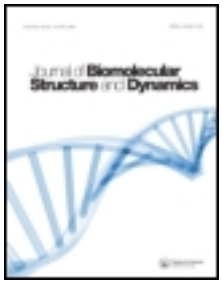

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Amino acid/water interactions study: a new amino acid scale

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Partition ratios of 8 free L-amino acids (Gln, Glu, His, Lys, Met, Ser, Thr, and Tyr) were measured in 10 different polymer/polymer aqueous two-phase systems containing 0.15 M NaCl in 0.01 M phosphate buffer, pH 7.4. The solute-specific coefficients representing the solute dipole/dipole, hydrogen-bonding and electrostatic interactions with the aqueous environment of the amino acids were determined by multiple linear regression analysis using a modified linear solvation energy relationship. The solute-specific coefficients determined in this study together with the solute-specific coefficients reported previously for amino acids with non-polar side-chains where used in a Quantitative Structure/Property Relationship analysis. It is shown that linear combinations of these solute-specific coefficients are correlated well with various physicochemical, structural, and biological properties of amino acids.

Keywords: amino acids; descriptors; protein structure; aqueous two-phase partitioning; biological activity

Introduction

It is well known that the aqueous environment plays an active role in protein folding, maintaining the protein structure and protein function *in vivo* (Ball, 2011; Berkowitz, Engen, Mazzeo, & Jones, 2012). Therefore, studies of protein/water interactions are important from both theoretical and practical viewpoints. Amino acids are the building blocks of proteins, and hence, analysis of their interactions with aqueous media is necessary to gain better insight into protein/water interactions. Amino acids are often grouped based on their ability to interact with water into hydrophobic (i.e. those with low affinity for water) and hydrophilic ones (i.e. those with high affinity for water). This classification is, however, overly simplistic, as amino acids have quite different physicochemical properties and interact with water very differently (Biswas, DeVido, & Dorsey, 2003).

Interactions of a solute with a solvent may be described in various terms. One of the most successful models suggested for the analysis and description of these interactions is the Abraham's solvation parameter model (Abraham, Gola, Kumarsingh, Cometto-Muniz, & Cain, 2000; Abraham, Ibrahim, & Zissimos, 2004). The model is based on the assumption that different types of solute/solvent interactions are mutually independent and may be described as:

$$\log \mathrm{SP} = z + \sum xX_\mathrm{s} + vV_\mathrm{s} \qquad (1)$$

where SP is a property of a series of solutes in a given solvent system (typically the partition ratio, solubility, or retention time in high-performance liquid chromatography [HPLC]), z is the intercept constant, X_s is a solute descriptor representing the solute effect on particular types of solute/solvent interactions, x is a coefficient representing the complementary effect of the solvent with regard to this interaction; V_s is McGowan's characteristic volume (see in Abraham et al. (2004)) of the solute; and v is the coefficient serving as a measure of the combined dispersion and cavity effect. The solute descriptors

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characterize the solute excess molar refraction, the solute combined dipolarity/polarizability, the overall solute hydrogen bond acidity, and the overall solute hydrogen bond basicity. The solute descriptor values are generally derived from experimental measurements (Abraham et al., 2000; Abraham et al., 2004), though they may be calculated as well (Abraham et al., 2002; Platts, Abraham, Butina, & Hersey, 1999). The coefficients are determined by multiple linear regression analysis.

The solute excess molar refraction may be calculated or derived from the refractive index and, according to Goss (2005), may be eliminated from Equation (1) without significant effect on the log SP value. The solute dipolarity/polarizability descriptor representing dipole/dipole and dipole-induced dipole solute/solvent interactions is commonly determined along with hydrogen-bond descriptors using organic solvent/water partitioning or liquid chromatography (Abraham et al., 2000; Abraham et al., 2004). These experimental methods are poorly suitable for the analysis of strongly ionized and zwitterionic compounds and biopolymers. Hence, the solute descriptors reported in the literature (Abraham et al., 2000; Abraham et al., 2002; Abraham et al., 2004; Goss, 2005; Platts et al., 1999; Valko, Du, Bevan, Reynolds, & Abraham, 2001) are limited to nonionic compounds.

In order to study solute descriptors for biological molecules, we suggested (Madeira, Reis, Rodrigues, Mikheeva, & Zaslavsky, 2010; Madeira et al., 2011) to apply the solvatochromic comparison method by Kamlet and Taft (Kamlet, Abboud, Abraham, & Taft, 1983; Kamlet, Abboud, & Taft, 1977; Kamlet & Taft, 1976; Taft & Kamlet, 1976) for characterization of the solvent properties of the media in the coexisting phases in multiple aqueous two-phase systems (ATPS) with a set of selected solvatochromic probes.

ATPS naturally arise in aqueous mixtures of different polymers or a single polymer and a specific salt. When two particular polymers, for example, dextran and Ficoll, are mixed in water above certain concentrations, the mixture separates into two immiscible aqueous phases. Each phase is preferentially rich in one of the polymers with the aqueous solvent in both phases providing media suitable for biological products (Albertsson, 1986; Zaslavsky, 1995). The advantage of ATPS over organic solvent/water biphasic systems is that the aqueous environment in ATPS is suitable for the analysis of ionizable solutes and biological macromolecules such as proteins.

It has been shown previously (Madeira et al., 2011) that the partition ratio of an ionizable solute in ATPS may be described as:

$$\log K_s = S_s\Delta\pi^* + A_s\Delta\beta + B_s\Delta\alpha + C_s c \qquad (2)$$

where K is the solute partition ratio; $\Delta\pi^*$ is the difference between the solvatochromic solvent dipolarity/polarizability of the coexisting phases, $\Delta\alpha$ is the difference between the solvatochromic solvent hydrogen-bond donor acidity of the phases, $\Delta\beta$ is the difference between the solvatochromic solvent hydrogen-bond acceptor basicity of the phases; c is the difference between the electrostatic properties of the phases; S_s, As, B_s and C_s are constants (solute-specific coefficients) that describe the susceptibility of the complementary solute/solvent interactions to the changes in the corresponding solvent properties.

