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Solvent properties governing protein partitioning in polymer/polymer aqueous two-phase systems

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ABSTRACT

Distribution coefficients of various proteins were measured in aqueous Dextran–Ficoll, Dextran–PES, and Ficoll–PES two-phase systems, containing 0.15 M NaCl in 0.01 M phosphate buffer, pH 7.4. The acquired data were combined with data for the same proteins in different systems reported previously [29,30] and known solvatochromic solvent properties of the systems [17] to characterize the protein–solvent interactions. The relative susceptibilities of proteins to solvent dipolarity/polarizability, solvent hydrogen bond acidity, solvent hydrogen bond basicity, and solvent ability to participate in ion–ion and ion–dipole interactions were characterized. These parameters, which are representative of solute–solvent interactions, adequately described the partitioning of the proteins in each system. It was found that the relative susceptibilities of proteins to solvent dipolarity/polarizability are interrelated with their relative susceptibilities to solvent hydrogen bond acidity and solvent hydrogen bond basicity similarly to those established previously for small nonionic organic compounds.

1. Introduction

The crucial importance of solvent interactions in stabilization, transport, and function of proteins has long been recognized. The view that hydrophobic effect is the major contributor to protein stability is widely held [1–3], though current studies of solvation recognize the importance of other types of solvent–protein interactions such as van der Waals, polar, ion–dipole, and hydrogen bonding interactions [4]. The view of solvation as a stabilizing force was further expanded to include the possibility that solvent interactions play a role in specifying protein structure and function, protein–protein, and protein–drug interactions [4–6].

The solvent–solute interactions are generally described by the linear free energy relationships, particularly by the Abraham model [7–10]:

$$\log \mathrm{SP} = z + rR_2 + s\pi_2{}^{\mathrm{H}} + a\sum \alpha_2{}^{\mathrm{H}} + b\sum \beta_2{}^{\mathrm{H}} + vV_{\mathrm{x}} \quad (1)$$

where SP is a property of a series of solutes in a given solvent system (typically the logarithm of a partition coefficient), z is the intercept constant, and solute descriptors are defined as follows: V_{x} is McGowan's characteristic volume [11] of the solute; R_2 is the solute excess molar refraction derived from the solute refractive index; $\pi_2{}^{\mathrm{H}}$ is the solute combined dipolarity/polarizability descriptor; $\sum\alpha_2{}^{\mathrm{H}}$ is the overall solute hydrogen bond acidity; and $\Sigma\beta_2{}^{\mathrm{H}}$ is the overall solute hydrogen bond basicity. The solute descriptors values are generally derived from experimental measurements [7,8], though they can be acquired from computational methods as well [9]. Whereas each solute descriptor denotes the solute effect on various solute–solvent interactions, the coefficients of each term: r, s, a, b, and v represent the complementary effects of the solvent in regards to these interactions.

The coefficient r reflects the tendency of the solvent to interact with solutes through π- and n-electron pairs. The coefficient s indicates the tendency of the solvent to interact with dipolar/polarizable solutes. Coefficient a denotes the hydrogen bond basicity of the solvent, and the coefficient b is a measure of the

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hydrogen bond acidity of the solvent. The coefficient v is a measure of the combined dispersion and cavity effect. It should be noted that hydrogen bond interactions occur through opposite pairs in solute and solvent interactions.

The Abraham's approach is generally applicable to nonionic organic compounds but it was never used for proteins due to the fact that their solute properties are unable to be measured experimentally.

The Abraham's method originated from the Kamlet–Taft solvatochromic comparison approach [12–15] which is based on the use of solvatochromic dyes to experimentally estimate the solvent dipolarity/polarizability (π^*), solvent hydrogen-bond donor acidity (α), and solvent hydrogen-bond acceptor basicity (β). According to this approach, the nonionic solute partition coefficient in a solvent biphasic system may be expressed as [16]:

$$\log K_s = z_0 + S_s\,\Delta\pi^* + b_s\,\Delta\beta + a_s\,\Delta\alpha + v_s\,\Delta\delta_H \quad (2)$$

where K_s is the solute partition coefficient; $\Delta\pi^*$, $\Delta\alpha$, and $\Delta\beta$ are the differences between the corresponding solvatochromic solvent properties of the coexisting phases; the δ_H term is the Hildebrand solubility parameter, which serves as a measure of the solvent/solvent interactions that are interrupted in creating a cavity for the solute [15]; s_s, a_s, b_s, and v_s are coefficients that measure the relative susceptibility of the solute partition coefficient to the indicated solvent properties. This approach was used [17] to estimate the solvent properties of aqueous media in both coexisting phases in aqueous two-phase systems formed by different pairs of nonionic polymers, such as Dextran, Ficoll, poly(ethylene glycol) (PEG), Ucon, and PES, and to estimate the solute–solvent interactions for nonionic organic solutes.

