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# Enthalpic Homogeneous Pair Interaction Coefficients of L-α-Amino Acids as a Hydrophobicity Parameter of Amino Acid Side Chains

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***Abstract:*** Enthalpies of dilution of aqueous solutions of L-α-cysteine, L-α-histidine, L-α-asparagine, L-α-glutamine, L-α-arginine, L-α-tryptophan, and L-α-glutamic acid in water at a temperature of 298.15 K have been measured. The values of dilution enthaply were used to determine enthalpic homogeneous pair interaction coefficients which characterize the interactions between zwitterions of the examined L-α-amino acids in water. Approachable literature data of hydrophobic scales have been analyzed to obtain average values. The obtained values of enthalpic pair interaction coefficients have been put together with an average hydrophobic scale.

## Introduction

L-α-Amino acids in aqueous solutions occur in the form of zwitterions ($RCHCO_2^-NH_3^+$) in which side chain $-R$ is linked with the zwitterion "head" $-CHCO_2^-NH_3^+$. In aqueous solutions, the polar "head" ($-CHCO_2^-NH_3^+$) of various L-α-amino acids interacts almost identically with the polar groups or ions and water molecules which form a hydration layer around it. The properties and behavior of particular L-α-amino acids in aqueous solutions are thus diversified only by their side chain ($-R$). The amino acids under investigation show different behaviors in relation both to the surrounding water molecules and to other molecules dissolved in the aqueous solution, depending on whether they possess a nonpolar, polar, or ionic chain. Water creates around polar or charged chains hydration sheaths which are characterized by a specified order. The order and size of the hydration layer depend on the charge or polarity of the amino acid side chain, while water molecules in the direct vicinity of nonpolar amino acid groups reinforce hydrogen bonds between themselves,[1−4] trying, so to say, to "push out" these nonpolar groups from their volume to decrease the contact surface with them. The number of hydrogen bonds of the water molecules surrounding nonpolar side chains is higher than that in bulk water.[5] The results of spectroscopic measurements performed by Hechte et al.[6] suggest that the size of clathrate compounds formed by water molecules around hydrophobic side chains of amino acids depends on the alkyl chain length and increases in the following sequence: Gly < Ala < Val < Ile ≅ Leu. NMR measurements carried out by Bagno et al.[7] have shown that the limitation of thermal movements of water molecules surrounding the amino acid hydrophobic side chains increases with increasing the alkyl group size. The latest theoretical studies on aqueous solutions of hydrophobic substances performed by Hirata[8] have confirmed the above views. The author has shown that the density of water molecules increases in the direct surrounding of nonpolar groups. This observation is consistent with the conclusions obtained in computer simulations of the radial distribution of water molecules in the direct vicinity of side substituents of amino acids.[9]

In many research centers, studies have been performed to determine a parameter which would allow one to describe nonpolar, polar, and ionic side chains.[10−33] These parameters

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***Table 1.*** Hydrophobicity Scales of Natural Amino Acids[a]