The solute-specific coefficients are determined from partition ratios for the solute in a set of ATPS with established solvent properties of the phases by multiple linear regression analysis.

Previously, we reported the solute-specific coefficients for amino acids with non-polar side-chains and demonstrated that these amino acids descriptors are well correlated with different physico-chemical properties of amino acids (Madeira, Bessa, Alvares-Ribeiro, 2013). Here, we extend the study to determine the descriptors for amino acids with polar and charged side-chains and explore the applicability of the established descriptors to the analysis of biological effects of free amino acids and various features of amino acids residues in protein structures.

Materials and methods

Materials

Polymers. Dextran 75 (lot 126567), weight-average molecular weight (Mw) 75 000 was purchased from USB (Cleveland, OH, USA). Polyethylene glycol 10,000 (lot BCBB0795), Mw = 10,000; Polyethylene glycol 8000 (lot 050M0215 V), Mw = 8000; Polyethylene glycol 6000 (lot BCBC7560), Mw = 6000; Polyethylene glycol 4000 (lot BCBD2874), Mw = 4000; Polyethylene glycol 1000 (lot 0001452731), Mw = 1000 and Polyethylene glycol 600 (lot BCBD8607 V), Mw = 600 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ucon 50-HB-5100 (lot SJ1955S3D2), Mw = 3930 was purchased from Dow-Chemical (Midland, MI, USA). Ficoll 70 (lot 10022579), Mw 70 000 was purchased from GE Healthcare Biosciences AB (Sweden). All polymers were used without further purification.

L-amino acids

Free L-amino acids were purchased from Sigma.

Other chemicals

O-phthaldialdehyde (OPA) reagent solution (complete) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All salts and other chemicals used were of analytical reagent grade.

Methods

Partitioning. Solutions of each compound were prepared in water at concentrations of 1–5 mg/mL. Varied amounts (e.g. 0, 10, 20, 30, 40, and 50 µL) of a given compound solution and the complementary amounts (e.g. 100, 90, 80, 70, 60, and 50 µL) of water were added to a set of the same polymer/buffer/salt mixtures using a Multipette Xstream pipette (Eppendorf, Hamburg, Germany). The final polymer compositions of the ATPS in the presence of 0.15 M NaCl and 0.01 M sodium phosphate buffer, pH 7.4 are listed in Table 1. Systems were vortexed and centrifuged for 30–60 min at 10,000 g in a mini-spin centrifuge (Eppendorf) to accelerate phase settling. Aliquots of 20 to 70 µL from the upper and lower phases were withdrawn with a Multipette Xstream pipette in duplicate for analysis. Two aliquots from both phases were diluted with OPA solution up to 250 µl in microplate wells. Following moderate shaking at room temperature (23 °C), a synergy-2 fluorescence plate reader with a 360-nm excitation filter and a 460-nm emission filter (Bio-Tek Instruments) was used to measure fluorescence intensity. Phases of blank systems at corresponding dilutions were measured for comparison. Calibration of OPA assay for each compound in both phases was performed in a similar manner.

The partition ratio, K, is defined as the ratio of the compound concentration in the upper phase to the compound concentration in the lower phase. The partition ratio value for each solute was determined as the slope of the plot of the solute concentration in the upper phase as a function of the solute concentration in the bottom phase obtained from six partition experiments carried out at different concentrations of the solute and at the fixed composition of the system. Deviation from the average K value was consistently below 5% and in most cases lower than 2%.

Computational methods. The linear regression analysis where performed using the JMP software version 7.0.1 and Sigma Plot 12.0. The computerized selection and fitting code was developed using the Matlab software, version R2010b (Madeira, Bessa, de Barros, 2013).

Results

Partition ratios of free amino acids examined in the ATPS listed in Table 1 are presented in Table 2. The multiple linear regression analysis of all the partition ratios values for each amino acid was performed using Equation (2), and statistically significant ($p < 0.1$) solute-specific coefficients were determined and listed in Table 3.

The coefficients for free amino acids with nonpolar side-chains reported previously (Madeira, Bessa, Alvares-Ribeiro, 2013; Madeira, Bessa, de Barros, 2013) are also presented in Table 3.

Contribution of a methylene (and methyl) group into coefficient S_s established previously (Madeira, Bessa, Alvares-Ribeiro, 2013) is constant and amounts to -0.41 ± 0.06. The estimates of this contribution from comparison of the S_s values for Ser with Thr and for Gln with Asn (or Glu with Asp) agree with the above value within the experimental error limits. Contribution of CH_2 (and CH_3) group into coefficient B_s is affected by the presence of neighboring polar moiety and its particular structure.

Comparison of the coefficients for Met and norvaline agrees with the accepted classification of Met as amino acid with nonpolar side-chain. The data obtained for proline, on the other hand, implies that the contribution of CH_2 groups in cyclic structure into coefficient S_s is dramatically different from those in aliphatic side-chains.

Table 1. Polymer[a] composition of ATPS and differences[b] between solvent features in the coexisting phases of ATPS (Madeira et al., 2013).

			Total composition[a]		Difference between solvent features of coexisting phases			
ATPS	Polymer1[a]	Polymer2[a]	Polymer 1	Polymer 2	$\Delta\pi^{*b}$	$\Delta\alpha^b$	$\Delta\beta^b$	c^c
S1	Dextran	Ficoll	12.94	18.06	0.003	−0.028	0.010	0.0481 ± 0.0005
S2	Dextran	PEG4000	13.67	6.15	−0.041	−0.024	0.007	−0.0371 ± 0.0003
S3	Dextran	PEG1000	20.00	13.57	−0.052	−0.061	0.018	−0.018 ± 0.006
S4	Dextran	PEG600	16.23	16.87	−0.040	−0.017	0.005	−0.0148 ± 0.0004
S5	Dextran	Ucon	12.39	10.08	−0.023	−0.181	0.015	0.041 ± 0.003
S6	Ficoll	PEG10000	22.99	9.90	−0.050	−0.014	−0.029	−0.1262 ± 0.0002
S7	Ficoll	PEG8000	24.67	10.42	−0.061	−0.026	0.000	−0.157 ± 0.005
S8	Ficoll	PEG6000	29.23	15.00	−0.106	0.000	0.039	−0.079 ± 0.002
S9	Ficoll	Ucon	19.12	15.47	−0.065	−0.138	0.045	0.085 ± 0.002
S10	PEG8000	Ucon	15.00	29.97	−0.117	−0.091	0.070	0.60 ± 0.02

[a]Polymer 1, predominant polymer in the bottom phase; polymer 2, predominant polymer in the top phase; all concentrations of polymers are in % wt.; [b]π^*, solvent dipolarity/polarizability; α, solvent hydrogen-bond donor acidity; β, solvent hydrogen-bond acceptor basicity; all differences are calculated as those between values measured in the top phase and those measured in the bottom phase; [c]*c,* characterizes the difference between the electrostatic properties of the coexisting phases.

