Transfer Enthalpies and Entropies of Amino Acids from Water to Urea Solutions

Mohammad Abu-Hamdiyyah* and Afaf Shehabuddin

Chemistry Department, Kuwait University, Kuwait

Transfer enthalpies, ΔH°_t, of 11 amino acids and triglycine from water to 2, 4, 6, and 8 M urea solutions were determined at 25 °C. Combination with ΔG°_t values of Nozaki and Tanford for these compounds gave transfer entropies. The side-chain contributions to ΔH°_t and ΔS°_t were then estimated. Leucine, alanine, threonine, and phenylalanine side chains have positive ΔH°_t values which increase linearly with urea concentration, whereas tyrosine, methionine, histidine, glutamine, tryptophan, and asparagine side chains have negative values which become more negative with urea concentration. Leucine, phenylalanine, tyrosine, alanine, methionine, and threonine side chains have positive ΔS°_t values which increase linearly with urea concentration, while tryptophan side chain has a small negative ΔS°_t value which becomes less negative with urea concentration. The other amino acid side chains and triglycine have negative ΔS°_t values which become more negative with urea concentration.

Introduction

The native state of a given protein is determined by the nature and sequence of its constituent amino acids as well as by the solvent environment (*1*). In the native state the nonpolar side chains of amino acids tend to aggregate to minimize contact with bulk water. This tendency is commonly called hydrophobic bonding and is believed to be controlled by water structure (*2*). However, addition of urea, a denaturing agent of proteins, weakens hydrophobic bonding so that the nonpolar side chains tend to deaggregate and become solvated. It is of paramount interest to learn about the differences in the enthalpic and entropic states of the side chains and their environment in the native and denaturated states of proteins. Such information could be obtained from studies using amino acids as models since they contain the same side chains as proteins. Nozaki and Tanford (*3*) determined the free energy of solution for 11 amino acids and two peptides in water and in 2, 4, 6, and 8 M urea solutions and estimated the change in free energy for transferring a side chain from water to urea solutions.

However, the transfer free energy is a measure of the overall change in the state of the side chain and its surrounding in the two media and does not provide the enthalpic and entropic details of the process involved. These details are important in order to understand more fully the changes that occur on transferring the solute from water to aqueous urea. Data on the enthalpies of solution of amino acids in aq. urea are very scarce in the literature. There is only one study (*4*) involving three amino acids at one urea concentration only. There is, on the other hand, another study (*5*) involving hydrocarbons which approximate nonpolar amino acid side chains, in which the enthalpies of solution of these hydrocarbons in aqueous urea were determined by the van't Hoff method at only one urea concentration. It is generally recognized (*6*) however, that the enthalpy of solution obtained by the van't Hoff method is not as reliable as that obtained directly. Secondly, the use of hydrocarbons as representatives of amino acid side chains involves uncertainties concerning possible head-group effects. For example ΔH°_t values for toluene and isobutane obtained by Wetlaufer et al. (*5*) by the van't Hoff method for the transfer from water to 7 M urea are both 1.5 kcal mol^{-1}. The corresponding ΔH°_t values for (phenylalanine–glycine) and for (leucine–glycine) obtained by Kresheck and Benjamin (*4*) are 0.83 and 0.18 kcal mol^{-1}, respectively. Finally, the study of the enthalpies of solution of amino acids as a function of urea concentration is important in order to learn how the side-chain contribution to ΔH°_t or ΔS°_t varies as urea concentration is increased. This information throws light on the structural changes that occur in the solvent on adding urea.

We therefore have determined the enthalpy of solution of glycine, L-, D-, and DL-alanine, L-leucine, L-phenylalanine, L-tryptophan, L-methionine, L-threonine, L-tyrosine, L-histidine, L-asparagine, L-glutamine, and triglycine, at 25 °C in water and in 2, 4, 6, and 8 M urea solutions. Transfer enthalpies for the amino acids and for their side chains as well as the dependence of these quantities on urea concentration were obtained. The enthalpic data were combined with the corresponding free-energy data of Nozaki and Tanford (*3*) and the corresponding transfer entropies were also obtained. Thus, the driving force for the transfer of amino acids from water to urea solution (up to 8 M) was determined.

Experimental Section

Chemicals. The amino acids, triglycine, and tris(hydroxymethyl)aminomethane were all purchased from Koch Light Laboratories Ltd. and were of puriss grade. Urea of analytical reagent grade quality (British Drug House) was used. Water was double distilled and deionized. Urea solutions were all freshly prepared. The amino acid samples and the triglycine were dried under vacuum at 50 °C for at least 12 h in preweighed ampules which were then covered and sealed under nitrogen.