| | Levitt[11] 1976 | Hoop[12] 1981 | Kyte[13] 1982 | Sweet[14] 1983 | Fauchere[15] 1983 | Sasagawa[16] 1984 | Rose[17] 1985 | Miyazawa[18] 1985 | Kidera[19]* 1985 | Parker[20]* 1986 | Eisenberg[21] 1986 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ala (A) | 0.5 | 0.5 | 1.8 | −0.40 | 0.31 | 0.13 | 0.74 | 5.33 | 0.27 | −2.10 | 0.67 |
| Arg (R) | −3.0 | −3.0 | −4.5 | −0.59 | −1.01 | 0.26 | 0.64 | 4.18 | −1.87 | −4.20 | −2.10 |
| Asn (N) | −0.2 | −0.2 | −3.5 | −0.92 | −0.60 | −0.45 | 0.63 | 3.71 | −0.81 | −7.00 | −0.60 |
| Asp (D) | −2.5 | −3.0 | −3.5 | −1.31 | −0.77 | 0.10 | 0.62 | 3.59 | −0.81 | −10.0 | −1.20 |
| Cys (C) | 1.0 | 1.0 | 2.5 | 0.17 | 1.54 | 1.57 | 0.91 | 7.93 | 1.05 | −1.40 | 0.38 |
| Gln (Q) | −0.2 | −0.2 | −3.5 | −0.91 | −0.22 | 0.36 | 0.62 | 3.87 | −1.10 | −6.00 | −0.22 |
| Glu (E) | −2.5 | −3.0 | −3.5 | −1.22 | −0.64 | 0.27 | 0.62 | 3.65 | −1.17 | −7.80 | −0.76 |
| Gly (G) | 0.0 | 0.0 | −0.4 | −0.67 | 0.00 | 0.22 | 0.72 | 4.48 | 0.16 | −5.70 | 0.00 |
| His (H) | 0.5 | 0.5 | −3.2 | −0.64 | 0.13 | 0.34 | 0.78 | 5.10 | −0.28 | −2.10 | 0.64 |
| Ile (I) | 1.8 | 1.8 | 4.5 | 1.25 | 1.80 | 1.38 | 0.88 | 8.83 | 0.77 | 8.00 | 1.90 |
| Leu (L) | 1.8 | 1.8 | 3.8 | 1.22 | 1.70 | 1.34 | 0.85 | 8.47 | 1.10 | 9.20 | 1.90 |
| Lys (K) | −3.0 | −3.0 | −3.9 | −0.67 | −0.99 | 0.05 | 0.52 | 2.95 | −1.70 | −5.70 | −0.57 |
| Met (M) | 1.3 | 1.3 | 1.9 | 1.02 | 1.23 | 0.88 | 0.85 | 8.95 | 0.73 | 4.20 | 2.40 |
| Phe (F) | 2.5 | 2.5 | 2.8 | 1.92 | 1.79 | 1.71 | 0.88 | 9.03 | 1.43 | 9.20 | 2.30 |
| Pro (P) | 1.4 | 0.0 | −1.6 | −0.49 | 0.72 | 0.48 | 0.64 | 3.87 | 0.75 | −2.10 | 1.20 |
| Ser (S) | −0.3 | −0.3 | −0.8 | −0.55 | −0.04 | 0.18 | 0.66 | 4.09 | −0.42 | −6.50 | 0.01 |
| Thr (T) | 0.4 | 0.4 | −0.7 | −0.28 | 0.26 | 0.12 | 0.70 | 4.49 | −0.63 | −5.20 | 0.52 |
| Trp (W) | 3.4 | 3.4 | −0.9 | 0.50 | 2.25 | 2.34 | 0.85 | 7.66 | 1.57 | 10.0 | 2.60 |
| Tyr (Y) | 2.3 | 2.3 | −1.3 | 1.67 | 0.96 | 1.23 | 0.76 | 5.89 | 0.56 | 1.90 | 1.60 |
| Val (V) | 1.5 | 1.5 | 4.2 | 0.91 | 1.22 | 0.38 | 0.86 | 7.63 | 0.40 | 3.70 | 1.50 |

| | Engelman[22] 1986 | Hellberg[23]* 1987 | Ooi[24] 1987 | Oobatake[25] 1988 | Radzicka[26] 1988 | Cowan[27] 1990 | Chmelik[28] 1991 | Norinder[29] 1991 | El Tayar[30] 1992 | Waterbeemd[31] 1994 | Yoshida[32]* 1998 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ala (A) | 1.6 | −0.07 | 2.50 | −0.54 | 1.81 | 0.35 | 0.30 | −33.19 | 0.23 | −2.999 | −0.28 |
| Arg (R) | −12.3 | −2.88 | −53.50 | −5.96 | −14.92 | −1.50 | −0.91 | −65.57 | −0.79 | −7.938 | −3.90 |
| Asn (N) | −4.8 | −3.22 | −27.75 | −3.55 | −6.64 | −0.99 | −0.48 | −155.49 | −0.48 | −4.563 | −3.25 |
| Asp (D) | −9.2 | −3.64 | −28.28 | −2.97 | −8.72 | −2.15 | −0.55 | −95.62 | −0.61 | −3.823 | −2.45 |
| Cys (C) | 2.0 | −0.71 | −5.12 | −1.64 | 1.28 | 0.76 | 0.86 | −45.79 | 0.45 | −3.172 | −0.80 |
| Gln (Q) | −4.1 | −2.18 | −27.91 | −3.92 | −5.54 | −0.93 | −0.30 | −118.12 | −0.11 | −4.927 | −2.35 |
| Glu (E) | −8.2 | −3.08 | −28.98 | −3.71 | −6.81 | −1.95 | −0.32 | −59.25 | −0.51 | −3.927 | −1.58 |
| Gly (G) | 1.0 | −2.23 | 0.00 | −0.59 | 0.94 | 0.00 | 0.00 | −55.68 | 0.00 | −3.308 | 0.16 |
| His (H) | −3.0 | −2.41 | −23.27 | −3.38 | −4.56 | −0.65 | 0.02 | −126.76 | 0.15 | −4.000 | −3.44 |
| Ile (I) | 3.1 | 4.44 | 6.36 | 0.32 | 4.92 | 1.83 | 1.53 | 64.19 | 1.20 | −1.542 | 1.34 |
| Leu (L) | 2.8 | 4.19 | 5.91 | 0.27 | 4.92 | 1.80 | 1.50 | 65.29 | 1.28 | −1.542 | 2.31 |
| Lys (K) | −8.8 | −2.84 | −20.58 | −2.19 | −5.55 | −1.54 | −0.74 | −28.92 | −0.7 | −3.299 | −2.77 |
| Met (M) | 3.4 | 2.49 | 0.40 | −0.60 | 2.35 | 1.10 | 1.14 | 14.59 | 0.90 | −2.851 | 0.14 |
| Phe (F) | 3.7 | 4.92 | −3.69 | −1.06 | 2.98 | 1.69 | 1.91 | 103.72 | 1.56 | −1.581 | 2.94 |
| Pro (P) | −0.2 | 1.22 | 2.19 | 0.32 | −0.52** | 0.84 | 0.53 | −5.10 | 0.38 | −2.655 | −0.77 |
| Ser (S) | 0.6 | −1.96 | −21.52 | −3.82 | −3.40 | −0.63 | −0.19 | −135.36 | 0.00 | −4.215 | −2.53 |
| Thr (T) | 1.2 | −0.92 | −14.25 | −1.97 | −2.57 | −0.27 | 0.09 | −114.03 | 0.17 | −3.906 | −1.73 |
| Trp (W) | 1.9 | 4.75 | −17.55 | −3.80 | 2.33 | 1.35 | 2.01 | 683.54 | 1.85 | −1.581 | 1.80 |
| Tyr (Y) | −0.7 | 1.39 | −33.25 | −5.64 | −0.14 | 0.39 | 0.85 | 78.78 | 0.89 | −2.248 | 0.11 |
| Val (V) | 2.6 | 2.69 | 4.85 | 0.13 | 4.04 | 1.32 | 1.07 | 28.77 | 0.71 | −2.071 | 2.19 |

