Estimation of the hydration of polar groups of α-amino acids by differential scanning calorimetry

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The heat capacity of hydration of zwitterions derived from aliphatic amino acids depends linearly on the surface area of the amino acid side radicals accessible to water molecules with the slope $b = 2.35 \pm 0.11$ J mol^{-1} K^{-1} Å^{-2} at 298 K. The linear correlation between hydration heat capacities of zwitterions of aliphatic amino acids and the corresponding aliphatic alcohols with a coefficient of approximately unity confirms the assumption that hydrophobic hydration does not depend on the nature of the surrounding groups. Using the assumption that the hydration of hydrocarbon radicals is independent of the neighboring groups, the b value has been used to calculate the contributions of polar groups. The contributions of OH, COOH, and CONH groups of the side radicals in polar amino acids in the zwitterion form are close to zero; in the case of organic nonionic molecules, these contributions are negative. The increments for polar groups obtained for the zwitterions can be used for the calculation of the heat capacities of proteins and polypeptides incorporating charged amino acid residues. The difference between hydrophilic and hydrophobic hydration mechanisms is manifested not only as different magnitudes and signs of heat capacities and temperature coefficients but also in the fact that the neighboring polar (charged) groups have an effect on hydrophilic hydration but have no effect on hydrophobic hydration.

Key words: amino acids; hydrophobic and hydrophilic hydration; calorimetry; thermodynamic stability of proteins.

The nonvalent interactions of amphiphilic molecules (*i.e*, those including both polar and nonpolar groups) with water (hydration) determine largely their physicochemical properties and physiological activity in solution.[1,2] Numerous facts indicate that the mechanisms of hydration of nonpolar and polar groups are different.[2] In the former case, during hydrophobic hydration (HH), hydrogen bonds arise between the water molecules that form a network around the nonpolar group; in the latter case, the arising H-bonds involve the polar groups of the molecule in question. The superposition (competition) of these mechanisms apparently determines the hydration of the whole amphiphilic molecule.

The problem of the effects of groups of atoms on the hydrophobic and hydrophilic hydration of molecules has been discussed time and again. It has been found that the hydration of hydrocarbon radicals is independent of the nature of surrounding groups,[3,4] *i.e.*, that the heat capacity of the CH_2 group in a solution is invariable (~95 J mol^{-1} K^{-1}). This means that the water environment of an alkyl group is not perturbed by polar groups in the molecules and that the principle of additivity of group contributions is applicable to the evaluation of the effects of hydration of the nonpolar part of the molecule.

There are no strict proofs of the validity of the additivity principle in the evaluation of the hydration of an amphiphilic molecule incorporating polar groups. Nevertheless, the contributions of various functional groups to hydration have been calculated with the assumption that additivity holds both for polar and nonpolar fragments.[5,6] Analysis of these studies indicates that the contribution values calculated for the same polar group in various series of compounds are quite scattered.[5–10] In fact, determination of the group contributions for amphiphilic molecules within the framework of the additivity approach[10] required the introduction of correcting terms that took into account the interaction of various groups.

In the present work, the heat capacity of hydration calculated by differential scanning calorimetry (DSC) was used as a measure of hydration.[11]

The additivity principle is significant for the calculation of the heat capacity of hydration for a polypeptide

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 9, pp. 2237—2242, September, 1996.

1066-5285/96/4509-2121 $15.00

chain in the globular or denatured form needed for the theoretical evaluation of thermodynamic functions for the thermal denaturation of a protein. At present, this is done[12,13] using increments of the heat capacity of the hydration of side amino acid residues calculated from the heat capacities of nonionic organic molecules assuming additivity.

The purpose of the present study has been to quantitatively compare the hydration of the polar groups of the side amino acid residues in the zwitterions derived from amino acids with that of nonionic organic molecules. It was shown that the principle of additivity of the contributions of polar groups is only partly applicable to the quantitative evaluation of the hydration of proteins and peptides.