Calorimetry. An LKB 8700-1 precision calorimetry system equipped with a 100-cm^3 reaction vessel was used. The temperature changes that occur on dissolution of the sample or on calibration were expressed in terms of $\Delta R/R_m$, the relative change in the resistance of the calorimeter thermistor, where ΔR is the corrected resistance change, $R_m = {}^1/_2(R_i + R_f)$, and R_i and R_f are the resistances at the beginning and the end of the main period, respectively. ΔR was obtained by using Dickenson's graphical method (*7*) for all of the compounds used except for leucine, tyrosine, and tryptophan. For the latter three amino acids, which were slow dissolving, the Gunn-modified Dickenson's method was used to estimate ΔR. The resistance–time plots were obtained initially manually and later via a recorder. The energy equivalent of the calorimeter was obtained before and after each dissolution measurement by electrical calibration.

Results and Discussion

Chemical Calibration with Tham. The system was checked by measuring the enthalpy of solution of Tham in 100 mL of 0.1 M aqueous hydrochloric acid. Six independent runs gave an average enthalpy of solution of –(7113 ± 8) cal mol^{-1} with the uncertainty equal to twice the standard deviation of the mean.

0021-9568/82/1727-0074$01.25/0

Table I. Enthalpy of Solution at Infinite Dilution, ΔH_s°, of Amino Acids in Aqueous Urea Solutions at 25 °C[a]

amino acid	[urea], M	ΔH_s°, cal mol^{-1}				
		0	2	4	6	8
glycine		3365 ± 10 (7)	3032 ± 14 (2)	2768 ± 2 (2)	2528 ± 10 (2)	2279 ± 14 (7)
L-alanine		1801 ± 2 (4)	1601 ± 4 (2)	1474 ± 2 (2)	1358 ± 10 (2)	1241 ± 14 (4)
D-alanine		1789 ± 8 (4)				1242 ± 12 (4)
DL-alanine		2183 ± 8 (6)				1625 ± 2 (4)
L-leucine		823 ± 18 (3)	724 ± 12 (2)	692 ± 8 (2)	655 ± 86 (2)	626 ± 128 (2)
L-phenylalanine		1986 ± 36 (3)	1655 ± 44 (2)	1412 ± 46 (2)	1164 ± 2 (2)	930 ± 30 (5)
L-tryptophan		2954 ± 6 (3)	2105 ± 42 (2)	1655 ± 12 (2)	1230 ± 28 (2)	836 ± 20 (3)
L-methionine		2770 ± 18 (4)	2384 ± 26 (2)	2078 ± 20 (2)	1810 ± 30 (2)	1525 ± 14 (3)
L-threonine		2338 ± 8 (4)	1992 ± 8 (2)	1787 ± 6 (2)	1576 ± 6 (2)	1390 ± 36 (4)
L-tryosine		5177 ± 24 (2)	4808 ± 2 (3)	4522 ± 32 (2)	4250 ± 24 (2)	3971 ± 10 (2)
L-histidine		3336 ± 38 (4)	2728 ± 40 (3)	2286 ± 10 (2)	1981 ± 24 (3)	1666 ± 60 (3)
L-asparagine		5008 ± 34 (3)	4179 ± 58 (2)	3676 ± 66 (2)	3261 ± 8 (2)	2865 ± 28 (3)
L-glutamine		5328 ± 14 (3)	4673 ± 24 (2)	4218 ± 36 (2)	3792 ± 12 (2)	3503 ± 14 (3)
triglycine		2768 ± 22 (5)	2068 ± 8 (2)	1432 ± 16 (2)	1001 ± 110 (2)	492 ± 36 (3)

[a] The uncertainty is equal to twice the standard deviation of the mean. The number of determinations is shown in parentheses.

Literature values for the enthalpy of this standard reaction are as follows (cal mol^{-1}): Parson and Rochester (*8*) –(7132 ± 24), Gunn (*9*) –(7107 ± 1), Cassel and Wen (*10*) –(7107 ± 4), de Visser and Somsen (*11*) –(7104 ± 2), Vandreeze and King (*12*) –(7110 ± 4), Irving and Wadso (*13*) –(7104 ± 4), and Skinner (*14*), using an LKB reaction calorimeter, –711 ± 2.

