following relation between B and X was found (Figure 2):

$$B = (-10.67 - 5.35X + 7.65X^2 - 3.56X^3) \times 10^{-4}$$

$$\sigma_B = 4.034 \times 10^{-5} \quad (2)$$

Further, another relationship between A and B (Figure 3) is given by

$$A = 0.8607 + 34.125B$$

$$\sigma_A = 1.377 \times 10^{-3} \quad (3)$$

The following equation was obtained:

$$\rho = [(8243.08 - 182.55X + 261.21X^2 - 121.39X^3) \times 10^{-4}] \exp[t(-10.67 - 5.35X + 7.65X^2 - 3.56X^3) \times 10^{-4}] \quad (4)$$

For the refractive indexes, the following equation was assumed:

$$n_D = A'e^{B't} \quad (5)$$

The work of calculation was made in a similar form as described before, and the following results were obtained:

$$B' = (-2.62 - 2.45X + 3.48X^2 - 1.50X^3) \times 10^{-4}$$

$$\sigma_{B'} = 1.694 \times 10^{-5} \quad (6)$$

$$A' = 1.4046 + 0.5B'$$

$$\sigma_{A'} = 0.000 \quad (7)$$

Equation 8 was obtained to relate the refractive index to the temperature and composition of the mixture.

$$n_D = [(14044.69 - 1.23X + 1.74X^2 - 0.75X^3) \times 10^{-4}] \exp[t(-2.62 - 2.45X + 3.48X^2 - 1.50X^3) \times 10^{-4}] \quad (8)$$

In this case the relationship between B' and X becomes slightly curved, owing to the relative importance of the association between the components of the mixture.

By means of eq 4 and 8, density and refractive index data at any given temperature and composition within the experimental ranges can be predicted. Density and refractive index data predicted through eq 4 and 8 compare well with experimental data (Tables II and III), and the average percent deviations are 5.5×10^{-2} and 0.8×10^{-2}, respectively.

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Glossary

A, B	constants in eq 1
A', B'	constants in eq 5
ρ	density of the mixture, g cm^{-3}
n_C	refractive index
X_{Mik}	molar fraction of methyl isobutyl ketone
t	temperature, °C
E	error percentage
σ_B, σ_A, $\sigma_{B'}$, $\sigma_{A'}$	standard deviations for eq 2, 3, 6, and 7, respectively

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Thermodynamic Studies of the Interactions of Guanidinium Chloride with Some Oligopeptides Containing L-Valine, L-Leucine, L-Tryptophan, and L-Tyrosine

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The solubilities of several oligopeptides containing L-valine, L-leucine, L-tryptophan, and L-tyrosine have been measured in water and guanidinium chloride solutions. Approximate Gibbs free energies of transfer of peptide backbone units and valyl, tryptophyl, and tyrosyl side chains from water to guanidinium chloride solutions have been calculated. Enthalpies of transfer for the same groups have been determined from calorimetric measurements. The results obtained show that the behavior of the aromatic side chains in guanidinium chloride solutions is fundamentally different from that of the aliphatic side chains. For the latter the entropic contributions to Gibbs free energies of transfer are relatively large; for the former they are relatively small. The difference in behavior indicates in all probability that aliphatic side chains interfere more strongly than aromatic ones with the structure of the solvent surrounding them.

In a previous paper we reported on the determination of thermodynamic quantities, i.e., changes of Gibbs free energy and enthalpy, for the transfer of oligoglycines and oligoleucines from water to guanidinium chloride and urea solutions of various concentrations (*1*). Both compounds are strong protein denaturants; i.e., they produce partial or complete unfolding (*2*). The results obtained in studies of oligopeptides should help in interpreting the values of the thermodynamic quantities of proteins for the same transfers. It is evident that in the case of proteins the transfer is a more complex process, involving not only unfolding but also large solvation changes. The latter contribute essentially to the thermodynamic quantities of transfer (*3*). Practically the only way to ascertain these contributions is to study oligopeptides of properly selected compositions.