Table 2. Distribution coefficients of amino acids in ATPS.

	ATPS									
Amino acids	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Gln	0.793 ± 0.004	0.817 ± 0.003	0.725 ± 0.003	0.79 ± 0.01	0.583 ± 0.006	0.811 ± 0.003	0.825 ± 0.006	0.662 ± 0.007	0.559 ± 0.005	0.335 ± 0.003
Glu	0.756 ± 0.003	0.643 ± 0.002	0.503 ± 0.002	0.611 ± 0.006	0.431 ± 0.003	0.720 ± 0.005	0.636 ± 0.005	0.497 ± 0.006	0.381 ± 0.006	0.216 ± 0.003
His	0.844 ± 0.008	0.778 ± 0.005	0.68 ± 0.01	0.788 ± 0.009	0.652 ± 0.001	0.710 ± 0.005	0.749 ± 0.007	0.682 ± 0.004	0.598 ± 0.006	0.368 ± 0.004
Lys	0.824 ± 0.005	0.869 ± 0.007	0.696 ± 0.009	0.845 ± 0.003	0.606 ± 0.001	0.811 ± 0.002	0.711 ± 0.003	0.482 ± 0.004	0.514 ± 0.003	0.336 ± 0.002
Met	0.819 ± 0.002	0.84 ± 0.01	0.81 ± 0.01	0.857 ± 0.006	0.692 ± 0.008	0.87 ± 0.02	0.882 ± 0.007	0.73 ± 0.01	0.67 ± 0.01	0.499 ± 0.003
Ser	0.801 ± 0.003	0.80 ± 0.01	0.658 ± 0.007	0.766 ± 0.007	0.582 ± 0.004	0.84 ± 0.01	0.85 ± 0.01	0.655 ± 0.009	0.567 ± 0.008	0.380 ± 0.001
Thr	0.802 ± 0.002	0.807 ± 0.003	0.725 ± 0.004	0.826 ± 0.006	0.614 ± 0.006	0.867 ± 0.008	0.803 ± 0.009	0.751 ± 0.008	0.585 ± 0.005	0.440 ± 0.005
Tyr	0.920 ± 0.008	0.997 ± 0.009	0.980 ± 0.009	0.96 ± 0.02	1.00 ± 0.01	1.04 ± 0.01	1.01 ± 0.01	0.995 ± 0.005	1.052 ± 0.004	0.644 ± 0.003

*Compositions of ATPS see in Table 1.

The number of compounds examined so far is very limited and does not allow one to draw conclusions in regard to the applicability of the additivity principle to contributions of various structural fragments to different solute-specific coefficients under consideration. Much more compounds of different structures must be examined.

Discussion

It should be stressed that the solute-specific coefficients in Equation (2) represent the susceptibility of a given type of solute/solvent interaction to changes in the particular solvent property governing the interactions in question.

We compared the solute-specific coefficient S_s values representing the sensitivity of solute-solvent dipole/dipole interactions to changes in the aqueous solvent dipolarity/ polarizability for all amino acids examined with their polarizability (Krishtal, Senet, & Van Alsenoy, 2009). The polarizability was chosen as a compound feature well known to affect its dipole/dipole interactions (Krishtal et al., 2009). The results are presented in Figure 1 as a plot of S_s values against the polariazability α-values. It should be noted that the α-values analyzed here were estimated for compounds in water and not in saline solution (Krishtal et al., 2009). The data for all amino acids examined are grouped in three categories described by different linear relationships. Here, amino acids with nonpolar aliphatic side chains (Ala, Val, Ile, Leu), with polar hydroxyl group containing side chains (Ser, Thr) and Gly can be described by the following equation:

$$S_s = 3.57_{\pm 0.08} - 0.036_{\pm 0.001}\alpha$$
$$N = 7;\ r_2 = 0.9929;\ SE = 0.06;\ F = 695.6 \quad (3)$$

The amino acids with amide group in the side chain (Asn, Gln), sulfur-containing aliphatic side chain (Met), and side chains with aromatic (Phe, Tyr) or cyclic (Pro) moiety are fit by

$$S_s = 4.9_{\pm 0.1} - 0.042_{\pm 0.002}\alpha$$
$$N = 6;\ r^2 = 0.9796;\ SE = 0.13;\ F = 192.5 \quad (4)$$

whereas all amino acids with charged side-chains (Asp, Glu, Arg, Lys, His) and Trp are described by the following equation:

$$S_s = 6.1_{\pm 0.05} - 0.0418_{\pm 0.0006}\alpha$$
$$N = 6;\ r^2 = 0.9976;\ SE = 0.06;\ F = 1664.2 \quad (5)$$

Table 3. Solute-specific coefficients for free amino acids.