Aqueous two-phase systems (ATPSs) arise in aqueous mixtures of different water-soluble polymers or a single polymer and a specific salt. When two specific polymers, e.g., Dextran and PEG, are mixed in water above certain concentrations, the mixture separates into two immiscible aqueous phases. There is a clear interfacial boundary separating two distinct aqueous-based phases, each preferentially rich in one of the polymers, with the aqueous solvent in both phases providing media suitable for biological products [18,19]. These systems are unique in that each of the phases contains over 80% water on a molal basis and yet are immiscible and differ in their solvent properties [18,20], therefore these systems can be used for differential distribution of biological solutes and particles [19], for characterization of protein surface properties [18,21] and changes in protein structure [22].

The underlying concept for one current explanation of partitioning in ATPSs is that the polymers engaged in the formation of an ATPS are essentially neutral to the solute being partitioned and are important only in regard to their effects on the solvent features of the aqueous media in the coexisting phases. This view is supported by experimental evidence which indicates that the solvent features of the aqueous media in the coexisting phases are different [18,20], and there are clear similarities between partitioning of solutes in ATPSs and in water–organic solvent systems [18,20,23–30].

This study analyzed the partitioning of various proteins in several ATPSs. The acquired data were combined with data reported for the same proteins in other ATPS [29,30] as well as with the solvatochromic solvent properties of the phases [17], and the partition coefficients of model compounds [28] to allow for a detailed analysis of protein partitioning in regard to protein–solvent interactions.

2. Experimental

2.1. Materials

2.1.1. Polymers

All polymers were used without further purification. Dextran 75 (lot 115195), weight-average molecular weight (M_w) ≅ 75 000 was purchased from USB (Cleveland, OH, USA). Ficoll 70 (lot 302970), M_w ≅ 70 000 was purchased from GE Healthcare Biosciences AB (Sweden). Reppal PES-100 (lot D702-09/01), M_w ≅ 100 000 was purchased from REPPE AB (Växjö, Sweden).

2.1.2. Proteins

Chicken egg lysozyme (#L-6876), bovine α-chymotrypsinogen A (#C-4879), bovine hemoglobin (#H-2500), bovine ribonuclease B (#R-7884), bovine ribonuclease A (#R-5000), bovine trypsinogen (#T-1143), human hemoglobin (#H-7379), horse myoglobin (#M-0630), and bovine β-lactoglobulin (#L-3908) were purchased from Sigma (Saint Louis, MO, USA). Porcine lipase (#18480), and human transferrin (#22508) were purchased from USB.

2.1.3. Other chemicals

O-Phthaldialdehyde (OPA) reagent solution (complete) was purchased from Sigma. All salts and other chemicals used were of analytical-reagent grade.

2.2. Methods

2.2.1. Partitioning

(a) Phase systems. A mixture of polymers was prepared as described previously [28–30] by dispensing appropriate amounts of the aqueous stock polymer solutions into a 1.2 mL microtube using a Hamilton Company (Reno, NV, USA) ML-4000 four-probe liquid-handling workstation. Appropriate amounts of stock buffer solutions were added to give the required ionic and polymer composition of the final system with total volume of 0.5 mL. All two-phase systems had the polymer compositions indicated in Table 1 and salt composition of 0.15 M NaCl in 0.01 M sodium phosphate buffer (NaPB), pH 7.4.