Enthalpies of Solution of Amino Acids and Triglycine. The measurement of the enthalpies of solution were all carried out in very dilute solutions (concentration range, 10^{-3} – 10^{-2} M), and thus the resulting enthalpy for each of these compounds is regarded as the enthalpy of solution at infinite dilution, ΔH°_s. Table I shows the average values of ΔH°_s for each of these compounds at the various urea concentrations with the number of measurements shown in parentheses.

Enthalpy values of some of the amino acids which were also determined by others are compared with corresponding values obtained in this work in Table II. Our result for glycine in water agrees reasonably well with the values obtained by Kresheck and Benjamin (*4*), Kresheck et al. (*15*) and Spink and Auker (*16*) but is less by about 10% than that obtained by Zittle and Schmidt (*17*). The value obtained by Bull et al. (*18*) is much lower; however, the value which they obtained through the solubility measurements is closer to our result. The enthalpy of solution of glycine in 6 M urea that we obtained is higher than that obtained by Kresheck and Benjamin (*4*) by about 7%. The results for DL-alanine are all similar. For L-alanine our result and that of Matsumoto and Amuga (*19*) are close, but both are lower than the result obtained by Bull et al. (*18*) by about 6%. For L-leucine and L-phenylalanine in water and in 6 M urea our results are similar to those obtained by Kresheck and Benjamin (*4*). For L-histidine and L-methionine our values are higher than those obtained by Bull et al. (*18*) by calorimetry but are similar to the values obtained from solubility measurements. Finally, the enthalpy of solution of L-asparagine obtained is lower than that obtained by Zittle and Schmidt by about 700 calories.

The enthalpies of solution at infinite dilution of L-, D- and DL-alanine were determined in water and in 8 M urea solution. The results show that L and D isomers have essentially identical enthalpies of solution in water and also in 8 M urea solution. However, the optically inactive form has a higher enthalpy of solution than the optically pure isomers in water and in 8 M urea solution.

The enthalpies of solution of these compounds are all endothermic in water. ΔH°_s values in aq. urea are endothermic but are smaller than those in water. The decrease in ΔH°_s with urea is greater in the range 0–2 M urea than that in the range 2–8 M urea. The variation of ΔH°_s with urea in the range 2–8 M urea is linear. The order of the rate of this decrease followed by the different amino acids is the same in both ranges and is as follows: leucine < alanine < threonine < phenylalanine < glycine < tyrosine < methionine < histidine glutamine < tryptophan < asparagine < triglycine. The greater the rate of decrease of ΔH°_s with urea concentration, the more hydrophilic the side chain.

Table II. ΔH_s° Values of Some Amino Acids at 25 °C as Determined by Others Compared with our Results[a]

amino acid	ref	ΔH_s°, cal mol^{-1}	
		water	6 M urea
glycine	*17*	3750	
	4	3376 ± 10	2365 ± 25
	15	3413 ± 56	
	16	3390 ± 100	
	18	2801	
	18	3312 (van't Hoff)	
	this work	3365 ± 10	2528 ± 10
DL-alanine	*17*	2040	
	15	2200	
	16	2210 ± 80	
	19	2235 ± 17	
	this work	2183 ± 8	
L-alanine	*18*	1911	
	18	1881 (van't Hoff)	
	this work	1801 ± 2	
	19	1759 ± 36	
L-leucine	*4*	823 ± 20	650 ± 15
	this work	823 ± 18	655 ± 86
L-phenylalanine	*4*	2010 ± 15	1180 ± 10
	this work	1986 ± 38	1164 ± 2
L-histidine	*17*	3300	
	18	2876	
	18	3435 (van't Hoff)	
	this work	3336 ± 38	
L-asparagine	*17*	5750	
	this work	5008 ± 30	
L-methionine	*18*	2386	
	18	2787 (van't Hoff)	
	this work	2770 ± 18	

[a] The uncertainty shown for our results is equal to twice the standard deviation of the mean.

Transfer Enthalpies and Entropies from Water to Urea Solutions. The change in enthalpy on taking 1 mol of amino acid from its infinitely dilute solution in water to the corresponding infinitely dilute solution in aqueous urea of a given concentration is ΔH°_t, the transfer enthalpy. The enthalpies of transfer of the 11 amino acids and of triglycine are all negative and decrease linearly with increasing urea concentration. The order of the rate of this decrease followed by the amino acids is the same as that obtained for ΔH°_s.