Experimental

Solutions were prepared using bidistilled water. Aliphatic alcohols were thoroughly dried with CaO, Mg alkoxide, and molecular sieves (4 Å) and purified by repeated distillation. Amino acids from the Sigma company (USA) were used.

The heat capacity of solutions was measured on a DASM-4 differential scanning calorimeter (Special Design Bureau, Pushchino, Russia). In each experiment, electrical calibration of the heat capacity scale was carried out based on the Joule—Lenz effect. The reliability of this calibration was additionally checked by using aqueous solutions of methanol as calorimetric standards.

Partial molar heat capacities of solutions were calculated from the equation

$$C_{p,2} = C_{p,1}(V_2/V_1) - M\Delta C_p/m, \qquad (1)$$

where $C_{p,2}$ and $C_{p,1}$ are the heat capacities of the dissolved compound and pure water, respectively; V_2 and V_1 are the partial molar volumes of the solution and pure water, respectively; ΔC_p is the measured difference between the heat capacities of water and the studied solution; M is the molecular weight of the dissolved substance; and m is the weight of the dissolved substance in the cell.

Results and Discussion

The heat capacity of hydration $C_{p,h} = C_{p,2} - C_p^g$ is the difference between the partial molar heat capacities of a compound in an aqueous solution ($C_{p,2}$) and in the gas phase (C_p^g). These values for aliphatic alcohols and for α-amino acids are presented in Tables 1 and 2, respectively. The heat capacities of solutions were measured by DSC under standard conditions (1 atm, 298 K), and the heat capacities of compounds in the gas phase were calculated in terms of the additive scheme with allowance for group contributions (see Ref. 14).

Table 1. Heat capacities of hydration ($C_{p,h}$) of aliphatic alcohols ROH

Alcohol	R	$C_{p,h}$ /J mol^{-1} K^{-1}
MeOH	Me	108.4
EtOH	CH_2Me	192.1
PrOH	CH_2CH_2Me	241.8
Pr^{i}OH	$CHMe_2$	283.1
Bu^{i}OH	CH_2CHMe_2	317.5
Bu^{s}OH	$CHMeCH_2Me$	336.4

Note. R is a nonpolar group.

Table 2. Heat capacities of hydration ($C_{p,h}$) of zwitterions derived from α-amino acids

Amino acid[a]	R[b]	$C_{p,2}$[c] J mol^{-1} K^{-1}	$C_{p,h}$ J mol^{-1} K^{-1}	S_R[d] /Å^2
	b = 2.35±0.11 J mol^{-1} K^{-1} Å^{-2} (298 K)[e]			
Gly	H	35.9	−56.0	25[31]
Ala	Me	138.3	25.7	67
Abu	CH_2Me	225.9	90.2	
Val	$CHMe_2$	303.3	145.7	117
Leu	CH_2CHMe_2	387.1	206.5	137
Ile	$CHMeCH_2Me$	395.6	215.0	140
Ser	CH_2OH	106.1	−19.6	80
Thr	CHOHMe	205.5	54.6	102
Asn	CH_2CONH_2	128.3	−27.9	113
Gln	$CH_2CH_2CONH_2$	181.6	2.4	144
	b = 2.33±0.13 J mol^{-1} K^{-1} Å^{-2} (298 K)[16,17]			
Gly	H	37.6	−51.5	25[31]
Ala	Me	141.2	28.6	67
Abu	CH_2Me	224.6	88.9	
Val	$CHMe_2$	305.8	148.2	117
Leu	CH_2CHMe_2	397.5	216.9	137
Ile	$CHMeCH_2Me$	383.8	203.2	140
Ser	CH_2OH	114.1	−11.6	80
Thr	CHOHMe	205.3	54.4	102
Asn[18]	CH_2CONH_2	125.3	−31.2	113
Gln[18]	$CH_2CH_2CONH_2$	181.6	7.8	144
Asp[b]	CH_2COOH	134.4	−16.0	106
Glu[b]	CH_2CH_2COOH	180.6	7.4	138
	b = 2.06±0.13 J mol^{-1} K^{-1} Å^{-2} (328 K)[16,17]			
Gly	H	76.8	−16.1	25[31]
Ala	Me	166.5	48.1	67
Val	$CHMe_2$	325.2	158.2	117
Leu	CH_2CHMe_2	411.9	220.2	137
Ile	$CHMeCH_2Me$	399.5	207.8	140
Ser	CH_2OH	157.9	26.0	80
Thr	CHOHMe	240.2	81.3	102
Asp[f]	CH_2COOH	180.4	23.4	106
Glu[f]	CH_2CH_2COOH	227.0	45.2	138

