

Thermodynamics of transfer of amino acids from water to ethanol-water mixtures

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Dissociation constants of L-tryptophan, L-histidine, L-aspartic acid, L-glutamic acid and L-serine have been determined in water and ethanol-water mixtures at 298 K potentiometrically by the Calvin-Bjerrum titration technique. These values have been used to calculate the free energies of transfer $\Delta G_i^\circ(i)$ involved in the dissociation equilibria. $\Delta G_i^\circ(RH_2^{2+})$ and $\Delta G_i^\circ(R^{2-})$ have been calculated ($RH^\pm$ being the zwitter ion) knowing the solubilities of these acids in ethanol-water mixtures. Contribution due to amino acid side chain to the free energy of transfer from 100% ethanol to water has been estimated in acidic, neutral and basic media. The equivalence of these values confirms the hydrophobicity scale first proposed by Nozaki and Tanford [*J Biol Chem*, 246 (1971) 2211].

Solubilities of amino acids decrease on addition of alcohol or dioxane to their aqueous solutions. The decrease is related to the structural factors as well as to the solute-solvent interactions. The solubility data in combination with the pK values of amino acids can be used to determine the free energy of transfer of the species involved in the dissociation equilibria^{1,2}. The free energies of transfer of charged species from one solvent to another may formally be divided into 'electrostatic' and 'non-electrostatic'^{3,4} contributions.

The measurement of the free energy of transfer of amino acids from aqueous to mixed solvents has biological significance⁵. Solubility measurements in ethanol and dioxane serve a broader purpose in that they provide a semiquantitative estimate of interactions inside the native protein molecule for hydrophobic moieties, relative to the interaction of the same moieties when exposed to water⁶. The ability of various solvent media for protein denaturation can be understood from solubility measurements. In addition, free energy of transfer of amino acid side chain from 100% organic solvent to water has been used to construct a "hydrophobicity scale"⁶. This concept and our earlier work on the physical properties of amino acids⁷ prompted us to undertake the present investigation. The earlier work on hydrophobicity was carried out in neutral medium. Since the hydrophobicity scale is evaluated from free energies of transfer of zwitter ionic ($RH^\pm$) form of amino acids, the question of interest was whether the same scale would be valid for acidic and basic media.

Materials and Methods

Ethanol was purified by the standard method⁸. Water used was distilled in an all-glass apparatus after prior deionization and distillation with alkaline permanganate. The mixed solvents were prepared by volume. The amino acids, viz., L-glutamic acid, L-aspartic acid, L-tryptophan, L-leucine, L-serine and L-histidine were obtained from M/s Aldrich Chemicals (AR grade).

The pH meter used for measurements was a digital Elico model (accuracy ± 0.01 pH units) with a glass and calomel electrode assembly. The Calvin-Bjerrum titration technique⁹ was adopted to determine the pK values of the amino acids. The H^+ ion concentration in any solution was obtained from the observed B values (pH meter reading) after taking into account the solvent corrections given by Bates¹⁰. The details regarding calculations, maintenance of inert atmosphere during titration, etc., have been reported earlier¹¹⁻¹³.

Results and Discussion

The dissociation equilibria of amino acids in acid and alkali can be represented by Eqs (1) and (2), respectively,



Since the concentration of zwitter ion is negligibly small (less than 0.1%) even at high percentages of alcohol, reactions (1) and (2) correctly represent the dissociation equilibria K_1 and K_2 . The thermodynamic values of K_1 and K_2 were derived from the expression:

$$pK^{\circ} = pK + 2A\sqrt{I}/(1 + Ba_i\sqrt{I})$$

where " a_i " is the ionic radius of a zwitter ion. The values of activity coefficient, γ , needed for the calculation of I in ethanol-water mixtures were obtained from the work of Bates¹⁰.

The constants A and B were calculated from the dielectric constant, ϵ , and densities, d , of ethanol + water mixtures⁷ at 25°C using the following relationships:

$$A = 1.8246 \times 10^6 d^{1/2}/(\epsilon T)^{3/2}$$

$$B = 50.29 d^{1/2}/(\epsilon T)^{1/2}$$

These values are listed in Table 1. The thermodynamic pK° values are listed in Table 2.