In this report the results obtained are presented in studies of transfer of several oligopeptides containing L-valine, L-leucine, L-tryptophan, and L-tyrosine from water to guanidinium chloride solutions. The procedure for determination of the thermodynamic

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Table I. Solubilities[b] of Oligopeptides in Guanidinium Chloride Solutions at 298.1 K

c(guanidinium chloride), mol/dm³	0	2	4	6
L-valine	5.74 ± 0.05	6.47 ± 0.05	6.62 ± 0.05	6.58 ± 0.05
L-valyl-L-valine	8.50 ± 0.08	16.3 ± 0.1	20.6 ± 0.1	17.9 ± 0.1
L-leucyl-L-valine	15.4 ± 0.1	31.4 ± 0.1	30.6 ± 0.1	28.5 ± 0.1
glycyl-L-leucine	6.94 ± 0.08	12.9 ± 0.1	13.5 ± 0.01	13.1 ± 0.1
glycylglycyl-L-leucine	18.9 ± 0.1		48.6 ± 0.2	44.8 ± 0.2
L-leucylglylcylglycine	49.8 ± 0.2	70.4 ± 0.3	69.2 ± 0.3	56.6 ± 0.2
glycyl-L-tryptophan	0.34 ± 0.01	0.88 ± 0.01	1.87 ± 0.02	2.10 ± 0.02
L-tryptophylglycine	3.72 ± 0.03	6.49 ± 0.05	12.1 ± 0.1	12.0 ± 0.1
L-leucyl-L-tryptophan	2.42 ± 0.03	5.78 ± 0.05	6.29 ± 0.05	9.47 ± 0.1
L-tryptophyl-L-leucine	0.55 ± 0.01	1.71 ± 0.02	7.49 ± 0.05	13.1 ± 0.1 (6.30)[a]
glycyl-L-tyrosine	4.41 ± 0.05	9.55 ± 0.1	15.7 ± 0.1	19.3 ± 0.1
L-tyrosylglycine	1.90 ± 0.02	3.80 ± 0.02	5.44 ± 0.05	
L-tyrosyl-L-leucine	0.25 ± 0.01	0.81 ± 0.01	2.19 ± 0.02	2.72 ± 0.02
L-tyrosylglycylglycine	1.00 ± 0.02	3.35 ± 0.03	5.51 ± 0.05	7.09 ± 0.05
L-leucyl-L-tyrosine	0.78 ± 0.01	1.74 ± 0.02	3.35 ± 0.02	4.23 ± 0.03

[a] The low solubility value. [b] g/100 g of solvent. Uncertainties given are estimates.

quantities was identical with that used in the previous study (*1*). Therefore only its main features will be repeated. Gibbs free energies of transfer were obtained from solubility measurements. Enthalpies of transfer were determined calorimetrically. Analysis of the results allows, among other things, ascertaining the contributions of aromatic amino acid residues to Gibbs free energies and enthalpies of transfer, which, considering their abundance in proteins, are extremely important.

Experimental Section

Materials. Peptides (Sigma) were used as delivered. Guanidinium chloride (Merck, laboratory grade) was washed with acetone; 6 mol/dm³ solution possessed an absorbance of less than 0.2 at 280 nm.

Solubility Measurements. Solubility measurements were performed at 298.1 ± 0.1 K as described previously (*1*). Except in experiments with L-tyrosine-containing peptides, the supernatent liquids were assayed by titration with 0.2 mol/dm³ KOH under streaming nitrogen. The concentration of saturated solutions of peptides containing L-tyrosine was determined spectrophotometrically at 278 nm. The molar absorbances at individual denaturant concentrations were obtained from measurements on solutions of known peptide concentrations. For aqueous solutions, in a few instances the dry weight method was also applied, and good agreement was observed with the results obtained by either titration or spectrophotometry. Two determinations were made for each solvent and solute. If the agreement between the solubility values was not within the limits of estimated uncertainty, a third experiment was performed.

In the measurement of the solubility of one of the peptides studied, i.e., L-tryptophyl-L-leucine in 6 mol/dm³ guanidinium chloride, two values of solubility have been obtained, a high and a low one (see Table I). This is due to the fact that the originally saturated solution after several hours suddenly separated into a gel and a new saturated solution containing much less peptide. No interpretation is available for this behavior, but very likely it involves different solvated species which may or may not possess different crystalline structures. However, inspection of Table I shows that the high value corresponds better with those found at lower guanidinium chloride concentrations than the low one. Therefore the high solubility value was used in the Gibbs free energy calculation. The solubility of L-tyrosylglycine in 6 mol/dm³ guanidinium chloride could not be determined, since at relatively high peptide concentrations gelation occurred.