(b) Partitioning experiments. An automated instrument for performing aqueous two-phase partitioning, Automated Signature Workstation, ASW (Analiza, Inc., Cleveland, OH, USA) was used for the partitioning experiments. The ASW system is based on the ML-4000 liquid-handling workstation (Hamilton Company) integrated with a FL600 fluorescence microplate reader (Bio-Tek Instruments, Winooski, VT, USA) and a UV–vis microplate spectrophotometer (SpectraMax Plus^{384}; Molecular Devices, Sunnyvale, CA, USA). Solutions of all proteins were prepared in water at concentrations of 1–5 mg/mL. Varied amounts (e.g., 0, 15, 30, 45, 60, and 75 μL) of protein solution and the corresponding amounts (e.g., 100, 85, 70, 55, 40 and 25 μL) of water were added to a set of the same polymer/buffer mixtures for a total mass of the system of 500 mg (~480 μL). The systems were then vortexed in a multi-pulse vortexer and centrifuged (Jouan, BR4i, Thermo Fisher Scientific, Waltham, MA, USA) for 30 min at 1160 × *g* at 23 °C to accelerate phase settling. The top phase in each system was removed, the interface discarded, and aliquots of 20–70 μL from the top and bottom phases were withdrawn in duplicate for analysis of protein content. These aliquots were combined with 250 μL of OPA solution in microplate wells. After moderate shaking for 2 min at room temperature fluorescence was determined with a fluorescence plate reader with a 360 nm excitation filter and a 460 nm emission filter, with a sensitivity setting of 100–125.

The distribution coefficient, K, is defined as the ratio of the sample concentration in the top phase to that in the bottom phase. The K value for each solute was determined as the slope of the concen-

Table 1
Polymer compositions[a] of the phases in the aqueous two-phase systems used for partitioning. The difference between the relative hydrophobic characters of the coexisting phases is represented by parameter E.[b] The difference between the ability of the media in the coexisting phases to participate in ion–ion and ion–dipole interactions is represented by parameter c[c].

Polymer 1	Polymer 2	Total composition		Top phase		Bottom phase		E[b]	c[c]
		Polymer 1	Polymer 2	Polymer 1	Polymer 2	Polymer 1	Polymer 2		
Dextran	Ficoll	12.94	18.06	3.23	28.31	21.57	9.03	0.0191 ± 0.0006	0.054 ± 0.002
Dextran	PEG	12.41	6.06	0.31	13.02	22.44	0.53	0.0271 ± 0.0008	−0.039 ± 0.003
Dextran	Ucon	12.39	10.08	0.16	18.30	26.51	0.59	0.085 ± 0.002	0.0017 ± 0.007
PES	Dextran	17.30	12.43	5.31	21.68	31.38	1.93	$-0.02205 \pm 4 \times 10^{-5}$	−0.0880 ± 0.0001
PEG	Ucon	15.00	29.97	0.34	52.12	32.39	3.78	0.123 ± 0.008	0.56 ± 0.03
Ficoll	PEG	15.06	7.90	9.55	11.65	23.97	1.83	0.0097 ± 0.0009	−0.092 ± 0.003
Ficoll	Ucon	13.01	9.93	2.90	16.42	24.50	2.54	0.05 ± 0.02	−0.05 ± 0.06
PES	Ficoll	17.31	14.86	10.31	20.20	25.35	7.80	−0.0126 ± 0.0007	−0.007 ± 0.002
PES	PEG	15.24	6.96	3.67	12.28	29.58	0.37	$-0.0147 \pm 6 \times 10^{-5}$	$-0.1302 \pm 2 \times 10^{-4}$
PES	Ucon	12.91	7.68	2.76	13.50	24.01	1.32	0.035 ± 0.006	−0.12 ± 0.01

[a] Polymer concentrations are given in wt.%.
[b] Parameter E calculated from experimental data on partitioning of sodium salts of dinitrophenyl-amino acids with aliphatic side chains described by Eq. (3) as reported in [28]; see text for physical meaning of parameter E.
[c] Parameter c calculated from experimental data on partitioning of sodium salts of dinitrophenyl-amino acids with aliphatic side chains described by Eq. (3) as reported in [28]; see text for physical meaning of parameter c.

tration in the top phase plotted as a function of the concentration in the bottom phase averaged over the results obtained from two to four partition experiments carried out at the specified ionic composition of the system (0.15 M NaCl in 0.01 M NaPB, pH 7.4). The deviation from the average K value was always less than 5%, and in most cases lower than 2%.