[a] "*" denotes a changed sign; "**" denotes an estimated value by Van de Waterbeemd.[31]

were found experimentally from the measurements of amino acid solubility in water and organic solvents, on the basis of distribution coefficients of the amino acids under investigation (or organic compounds identifying their side substituents) in the systems gas phase−water or organic solvent−water, by chromatographic methods as well as by means of theoretical calculations. The authors called the resultant values parameters or scales of hydrophobicity (hydrophilicity or lipophilicity). These terms relate to two kinds of interactions between dissolved molecules, that is, interactions through hydrogen bonds and dipole−dipole or dipole−ion interactions and weak dispersion interactions of nonpolar groups associated with hydrophobic effects.

The hydrophobicity (lipophilicity) parameters used in the above cited studies systematize only to a slight extent the properties of amino acids or their side substituents. The lack of a linear correlation between these physicochemical dates (probably an incidental exception are the parameters obtained by Levitt[11] and Hopp and Woods[12] for which $R^2 = 0.9696$) results probably from the fact that these authors used various measuring techniques, different organic solvents, or chromatographic column packing in which some amino acids behave abnormally.[33] The obtained numerical values, describing the properties of some amino acids or their side substituents, are quite controversial.

Analyzing the cited lipophilicity scales (Table 1), it appears that tryptophane, according to some studies,[11,12,15,16,19−21,28−30] shows the most hydrophobic side chain, while other studies include it among amino acids possessing a hydrophilic side substituent.[13,24,25]

Similarly, tyrosine is usually classified by biochemists as an amino acid with a polar side chain which is regarded according to some authors as strongly hydrophobic[11,12,14,29] and as strongly hydrophilic by others[24,25] (Table 1). As many as 16 mentioned

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scales accepted L-tyrosine as a more hydrophobic amino acid than L-alanine (Table 1). From the analysis of most lipophilicity scales, it follows that cysteine is more hydrophobic than alanine,[11−16,18−20,22,27,28,30] while in accordance with the lipophilicity scale developed by Rose et al.,[17] it is the most hydrophobic amino acid. It is known, however, that the value of the parameter which specifies the hydrophobicity of cysteine or its side substituent should be between the values obtained for glycine and alanine (Gly < Cys < Ala). Erroneous results in the determination of the lipophilicity of cysteine are probably due to insufficient deaeration of its solutions. The abnormally high value of the hydrophobicity of the cysteine side constituent is explained by Fauchere with an experimental error consisting in too long times of solution shaking (Fauchere's private communication).[30]

It is also difficult, using the hydrophobicity scales under discussion, to classify amino acids with polar and ionic side substituents such as asparagine, aspartic acid, glutamine, glutamic acid, arginine, or lysine.

To find a parameter which would allow one to systematize amino acid side chains more precisely, the enthalpy of dilution of L-α-amino acid in water was measured by the calorimetric method. The obtained values of the dilution enthalpy were used to find the enthalpic pair interaction coefficients of L-α-amino acid zwitterions in water similarly as in previous studies.[34,35] The enthalpic coefficients derived from McMillan−Mayer's theory,[36] modified by Franks et al.[37] and by Friedman and Krishman,[38] characterize the total energetic effects of interactions between the examined molecules with the competitive participation of water molecules. Thus, these coefficients illustrate the differences in amino acid molecule interactions both with the homogeneous amino acid molecules and with the water molecules around them, and, consequently, they may play the part of a parameter which differentiates the hydrophobic/hydrophilic properties of amino acid side chains, and it can contribute to a better understanding of the effects causing the reasons of protein folding.