Calorimetry. Calorimetric experiments were carried out in an LKB batch microcalorimeter 10700-2 at 298.1 K. The procedure applied was the same as in the previous study (*1*).

Table II. Approximate Gibbs Free Energies of Transfer $\Delta G_{tr}'$ of Oligopeptides on a Concentration Basis from Water to Guanidinium Chloride Solutions at 298.1 K

c(guanidinium chloride), mol/dm³	$-\Delta G_{tr}'$,[a] J/mol		
	2	4	6
L-valine	380 ± 40	610 ± 40	620 ± 40
L-valyl-L-valine	1555 ± 40	2140 ± 40	1935 ± 40
L-leucyl-L-valine	1550 ± 40	1590 ± 40	1510 ± 40
glycyl-L-leucine	1520 ± 40	1720 ± 40	1735 ± 40
glycylglycyl-L-leucine		2060 ± 35	1985 ± 35
L-leucylglycylglycine	665 ± 20	710 ± 20	450 ± 20
L-tryptophan[b]	2800	4180	5035
glycyl-L-tryptophan	2420 ± 90	4370 ± 100	4750 ± 100
L-tryptophylglycine	1550 ± 50	2985 ± 60	3055 ± 60
L-leucyl-L-tryptophan	2170 ± 30	2470 ± 40	3500 ± 50
L-tryptophyl-L-leucine	2870 ± 70	6490 ± 60	7860 ± 70
L-tyrosine[b]	1780	2610	3095
glycyl-L-tyrosine	2055 ± 60	3425 ± 70	4040 ± 80
L-tyrosylglycine	1835 ± 70	2840 ± 40	
L-tyrosylglycylglycine	3115 ± 80	4470 ± 100	5195 ± 100
L-leucyl-L-tyrosine	2115 ± 80	3840 ± 90	4520 ± 100
L-tyrosyl-L-leucine	2950 ± 80	5530 ± 90	6165 ± 110

[a] Error limits indicate the possible absolute uncertainties. [b] From ref 3. No error limits given.

Results and Discussion

The solubilities of oligopeptides in water and guanidinium chloride solutions determined in this study are given in Table I. Included are also the results obtained with L-valine which were not available in the literature. From the solubilities the approximate Gibbs free energies of transfer $\Delta G_{tr}'$ were calculated by using eq 1, where S_d and S_w are the concentrations

$$-\Delta G_{tr}' = RT \ln (S_d/S_w) \quad (1)$$

(mol/dm³) of saturated solutions of the oligopeptide in the given guanidinium chloride solution and water, respectively. $-\Delta G_{tr}'$ refers to the transfer of a solute from water to the denaturant solution and does not include the contribution of solute–solute self-interaction to Gibbs free energy of transfer. The exact relation contains a second term on the right-hand side of eq 1, i.e., $RT \ln (y_d/y_w)$, where y_d and y_w are the activity coefficients of the solute in the denaturant solution and water, respectively (*2*).

Table II presents the values of $\Delta G_{tr}'$ calculated from solubilities. They are all negative, reflecting the fact that the solubilities of the oligopeptides studied as well as of L-valine are higher in guanidinium chloride solutions than in water (see Table I). From the values in Table II the contributions to $\Delta G_{tr}'$ from individual units composing the peptides can be calculated.

Table III. Approximate Gibbs Free Energies of Transfer, $\Delta G_{tr}'$, of Peptide Structural Units from Water to Guanidinium Chloride Solutions at 298.1 K[c]

			$-\Delta G_{tr}'$,[a] J/mol		
c(guanidinium chloride), mol/dm³			2	4	6
A	B	C			
Val	Gly	L-valyl group	400 ± 45	780 ± 50	1310 ± 50
Gly-Gly-Leu	Gly-Leu	peptide group		340 ± 70	250 ± 50
Trp	Gly	L-tryptophyl group[b]	2655	4095	5160
Tyr	Gly	L-tyrosyl group[b]	1635	2525	3220

[a] Error limits indicate the possible absolute uncertainties. [b] From ref 3. No error limits given. [c] Abbreviations used: Val, L-valine; Gly, glycine; Leu, L-leucine; Trp, L-tryptophan; Tyr, L-tyrosine. $\Delta G_{tr}'(C) = \Delta G_{tr}'(A) - \Delta G_{tr}'(B)$.