Amino acid	S_s	A_s	B_s	C_s
Gly[a]	2.4 ± 0.3	–	0.9 ± 0.2	−0.17 ± 0.08
Ala[a]	1.9 ± 0.2	–	0.7 ± 0.2	−0.22 ± 0.06
Val[a]	1.2 ± 0.2	–	0.5 ± 0.2	−0.27 ± 0.06
Nor-Val[a]	1.2 ± 0.2	–	0.5 ± 0.2	−0.26 ± 0.06
Leu[a]	0.7 ± 0.2	–	0.4 ± 0.1	−0.28 ± 0.05
Nor-Leu[a]	0.8 ± 0.2	–	0.3 ± 0.1	−0.29 ± 0.05
Ile[a]	0.8 ± 0.2	–	0.4 ± 0.1	−0.25 ± 0.05
Arg[b]	1.86 ± 0.09	–	0.65 ± 0.08	−0.25 ± 0.03
Tyr	0.3 ± 0.1	–	−0.2 ± 0.1	−0.27 ± 0.04
Ser	1.8 ± 0.2	–	1.0 ± 0.2	−0.21 ± 0.05
Thr	1.4 ± 0.2	–	1.0 ± 0.1	−0.17 ± 0.04
Met	1.3 ± 0.2	–	0.7 ± 0.1	−0.16 ± 0.04
Phe[a]	0.6 ± 0.2	–	0.2 ± 0.1	−0.15 ± 0.05
Trp[a]	0.5 ± 0.3	1.7 ± 0.9	−0.3 ± 0.2	−0.24 ± 0.09
His	2.5 ± 0.2	1.6 ± 0.6	0.8 ± 0.1	−0.31 ± 0.06
Lys	2.5 ± 0.2	–	0.8 ± 0.2	−0.19 ± 0.07
Pro[a]	2.1 ± 0.3	–	–	−0.80 ± 0.08
Asp[b]	3.5 ± 0.2	–	1.5 ± 0.2	−0.17 ± 0.06
Asn[b]	2.4 ± 0.1	–	1.03 ± 0.09	−0.11 ± 0.03
Glu	3.2 ± 0.2	–	1.6 ± 0.2	−0.24 ± 0.07
Gln	1.8 ± 0.2	–	1.0 ± 0.1	−0.29 ± 0.05

[a]Data from Madeira, Bessa, Alvares-Ribeiro et al. (2013); [b]data from Madeira, Bessa, de Barros et al. (2013).

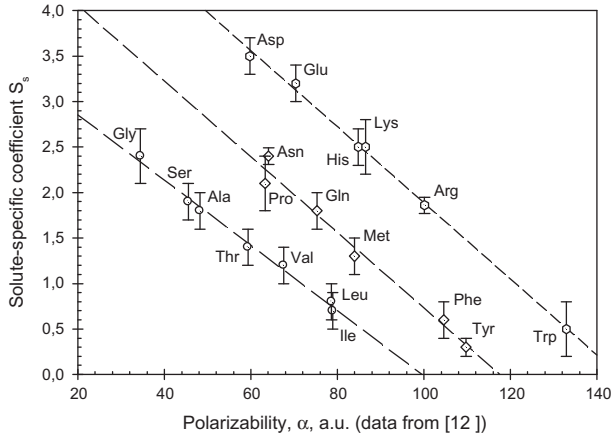

Figure 1. Solute-specific coefficient (S_s) values for all amino acids examined plotted vs. free amino acid polarizability (Krishtal et al., 2009) values.

where N is the number of amino acids; r^2 is the correlation coefficient; SE is the standard deviation; F is the ratio of variance.

It should be noted that the two last relationships (Equations (4) and (5)) are parallel to each other. Since polarizability is well known to be linearly related to the molecular volume, it seems reasonable to suggest that the established relationships results from the interrelationship of coefficient S_s with the solute volume (i.e. with the solvent cavity required to be formed in the solvent media to accommodate the solute (Madeira et al., 2010) and with the solute ability to participate in dipole/dipole interactions. It should be mentioned that there is no relationship between the amino acids polarizability and solute-specific coefficient B_s.

Physicochemical, structural, and biological properties of amino acids

Current literature contains information about more than 500 amino acid attributes derived from various experiments (Campen et al., 2008). Among the amino acids attributes are a variety of hydrophobicity scales, different measures of side chain bulkiness, polarity, volume, etc. For example, 494 distinct numerical indices representing various physicochemical and biochemical properties of amino acids and pairs of amino acids are included in the AAIndex database (http://www.genome.jp/aaindex/). The solute-specific coefficients listed in Table 3 form the first scale for amino acids based on amino acid/water interactions, and hence, it is of interest to compare this new scale with other amino acid scales developed over the years.

Previously (Madeira, Bessa, Alvares-Ribeiro, 2013), we showed that linear combinations of the solute-specific coefficients S_s and B_s may be used to describe partition ratio of amino acid in octanol–water, contribution of side chain into free energy of transfer from water to ethanol or dioxane, and retention time of peptides in HPLC (pH 7.0) for amino acids with nonpolar side-chains. We could not examine some of these relationships due to the lack of the corresponding data for amino acids with polar side chains. However, for many of the amino acids listed in Table 3, the following relationships were established.

For partition ratios of free amino acids Ala, Asn, Gln, Gly, Ile, Leu, Phe, Pro, Ser, Thr, Trp, and Val in octanol–water, pH 7.0 (Abraham & Leo, 1987) (see in Table 4):

$$\log D^{\mathrm{pH7.0}} = -0.9_{\pm 0.1} - 0.71_{\pm 0.09} S_s - 0.9_{\pm 0.15} B_s - 0.4_{\pm 0.1} A_s$$
$$N = 12;\ r^2 = 0.969;\ SE = 0.15;\ F = 84.2 \quad (6)$$

where all the parameters as defined above. It should be indicated that the estimates of log $D^{\mathrm{pH\ 7.0}}$ values for amino acids with charged side chains Arg, Asp, Glu, His, and Lys were not used as it is known that they might be affected by the particular ionic composition of the buffer used in octanol-buffer system. The log $D^{\mathrm{pH\ 7.0}}$ values for Met and Tyr do not fit the above correlation likely due to that their partition ratios were measured under conditions different from those used for the other amino acids (Abraham & Leo, 1987), and they were not used for deriving Equation (6).