## Experimental Section

L-α-Cysteine (Cys), L-α-histidine (His), L-α-glutamine (Gln), L-α-arginine (Arg), L-α-tryptophan (Trp), L-α-glutamic acid (Glu) (all Merck ≥99%), and L-α-asparagine (Asn) (Aldrich 99%) were crystallized from water−methanol mixtures and dried under reduced pressure at 323 K. The water used in the experiments was deionized, distilled. and degassed.

The enthalpies of dilution were determined with an isothermal calorimeter SETARAM MS-80D at 298.15 K, equipped with cells having a diameter of 17 mm. The measurements were carried out in a stainless steel reversal mixing vessel with a capacity of 10 $cm^3$ with a small compartment of 0.6 $cm^3$ or 6.7 $cm^3$ in the volume. The degassed solutions and calorimetric vessels were weighed by means of a Sartorius RC 210D balance. Moreover, the solutions of L-α-cysteine were several times saturated with argon and degassed to avoid the oxidation of cysteine to cystine by the oxygen dissolved in aqueous solution. Even a trace oxidation of cysteine was reflected by the thermogram recording. The apparatus and procedure used were the same as those described in earlier work.[34,35]

## Results and Discussion

The determined enthalpies of dilution, $\Delta H_{\mathrm{dil}}$, of the aqueous solutions of cysteine, histidine, asparagine, glutamine, arginine, tryptophan, and glutamic acid in water are presented in Table 2. The resultant enthalpies of dilution were used to find the enthalpic interaction coefficients according to the method proposed by Desnoyers et al.[39] The experimental values of the enthalpies of dilution were described by the second-degree polynomial:

$$\Delta H_{\mathrm{dil}} = (m_{\mathrm{f}} - m_{\mathrm{i}})h_{\mathrm{AA}} + (m_{\mathrm{f}}^2 - m_{\mathrm{i}}^2)h_{\mathrm{AAA}} + ... \qquad (1)$$

where $h_{\mathrm{AA}}$ and $h_{\mathrm{AAA}}$ are the enthalpic interaction coefficients of pairs and triplets of the examined L-α-amino acids in aqueous solution, respectively, and $m_{\mathrm{f}}$ and $m_{\mathrm{i}}$ are final and initial molalities of amino acids.

The enthalpic pair interaction coefficients calculated from eq 1 using the least-squares method are given in Table 3. The $h_{\mathrm{AA}}$ coefficients for all examined L-α-amino acids in this paper have negative values.

The enthalpic coefficient values of the homogeneous pairs interaction reflect the summary process of interaction between two statistical amino acid molecules in solution proceeding with the participation of water molecules. To provide a direct interaction between two zwitterion "heads", some water molecules must be removed from the hydration sheaths of zwitterion "heads" since they are a hindrance to their direct contact (Figure 1). The water molecules, pushed out from the hydration sheaths, assume a typical order for "bulk water". Thus, the enthalpy coefficients of the homogeneous pairs interaction are a measure of the global effect constituting a sum of the following processes: a direct interaction between homogeneous zwitterion "heads" of amino acids (an exothermic process), and a partial dehydration of the hydration sheaths of the interacting zwitterions (an endothermic processes).

In the case of amino acids possessing polar or ion groups in side chains, the global effect is increased by processes connected with the interaction of these groups between themselves as well as with the zwitterion "head" of the second amino acid of statistical pair (exothermic effects). These effects are weakened by endothermic processes associated with the partial dehydration of the polar or ion groups under discussion.

To the global interaction effect of amino acids with nonpolar side chains is added a process brought about by the hydrophobic hydration of these nonpolar groups. The water molecules surrounding the nonpolar groups because of their influence strengthen hydrogen bonds between themselves.[3,4,35,40,41] Because of the cooperativeness of hydrogen bonds, the effect of the strengthened interactions between water molecules is transferred on the water molecules hydrating the polar or ion groups in the discussed amino acid molecules.

This brings about a reinforcement of interaction between the water molecules of the hydration sheath and polar or ion groups. To provide a direct interaction between a pair of polar or ion groups of the discussed molecules, it is necessary to supply more energy to remove some water molecules from their hydration

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**Table 2.** Enthalpies of Dilution $\Delta H_{dil}$ of Aqueous Solutions of L-α-Amino Acid in Water at 298.15 K