[a] Gly is glycine, Ala is L-alanine, Abu is L-α-aminobutyric acid, Val is L-valine, Leu is L-leucine, Ile is L-isoleucine, Ser is L-serine, Thr is L-threonine, Asn is L-asparagine, Asp is L-aspartic acid, Gln is L-glutamine, Glu is L-glutamic acid.

[b] The side radical in the amino acid.

[c] Partial molar heat capacity of aqueous solutions of amino acids.

[d] The surface area of the radical R accessible to water molecules, see Ref. 30 (for all amino acids except for Gly).

[e] Data obtained in the present study.

[f] The presented heat capacities refer to the completely protonated form of R.

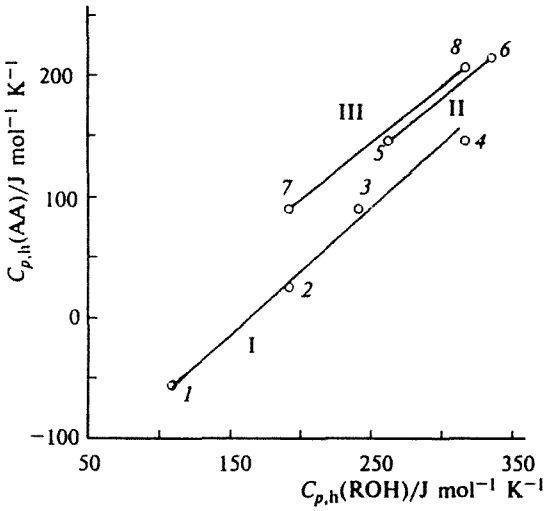

Fig. 1. Relationship between the heat capacities of hydration ($C_{p,h}$) of alcohols (ROH) and amino acids (AA). The points correspond to pairs of compounds: glycine, methanol (*1*); alanine, ethanol (*2*); aminobutyric acid, propanol (*3*); valine, isobutyl alcohol (*4*); valine, isopropyl alcohol (*5*); isoleucine, *sec*-butyl alcohol (*6*); aminobutyric acid, ethanol (*7*); leucine, isobutyl alcohol (*8*); I—III are series of compounds (see the text).