2.2.2. Electrophoresis

All protein preparations were characterized by SDS-PAGE electrophoresis in a microfluidic chip using Bioanalyzer 2100, Protein 200 Plus Assay (Agilent Technologies, USA) under non-reduced conditions. All the proteins were observed as single bands in the electrophoregrams.

3. Results and discussion

3.1. Background

As mentioned above the composition of the coexisting phases for all the ATPS used here and previously [28–30] is shown in Table 1. The distribution coefficient data for proteins reported previously are presented in Table 2 [29,30]. Solvent solvatochromic parameters for all the ATPS [17] used are listed in Table 3.

Each ATPS analyzed was also previously characterized [28] in terms of the free energy of transfer of a CH_2 group between the coexisting phases. This approach [18,11,27,31–36] is based on the partitioning of a homologous series of solutes with varied aliphatic alkyl chain length, which can be described as:

$$\log K_j = c + E * N(CH_2)_j \quad (3)$$

where K_j is the partition coefficient of a jth member of the homologous series with the corresponding $N(CH_2)_j$ length of the aliphatic chain of a given solute. E is an average $\log K$ increment per CH_2 group; and c represents the total contribution of the non-alkyl part of the solute structure into $\log K_j$. The c and E values for each system are listed in Table 1.

The relationship between the parameter E and the solvatochromic solvent parameters of the ATPS has previously been established as [17]:

$$E = -0.001\ (\pm 0.004) - 0.34(\pm 0.05)\,\Delta\pi^* - 0.46(\pm 0.04)\,\Delta\alpha \quad (4)$$

$N = 9$; $r^2 = 0.9620$; SD = 0.011 ; $F = 75.9$where E, $\Delta\pi^*$, and $\Delta\alpha$ are as defined above; N is the number of ATPS; F is the ratio of variance; SD the standard deviation; and r^2 the correlation coefficient.

It was also established that the partition coefficient of a nonionic compound in a set of different ATPSs may be expressed as:

$$\log K_s = z_0 + s_s\,\Delta\pi * + a_s\,\Delta\alpha + b_s\,\Delta\beta \quad (5)$$

where all parameters are defined above.

4. Results

The distribution coefficients of proteins examined in the aqueous Dex–Ficoll, PES–Dex, and PES–Ficoll ATPS are presented in Table 2.

An important feature of ATPS is the difference in concentrations of phosphate buffer and NaCl in the coexisting phases [18]. Buffers and salts are known to affect the polymer composition of the phases and their solvent properties so much that, for example, the same Dex–PEG systems with different concentrations of salt and/or buffer additives are to be considered as different ATPSs with different solvent properties of the phases [18, pp. 155–220]. The difference between the electrostatic properties of the coexisting phases translates to different ion–ion, ion–dipole and possibly dipole–dipole solute–solvent interactions. A linear solvation energy relationship (Eq. (2) or (5)) is applicable to the partitioning of nonionic solutes, but an added advantage of ATPS is the possibility to examine the partitioning of ionizable compounds. Hence it appears that an additional parameter capable of quantifying the difference between the electrostatic properties of the phases is necessary. Zaslavsky [18, pp. 208–216] proposed to use the contribution of an ionic group into the solute partition coefficient as an empirical measure of the difference in question. The experimental results here were obtained with sodium salts of p-dinitrophenyl-amino acids, i.e. compounds possessing a DNP–NH–CH–COO^-Na^+ group. This moiety is bulky and contains a substituted aromatic ring. Use of this particular group as a probe for electrostatic ion–ion, ion–dipole and dipole–dipole interactions obviously has some limitations. Only to a first approximation can the free energy of transfer of this group between the coexisting phases of an ATPS be viewed as a measure of the ability of aqueous media to participate in a particular kind of intermolecular interactions. This parameter is represented by parameter c in Eq. (3), and it is included in the modified linear solvation energy relationship equation as follows:

$$\log K_s = z_0 + s_s\,\Delta\pi^* + a_s\,\Delta\alpha + b_s\,\Delta\beta + c_s c \quad (6)$$

where c is the contribution of a DNP–NH–CH–COO^-Na^+ group into $\log K$ (see Eq. (3)), coefficient c_s characterizes the relative suscep-

Table 2
Distribution coefficients, *K*, determined for proteins in the Dextran–Ficoll, PES–Dextran, PES–Ficoll and in other ATPS indicated (as reported previously [30]).