The carboxyl group dissociation is diminished in ethanol-water medium, but not to such an extent as found for acids like acetic, succinic, adipic, etc.¹³. Correspondingly the ammonium group dissociation is expected to be only slightly changed in ethanol-water. The variation in pK_2 with respect to $1/\epsilon$ and mole fraction was investigated but no systematic or uniform trend was exhibited and the pattern of curves was also not the same for all the amino acids investigated.

For reaction (1), one can write,

$$\Delta G_i^{\circ}(1) = \Delta G_i^{\circ}(H^+) + \Delta G_i^{\circ}(RH^{\pm}) \rightarrow \Delta G_i^{\circ}(RH_2^{2+}) \quad \dots (3)$$

or

$$\Delta G_i^{\circ}(RH_2^{2+}) = \Delta G_i^{\circ}(H^+) + \Delta G_i^{\circ}(RH^{\pm}) - \Delta G_i^{\circ}(1) \quad \dots (4)$$

Similarly, for reaction (2) one can write,

$$\Delta G_i^{\circ}(R^-) = \Delta G_i^{\circ}(2) - \Delta G_i^{\circ}(H^+) + \Delta G_i^{\circ}(RH^{\pm}) \quad \dots (5)$$

$\Delta G_i^{\circ}(i)$ is the Gibbs free energy change accompanying transfer of species (i) from the standard state in reference solvent water (W) to that in the solvent (S) of the process $i(W) \rightarrow i(S)$.

The values of $\Delta G_i^{\circ}(RH^{\pm})$ and $\Delta G_i^{\circ}(R^-)$ from water to mixed aquo-organic solvents can be calculated from the knowledge of $\Delta G_i^{\circ}(1)$, $\Delta G_i^{\circ}(2)$ and $\Delta G_i^{\circ}(H^+)$. $\Delta G_i^{\circ}(1)$ and $\Delta G_i^{\circ}(2)$ were calculated from pK_1 and pK_2 by the expressions:

$$\Delta G_i^{\circ}(1) = 5.68 (pK_1^{\circ S} - pK_1^{\circ W})(\text{kJ mol}^{-1})$$

and

$$\Delta G_i^{\circ}(2) = 5.68 (pK_2^{\circ S} - pK_2^{\circ W})(\text{kJ mol}^{-1})$$

We have experimentally determined pK_1 and pK_2 values for L-tryptophan, L-aspartic acid and glutamic acid. $\Delta G_i^{\circ}(RH^+)$ for these were obtained from reported solubility data¹ of these acids in ethanol-water mixtures. Utilising Eq. (3), we estimated $\Delta G_i^{\circ}(RH_2^{2+})$. $\Delta G_i^{\circ}(H^+)$ values for the various ethanol-water mixtures were taken from the work of Dey *et al.*^{1,2}. The $\Delta G_i^{\circ}(RH_2^{\pm})$ and $\Delta G_i^{\circ}(R^-)$

Table 1—Mole fractions of ethanol, dielectric constants and the values of Debye-Huckel constants A and B at various ethanol-water mixtures at 298 K

% of ethanol		Mol. fr.	D.C.	A	B
(v/v)	(w/w)				
0	0	0.00	78.13	0.5128	0.3038
20	16.16	0.070	70.3	0.5936	0.3428
40	33.24	0.163	60.4	0.7340	0.3641
60	52.03	0.2979	48.5	0.9976	0.3973
80	73.45	0.5197	37.0	1.4556	0.4424

Table 2—Thermodynamic pK (± 0.02 to ± 0.04) values for various L-amino acids in ethanol-water mixtures at 298 K

		% of ethanol (v/v)				
		0	20	40	60	80
1. Glutamic acid	pK_1	4.42	4.56	4.75	4.90	5.36
	pK_2	9.82	9.80	9.95	10.15	10.86
2. Aspartic acid	pK_1	3.93	4.35	4.86	5.30	5.70
	pK_2	9.77	9.85	10.10	10.30	10.51
3. L-Tryptophan	pK_1	2.60	2.70	3.11	3.68	4.10
	pK_2	9.61	9.45	9.11	8.82	8.61
4. L-Histidine	pK_2	9.22	9.20	9.20	9.22	9.34
	pK_2	9.32	9.27	9.30	9.40	9.71

pK_1 and pK_2 correspond to the pK values of second $-COOH$ and NH_3^+ groups respectively for glutamic and aspartic acids.