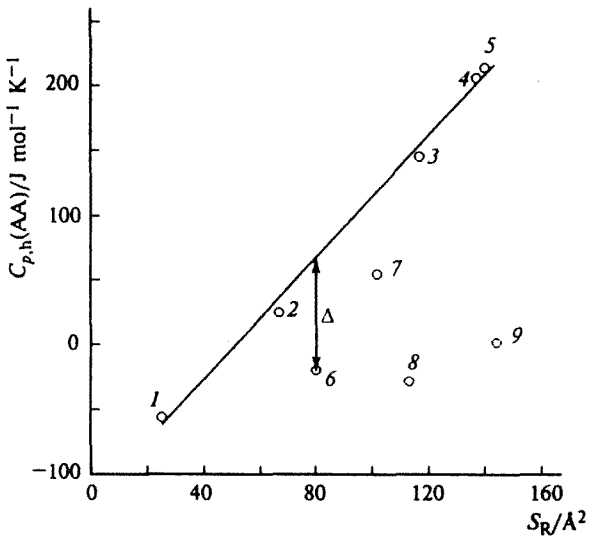

Fig. 2. Dependence of the heat capacities of hydration ($C_{p,h}$(AA)) of amino acids on the surface area of the side radical accessible to water (S_R): glycine (*1*); alanine (*2*); valine (*3*); leucine (*4*); isoleucine (*5*); serine (*6*); threonine (*7*); asparagine (*8*); glutamine (*9*); Δ is the distance between the calculated straight line corresponding to aliphatic amino acids and the experimental values of heat capacity of hydration of nonaliphatic amino acids ($C_{al} - C_{p,h}$).

The additivity of the group contributions for various nonpolar groups R means that there is a linear correlation between any two series of compounds XR and YR (R is the variable alkyl group, and X and Y are invariable groups), and the correlation coefficient is equal to unity.

In fact, for several series of zwitterions derived from aliphatic amino acids and for aliphatic alcohols (Fig. 1), linear correlations with slopes close to unity were observed. For series I (compounds with X = $H_3N^+CHCOO^-$; Y = $HOCH_2$; R = H, Me, CH_2Me, or $CHMe_2$), the slope is 0.98±0.06, r = 0.995. In the case of series II (two pairs of compounds with X = $H_3N^+CH(CHMe)COO^-$; Y = HOCHMe; R = Me or CH_2Me), the coefficient of proportionality is 0.95. For series III (compounds with X = $H_3N^+CHCH_2COO^-$; Y = $HOCH_2$; R = Me or $CHMe_2$), the slope is 0.93. Despite the fact that X includes charged groups, while Y does not contain them, the data presented confirm the expediency of using the additivity principle for the contributions of nonpolar groups in the calculations of the heat capacities of amphiphilic compounds, even with no allowance for the difference in the steric effects of the groups X and Y on the interaction of R with water.

The mutual shielding of the neighboring groups during the interaction with water is determined by the surface area accessible to water (ASA), calculated for a particular molecule using a known algorithm.[15] The dependence of $C_{p,h}$ on the ASA for side radicals (S_R) in aliphatic α-amino acids (Gly, Ala, Val, Leu, and Ile) and in polar amino acids (Thr, Ser, Asn, and Gln) is presented in Fig. 2. The linear dependence C_{al} = $a + bS_R$ for aliphatic amino acids indicates that hydrate shells of the same type are formed around aliphatic radicals. The proportionality coefficient b reflects the increment of purely hydrophobic hydration per 1 Å^2 (b = 2.35±0.11 J mol^{-1} K^{-1} Å^{-2} at 298 K). Therefore, by multiplying b by the ASA of an alkyl group, one can estimate the hydrophobic hydration of this group. Assuming that the mean ASA for a CH_2 group is 30 Å^2, we find that $C_{p,h}$ is equal to ~71 J mol^{-1} K^{-1} at 298 K. The addition of this value to the heat capacity of a methylene group in the gas phase (23 J mol^{-1} K^{-1}) results in the heat capacity of the CH_2 group in an aqueous solution being equal to 94 J mol^{-1} K^{-1}, which is in good agreement with published data. Analysis of the studies dealing with the determination of group contributions indicates that the scattering of the contribution of a nonpolar group on going from one compound to another (for example, 0.1σ—0.2σ, where s is the mean standard deviation)[10] is much smaller than the similar parameter for polar groups and correcting terms (0.5σ—1σ). Analysis of the published data[16—18] on the heat capacity of the hydration of amino acids (see Table 2) gives b = 2.33±0.13 J mol^{-1} K^{-1} Å^{-2} at 298 K, which coincides with our results. Increasing in the temperature, which is known to result in weakening of the HH, leads to a decrease in the coefficient b (at 328 K, b = 2.06±0.13 J mol^{-1} K^{-1} Å^{-2}).