| $m_i$ mol kg$^{-1}$ | $m_f$ mol kg$^{-1}$ | $\Delta H_{dil}$ J mol$^{-1}$ | $m_i$ mol kg$^{-1}$ | $m_f$ mol kg$^{-1}$ | $\Delta H_{dil}$ J mol$^{-1}$ |
|---|---|---|---|---|---|
| L-α-cysteine | | | | | |
| 1.4095 | 0.7122 | 34.6 | 0.7112 | 0.4212 | 28.9 |
| 1.4095 | 0.1410 | 84.8 | 0.7112 | 0.0390 | 73.3 |
| 1.3558 | 0.6852 | 43.3 | 0.6095 | 0.3327 | 27.1 |
| 1.3558 | 0.1285 | 95.3 | 0.6095 | 0.0388 | 58.8 |
| 1.2225 | 0.6852 | 38.0 | 0.5598 | 0.3104 | 27.5 |
| 1.2225 | 0.1208 | 81.3 | 0.5598 | 0.0330 | 53.2 |
| 1.0010 | 0.5212 | 41.1 | 0.3256 | 0.1845 | 16.9 |
| 1.0010 | 0.0123 | 86.5 | 0.3256 | 0.0286 | 37.2 |
| 0.9138 | 0.4521 | 35.3 | 0.2476 | 0.1583 | 9.8 |
| 0.9138 | 0.0855 | 84.2 | 0.2476 | 0.0231 | 25.7 |
| L-α-histidine | | | | | |
| 0.1979 | 0.0995 | 57.4 | 0.1508 | 0.0215 | 77.2 |
| 0.1979 | 0.0198 | 106.0 | 0.1508 | 0.0132 | 84.6 |
| 0.1979 | 0.0132 | 111.1 | 0.1388 | 0.0754 | 37.6 |
| 0.1855 | 0.0956 | 53.0 | 0.1388 | 0.0133 | 76.3 |
| 0.1855 | 0.0181 | 99.7 | 0.1256 | 0.0721 | 32.5 |
| 0.1745 | 0.0851 | 53.7 | 0.1256 | 0.0114 | 69.7 |
| 0.1745 | 0.0171 | 93.6 | 0.1098 | 0.0621 | 29.1 |
| 0.1508 | 0.0833 | 40.7 | 0.1098 | 0.0109 | 60.8 |
| L-α-asparagine | | | | | |
| 0.1659 | 0.0826 | 140.3 | 0.1121 | 0.0594 | 90.3 |
| 0.1451 | 0.0821 | 107.4 | 0.1006 | 0.0062 | 163.2 |
| 0.1451 | 0.0733 | 121.8 | 0.0996 | 0.0331 | 115.8 |
| 0.1451 | 0.0101 | 233.8 | 0.0859 | 0.0429 | 74.3 |
| 0.1451 | 0.0084 | 235.3 | 0.0844 | 0.0056 | 139.3 |
| 0.1405 | 0.0136 | 218.0 | 0.0770 | 0.0055 | 124.8 |
| 0.1375 | 0.0456 | 158.1 | 0.0749 | 0.0374 | 65.0 |
| 0.1225 | 0.0853 | 63.3 | 0.0706 | 0.0435 | 47.6 |
| L-α-glutamine | | | | | |
| 0.2238 | 0.1421 | 93.0 | 0.1606 | 0.0873 | 87.1 |
| 0.2238 | 0.0133 | 247.1 | 0.1606 | 0.0182 | 169.6 |
| 0.2203 | 0.1154 | 122.9 | 0.1453 | 0.0854 | 71.8 |
| 0.2203 | 0.0121 | 245.7 | 0.1453 | 0.0099 | 164.2 |
| 0.2105 | 0.1219 | 101.8 | 0.1335 | 0.0687 | 78.2 |
| 0.2105 | 0.0135 | 234.6 | 0.1261 | 0.0711 | 66.3 |
| 0.1896 | 0.0987 | 107.2 | 0.1261 | 0.0081 | 142.6 |
| 0.1896 | 0.0121 | 209.7 | 0.1018 | 0.0075 | 115.7 |
| L-α-arginine | | | | | |
| 0.5488 | 0.2834 | 322.7 | 0.3092 | 0.0301 | 479.3 |
| 0.5488 | 0.2365 | 356.1 | 0.3092 | 0.0205 | 483.2 |
| 0.5488 | 0.0532 | 660.7 | 0.2623 | 0.0148 | 388.6 |
| 0.5488 | 0.0335 | 690.9 | 0.2251 | 0.1098 | 195.2 |
| 0.3984 | 0.1975 | 279.4 | 0.2251 | 0.0225 | 326.2 |
| 0.3894 | 0.0253 | 530.8 | 0.1945 | 0.0143 | 309.9 |
| 0.3198 | 0.1657 | 246.9 | 0.1569 | 0.0784 | 123.9 |
| 0.3092 | 0.1564 | 224.1 | 0.1068 | 0.0101 | 177.1 |
| L-α-tryptophan | | | | | |
| 0.05587 | 0.03452 | 28.8 | 0.03383 | 0.02586 | 11.1 |
| 0.05587 | 0.02744 | 38.0 | 0.03383 | 0.01702 | 23.5 |
| 0.05587 | 0.01623 | 52.3 | 0.03383 | 0.01258 | 28.9 |
| 0.04374 | 0.03382 | 13.8 | 0.03003 | 0.01576 | 19.1 |
| 0.04374 | 0.02913 | 19.9 | 0.03003 | 0.01008 | 27.2 |
| 0.04374 | 0.01442 | 39.0 | 0.02877 | 0.01498 | 19.0 |
| 0.04065 | 0.02865 | 16.8 | 0.02877 | 0.01198 | 22.3 |
| 0.04065 | 0.02008 | 27.7 | 0.02372 | 0.01422 | 13.3 |
| 0.04065 | 0.01213 | 38.0 | 0.02372 | 0.01056 | 17.8 |
| L-α-glutamic acid | | | | | |
| 0.04582 | 0.03022 | 32.3 | 0.03925 | 0.01987 | 40.6 |
| 0.04582 | 0.02234 | 43.4 | 0.03925 | 0.01232 | 51.6 |
| 0.04582 | 0.01632 | 59.9 | 0.03714 | 0.02311 | 33.1 |
| 0.04392 | 0.02881 | 31.7 | 0.03714 | 0.01855 | 37.1 |
| 0.04392 | 0.01616 | 59.8 | 0.03714 | 0.01321 | 54.1 |
| 0.04392 | 0.01533 | 58.3 | 0.03511 | 0.02008 | 33.0 |
| 0.04296 | 0.03002 | 31.1 | 0.03511 | 0.01675 | 39.2 |
| 0.04296 | 0.01654 | 53.4 | 0.03148 | 0.01823 | 31.5 |
| 0.03925 | 0.02876 | 23.1 | 0.03148 | 0.01392 | 36.8 |