Table IV. Approximate Gibbs Free Energies of Transfer, $\Delta G_{tr}'$, of L-Tryptophyl-L-tryptophan and L-Tyrosyl-L-tyrosine from Water to Guanidinium Chloride Solutions at 298.1 K[b]

				$-\Delta G_{tr}'$,[a] J/mol		
c(guanidinium chloride), mol/dm³				2	4	6
A	B	C	D			
Gly-Trp	Trp-Gly	Gly-Gly	L-tryptophyl-L-tryptophan	3760 ± 150	7220 ± 170	7520 ± 170
Leu-Trp	Trp-Leu	Leu-Leu		2570 ± 160		7620 ± 200
Gly-Tyr	Tyr-Gly	Gly-Gly	L-tyrosyl-L-tyrosine	3680 ± 140	6130 ± 120	
Leu-Tyr	Tyr-Leu	Leu-Leu		2600 ± 220		6950 ± 290

[a] Error limits indicate the possible absolute uncertainties. [b] $\Delta G_{tr}'(D) = \Delta G_{tr}'(A) + \Delta G_{tr}'(B) - \Delta G_{tr}'(C)$.

Moreover, by proper combination of the values of $\Delta G_{tr}'$ it is also possible to arrive at values of $\Delta G_{tr}'$ for oligopeptides whose solubility is too small to allow accurate determination. As previously, the calculations are based on the principle of additivity; i.e., it is assumed that the total Gibbs free energy of transfer for a solute is the sum of the component Gibbs energies (*3*). Thus, e.g., the $\Delta G_{tr}'$ of an amino acid is considered as the sum of the $\Delta G_{tr}'$ of glycine plus the $\Delta G_{tr}'$ of its side chain so that the latter is evaluated as the difference between $\Delta G_{tr}'$ for the amino acid and $\Delta G_{tr}'$ for glycine. Table III lists the values of $\Delta G_{tr}'$ obtained by using the additivity principle. We shall briefly explain the procedure used in the calculations and give some comments on the values themselves. For peptides containing glycine and L-leucine, the values of $\Delta G_{tr}'$ determined in the previous study were used (*1*). The values of $\Delta G_{tr}'$ for the valyl side chain are all negative, increasing with increasing guanidinium chloride concentration. The range of the values classifies guanidinium chloride solutions as moderately good solvents. Since the values for the leucyl side chain have been determined in the previous study, it is tempting to speculate on the values of $\Delta G_{tr}'$ for the CH_2 group. They should be equal to the difference between the values of $\Delta G_{tr}'$ for the leucyl and valyl side chains. For 2 mol/dm³ we find the difference to be −453 J/mol, and for 6 mol/dm³ −580 J/mol. The values are much too large to be taken seriously. We can invoke, without further discussion, previous findings that for small groups like CH_2 the additivity principle is not valid (*3*). An interesting set of $\Delta G_{tr}'$ values can be obtained by subtracting from the values of $\Delta G_{tr}'$ for L-valyl-L-valine those for L-valine. They should comprise the contributions of the peptide unit, CH_2CONH, and the valyl side chain. The latter being known, the contributions of the peptide unit to $\Delta G_{tr}'$ of L-valyl-L-valine can be estimated. The values found in this way for 2, 4, and 6 mol/dm³ are −771, −823, and −5 J/mol, which show that the additivity principle is not valid.

The differences in $\Delta G_{tr}'$ from water to guanidinium chloride solutions between glycylglycyl-L-leucine and glycyl-L-leucine should roughly be equal to the differences in $\Delta G_{tr}'$ between diglycine and glycine, i.e., the $\Delta G_{tr}'$ for the peptide unit. We infer from Table III that the differences for the first pair in 4 and 6 mol/dm³ are −340 and −250 J/mol. For the second pair they were found to be −385 and −405 J/mol. Thus semiquantitative agreement may be claimed. Examination of Table II reveals also another interesting feature, i.e., large differences between the values of $\Delta G_{tr}'$ for L-leucylglycylglycine and glycylglycyl-L-leucine, reflecting apparently the importance of the amino acid sequence.