The millimeter absorption spectroscopy data[19,20] imply that the indices of the hydrophobic hydration of aliphatic amino acids, determined by the dynamic mo-

bility of water molecules in the hydrate shell, also vary linearly as functions of the ASA with a correlation coefficient of 0.997. Similar results have been obtained in a study of the adiabatic compressibility of solutions of the homologous series of amino acids from glycine to norleucine.[21] The hydrophobic network of additionally stabilized H-bonds is probably a uniform layer, whose area is determined by the area of direct contact with nonpolar groups.

Thus, the assumption that the surrounding groups make independent contributions to hydrophobic hydration and that these contributions can be calculated from the ASA values of the corresponding nonpolar fragments can be considered to be valid. Based on this, let us derive the equation determining the contributions of polar groups.

It can be seen from Fig. 2 that the experimental values of $C_{p,h}$ for all the nonaliphatic amino acids lie below the straight line corresponding to C_{al} ($\Delta = C_{al} - C_{p,h} > 0$, see Fig. 2). A comparison of two compounds containing polar and nonpolar groups and having identical ASA, makes it possible to infer that the heat capacity of hydration for a nonpolar group (ΔC_{np}) is always greater than that for a polar group (ΔC_p), *i.e.*, that at $S_{np} = S_p$, the inequality $\Delta C_{np} > \Delta C_p$ holds (S_{np} and S_p are the ASA values of the nonpolar and polar groups, respectively).

The contribution of the polar groups R incorporated in the side radicals of α-amino acids to hydration can be quantitatively evaluated in the following way. Let us represent the heat capacity of the hydration $C_{p,h}$ of the whole amino acid molecule by the sum of the heat capacities of hydration of the invariable part ΔC_0 ($H_3N^+CHCOO^-$), of nonpolar groups ΔC_{np}, and of polar groups ΔC_p in the side radical. Taking into account the linear dependence of HH on ASA, we obtain

$$C_{p,h} = \Delta C_0 + \Delta C_p + \Delta C_{np} = \Delta C_0 + \Delta C_p + bS_{np}. \quad (2)$$

Since for the whole side radical, the ASA $S_R = S_p + S_{np}$ and since by the definition of the ΔC_0 value, the equality $\Delta C_{al} = \Delta C_0 + bS_R$ holds, Eq. (2) can be written as follows:

$$C_{p,h} = \Delta C_0 + \Delta C_p + b(S_R - S_p) = C_{al} + \Delta C_p - bS_p. \quad (3)$$

With allowance for the equality $\Delta = C_{al} - C_{p,h}$, from Eq. (3) we obtain a formula for the calculation of the contribution of the polar constituent to the heat capacity of hydration from the Δ values measured (see Fig. 2):

$$\Delta C_p = bS_p - \Delta. \quad (4)$$

The values for the contributions of OH and $CONH_2$ groups calculated from Eq. (4) for Ser, Thr, Gln, and Asn are listed in Table 3; the contributions calculated by the same method for OH, $CONH_2$, and COOH groups using published data are also presented. The heat capacities found for the $H_3N^+CHCOO^-$ fragment are given in Table 4 (the average value is −119 J mol^{-1} K^{-1} at 298 K). The heat capacities for polar noncharged groups are equal to zero or have small negative values. The specific heat capacities of hydration for polar groups $\Delta C_p/S_p$ (see Table 3) are much smaller than the corresponding parameter for the HH (b = 2.35 at 298 K and 2.06 at 328 K). Conversely, the heat capacities of the nonpolar groups incorporated in noncharged organic mol-

Table 3. Contributions of polar groups (ΔC_p) to the hydration of zwitterions derived from amino acids