values of L-histidine, L-leucine, L-serine and DL-alanine in ethanol-water mixtures have been reported earlier by Dey and Lahiri¹. We have used their $\Delta G_i^{\circ}(RH_2^{\pm})$ values and attempted to obtain $\Delta G_i^{\circ}(R^-)$ from our experiment pK_2 values. The estimated $\Delta G_i^{\circ}(R^-)$ are in a satisfactory agreement with Dey and Lahiri's values; for example, values of $\Delta G_i^{\circ}(R^-)$ (in kJ mol^{-1}) for L-histidine and L-serine at 20% and 40% ethanol obtained by us are 3.24, 5.76 and 4.31 and 8.52 and the values reported by Dey and Lahiri¹ are 3.40, 5.82 and 4.53, 8.60 respectively. Free energies of transfer of L-tryptophan and L-aspartic acid from water to water-ethanol at 298 K are given in Tables 3a and 3b.

Free energy terms

$\Delta G_i^{\circ}(RH^+)$ and $\Delta G_i^{\circ}(R^-)$ values increase with increase in [alcohol] while $\Delta G_i^{\circ}(RH_2^{\pm})$ decreases with increase in [alcohol]. It is known that the free energies of transfer of cations and anions from water to aquo-organic mixtures are predominantly negative and positive respectively, i.e., cations are stabilised in water whereas anions are destabilised¹⁴. The variation of these quantities with respect to the alcohol solvent composition, however, depends on the hydrophobicity of the amino acid.

The free energy calculated in this way can be expressed as the sum of electrostatic, ΔG_i^{el} , and nonelectrostatic (chem), ΔG_i^{chem} , contributions.

$$\Delta G_i^{\text{chem}} = \Delta G_i^{\text{el}}(1) - \Delta G_i^{\text{el}} \quad \dots (6)$$

The electrostatic free energy contribution was calculated from the Born equation⁴.

$$\Delta G^{\text{el}} = 4.2 \times 122 \left(\frac{1}{\epsilon} - 0.0128 \right) \left(\frac{1}{\gamma_{\text{H}^+}} + \frac{1}{\gamma_{\text{A}^-}} \right)$$

$$\text{kJ/mol} = 5.70 (pK^{\text{s}} - pK^{\text{w}}) \quad \dots (7)$$

The radius (γ_{A^-}) of an amino acid anion was calculated¹⁵ as $\left(\frac{3V}{4\pi} \right)^{1/3}$ where V is the molar volume

of the acid anion. The apparent molal volumes, V ($\text{cm}^3 \text{mol}^{-1}$), of amino acids are reported in literature¹⁶. The values of V and the calculated values of ionic radius in Å, are given below: alanine (60.47, 2.43), aspartic acid (73.83, 2.60), glutamic acid (85.88, 2.70), histidine (98.79, 2.86), tryptophan (143.91, 3.25) and leucine (107.74, 2.95).

The experimentally found ΔpK° values were compared with the values calculated using the Born equation. The ΔpK° values are a function of the reciprocal of dielectric constant. All the amino acids exhibit deviations from the theoretical line

Table 3(a)—Free energy (kJ mol^{-1}) of transfer of L-tryptophan from water to ethanol-water mixtures at 298 K

	% of ethanol (v/v)			
	20	40	60	80
$(\Delta G^{\circ})_i(1)$	0.57	2.89	6.13	8.52
$(\Delta G^{\circ})_i(\text{H}^+)$	-1.55	-3.15	-4.32	-5.09
$(\Delta G^{\circ})_i(\text{RH}^{\pm})$	0.218	-0.16	-0.714	-0.84
$(\Delta G^{\circ})_i(\text{RH}_2^{\pm})$	-1.91	-6.20	-11.17	-14.45
$\Delta G_i^{\circ}(2)$	-0.89	-2.90	-4.54	-5.65
$\Delta G_i^{\circ}(\text{R}^-)$	0.90	-0.02	-0.93	-1.6

Table 3(b)—Free energy of transfer of L-aspartic acid from water to ethanol-water mixtures at 298 K

	% of ethanol			
	20	40	60	80
$\Delta G_i^{\circ}(\text{RH}_2)$	-0.42	-1.30	-1.60	-2.25
$\Delta G_i^{\circ}(\text{R}^-)$	3.38	7.61	11.21	18.17
$\Delta G_i^{\circ}(\text{RH}_2^{\pm})$	-2.05	-4.94	-6.81	-7.18
$\Delta G_i^{\circ}(\text{R}^-)$	3.94	8.57	12.88	16.31

ΔG° values are expressed in kJ mol^{-1}

based on the Born equation. The deviations were more pronounced at higher mol percent of ethanol. Amongst the amino acids, L-tryptophan showed the maximum deviation.