Table V. Enthalpies of Transfer ΔH_{tr} of Oligopeptides from Water to Guanidinium Chloride Solutions[a]

	ΔH_{tr},[a] J/mol		
c(guanidinium chloride), mol/dm³	2	4	6
L-valine	640 ± 70	1540 ± 160	2380 ± 220
L-valyl-L-valine	540 ± 100	2300 ± 320	4310 ± 510
L-leucyl-L-valine	100 ± 80	1390 ± 300	2970 ± 650
glycyl-L-leucine	−1665 ± 140	−1785 ± 150	−1250 ± 120
glycylglycyl-L-leucine	−3560 ± 200	−5200 ± 210	−5660 ± 240
L-leucylglycyl-glycine	−3530 ± 200	−6230 ± 210	−7630 ± 390
L-tryptophan	−4830 ± 120	−7130 ± 250	−8055 ± 500
glycyl-L-tryptophan	−3040 ± 350	−5520 ± 810	−6150 ± 1360
L-tryptophylglycine	−4790 ± 110	−9150 ± 350	−10875 ± 630
L-leucyl-L-tryptophan	−4540 ± 150	−6550 ± 500	−7930 ± 800
L-tryptophyl-L-leucine	−6800 ± 150	−10800 ± 500	−9700 ± 1100
glycyl-L-tyrosine	−5470 ± 180	−7910 ± 210	−9070 ± 240
L-tyrosylglycine	−4980 ± 50	−4900 ± 50	−6400 ± 70
L-tyrosylglycylglycine	−8830 ± 200	−13300 ± 260	−15450 ± 290
L-leucyl-L-tyrosine	−4560 ± 40	−6210 ± 50	−6880 ± 60
L-tyrosyl-L-leucine	−5210 ± 370	−7380 ± 390	−6430 ± 350

[a] Uncertainties given are estimates.

The values of $\Delta G_{tr}'$ for the tryptophyl and tyrosyl side chains obtained as the difference in $\Delta G_{tr}'$ between the corresponding acid and glycine are from the article of Nozaki and Tanford (*3*), and we need only emphasize that guanidinium chloride solutions are good solvents for both side chains. The difference in $\Delta G_{tr}'$ between L-tyrosylglycylglycine and L-tyrosylglycine should be roughly the same as that between diglycine and glycine. The values found in 2 and 4 mol/dm³ are −1280 and −1630 J/mol. They are much larger than the differences between diglycine and glycine, −230 and −385 J/mol, respectively, reflecting failure of the additivity principle. By adding the values of $\Delta G_{tr}'$ of glycyl-L-tyrosine and L-tyrosylglycine and by subtracting from the sum the values of $\Delta G_{tr}'$ for diglycine, we obtained the approximate values of $\Delta G_{tr}'$ for L-tyrosyl-L-tyrosine. The same procedure with the corresponding values of L-leucine containing peptides should yield about the same values of $\Delta G_{tr}'$ for the

Table VI. Enthalpies of Transfer, ΔH_{tr}, of Peptide Structural Units from Water to Guanidinium Chloride Solutions at 298.1 K[b]

			$-\Delta H_{tr}$,[a] J/mol		
c(guanidinium chloride), mol/dm³			2	4	6
A	B	C			
Val	Gly	L-valyl group	−2310 ± 120	−4340 ± 280	−5680 ± 320
Gly-Gly-Leu	Gly-Leu	peptide group	1890 ± 550	3420 ± 620	4410 ± 760
Trp	Gly	L-tryptophyl group	3160 ± 180	4330 ± 330	4760 ± 600

[a] Error limits indicate the possible absolute uncertainties. [b] $\Delta H_{tr}(C) = \Delta H_{tr}(A) - \Delta H_{tr}(B)$.

Table VII. Enthalpies of Transfer, ΔH_{tr}, of L-Tryptophyl-L-tryptophan and L-Tyrosyl-L-tyrosine from Water to Guanidinium Chloride Solutions at 298.1 K[b]

				$-\Delta H_{tr}$,[a] J/mol		
c(guanidinium chloride), mol/dm³				2	4	6
A	B	C	D			
Gly-Trp	Trp-Gly	Gly-Gly	L-tryptophyl-L-tryptophan	4110 ± 610	9820 ± 1340	10 010 ± 2290
Leu-Trp	Trp-Leu	Leu-Leu		10400 ± 480	17590 ± 480	16 980 ± 2150
Gly-Tyr	Tyr-Gly	Gly-Gly	L-tyrosyl-L-tyrosine	6730 ± 380	7960 ± 410	8 450 ± 610
Leu-Tyr	Tyr-Leu	Leu-Leu		8830 ± 590	12830 ± 590	12 660 ± 680

[a] Error limits indicate the possible absolute uncertainties. [b] $\Delta H_{tr}(D) = \Delta H_{tr}(A) + \Delta H_{tr}(B) - \Delta H_{tr}(C)$.

dipeptide. Examination of Table IV shows that considering the individual contributions of the component units the values of $\Delta G_{tr}'$ appear to be reasonable and consistent. On the other hand, the values of $\Delta G_{tr}'$ for L-tryptophyl-L-tryptophan obtained by the same procedure are in general considerably lower than could be expected on the basis of the additivity rule. The poorer agreement is perhaps due to the fact that the tryptophyl side chain is bulkier than the tyrosyl one.