Group (amino acid)	Δ^a	S_p [30] /Å^2	$\Delta C_p = bS_p - \Delta$ /J mol^{-1} K^{-1}	$\Delta C_p/S_p$ /J mol^{-1} K^{-1} Å^{-2}
		b = 2.35±0.11 J mol^{-1} K^{-1} Å^{-2} (298 K)[b]		
OH (Ser)	78±4	36	−2±6	−0.1
OH (Thr)	65±3	28	1±5	<0.1
$CONH_2$ (Gln)	216±8	91	−2±12	<0.1
$CONH_2$ (Asn)	173±7	69	−11±10	−0.2
		b = 2.33±0.13 J mol^{-1} K^{-1} Å^{-2} (298 K)[16,17]		
OH (Ser)	81±6	36	−3±8	0.1
OH (Thr)	66±3	28	−1±6	<0.1
$CONH_2$ (Gln)	203±8	91	13±11	0.1
$CONH_2$ (Asn)	184±7	69	−21±10	−0.3
COOH (Asp)	146±6	58	−10±9	−0.2
COOH (Glu)	197±7	77	−17±10	−0.2
		b = 2.06±0.13 J mol^{-1} K^{-1} Å^{-2} (328 K)[16,17]		
OH (Ser)	62±6	36	12±8	0.3
OH (Thr)	52±3	28	5±5	0.2
COOH (Asp)	118±5	58	1±7	<0.1
COOH (Glu)	163±6	77	−4±9	<0.1

[a] $\Delta = C_{al} - C_{p,h}$. [b] Data obtained in the present study.

Table 4. Heat capacities of the hydration of groups in zwitterions derived from amino acids compared to the heat capacities of groups in nonionic organic compounds ($C_{p,h}$/J mol^{-1} K^{-1}) at 298 K

Group	Zwitterions derived from amino acids[a]	Amphiphilic organic nonionic molecules		
		Ref. 17	Ref. 10	Ref. 5
CH_2	70.5 (61.8)	60.1	64.5	68.2
OH	−2, 1, 3, −1	1.2	−44.4	−21.7
$CONH_2$	−2, −11, 13, −21	—	−84.6	−51
COOH	−10, −17	−33.7	−84.7	−46
$H_3N^+CHCOO^-$	−119 (−77)	−87	—	−177
CONHCH	−32	—	—	−43.5

[a] Data obtained in the present study; the values given in parentheses correspond to 328 K.

ecules assume fairly great negative values (see Table 4). The divergence established is not unique to the calculation method described by us, because Hakin *et al.*[17] who carried out the calculations using the linear regression method assuming that

$$C_{CH_3} = 1.5C_{CH_2},\ C_H = 0.5C_{CH_2}, \qquad (5)$$

obtained similar results (see Table 4).

According to the data obtained by IR spectroscopy[22] (10^2—10^4 cm^{-1}), millimeter spectroscopy[23] (1—10 cm^{-1}), theoretical calculations,[24] and by other methods, the rotational and vibrational mobility of water molecules is characterized by a broad frequency spectrum corresponding to the vibrational, librational, and rotational degrees of freedom. At room temperature, only those degrees of freedom that are excited at this temperature contribute to the heat capacity. The heat capacity of hydration of nonpolar groups (for the CH_2 group: 70.5 J mol^{-1} K^{-1} at 298 K and 61.8 J mol^{-1} K^{-1} at 328 K, *i.e.*, the temperature coefficient is negative) is much greater than that of polar groups; this makes it possible to link the HH to the degrees of freedom that correspond to vibrations of the network of the weak H-bonds in hydrophobically structurized water.[25] The negative value of the heat capacity of hydration (−119 J mol^{-1} K^{-1} for $H_3N^+CHCOO^-$ at 298 K and −77 J mol^{-1} K^{-1} at 328 K, *i.e.*, positive temperature coefficient of heat capacity) indicates that at relatively low temperatures, the number of excited degrees of freedom is smaller than that for the water bulk, but with an increase in the temperature, the proportion of excited degrees of freedom increases as the strong chain H-bonds between ions become weaker. The heat capacities of hydration of various polar groups (see Table 4) are close; however, those for noncharged molecules and for zwitterions are substantially dissimilar. The electrostatic interaction of oppositely charged ions determines largely the physicochemical properties of zwitterion molecules, for example, due to electrostriction.[26,27] Ionization of terminal groups causes the heat capacities of aliphatic α-amino acids to be lower than those calculated theoretically for the noncharged form by approximately −60 J mol^{-1} K^{-1} (see Ref. 28). Thus, redistribution of water molecules over degrees of freedom may be caused by, among other things, electrostatic field created by zwitterions, because the field has an effect on the interaction of polar groups with the water incorporated in the hydrate shell.