The value of $\Delta G_i^{\circ}(\text{chem})$ were evaluated from equation (6). These are presented in Table 4. Values of $\Delta G_i^{\circ}(1)$ (chem) are positive for L-tryptophan and increase with the ethanol content. $\Delta G_i^{\circ}(2)$ (chem) values, on the other hand, are negative and decrease significantly with the increase in [alcohol]. The favourable nature of the free energy term may be due to the hydrophobic nature of the side chain of L-tryptophan. L-Serine also shows negative values of $\Delta G_i^{\circ}(2)$ (chem), though its magnitude is much less than that of L-tryptophan. L-Aspartic acid shows positive values.

The different behaviour of these acids may be considered in terms of their positions in the hydrophobic scale. Solubilities of L-amino acids in ethanol-water mixtures have been measured by Tanford⁵, and Dey and Lahiri¹. The values reported by the two groups agree for aqueous medium but show disagreement for mixtures with greater ethanol content. Obviously, $\Delta G_i^{\circ}(\text{RH}^{\pm})$ values obtained by these two groups of workers do not agree. Since these values are useful for the final determination of $\Delta G_i^{\circ}(\text{RH}^{\pm})$ and $\Delta G_i^{\circ}(\text{R}^{2-})$, we decided to use $\Delta G_i^{\circ}(\text{RH}^{\pm})$ obtained from the solubility measurements of Nozaki and Tanford⁶. We, therefore, reestimated $\Delta G_i^{\circ}(\text{RH}_2^{\pm})$ and $\Delta G_i^{\circ}(\text{R}_2^{\pm})$ values reported by Dey and Lahiri¹ by taking $\Delta G_i^{\circ}(\text{RH}^{\pm})$ from the work of Nozaki and Tanford⁶.

The hydrophobicity scale was established from the knowledge of the contributions of amino acid side chains to the free energies of transfer from 100% organic solvent (ethanol) to water. If, for any amino acid the free energy change is represented as $\Delta G_i^{\circ}(\text{amino acid})$ and for glycine, an amino acid without a side chain, as $(\Delta G^{\circ})_i(\text{glycine})$, then $(\Delta f) = \Delta G_i^{\circ}(\text{amino acid}) - \Delta G_i^{\circ}(\text{glycine})$. Δf is the contribution of the side chain and is the measure of hydrophobicity. Nozaki and Tanford⁶ obtained (Δf) values from solubility measurements of amino acids, i.e., from the magnitudes of $\Delta G_i^{\circ}(\text{RH}^{\pm})$.

We have attempted to obtain (Δf) values from the magnitudes of $\Delta G_i^{\circ}(\text{RH}_2^{\pm})$ and $\Delta G_i^{\circ}(\text{R}^-)$. We assumed that the hydrophobic nature of the side chain would be independent of the nature of the basic unit (common to all the amino acids). Evidently, (Δf) would be the same for an amino acid, even if it is derived from acidic, neutral or basic form. The observed values are set out in Table 5. The (Δf) values are almost the same within the limits of experimental accuracy for acidic, neutral and basic media. This observation gives sup-

Table 4—Electrostatic, $\Delta G_i^{\circ}(\text{el})$, and chemical, $\Delta G_i^{\circ}(\text{chem})$, contributions to free energy (kJ mol^{-1}) of transfer of dissociation $\Delta G_i^{\circ}(\text{disso})$, from water to ethanol-water mixtures of some amino acids at 298 K