Protein	M_w (kDa)	pI[a]	Dex–Ficoll	Dex–PEG	PEG–Ucon	Dex–Ucon	PES–PEG	Ficoll–PEG	Ficoll–Ucon	PES–Dex	PES–Ficoll	PES–Ucon
RNAse A	13.7	9.6	1.16	0.489	~0.014[b]	0.247	0.604	0.466	0.25	1.275	1.365	0.506
RNAse B	~15.0	~9.45	1.069	0.455	–	0.265	0.703	0.440	0.237	1.452	1.435	0.63
Chymotrypsinogen	~25.7	8.97	2.02	2.71	0.0098	1.78	2.98	1.04	0.638	1.313	1.993	1.80
Trypsinogen	23.7	9.3	1.883	0.89	0.015	0.702	0.967	0.580	0.345	1.071	1.556	0.779
Lysozyme	14.3	11.4	2.31	2.36	0.036	2.95	1.29	0.91	1.00	0.416	1.064	1.28
Hemoglobine bovine	64.5	6.8	1.191	0.074	[b]	0.053	0.148	0.094	0.052	2.199	2.317	0.208
Hemoglobine human	64.5	6.8	1.414	0.131	[b]	0.117	0.22	0.153	0.074	1.734	2.298	0.282
Lactoglobulin	18.4	5.2	0.408	0.071	[b]	0.033	0.213	0.112	0.044	6.019	2.36	0.176
Transferrin	77.0	5.7	0.58	0.0084	[b]	0.0015	0.052	0.019	0.0035	7.145	3.035	0.042
Myoglobin	17.6	7.3	0.685	0.161	0.065	0.080	0.340	0.258	0.154	1.96	1.414	0.310
Lipase	48	5.2	0.904	0.716	0.358	0.658	0.75	0.733	0.634	1.268	1.042	0.78
γ-Globulin human	~160	~6.8	5.587	0.043	–	0.014	0.0746	0.0166	0.0054	2.373	8.257	0.0816
γ-Globulin bovine	~180	~6.5	4.48	0.024	–	0.007	0.0573	0.00399	0.0051	3.107	8.977	0.0816

[a] pI – isoelectric point.
[b] Protein partially or completely precipitates at the interface of the ATPS.

Table 3
Solvatochromic solvent parameters characterizing solvent polarity (π^*), solvent hydrogen-bond donor acidity (α), and solvent hydrogen-bond acceptor basicity (β), and the differences,[a] $\Delta\pi^*$, $\Delta\alpha$, and $\Delta\beta$, between these characteristics of the media in the coexisting phases of the aqueous two-phase systems (data from [17]).

#	Polymer 1[b]	Polymer 2[b]	Top phase			Bottom phase			$\Delta\pi^*$[a]	$\Delta\alpha$[a]	$\Delta\beta$[a]
			π^*	α	β	π^*	α	β			
1	Dextran	Ficoll	1.188	0.984	0.633	1.150	1.048	0.678	0.038	−0.064	−0.045
2	Dextran	PEG	1.099	1.078	0.632	1.167	1.096	0.624	−0.068	−0.018	0.008
3	Dextran	Ucon	1.112	0.882	0.659	1.179	1.023	0.597	−0.068	−0.141	0.062
4	PES	Dextran	1.275	0.995	0.596	1.163	0.997	0.681	0.113	−0.002	−0.084
5	PEG	Ucon	1.035	0.628	0.757	1.158	0.766	0.697	−0.123	−0.138	0.060
6	Ficoll	PEG	1.116	0.999	0.612	1.167	0.976	0.634	−0.051	0.023	−0.022
7	Ficoll	Ucon	1.146	0.850	0.667	1.031	1.063	0.644	0.115	−0.213	0.023
8	PES	Ficoll	1.137	1.050	0.668	1.196	0.996	0.628	−0.059	0.054	0.040
9	PES	PEG	1.116	1.032	0.595	1.175	0.962	0.697	−0.059	0.069	−0.102
10	PES	Ucon	1.133	0.640	0.904	1.209	0.634	0.962	−0.076	−0.059	0.006

[a] All differences are calculated between values measured in the top phases and those measured in the bottom phases.
[b] Polymer 1 – predominant polymer in the bottom phase; Polymer 2 – predominant polymer in the top phase (polymer composition of each phase – see Table 1).

tibility of the compound partition coefficient to the electrostatic ion–ion, ion–dipole, and dipole–dipole interactions with aqueous media, and all the other terms are as defined above.

