Delta G^{*\mathrm{tr}}$ for the peptide group obtained from solubility studies are 80 (2 M urea), −220 (6 M urea), and −430 (8 M urea) J/mol (cf. table 2). The differences with the osmometric values could be due to inadequate activity coefficient values. The values of $\Delta G^{*\mathrm{tr}}$ of the peptide group to 8 M urea obtained by solubility studies of acetyltetraglycine ethyl ester (ATGEE) [5], assuming the additivity of constituent contributions, are around −2000 J/mol which is comparable with the value −1445 J/mol determined from the difference between triglycine and diglycine in solubility studies [1]. The situation appears more complicated with the carbobenzoxyglycine peptides [5]. The values of $\Delta G^{*\mathrm{tr}}$ to 8 M urea from solubility studies of CBZ-glycine, -diglycine and -triglycine amides are −3630, −4000 and −4000 J/mol. The $\Delta G^{*\mathrm{tr}}$ value for the peptide group is thus small, approx. −400 J/mol, or zero. In this case the additivity principle does not appear to apply.

The trend in the Gibbs free energy of interaction in the case of alkylureas is to become more and more positive in the order methylurea, N,N'-dimethylurea, ethylurea (cf. table 4). For ethylurea solutions ΔG_{cav}^{tr} is more negative than for N,N'-dimethylurea solutions. As ΔG_{int}^{tr} is more positive for ethylurea solutions, there is little difference in ΔG^{tr} between the two alkylureas. What this behavior is due to could not even by speculated upon. However, owing to the existing trend the enthalpy-entropy compensation is less and less expressed. Thus, e.g., the total (positive) ΔG^{tr} for ethylurea solutions is nearly equal to the positive $(-T\Delta S^{tr})$ contribution (cf. fig. 1c). In accord with this ΔG_{int}^{tr} is larger by $-\Delta G_{cav}^{tr}$ than ΔG^{tr} (cf. fig. 2c and tables 2 and 3). Inspection of table 3 also reveals that generally cavity formation in alkylurea solutions is thermodynamically more favorable than in water or urea solutions. Namely, ΔG_{cav} in alkylurea solutions is less positive. Therefore, ΔG_{cav}^{tr} to these solutions from water is negative as expected for solvents that have lower surface tension than water.

Considering the behavior of urea and alkylurea solutions the main difference between them as regards the thermodynamic functions of transfer is the enthalpy term, (cf. figs. 1a–d). Its change from negative (urea) to positive (N,N'-dimethylurea) values is accompanied by loss of the enthalpy-entropy compensation attributed to diminished interactions between charged groups and alkylureas as well as to the hydrophobic interaction [1]. The same interactions also produce more positive values of $T\Delta S^{tr}$ [31]. However, there is another effect which must not be overlooked, i.e., that of alkylureas on the structure of water. It is possible that solvation changes occur which are accompanied by negative entropy changes. They should reflect increased order in the alkylurea solutions involving very likely the charged groups of (di)glycine. Unfortunately, no experimental data on the structure of these ternary systems, e.g., hydrogen bonding, are available.

The comparison with the results obtained from solubility measurements [1] (cf. table 2) reveals that the values ascertained by osmometry are comparable. However, since the values of ΔG^{*tr} from solubility studies do not contain the activity coefficient contribution, a detailed discussion is not feasible. It is interesting though that ΔG^{*tr} from water to 3 M urea, ethylurea and N,N'-dimethylurea are -770, -650 and -430 J/mol, respectively [5]. Only further studies could elucidate this behavior. If one now compares the peptide group contribution to ΔG^{*tr} from water to alkylurea solutions ascertained by osmometry and solubility studies, one observes relatively poor agreement, e.g., for 8 M methylurea 320 and 105 J/mol, for 4 M N,N'-dimethylurea -260 and 315 J/mol, etc. This is not surprising considering the fact that the values from solubility do not contain the activity coefficient correction.

In summary, it is justified to claim that the application of SPT and classical expressions for calculation of the Gibbs free energy of transfer of glycine and diglycine at infinite dilution from water to aqueous alkylurea solutions has yielded reasonable results, many simplifications notwithstanding. By separating the transfer into two independent processes, i.e., cavity formation and solute-solvent interaction, the contributions of the two processes could be calculated. Comparison with experimental data obtained by vapor pressure osmometry revealed satisfactory agreement. Possible refinement of the calculated quantities will be feasible when details become known about interactions in the system studied.

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