To determine the contribution of the peptide unit CONHCHR to the heat capacity of hydration (C^0), let us assume a model, according to which closely spaced H_3N^+ and COO^- terminal groups make an additional contribution to the hydration of the peptide units in a dipeptide or tripeptide but have no effect on the hydration of subsequent peptide units in a tetrapeptide, *etc.* This assumption has been justified by measurements of molar volumes, heat capacities, and adiabatic compressibilities of oligopeptide homologs. Let us present the heat capacities of hydration of a dipeptide (C^2), a tripeptide (C^3), and higher peptides in the following form

$$\begin{aligned} C^2 &= C^1 + C^0 + \delta, \\ C^3 &= C^1 + 2C^0 + \delta + \delta', \\ C^4 &= C^1 + 3C^0 + \delta + \delta', \\ C^5 &= C^1 + 4C^0 + \delta + \delta', \end{aligned} \qquad (6)$$

where C^1 is the heat capacity of the amino acid, and δ and δ′ are the additional contributions to hydration of a dipeptide and tripeptide, respectively. From relations (6), we obtain the following expressions for determining the contribution of a peptide unit and the corrections δ and δ′:

$$\begin{aligned} C^0 &= C^5 - C^4 = C^4 - C^3, \\ \delta &= C^3 - C^2 - C^0, \\ \delta' &= C^2 - C^1 - C^0. \end{aligned} \qquad (7)$$

The results of the calculations for the polymers of Ala and Gly are presented in Table 5.

The difference in the ΔC_p values for the polar groups incorporated in nonionic organic molecules and in zwitterions as well as the corrections δ and δ′ assume fairly great values comparable to the contributions of the groups or even exceeding them (see Tables 4 and 5). The surface of a globular protein contains charged side groups

Table 5. Heat capacity of hydration of a peptide unit (C^0) and corrections for this value (δ and δ')

Amino acid	R	δ	δ'	C^0/J mol^{-1} K^{-1}	
				CONHCHR	CONHCH
Gly	H	−25.8	−22.9	31.1	−28
Ala	Me	−18	−12.8	122.0	−35

Note. R is the side radical in the amino acid.

of the Arg, Lys, Glu, and Asp amino acid residues; therefore, calculations of hydration should be conducted using the ΔC_p values obtained by us for the amino acid residues located close to charged groups, rather than the corresponding values for the noncharged analogs[5] used in the calculations of the thermodynamic stability of proteins.[12,13]

The substantial nonadditivity of the hydration of polar groups in amino acids and peptides has been shown based on the values of group contributions to adiabatic compressibility.[21,29] Our data obtained by DSC confirm the nonadditivity of the hydration of polar groups, in contrast to nonpolar groups. Thus, the difference between the mechanisms of hydrophilic and hydrophobic hydration is manifested not only as different signs and magnitudes of the heat capacities of hydration and temperature coefficients but also in the fact that the surrounding polar (charged) groups have an effect on hydrophilic hydration and have no effect on hydrophobic hydration.

This work was carried out with the financial support of the Russian Foundation for Basic Research (Project No. 95-03-08958a).

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Received January 12, 1996;
in revised form April 9, 1996