Next, we evaluated how the determined in our study solute-specific coefficients for amino acids correlate with the retention time t_R of decapeptides of Ac–X–G–A–K–G–A–G–V–G–L-amide sequence where position X was substituted by different amino acids at pH 7.0, 0.05 M NaCl (Kovacs, Mant, & Hodges, 2006). This analysis revealed that the t_R value (see in Table 4) can be described as:

$$t_R = 75_{\pm 1.8} - 12_{\pm 1.5} S_s - 12_{\pm 2.4} B_s$$
$$N = 15;\ r^2 = 0.9472;\ SE = 2.9;\ F = 107.2 \quad (7)$$

where all the parameters are as defined above. In this case, data for amino acids with charged side chains Arg, Asp, Glu, His, and Lys were not used to derive Equation (7) as the chromatographic behavior of peptides with these amino acids in X position was clearly ionic composition dependent (Kovacs et al., 2006). The data for Tyr were not used also.

The relationships found between the descriptors representing the amino acids interactions with aqueous environment and physicochemical properties of amino acids imply that the descriptors might be used to describe the biological effects of amino acids. The validity of this hypothesis was tested next.

The only quantitative data on biological effects of a wide variety of free amino acids we could find in the literature are gustatory responses in humans (Asao, Iwamura, Akamatsu, & Fujita, 1987; Iwasaki, Kasahara, & Sato, 1985; Schiffman, Hornack, & Reilly, 1979).

Table 4. Partition coefficients of free amino acids in octanol/water, pH 7.0 (log D), retention time[a] in HPLC; taste quality of free amino acids in humans, surface area of amino acids, and structural properties displayed by amino acid residues in proteins.

Amino acid	log D (Abraham & Leo, 1987)	t_R, sec (Kovacs et al., 2006)	Log(1/T)[b] (Asao et al., 1987)	Log(1/T^{Σ})[c] (Schiffman et al., 1979)	Log(1/I^{Σ})[d] (Iwasaki et al., 1985)	Exposed/Buried ratio[e] (Bordo & Argos, 1991)	β-structure propensity (Wathen & Jia, 2010)	Top-IDP (Campen et al., 2008)	Surface area, A^2 (Chothia, 976)
Ala	−2.89	42.4		1.79	0.46	1.37	0.77	0.06[f]	115
Arg	−4.20*	42.4*	1.13*	2.92	0.76	16.80	0.93	0.180[f]	225
Asn	−3.41	39.3		2.79		8.20	0.60	0.007	160
Asp	<4.25*	37.5*		3.74		9.00	0.54	0.192[f]	150
Gln	−3.15	39.9		2.01*	0.34	8.10	0.77	0.318[f]	180
Glu	<4.19*	37.9*		4.2		23.25	0.72	0.736[f]	190
Gly	−3.25	38.3		1.51	0.26	1.42	0.63	0.166[f]	75
His	−3.56*	43.0*	1.32	2.91	0.61	3.47	0.91	0.303[f]	195
Ile	−1.72	61.1	1.96	2.13	0.81	0.83	1.77*	−0.486	175
Leu	−1.61	62.9	1.92	2.19	0.66	0.84	1.18	−0.326	180
Lys	<−4.44*	36.3*	1.07	3.15		46.50*	0.79	0.586[f]	200
Met	−1.84*	55.6		2.43	0.62	2.20*	1.00	−0.397	185
Phe	−1.63	68.4	2.19	2.18	0.87	1.00	1.42	−0.697	210
Pro	−2.50	48.7	1.59	1.82*	0.45	6.00	0.44	0.987[f]	145
Ser	−3.30	39.5		1.68	0.30	3.50	0.82	0.341[f]	115
Thr	−2.91	42.4		1.59	0.30	4.44	1.18	0.059	140
Trp	−1.75	71.3	2.30	2.64		1.11	1.31	−0.884	255
Tyr	−2.05*	54.3*	2.30			3.35	1.43	−0.51	230
Val	−2.08	53.3	1.68	2.38	0.67	0.80	1.94*	−0.121	155
NVal		55.2	1.32*						203
NLeu		64.2	1.70						233

*Data for amino acids not used in correlationships (see text); [a]retention time t_R of decapeptides of Ac–X–G–A–K–G–A–G–V–G–L-amide sequence where position X was substituted by different amino acid (Kovacs et al., 2006); [b]T, bitterness threshold (in mole/L); [c]T^{Σ}, overall taste detection threshold in young human subjects of 17–27 years of age (in mole/L); [d]I^{Σ}, overall taste intensity; [e]Calculated from data in Bordo and Argos (1991); [f]Disorder-promoting amino acid residues (Campen et al., 2008).

The bitterness threshold in humans (T) for His, Ile, Leu, Lys, Pro, Phe, Tyr, Trp, Val and nor-Leu reported in Asao et al. (1987) (see Table 4) are described as follows:

$$\log(1/T) = 2.31_{\pm 0.07} - 0.33_{\pm 0.06} S_s - 0.4_{\pm 0.15} B_s$$
$$N = 10;\ r^2 = 0.9268;\ SE = 0.12;\ F = 44.3 \quad (8)$$

where T is the bitterness threshold (Asao et al., 1987), all the other parameters are as defined above. Data for Arg and nor-Val do not fit the above correlation and they were not used for deriving Equation (8).

The overall taste (bitter, sweet, and "other") detection threshold (T^{Σ}) in humans presented by Schiffman et al. (1979) (young subjects) (see Table 4) is described as:

$$\log(1/T^{\Sigma}) = -0.7_{\pm 0.4} + 0.67_{\pm 0.09} S_s + 1.2_{\pm 0.18}(SA/100)$$
$$N = 16;\ r^2 = 0.8591;\ SE = 0.31;\ F = 39.5 \quad (9)$$

where T^{Σ} is the overall taste detection threshold in young subjects (17–27 years of age) (Schiffman et al., 1979); SA is the amino acid surface area in Å^2 (see in Table 4); all the other parameters are as defined above. It should be noted that the data for Gln and Pro were not used for deriving Equation (9), though they do fit the above relationship taking into account that the standard deviations in log units for all amino acids ranged from 0.23 to 1.04 (Schiffman et al., 1979) due to individual sensitivity and differences in the taste detection ability.