Amino acid	% of ethanol (v/v)	$\Delta G_i^{\circ}(\text{el})$	$\Delta G_i^{\circ}(\text{dis})$		$\Delta G_i^{\circ}(\text{chem})$	
			$\Delta G_i^{\circ}(1)$	$\Delta G_i^{\circ}(2)$	$\Delta G_i^{\circ}(1)$	$\Delta G_i^{\circ}(2)$
L-Tryptophan	20	0.47	0.57	-0.89	0.10	1.36
	40	1.28	2.89	-2.90	1.61	-4.18
	60	2.13	6.13	-4.54	4.00	-6.64
	80	4.81	8.52	-5.65	3.71	-10.46
L-Serine	20	0.54		-0.28		-0.82
	40	1.45		-0.11		-1.56
	60	2.40		0.45		-2.85
	80	5.41		2.78		-2.63
L-Aspartic acid	20	0.53	2.44	0.45	1.91	0.08
	40	1.44	5.34	1.87	3.90	-0.43
	60	2.38	7.84	3.01	5.46	0.63
	80	5.36	10.11	4.20	4.75	-1.16
L-Glutamic acid	20	0.52	0.79	-0.11	0.17	-0.63
	40	1.41	1.87	0.74	0.46	0.67
	60	2.34	2.73	1.87	0.39	0.47
	80	5.27	5.34	5.90	0.07	0.63

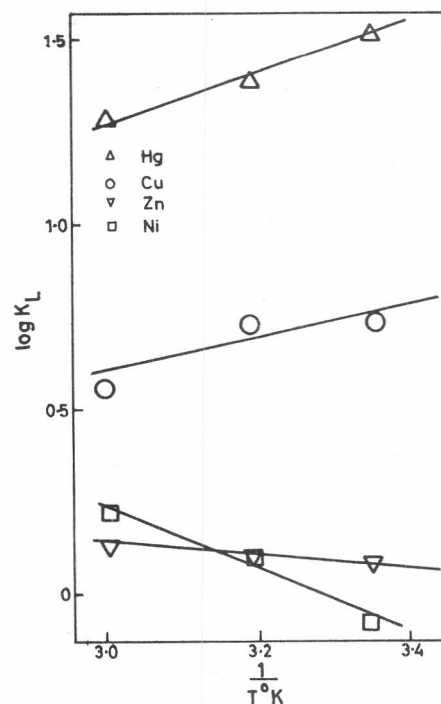
Table 5—(Δf_i) (kJ mol^{-1}) for transfer of amino acid side chain from 100% ethanol to water obtained from acid, neutral and basic states of amino acids

	Acid	Neutral	Basic	Average
L-Tryptophan	14.28	14.20*	14.07	14.18
L-Leucine	7.42	7.50*	7.64	7.52
L-Histidine	2.42	2.28*	2.13	2.28
L-Serine	-1.42	-1.26*	-1.70	-1.46
L-Aspartic acid	5.38	5.60	5.42	5.46
L-Glutamic acid	5.42	5.40	5.80	5.54

*Values from Nozaki and Tanford (1971)

port to our assumption and also confirms the hydrophobic scale⁶.

(Δf) on hydrophobicity scale was plotted against the number of C-atoms on the side chain of an amino acid. Out of 10 amino acids, eight exhibit a satisfactory linear relationship (Fig. 1). Values of (Δf) for tyrosine, alanine, threonine and valine are taken from the work of Nozaki and Tanford⁶. All these eight have only two dissociable groups, i.e., $-\text{COOH}$ and $-\text{NH}_3^+$. Histidine and tyrosine exhibit a significant deviation possibly because (i) these have three dissociable groups, and (ii) in these zwitterionic species is not formed due to the dissociation of $-\text{COOH}$ group, but due to the $-\text{NH}^+$ dissociation. Glutamic and aspartic acids, though containing three dissociable groups, fall on a straight line. The difference in these two and histidine is that in the former the cations are formed from anionic species and not from the cationic species. The free energies of transfer of cations and anions from water

Fig. 1—Variation of (Δf) against number of carbon atoms in the hydrophobic part of an amino acid [tryptophan (1), leucine (2), histidine (3), serine (4), aspartic acid (5), glutamic acid (6), tyrosine (7), alanine (8), threonine (9) and valine (10); (Δf) values for the first six amino acids are from Table 5 and for the rest from ref. 6]

to aquo-organic mixtures are usually negative and positive respectively.

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