**Table 3.** Enthalpic Pair Interaction Coefficients $h_{AA}$ for L-α-Amino Acids in Water and the Averaged Scale of Hydrophobicity ($P_{hydr-pho}$) of Amino Acid Side Chains

| amino acid | $h_{AA}$ J kg mol$^{-2}$ | $P_{hydr-pho}$ |
|---|---|---|
| Gly (G) | −424[34] | 0 |
| Ala (A) | 206[34] | 46 |
| Val (V) | 871[34] | 123 |
| Leu (L) | 1243[34] | 165 |
| Ile (I) | 1319[35] | 172 |
| Phe (F) | 1229[35] | 179 |
| Pro (P) | 377[35] | 47 |
| Met (M) | 659[35] | 119 |
| Ser (S) | −709[34] | −57 |
| Thr (T) | −99[34] | −9 |
| His (H) | −631 ± 7 | −34 |
| Gln(Q) | −1254 ± 9 | −81 |
| Asn (N) | −1786 ± 9 | −110 |
| Arg (R) | −1896 ± 46 | −130 |
| Cys (C) | −130 ± 4 | 78 |
| Trp (W) | −1368 ± 25 | 147 |
| Glu (E) | −2165 ± 623 | −106 |
| Tyr (Y) | | 64 |
| Asp (D) | | −123 |
| Lys (K) | | −100 |

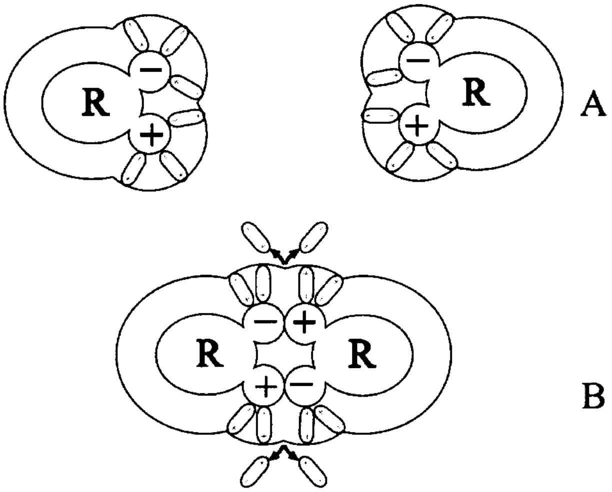

**Figure 1.** Model of interaction between zwitterion "heads". (A) Hydrated amino acid zwitterions. (B) Direct interaction of zwitterion "heads" with the water molecules pushed out from hydration sheaths.

layers, constituting a hindrance to their direct contact. Thereby, the exothermic effect of direct interactions between ion or polar groups of the amino acid molecule pair is weakened and even dominated[34,35] by the endothermic effects of the partial dehydration of the hydration sheaths of the interacting molecules (the values of the enthalpy coefficients of pair interaction, $h_{AA}$, become positive).

The enthalpic interaction coefficient of homogeneous pairs of the simplest amino acid, glycine, in water has a negative value (Table 3). It indicates a predominant exoenergetic effect of direct interactions between two-ion "heads" of glycine ($-C^{\alpha}$-$HCO_2^{-}NH_3^{+}$) over the endothermic effects of partial dehydration of hydration sheaths of their ionic "heads" and the influence exerted by the hydrophobic group $C^{\alpha}H$. The extension of the amino acid zwitterion side substituent with amide groups in asparagine and glutamine, with imidazol group in histidine, with guanidine group in arginine, or with carboxylic group in glutamic acid results in increased negative values of the enthalpic interaction coefficients of homogeneous zwitterion pairs (Table 3). Thus, the exothermic contribution associated with the direct interaction of these polar or ionic side substituents to the total effect of homogeneous zwitterion interactions is higher than the endothermic dehydration effects of hydration sheaths of these polar groups and the effects brought about by