Analysis of the distribution coefficients for all the proteins presented in Table 2 in terms of parameters $\Delta\pi^*$, $\Delta\alpha$ and $\Delta\beta$ (Table 3) and parameter c (Table 1), shows an existence of the linear correlations for all the proteins according to Eq. (6). The regression coefficients s_s, a_s, b_s and c_s, which were determined by multiple linear regression of the solvent parameters in all the ATPS on the logarithm of the protein partition coefficients, are presented in Table 4. The regression coefficients represent the relative susceptibilities of the compound partition coefficient to the corresponding solvent properties. Near perfect agreement between the distribution coefficients directly measured and those *K*-values calculated using Eq. (6) is shown in Fig. 1. These results imply that (a) partition behavior of proteins as well as small organic solutes in two-polymer ATPS is governed by the interactions of the solutes with the aqueous media in the coexisting phases; and (b) the approach presented here may be used for development of a general model to describe and predict the partition of solutes in ATPS.

The regression coefficients presented in Table 4 indicate that the contribution of the solvent hydrogen bond donor acidity into protein–aqueous media interactions significantly exceeds that of the solvent hydrogen bond acceptor basicity for essentially all the proteins examined. Similar tendency appears to hold for non-ionic organic solutes examined previously [17]. The regression coefficients for these solutes are given in Table 4 for comparison. Contribution of the dipole–dipole and induced dipole–dipole interactions of proteins with aqueous media into protein–solvent interactions appears to exceed those from the hydrogen bond interactions. Analysis of the regression coefficients presented in Table 4 indicates that they are interrelated as:

for proteins

$$s_s = -0.1_{\pm 0.2} + 0.62_{\pm 0.04} b_s + 0.86_{\pm 0.09} a_s \quad (7a)$$

$$N = 13; \quad r^2 = 0.9968; \quad SD = 0.4; \quad F = 1569.7$$

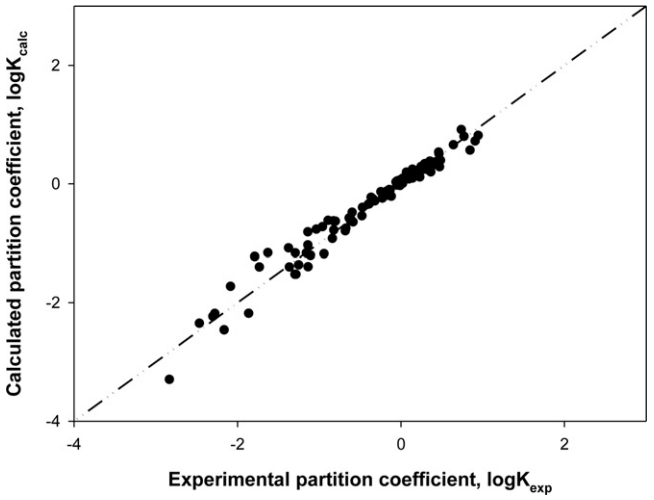

Fig. 1. Distribution coefficients, *K*, experimentally measured for proteins (see Table 2) in all different ATPSs (Table 1) plotted against *K*-values calculated from Eq. (6) using measured solvatochromic solvent properties of the coexisting phases (Table 3), electrostatic properties (parameter *c* in Table 1) and regression coefficients (presented in Table 4).

Table 4
Regression coefficients in Eq. (6) $\log K_s = z_0 + s_s\,\Delta\pi^* + a_s\,\Delta\alpha + b_s\,\Delta\beta + c_s c$ for indicated compounds.