Results obtained in calorimetric measurements are presented in Table V. With the exception of the L-valine-containing peptides, the enthalpies of transfer ΔH_{tr} from water to guanidinium chloride solutions are all negative. The contributions of the individual structural units were evaluated by assuming the additivity principle. ΔH_{tr} was thus evaluated as the difference in ΔH_{tr} between L-valine and glycine, etc. The values of ΔH_{tr} obtained in this way are assembled in Table VI. Examination of the values leads to some important conclusions. The values of ΔH_{tr} for the valyl side chain are positive and large, which is in agreement with its hydrophobic nature. However, the corresponding values of $\Delta G_{tr}'$ being negative and relatively small, the entropic contributions $T\Delta S$ to $\Delta G_{tr}'$ must also be positive and not much larger than the enthalpic contributions. This finding is in complete agreement with that for the leucyl side chain, described previously (*1*). Then without too much risk, the following generalization can be made. For aliphatic side chains in guanidinium chloride solutions, the enthalpic and entropic effects are large and widely compensating each other. Let us remember that according to the current dogma the entropic effects reflect changes in the structure of solvent surrounding the solute (*4*). As in the case of $\Delta G_{tr}'$, from the values of ΔH_{tr} for glycylglycyl-L-leucine and glycyl-L-leucine the values of ΔH_{tr} for the peptide backbone unit were calculated. Comparison of the values with those obtained from the differences in ΔH_{tr} between diglycine and glycine again reveals semiquantitative agreement. The latter values in 2, 4, and 6 mol/dm³ guanidinium chloride are −2050, −2050, and −3720 J/mol, respectively (*1*).

The values of ΔH_{tr} for the tryptophyl side chain are negative and relatively large. Comparison with the corresponding values for $\Delta G_{tr}'$ shows that they are very close. This naturally means that the enthalpic effects are much larger than the entropic ones. Thus, the behavior of the tryptophyl side chain is distinctly different from that of an aliphatic side chain. The conclusion is further corroborated by considering the peptides containing the tyrosyl side chain. Unfortunately, owing to low solubility of L-tyrosine in water and aqueous guanidinium chloride solutions, the values of ΔH_{tr} cannot be determined calorimetrically. However, approximate values of ΔH_{tr} can be obtained for L-tyrosyl-L-tyrosine by using the same procedure as in the case of $\Delta G_{tr}'$. Although there is only semiquantitative agreement between the values of ΔH_{tr} based on diglycine and dileucine (see Table VII), it is possible to assume after examining the values of $\Delta G_{tr}'$ for L-tyrosine and the tyrosyl side chain that the enthalpic contribution to them is predominant. The agreement between the values of ΔH_{tr} for L-tryptophyl-L-tryptophan based on diglycine and dileucine, respectively (Table VII), is again relatively poor. In spite of that, it can be inferred from the data that the enthalpic contributions to $\Delta G_{tr}'$ are the dominant ones. This should then be a feature characteristic of aromatic residues.

In conclusion, it can be claimed that the results of this study have confirmed the findings about the aliphatic side chains from the previous study (*1*). In addition, it has been shown that the behavior of the aromatic side chains in guanidinium chloride solutions is fundamentally different from that of the aliphatic side chains. For the latter the entropic contributions to Gibbs free energies of transfer are relatively large; for the former they are relatively small. The difference in behavior reflects in all probability the fact that aliphatic side chains interfere more strongly with the structure of solvent surrounding them than aromatic side chains. The view is also in agreement with the observation that aromatic hydrocarbons are more readily accommodated in water than aliphatic hydrocarbons (*5*, *6*). The findings presented in this study thus not only have provided the values of thermodynamic quantities of transfer for the aromatic amino acids composing proteins which are needed to account for the same quantities for proteins, but also give some important clues to the denaturing action of guanidinium chloride solutions.

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