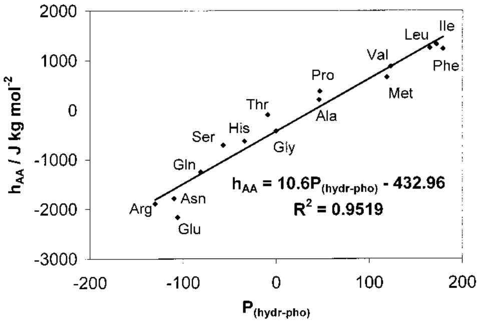

***Figure 2.*** Relationship between the averaged hydrophobicity parameters $P_{(\mathrm{hydr-pho})}$ and the enthalpic homogeneous pair interaction coefficients $h_{\mathrm{AA}}$ of the natural amino acids in water.

***Table 4.*** Parameters Describing Amino Acid Side Chains

| amino acid | $\Lambda$ | $\Lambda_{\mathrm{norm}}$ | $n_{(\mathrm{da})}$ | $V_{\mathrm{A}}^{\infty}$ cm$^3$ mol$^{-1}$ | $V_{\mathrm{R}}^{\infty}$ cm$^3$ mol$^{-1}$ |
|---|---|---|---|---|---|
| Gly | 0.03 | 0.0336 | 0 | 43.28 | 0 |
| Ala | −0.02 | 0.0522 | 0 | 60.48 | 17.20 |
| Val | −0.13 | 0.0933 | 0 | 90.78 | 47.50 |
| Leu | 0.12 | 0.0000 | 0 | 107.68 | 64.40 |
| Ile | 0.04 | 0.0298 | 0 | 105.73 | 62.45 |
| Phe | −0.05 | 0.0634 | 0 | 121.28 | 78.00 |
| Pro | −0.31 | 0.1604 | 0 | 82.48 | 39.20 |
| Met | −0.33 | 0.1679 | 0 | 105.18 | 61.90 |
| Ser | −0.4 | 0.1940 | 3 | 60.68 | 17.40 |
| Thr | −0.53 | 0.2425 | 3 | 76.88 | 33.60 |
| His | −1.06 | 0.4403 | 2 | 98.98 | 55.70 |
| Gln | −1.19 | 0.4888 | 5 | 93.78 | 50.50 |
| Asn | −1.24 | 0.5075 | 5 | 77.78 | 34.50 |
| Arg | −2.56 | 1.0000 | 7 | 127.32 | 84.04 |
| Cys | −0.11 | 0.0858 | 3 | 73.38 | 30.10 |

the presence of methylene groups ($CH_2$) in the side chain. Similarly, the replacement of the hydrogen atom in the alkyl chain of alanine with a group (−SH) also results in an increased negative value of the enthalpic interaction coefficient between homogeneous cysteine molecules ($h_{(\mathrm{Ala-Ala})} > h_{(\mathrm{Cys-Cys})}$, Table 3). The enthalpic pair interaction coefficient between tryptophan molecules reflects a negative value which evidently certifies the exothermic global effect of the interaction of two tryptophan zwitterions with water molecules. This negative value of enthalpic coefficients $h_{(\mathrm{Trp-Trp})}$ is certainly caused by an exothermic effect $\pi-\pi$ interaction together with the indole rings and with weak endothermic effects of partial dehydration of hydrophobic aromatic substituents putting into the global process of the interaction of tryptophan zwitterions in water.

The differences in the behavior of free amino acids as well as amino acid residues in the polypeptide chains of proteins are due to the dissimilarity of particular side chains of L-α-amino acids.

Analyzing 22 scales of hydrophobicity amino acid side chains (Table 1),[11−32] which differ not only in values describing each particular amino acid but also in hydrophobic−hydrophilic property classification of individual substituents according to authors of cited scales, I attempted to unify the classification of amino acids described in these scales. A reference point was the "0" value ascribed to the substituent of glycine, −H. In all considered classifications, the values related to nonpolar side chains, regardless of their units and numerical values in particular scales, assume the values of growing integers, starting from "0" or "1" for a substituent with the lowest hydrophobicity (Table 1). Hydrophilic side chains of amino acids considered in the cited scales assume the values of negative integers, starting from "0" or "−1" for a substituent with the lowest hydrophilicity (Table 1).

By adding up the numerical values of the lipophilicity of amino acids or their side substituents appearing in the analyzed scales, an "averaged" scale of hydrophobicity of amino acids or their side chains ($P_{\mathrm{hydr-pho}}$) was obtained (Table 3). The developed scale (Table 3), with the exception of the cysteine and tryptophan side substituents, well correlates (Figure 2) with the obtained values of enthalpic interaction coefficients of homogeneous amino acid pairs (Table 3).

Thus, on the basis of the above statement, one may conclude that the obtained enthalpic homogeneous pair interaction coefficients of L-α-amino acids in water make it possible to systematize amino acid side chains according to their affinity to water or their hydrophobic−hydrophilic properties. Thus, the enthalpic coefficients $h_{\mathrm{AA}}$ may play the role of the parameter describing the hydrophobicity of amino acid side chains.