When our enthalpy data were combined with the free-energy data of Nozaki and Tanford, $T\Delta S^\circ_t$ values for the amino acids were obtained. All amino acids have negative $T\Delta S^\circ_t$ values which become more negative with increasing urea concentra-

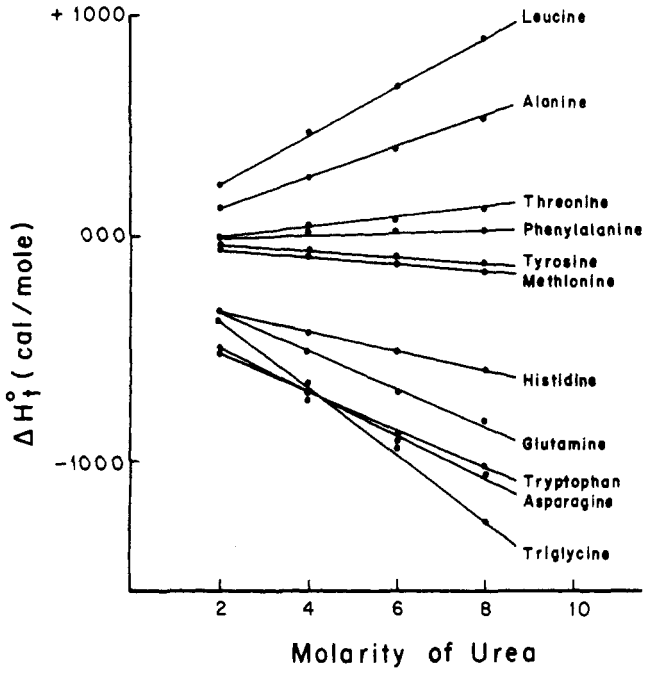

Figure 1. Amino acid side-chain contribution to ΔH°_t.

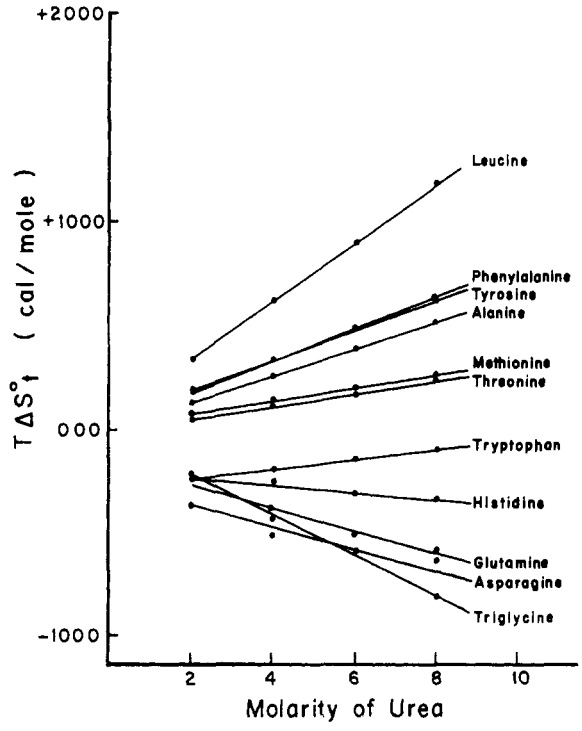

Figure 2. Amino acid side-chain contribution to $T\Delta S^\circ_t$.

tion except leucine, which has a small positive value which increases with urea concentration.

Side-Chain Contribution. Assuming that the enthalpy of solution of the amino acid is the sum of the effects of the side chain and of the zwitterionic head and that the latter effect is approximately equal to that of glycine (*3*), we obtained the contributions of the various amino acid side chains to ΔH°_t, and they are shown in Figure 1. It shows that the amino acid side chains fall into two groups; those of leucine, alanine, threonine, and phenylalanine which have positive contributions to ΔH°_t which increase with urea concentration and those of tyrosine, methionine, histidine, glutamine, tryptophan, and asparagine which have negative contributions to ΔH°_t and become more negative with increasing urea concentration.

The contributions of amino acid side chains to $T\Delta S^\circ_t$ are shown in Figure 2. It is found that the side-chain contributions are linear with urea concentration. Again, amino acid side chains fall into two groups: those of leucine, phenylalanine, tyrosine, alanine, methionine, and threonine which have positive contributions to $T\Delta S^\circ_t$ and become more positive with urea concentration and those of histidine, asparagine, glutamine, and triglycine which have negative contributions which become more negative with increasing urea concentration. The tryptophan side chain has a negative contribution to $T\Delta S^\circ_t$ but behaves like the side chains of the first group regarding the effect of urea concentration.