Solute	z_0	s_s	a_s	b_s	c_s	N	F	SD	r^2	Outlier[a]
RNAse A	0.07 ± 0.03	2.7 ± 0.4	3.9 ± 0.3	0.3 ± 0.6	3.1 ± 0.4	8	58	0.05	0.987	PES–Ucon
RNAse B	0.08 ± 0.06	2.8 ± 0.7	4.0 ± 0.7	0.2 ± 1.1	2.9 ± 0.8	8	15	0.1	0.953	PES–Ucon
Chymotrypsinogen	0.27 ± 0.03	−2.3 ± 0.4	0.3 ± 0.4	−2.2 ± 0.6	1.2 ± 0.4	8	23	0.06	0.969	Ficoll–PEG
Trypsinogen	0.16 ± 0.06	−0.08 ± 0.7	1.9 ± 0.6	−1.0 ± 1.1	2.7 ± 0.7	8	9	0.1	0.920	Ficoll–PEG
Lysozyme	0.07 ± 0.05	−4.0 ± 0.5	−2.6 ± 0.5	−3.1 ± 0.8	2.7 ± 0.5	9	22	0.005	0.957	–
Hemoglobine bovine	0.2 ± 0.2	8.5 ± 2.2	9.6 ± 2.0	3.0 ± 3.3	7.6 ± 2.3	8	9	0.3	0.927	PES–Ucon
Hemoglobine human	0.2 ± 0.2	6.3 ± 1.9	7.8 ± 1.8	2.0 ± 2.9	7.1 ± 2.0	8	8	0.3	0.917	PES–Ucon
Lactoglobulin	0.1 ± 0.1	13.6 ± 1.7	14.2 ± 1.6	5.9 ± 2.2	3.7 ± 1.5	7	24	0.2	0.980	Dex–Ucon; PES–Ucon
Transferrin	0.1 ± 0.3	16.7 ± 3.3	18.7 ± 3.1	5.6 ± 5.0	10.5 ± 3.6	8	15	0.5	0.952	PES–Ucon
Myoglobin	0.01 ± 0.09	6.4 ± 1.0	6.8 ± 1.0	2.6 ± 1.6	3.0 ± 1.1	8	20	0.1	0.963	PES–Ucon
Lipase	0.01 ± 0.01	1.8 ± 0.2	1.8 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	7	32	0.02	0.985	Dex–Ucon; PES–Ucon
γ-Globulin human	0.6 ± 0.3	11.2 ± 3.4	15.3 ± 3.1	1.6 ± 5.2	17.2 ± 3.6	8	12	0.5	0.941	PES–Ucon
γ-Globulin bovine	0.6 ± 0.3	13.2 ± 3.0	17.3 ± 2.8	3.9 ± 4.6	15.3 ± 3.4	7	17	0.4	0.971	Ficoll–PEG; PES–Ucon
Benzyl alcohol	0.14 ± 0.03	−0.8 ± 0.3	−0.3 ± 0.3	1.9 ± 0.5		9	31	0.05	0.949	–
3-Hydroxybenzaldehyde	0.00 ± 0.01	−2.5 ± 0.1	−2.9 ± 0.1	−0.8 ± 0.2		8	369	0.02	0.996	PES–Dex
Nitrobenzene	−0.18 ± 0.02	−1.7 ± 0.3	−2.3 ± 0.3	−0.2 ± 0.4		8	59	0.04	0.978	PEG–Ucon
Phenol	0.03 ± 0.01	−2.2 ± 0.2	−2.5 ± 0.2	−0.8 ± 0.3		9	142	0.03	0.988	–
4-Hydroxyacetanilide	0.02 ± 0.02	−2.9 ± 0.3	−3.1 ± 0.3	−1.2 ± 0.4		8	70	0.04	0.981	PEG–Ucon
Caffeine	−0.007 ± 0.003	−0.65 ± 0.04	−0.87 ± 0.04	−0.06 ± 0.07		9	381	0.007	0.996	–
p-NP-β-D-fucopyranoside	−0.02 ± 0.03	−1.6 ± 0.3	−1.7 ± 0.3	−0.4 ± 0.6		8	20	0.06	0.938	Dex–Ucon
p-NP-β-D-galactopyranoside	−0.03 ± 0.04	−1.5 ± 0.4	−1.6 ± 0.4	−0.2 ± 0.7		8	13	0.07	0.904	Dex–Ucon
p-NP-β-D-glucopyranoside	−0.02 ± 0.03	−1.5 ± 0.3	−1.6 ± 0.3	−0.3 ± 0.5		8	19	0.05	0.934	Dex–Ucon
p-NP-α-D-glucopyranoside	−0.02 ± 0.03	−1.4 ± 0.3	−1.6 ± 0.3	−0.2 ± 0.5		8	22	0.05	0.943	Dex–Ucon
p-NP-α-D-mannopyranoside	−0.05 ± 0.04	−1.8 ± 0.4	−2.1 ± 0.4	−0.3 ± 0.7		8	17	0.07	0.926	Dex–Ucon