To find additional information about factors characterizing the behavior of particular side chains of the amino acids under investigation, which affects the values of enthalpic pair interaction coefficients, it seemed reasonable to analyze the enthalpic coefficients by means of a multi-parameter linear regression. I used to that end the equation proposed by Abraham−Kamlet−Taft[42,43] as modified by myself. Because of the lack of parameters describing amino acids or their side chains, I replaced parameter $\pi^*$ which illustrates dipolarity/polarizability with polarity parameter $\Lambda$ of amino acid molecules (Table 4) as proposed by Tayar et al.,[30] while parameter α describing donor−proton properties of a molecule and parameter $\beta$ showing proton−acceptor properties were replaced with parameter $n_{(\mathrm{da})}$ (Table 4) describing the number of hydrogen bonds which can be formed by the side substituent of the amino acid as a donor and acceptor of proton.[44] The collection values of $n_{(\mathrm{da})}$ were extended with the value which describes the side substituent of cysteine omitted by Charton and Charton.[44] I have standardized the polarity parameter $\Lambda_{(\mathrm{norm})}$[30] (Table 4), in which $\Lambda$ assumes both positive and negative values for various amino acid side substituents. The values of limiting molar partial volumes for the discussed amino acid side chains $V_{\mathrm{R}}^{\infty}$ (Table 4) were calculated from the difference between the values of limiting partial molar volumes of particular amino acids, found in our laboratory, and that of glycine.[45]

Finally, the modified equation assumes the following form:

$$h_{\mathrm{AA}} = (h_{\mathrm{AA}})_0 + a\Lambda_{(\mathrm{norm})} + bn_{(\mathrm{da})} + cV_{\mathrm{R}}^{\infty} \tag{2}$$

The above parameters being independent variables of this equation are noncollinear, which allow one to make a statistically true separation of contributions in the correlation equation. The determined enthalpic pair interaction coefficients between homogeneous molecules of 15 amino acids correlated with the above presented factors are described by the following equation:

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(42) Abraham, M. H.; Kamlet, M. J.; Taft, R. W. *J. Solution Chem.* **1982**, *2*, 923.
(43) Kamlet, M. J.; Abboud, J.-L. M.; Abraham, M. H.; Taft, R. W. *J. Org. Chem.* **1983**, *48*, 2877.
(44) Charton, M.; Charton, B. I. *J. Theor. Biol.* **1982**, *99*, 629.
(45) Palecz, B., manuscript in preparation.

$$h_{AA} = (-150.7 \pm 133.2) - (2904.5 \pm 516.7)\Lambda_{(norm)} - (133.8 \pm 55.5)n_{(da)} + (22.65 \pm 2.90)V_R^{\infty} \quad (3)$$

$$n = 15, \qquad R^2 = 0.9671$$

The above equation describes the contributions of particular parameters in the total variation of the function being described; in this case, they are the enthalpic pair interaction coefficients $h_{AA}$. The obtained results show that the electrostatic interactions characterized by the expression $a\Lambda_{(norm)}$ and describing the ion−dipole and dipole−dipole interactions between amino acid molecules have negative values indicating the exothermic contribution in the total value of the enthalpic coefficient $h_{AA}$. Similarly, the negative value of the expression $bn_{(da)}$ indicates exothermic interactions caused by the formation of hydrogen bonds by the polar side groups of amino acids. The above effects are overlaid with the endothermic processes (described by $dV_R^{\infty}$) associated probably with the formation of a cavity in the water structure to accommodate in it the given pair of substituents, and with partial dehydration of polar and ionic groups in the molecules of interacting amino acids. The free term in this equation $(h_{AA})_0$ is not only a selectable parameter, but it describes probably the direct interactions between the pair of hydrated ionic "heads" of amino acids. The sign and order of magnitude of this expression are similar to the value of the enthalpic coefficient of interaction between homogeneous zwitterions of glycine ($h_{Gly-Gly} = -424$ J kg mol $^{-2}$).

This mathematical relationship well describes the determined enthalpic homogeneous interaction coefficients $h_{AA}$ of the amino acids under discussion.

## Conclusions

Enthalpic homogemous pair interaction coefficients calculated on the basis of dilution enthalpy measurements of aqueous amino acids solutions are a measure of interaction effects between a homogeneous pair of zwitterions taking place with the cooperation of water molecules. Therefore, enthalpic coefficients $h_{AA}$ do not contain contributions connected with specific interactions of amino acid molecules with other organic chemical molecules (that is solvents or chromatographic column fillers) which participate in determinated physicochemical parameters and thus cause wrong interpretation.

In this way, enthalpic homogeneous pair interaction coefficients of L-α-amino acids in water differentiating amino acid side chains in relation to their hydrophobic−hydrophilic properties or their affinity to water can play the role of the parameter describing hydrophobicity (lipophilicity) of amino acid side chains and may help in a better understanding of the protein folding process.

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