Transfer Driving Force. Knowing the enthalpy contribution to the transfer free energy of amino acids, or their side chains, from water to aqueous urea solutions enables us to determine whether the driving force is entropic or enthalpic in origin. Regarding the transfer of amino acids, we found that for glycine and alanine the transfer is unfavorable because of entropy ($-T\Delta S^\circ_t > -\Delta H^\circ_t$). For leucine the transfer is favorable because of entropy and enthalpy (ΔH°_t is negative and $T\Delta^\circ_t$ is positive). For the rest of the amino acids and triglycine, the transfer is favorable because of enthalpy ($-\Delta H^\circ_t > -T\Delta S^\circ_t$).

With respect to the transfer of the amino acid side chains, it is unfavorable for alanine because ΔH°_t is more positive than $T\Delta S^\circ_t$. For the side chains of leucine, phenylalanine, and threonine, the transfer is favorable because of entropy (both ΔH°_t and $T\Delta S^\circ_t$ are positive but $T\Delta S^\circ_t > \Delta H^\circ_t$). For methionine and tyrosine side chains the transfer is favorable because ΔH°_t is negative and $T\Delta S^\circ_t$ is positive. Finally, for the side chains of tryptophan, histidine, asparagine, glutamine, and triglycine, it is favorable because of enthalpy (both ΔH°_t and $T\Delta S^\circ_t$ are negative but $\Delta H^\circ_t > -T\Delta S^\circ_t$). When the driving force for the favorable transfer is enthalpic, it is indicative of the dominance of the enthalpic interaction of the amino acid (or the side chain) with aqueous urea. This is true for all of the amino acids, except for glycine and alanine, and for triglycine as well as for all of the side chains that contain polar groups or substituents which are acidic, basic, or both.

However, when the favorable transfer is governed by entropy, it must be due to the greater degrees of freedom of the nonpolar group and its surrounding in aqueous urea than in water. This is related to the difference in the structure of these two solvent systems.

Literature Cited

(1) Tanford, C. *Adv. Protein Chem.* **1968**, *23*, 121; **1970**, *24*, 2.
(2) Kauzmann, W. *Adv. Protein Chem.* **1959**, *14*, 1.
(3) Nozaki, Y.; Tanford, C. *J. Biol. Chem.* **1965**, *240*, 3568.
(4) Kresheck, G. C.; Benjamin, L. *J. Phys. Chem.* **1964**, *68*, 2476.
(5) Wetlaufer, D. B.; Malik, S. K.; Stoller, L.; Coffin, R. L. *J. Am. Chem. Soc.* **1964**, *86*, 508.
(6) Gill, S. J.; Nickolas, N. F.; Wadso, I. *J. Chem. Thermodyn.* **1976**, *8*, 445.
(7) Gunn, S. R. *J. Chem. Thermodyn.* **1971**, *3*, 19.
(8) Parsons, G. H.; Rochester, G. H. *J. Chem. Soc., Faraday Trans. 1* **1975**, *71*, 1069.
(9) Gunn, S. R. *J. Chem. Thermodyn.* **1970**, *2*, 535.
(10) Cassel, K. B.; Wen, W. Y. *J. Phys. Chem.* **1972**, *76*, 1389.
(11) deVisser, C.; Somen, G. *J. Solution Chem.* **1974**, *3*, 847.
(12) Vanderzee, C. E.; King, D. L. *J. Chem. Thermodyn.* **1972**, *4*, 675.
(13) Irving, R. J.; Wadso, I. *Acta Chem. Scand.* **1964**, *18*, 195.
(14) Skinner, H. A. In "Biological Microcalorimetry"; Brown, H. D., Ed.; Academic Press: New York, 1969; p 7.
(15) Kresheck, G. C.; Schneider, H.; Scheraga, H. A. *J. Phys. Chem.* **1965**, *69*, 3132.
(16) Spink, H.; Auker, M. *J. Phys. Chem.* **1970**, *74*, 1742.
(17) Zittle, C. A.; Schmidt, C. L. A. *J. Biol. Chem.* **1935**, *108*, 161.
(18) Bull, H. B.; Breese, K.; Swenson, C. A. *Biopolymers* **1978**, *17*, 1091.
(19) Matsumoto, M.; Amaya, K. *Chem. Lett.* **1978**, *1*, 87.

Received for review November 7, 1980. Revised manuscript received September 1, 1981. Accepted October 9, 1981.