The overall taste intensity (I^{Σ}) of amino acids presented in Iwasaki et al. (1985) (see Table 4) is described as:

$$\log(I^{\Sigma}) = 0.8_{\pm 0.18} - 0.6_{\pm 0.1} B_s + 0.7 \pm 0.18 C_s + 0.2_{\pm 0.06}(SA/100)$$
$$N = 12;\ r^2 = 0.9117;\ SE = 0.075;\ F = 27.7 \quad (10)$$

where I^{Σ} is the overall taste intensity (Iwasaki et al., 1985); all the other parameters are as defined above.

The above relationships (Equations (8)–(10)) indicate that different amino acids features govern the taste detection and taste intensity. The overall taste detection depends upon the dipole dipole and hydrogen-bonding interactions of amino acids and their surface area (Equation (9)), the bitterness threshold depends on the dipole/dipole and hydrogen-bonding interactions of amino acids (Equation (8)), while the overall taste intensity is also governed by hydrogen bonding and amino acid surface area (Equation (10)).

We also examined the possibility to use the established descriptors of amino acids for the analysis of properties displayed by the amino acids residues in protein structure.

The ratio of percentage of appearing as a solvent exposed residue to that of appearance as a buried residue (Bordo & Argos, 1991) (defined as buried if the solvent-accessible surface $\leqslant 10$ Å^2 and as exposed if the solvent-accessible surface $\geqslant 30$ Å^2) is described for amino acids with nonpolar side-chains (Gly, Ala, Ile, Leu, Phe, Pro, Trp, and Val) as:

$$\log(\text{exposed/buried ratio}) = 1.2_{\pm 0.3} + 2.3_{\pm 0.2} S_s - 1.7_{\pm 0.3} B_s - 5.7_{\pm 0.5} A_s$$
$$N = 8;\ r^2 = 0.9831;\ SE = 0.3;\ F = 77.4 \quad (11a)$$

and for amino acids with polar and charged side-chains (Arg, Tyr, Ser, Thr, His, Asp, Asn, Glu, Gln) as:

$$\log(\text{exposed/buried ratio}) = -31.8_{\pm 7.7} + 4.9_{\pm 2} B_s - 12.8_{\pm 2.5} A_s + 16.8_{\pm 3.5}(SA/100)$$
$$N = 9;\ r^2 = 0.8761;\ SE = 3.1;\ F = 11.8 \quad (11b)$$

where exposed/buried ratio values calculated from data in Bordo and Argos (1991) are listed in Table 4; all the other parameters are as defined above. The data for Lys and Met do not fit the above correlation and were not used for deriving Equation (11b).

It seems reasonable that the exposed/buried ratio for amino acids residues with nonpolar side chains (Equation (11a)) is described by the amino acids descriptors much better than for residues with polar and charged side chains (Equation (11b)) likely due to the lack of specific interactions between residues with nonpolar side chains within protein structure. It is also important to note that the underlying assumption that the amino acids residues in a polypeptide chain maintain the features of free amino acids displayed in their interactions with aqueous environment appears to be valid.

Propensity of amino acids residues for being included into the β-sheet structure in proteins (Wathen & Jia, 2010) (see Table 4) can be described as:

$$P(\beta)_s = 1.2_{\pm 0.17} - 0.24_{\pm 0.035} S_s + 0.6_{\pm 0.19} C_s + 0.18_{\pm 0.069}(SA/100)$$
$$N = 17;\ r^2 = 0.8794;\ SE = 0.12;\ F = 31.6 \quad (12)$$

where $P(\beta)_s$ is the β-structure propensity of amino acids presented in(Wathen & Jia, 2010); all the other parameters are as defined above; $P(\beta)_r$ values for Val and Ile were outliers and were not used for deriving Equation (13).

Finally, we compared the amino acid descriptors determined in this study with two different protein intrinsic disorder scales (Campen et al., 2008). The intrinsic disorder-related attributes considered are

normalized flexibility parameters (*B*-values) for each residue surrounded by one rigid neighbor (Vihinen, Torkkila, & Riikonen, 1994) and the TOP-IDP scale (Campen et al., 2008) (see Table 4). The following relationships were established:

$$Top - IDP = -1.2_{\pm 0.13} + 0.2_{\pm 0.1} S_s + 0.4_{\pm 0.20} B_s - 2.1_{\pm 0.4} C_s$$
$$N = 19;\ r^2 = 0.870;\ SE = 0.193;\ F = 33.56 \quad (13)$$

$$B - value = 0.94 \pm 0.013 + 0.026 \pm 0.005 S_s - 0.027_{\pm 0.0086} A_s - 0.08_{\pm 0.032} C_s$$
$$N = 19;\ r^2 = 0.755;\ \mathrm{SE} = 0.019;\ \mathrm{F} = 15.42 \quad (14)$$

where all the parameters are as defined above.

It can be concluded that the developed in our study scale provides a dissent description of intrinsic disorder propensity in proteins. This observation is further illustrated by the plot of the Top-IDP values against the S_s descriptor values shown in Figure 2, where black and open symbols correspond to order- and disorder-promoting residues, respectively. Classification of amino acid residues as order- and disorder-promoting is based on the earlier statistical analysis of intrinsically disordered proteins (IDPs) and intrinsically disordered protein regions (IDPRs) Campen et al., 2008. In fact, it has been established that IDPs/IDPRs differ from structured globular proteins and domains with regard to many attributes, including amino acid composition, sequence complexity, hydrophobicity, charge, flexibility, and type and rate of amino acid substitutions over evolutionary time (Campen et al., 2008; Dunker et al., 1998; Dunker et al., 2001; Garner, Cannon, Romero, Obradovic, & Dunker, 1998; Radivojac et al., 2007; Romero et al., 2001; Uversky, 2011; Uversky & Dunker, 2010; Uversky, Gillespie, & Fink, 2000; Vacic, Uversky, Dunker, & Lonardi, 2007; William, 2001). In direct relation to our study, IDPs/IDPRs were shown to be significantly depleted in a number of order-promoting residues, including bulky hydrophobic (Ile, Leu, and Val) and aromatic amino acids (Trp, Phe, Tyr), which would normally form the hydrophobic core of a folded globular protein and also possess low content of Cys and Asn residues. On the other hands, IDPs/IDPRs were shown to be substantially enriched in disorder-promoting amino acids (Ala, Arg, Gly, Gln, Ser, Pro, Glu, and Lys), which mostly are polar or change residues, and structure-breaking Pro and Gly residues (Dunker et al., 2001; Radivojac et al., 2007; Romero et al., 2001; William, 2001). Since open and black symbols in Figure 2 almost do not overlap (except to Asn, which according to Top-IDP is at the boundary of order-disorder promoting capabilities (Campen et al., 2008), this clearly shows that the amino acid attributes determined in this study can be used for discrimination between order- and disorder-promoting residues.