[a] Outlier – ATPS in which K-value for a given solute does not fit Eq. (6).

and for small organic neutral compounds

$$s_s = 0.0_{\pm 0.2} + 0.9_{\pm 0.1} b_s - 0.2_{\pm 0.1} a_s \quad (7b)$$

$$N = 11; \quad r^2 = 0.9331; \quad \mathrm{SD} = 0.2; \quad F = 55.8$$

where parameters s_s, a_s, and b_s are as defined above; N is the number of solutes; all the other parameters as defined above.

It is important to note that solutes examined in ATPSs and characterized by coefficients s_s, a_s, and b_s include different proteins as well as glycopyranosides and other different nonionic compounds, such as benzyl alcohol and caffeine (see Table 4). The correlation described by Eq. (7), and illustrated graphically in Fig. 2, is likely due to the fact that distribution of the solutes was examined between coexisting phases of ATPS, i.e. phases of different compositions and different solvent properties but of the same aqueous nature. The one particular feature of aqueous media is the cooperative effect in hydrogen bonding in water [37–40]. Therefore it seems reasonable that the relative solute susceptibility to the solvent dipolarity/polarizability in aqueous media would be correlated with the relative solute susceptibilities to the solvent hydrogen bond acidity and hydrogen-bond basicity of the media.

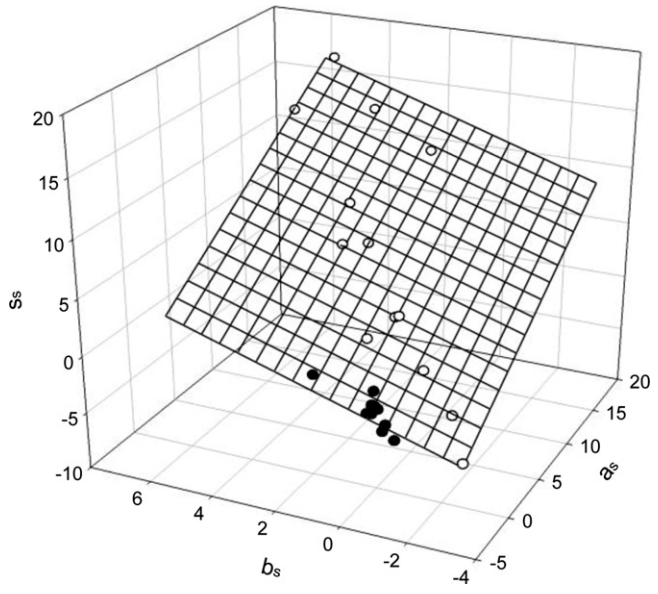

Fig. 2. Relationship between the relative susceptibility of the solute to the dipolarity/polarizability of the solvent and its susceptibilities to hydrogen bond acidity and hydrogen bond basicity of the solvent (unfilled symbols – proteins; filled symbols – nonionic organic compounds). It is also graphically shown the correlation for proteins.

5. Conclusions

Distribution coefficients of various proteins were measured in aqueous Dextran–Ficoll, Dextran–PES, and Ficoll–PES two-phase systems, containing 0.15 M NaCl in 0.01 M phosphate buffer, pH 7.4. The acquired data were combined with data reported by us previously and used to explore the possibility to use the LFER equation to correlate the partitioning data. This equation includes the relative susceptibilities of solutes to solvent dipolarity/polarizability, solvent hydrogen bond acidity, solvent hydrogen bond basicity, and solvent ability to participate in ion–ion and ion–dipole interactions adequately described the protein partitioning in each aqueous two-phase system used. It was found that the relative susceptibilities of proteins to solvent dipolarity/polarizability are interrelated with their relative susceptibilities to solvent hydrogen bond acidity and solvent hydrogen bond basicity similarly to those found for small nonionic organic compounds.

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