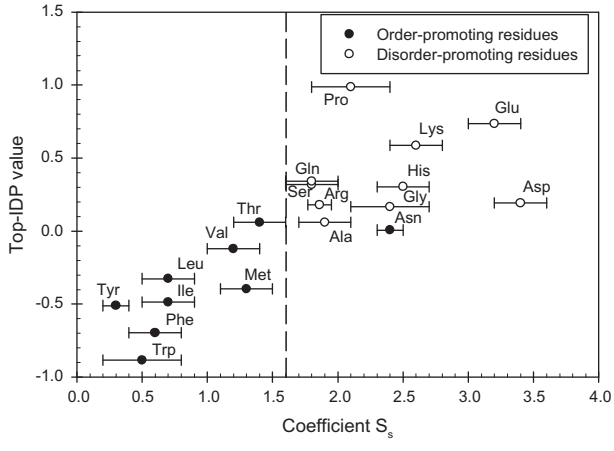

Figure 2. Solute-specific coefficient (S_s) values for all amino acids examined plotted vs. the Top-ID values for amino acid residues in proteins (Campen et al., 2008).

The rather unexpected finding that the amino acids electrostatic interactions (represented by the C_s parameter in Equations (13)–(15)) provide the most significant contribution is hard to explain currently. Descriptors for amino acids residues in peptides must be determined for this purpose. These studies are currently in progress in our laboratory.

The above results may be discussed in much more detail, but it is beyond the scope of the present work. The purpose of the study was to explore if the properties of free amino acids described by the descriptors established by partitioning in multiple ATPS with characterized solvent properties may be used for the analysis of physicochemical properties of amino acids, their biological effects, and for the analysis of properties displayed by the amino acids residues in protein structures. The relationships described by Equations (6)–(15) enable us to conclude that the aforementioned descriptors characterize properties of amino acids displayed in their interactions with aqueous environment important for their biological effects.

The fact that the descriptors established in this work for free amino acids are capable to characterize certain properties displayed by the amino acids residues in protein structures imply that these descriptors are related to those of amino acids residues in a polypeptide chain. This issue is currently being investigated in our laboratories.

Conclusions

Solute-specific coefficients for L-amino acids with polar and charged side chains were obtained. These coefficients reflect the ability of the solute to interact with aqueous environment, through dipole/dipole, electrostatic, and

hydrogen-bonding interactions. It is shown that linear combinations of these descriptors are correlated with physicochemical, structural, and biological properties of amino acids and thus may be used as the solute descriptors in Quantitative Structure/Property Relationship analysis.

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References

Abraham, M. H., Gola, J. M. R., Kumarsingh, R., Cometto-Muniz, J. E., & Cain, W. S. (2000). Connection between chromatographic data and biological data. *Journal of Chromatography B: Biomedical Sciences and Applications, 745*, 103–115.

Abraham, M. H., Ibrahim, A., & Zissimos, A. M. (2004). Determination of sets of solute descriptors from chromatographic measurements. *Journal of Chromatography A, 1037*, 29–47.

Abraham, M. H., Ibrahim, A., Zissimos, A. M., Zhao, Y. H., Comer, J., & Reynolds, D. P. (2002). Application of hydrogen bonding calculations in property based drug design. *Drug Discovery Today, 7*, 1056–1063.

Abraham, D. J., & Leo, A. J. (1987). Extension of the fragment method to calculate amino acid zwitterion and side chain partition coefficients. *Proteins, 2*, 130–152.

Albertsson, P. A. (1986). *Partition of cell particles and macromolecules* (3rd ed.). New York, NY: Wiley.

Asao, M., Iwamura, H., Akamatsu, M., & Fujita, T. (1987). Quantitative structure-activity relationships of the bitter thresholds of amino acids, peptides, and their derivatives. *Journal of Medicinal Chemistry, 30*, 1873–1879.

Ball, P. (2011). Biophysics: More than a bystander. *Nature, 478*, 467–468.

Berkowitz, S. A., Engen, J. R., Mazzeo, J. R., & Jones, G. B. (2012). Analytical tools for characterizing biopharmaceuticals and the implications for biosimilars. *Nature Reviews Drug Discovery, 11*, 527–540.

Biswas, K. M., DeVido, D. R., & Dorsey, J. G. (2003). Evaluation of methods for measuring amino acid hydrophobicities and interactions. *Journal of Chromatography A, 1000*, 637–655.

Bordo, D., & Argos, P. (1991). Suggestions for "safe" residue substitutions in site-directed mutagenesis. *Journal of Molecular Biology, 217*, 721–729.

Campen, A., Williams, R. M., Brown, C. J., Meng, J., Uversky, V. N., & Dunker, A. K. (2008). TOP-IDP-scale: A new amino acid scale measuring propensity for intrinsic disorder. *Protein and Peptide Letters, 15*, 956–963.

Chothia, C. (1976). The nature of the accessible and buried surfaces in proteins. *Journal of Molecular Biology, 105*, 1–12.

Dunker, A. K., Garner, E., Guilliot, S., Romero, P., Albrecht, K., Hart, J., … Villafranca, J. E. (1998). Protein disorder and the evolution of molecular recognition: Theory, predictions and observations. *Pacific Symposium on Biocomputing*, 473–484.

Dunker, A. K., Lawson, J. D., Brown, C. J., Williams, R. M., Romero, P., Oh, J. S., … Obradovic, Z. (2001). Intrinsically disordered protein. *Journal of Molecular Graphics and Modelling, 19*, 26–59.

Garner, E., Cannon, P., Romero, P., Obradovic, Z., & Dunker, A. K. (1998). Predicting disordered regions from amino acid sequence: Common themes despite differing structural characterization. *Genome Inform Ser Workshop Genome Inform, 9*, 201–213.

Goss, K.-U. (2005). Predicting the equilibrium partitioning of organic compounds using just one Linear Solvation Energy Relationship (LSER). *Fluid Phase Equilibria, 233*, 19–22.

Iwasaki, K., Kasahara, T., & Sato, M. (1985). Gustatory effectiveness of amino acids in mice: Behavioral and neurophysiological studies. *Physiology & amp; Behavior, 34*, 531–542.

Kamlet, M. J., Abboud, J. L. M., Abraham, M. H., & Taft, R. W. (1983). Linear solvation energy relationships. 23. A comprehensive collection of the solvatochromic parameters, π^*, α, and β, and some methods for simplifying the generalized solvatochromic equation. *Journal of Organic Chemistry, 48*, 2877–2887.

Kamlet, M. J., Abboud, J. L., & Taft, R. W. (1977). The solvatochromic comparison method. 6. The π scale of solvent polarities. *Journal of the American Chemical Society, 99*, 6027–6038.

Kamlet, M. J., & Taft, R. W. (1976). The solvatochromic comparison method. I. The β-scale of solvent hydrogen-bond acceptor (HBA) basicities. *Journal of the American Chemical Society, 98*, 377–383.

Kovacs, J. M., Mant, C. T., & Hodges, R. S. (2006). Determination of intrinsic hydrophilicity/hydrophobicity of amino acid side chains in peptides in the absence of nearest-neighbor or conformational effects. *Peptide Science, 84*, 283–297.

Krishtal, A., Senet, P., & Van Alsenoy, C. (2009). Local softness, softness dipole, and polarizabilities of functional groups: Application to the side chains of the 20 amino acids. *The Journal of Chemical Physics, 131*, 044312.

Madeira, P. P., Bessa, A., Alvares-Ribeiro, L., Aires-Barros, M. R., Rodrigues, A. E., & Zaslavsky, B. Y. (2013). Analysis of amino acid-water interactions by partitioning in aqueous two-phase systems. I–amino acids with non-polar side-chains. *Journal of Chromatography A, 1274*, 82–86.

Madeira, P. P., Bessa, A., de Barros, D. P., Teixeira, M. A., Alvares-Ribeiro, L., Aires-Barros, M. R., … Zaslavsky, B. Y. (2013). Solvatochromic relationship: Prediction of distribution of ionic solutes in aqueous two-phase systems. *Journal of Chromatography A, 1271*, 10–16.

Madeira, P. P., Reis, C. A., Rodrigues, A. E., Mikheeva, L. M., Chait, A., & Zaslavsky, B. Y. (2011). Solvent properties governing protein partitioning in polymer/polymer aqueous two-phase systems. *Journal of Chromatography A, 1218*, 1379–1384.

Madeira, P. P., Reis, C. A., Rodrigues, A. E., Mikheeva, L. M., & Zaslavsky, B. Y. (2010). Solvent properties governing solute partitioning in polymer/polymer aqueous two-phase systems: Nonionic compounds. *Journal of Physical Chemistry B, 114*, 457–462.

Platts, J. A., Abraham, M. H., Butina, D., & Hersey, A. (1999). Estimation of molecular linear free energy relationship descriptors by a group contribution approach. 2. Prediction of partition coefficients. *Journal of Chemical Information and Computer Sciences, 40*, 71–80.

Radivojac, P., Iakoucheva, L. M., Oldfield, C. J., Obradovic, Z., Uversky, V. N., & Dunker, A. K. (2007). Intrinsic disorder and functional proteomics. *Biophysical Journal, 92*, 1439–1456.

Romero, P., Obradovic, Z., Li, X., Garner, E. C., Brown, C. J., & Dunker, A. K. (2001). Sequence complexity of disordered protein. *Proteins, 42*, 38–48.

Schiffman, S. S., Hornack, K., & Reilly, D. (1979). Increased taste thresholds of amino-acids with age. *American Journal of Clinical Nutrition, 32*, 1622–1627.

Taft, R. W., & Kamlet, M. J. (1976). The solvatochromic comparison method. 2. The α-scale of solvent hydrogen-bond donor (HBD) acidities. *Journal of the American Chemical Society, 98*, 2886–2894.

Uversky, V. N. (2011). Intrinsically disordered proteins from A to Z. *International Journal of Biochemistry & Cell Biology, 43*, 1090–1103.

Uversky, V. N., & Dunker, A. K. (2010). Understanding protein non-folding. *Biochimica et Biophysica Acta, 1804*, 1231–1264.

Uversky, V. N., Gillespie, J. R., & Fink, A. L. (2000). Why are "natively unfolded" proteins unstructured under physiologic conditions? *Proteins, 41*, 415–427.

Vacic, V., Uversky, V. N., Dunker, A. K., & Lonardi, S. (2007). Composition Profiler: A tool for discovery and visualization of amino acid composition differences. *BMC Bioinformatics, 8*, 211.

Valko, K., Du, C. M., Bevan, C., Reynolds, D. P., & Abraham, M. H. (2001). Rapid Method for the Estimation of Octanol / Water Partition Coefficient (Log Poct) from Gradient RP-HPLC Retention and a Hydrogen Bond Acidity Term (Sigma alpha2H). *Current Medicinal Chemistry, 8*, 1137–1146.

Vihinen, M., Torkkila, E., & Riikonen, P. (1994). Accuracy of protein flexibility predictions. *Proteins-Structure Function and Genetics, 19*, 141–149.

Wathen, B., & Jia, Z. C. (2010). Protein beta-sheet nucleation is driven by local modular formation. *Journal of Biological Chemistry, 285*, 18376–18384.

Williams, R. M., Obradovi, Z., Mathura, V., Braun, W., Garner, E. C., Young, J., … Dunker, A. K. (2001). The protein non-folding problem: Amino acid determinants of intrinsic order and disorder. *Pacific Symposium on Biocomputing*, 89–100.

Zaslavsky, B. Y. (1995). *Aqueous two-phase partitioning: Physical chemistry and bioanalytical applications*. New York, NY: Marcel Dekker.