MEASUREMENT OF RELATIVE HYDROPHOBICITY OF AMINO ACID SIDE-CHAINS BY PARTITION IN AN AQUEOUS TWO-PHASE POLYMERIC SYSTEM: HYDROPHOBICITY SCALE FOR NON-POLAR AND IONOGENIC SIDE-CHAINS

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SUMMARY

The effect of ionic strength on the partition of twenty dinitrophenylated amino acids in buffered ficoll–dextran phase system was examined. The relative hydrophobicities of the amino acid side-chains were estimated. The results obtained for apolar side-chains are in agreement with those reported in the literature. It is shown that the relative hydrophobicity of any molecular fragment should be expressed in terms of equivalent number of CH_2 groups and not in terms of the free energy of transfer of a given moiety from one phase to the other of the phase system used in a particular study. A new hydrophobicity scale for apolar and ionogenic side-chains is established and it is shown that this scale depends on the ionic strength and/or ionic composition of the medium.

INTRODUCTION

Proteins are amphiphilic molecules that contain amino acids with hydrophobic side-chains and those with ionic and uncharged polar side-chains. The ratio of hydrophilic to hydrophobic amino acid residues is believed to be an important factor for the tertiary protein structure and to serve as an index of the protein localization and function *in vivo*[1,2]. Several attempts at deriving scales for amino acid hydrophobicity have been reported[3–6]. Nozaki and Tanford[3] measured the solubilities of different amino acids in water and in progressively increasing concentrations of organic solvents, such as ethanol and dioxan in water. The solubilities of the amino acids were extrapolated to pure organic solvents and the free energy of transfer for the amino acid from pure organic solvent to water was calculated. Using glycine as a reference, and subtracting its free energy of transfer from that of all the other amino acids, it was possible to formulate a hydrophobicity scale for amino acid residues with apolar side-chains[3]. Similar data were obtained by Fendler *et al.*[4] using hexane as the organic solvent. Bull and Breese[5] studied the effect of amino acids on the surface tension of water and constructed a hydrophobicity scale comparable to that of Nozaki and

Tanford[3]. Nandi[6] reported the partition coefficients of N-acetyl ethyl esters of a number of amino acids between water and different organic solvents.

The principal shortcoming of all of these approaches[3–6] is that they cannot be applied to molecular moieties containing polar ionogenic groups. The only promising means for the study of the relative hydrophobicities of polar and ionogenic compounds at present appears to be the technique based on the partition of solutes in an aqueous two-phase ficoll–dextran system[7]. This approach has been used earlier to measure the relative hydrophobicities of proteins[8] and cells[9].

The partition in aqueous two-phase polymeric systems technique was used in this study in order to measure the relative hydrophobicities of various polar and apolar amino acid side-chains. The results are compared with those reported in the literature[3–6] and it is shown that the hydrophobic characther of a given molecular moiety should be expressed in terms of equivalent number of CH_2 groups. A new combined hydrophobicity scale for hydrophilic and hydrophobic amino acid side-chains is formulated.

EXPERIMENTAL

Materials

All chemicals were of analytical-reagent grade unless indicated otherwise.

Ficoll-400 (lot 11069) was obtained from Pharmacia (Sweden). Dextran (M_w *ca.* 70,000) was obtained from Minmedprom (U.S.S.R.) under the trade-name Polyglucinum (lot 580870).

Dinitrophenylated amino acids (DNP-L-Ala, DNP-L-Arg, DNP-L-Asp, DNP-DL-Glu, DNP-Gly, DNP-L-Ile, DNP-DL-Leu, DNP-L-Phe, DNP-L-Pro, DNP-L-Ser, DNP-L-Thr, DNP-L-Trp and DNP-L-Val) were obtained from Serva (G.F.R.) and DNP-L-Asn, DNP-L-Gln, DNP-DL-Met and mono-O-DNP-L-Tyr from Reanal (Hungary). α-DNP-L-Lys was kindly provided by Dr. S. M. Andreev.

2,4-Dinitrofluorobenzene was obtained from Calbiochem-Behring Corp. (U.S.A.). L-Norleucine was obtained from Reanal, DL-norvaline from Chemapol (Czechoslovakia) and DL-2-amino-*n*-octoic acid from BDH (Great Britain). The amino acids were dinitrophenylated as described in ref. 10. All DNP derivatives of amino acids were checked for purity by thin-layer chromatography and their sodium salts were prepared by titration. The following abbreviations are used: norvaline = NVal; norleucine = NLeu; 2-amino-*n*-octoic acid = NAO.

Methods

Buffered ficoll–dextran aqueous two-phase systems were prepared as described previously[7–9]. All of the phase systems used had the same polymer composition [12.5% (w/w) ficoll and 10.8% (w/w) dextran], but differed in salt composition as indicated in the caption to Fig. 1.

The partition experiments were carried out as described elsewhere[7–9]. The phases were allowed to settle at room temperature for 23–24 h, then aliquots of both phases (0.1–0.15 ml) were carefully pipetted from the phase system and each was diluted by addition of an appropriate volume of water. The absorbance of each diluted aliquot was measured at 360 nm against a correspondingly diluted top or bottom phase blank.

The partition coefficient, K, is defined as the ratio of sample concentration (or absorbance) in ficoll-rich (bottom) phase to sample concentration in the dextran-rich (top) phase.

The partition coefficient for each solute was determined at four or five different ionic strengths as the mean of two measurements on two or more dilutions from each partition carried out two to four times at a given ionic strength. The deviation from the average K value did not exceed 3 % for any of the substances studied.

RESULTS

Fig. 1 shows the relationships between the logarithm of the partition coefficient and the ionic strength of the system for some of the compounds studied. It has been shown earlier[7,8] that these relationships can be described by the equation

$$\ln K = A + BI$$

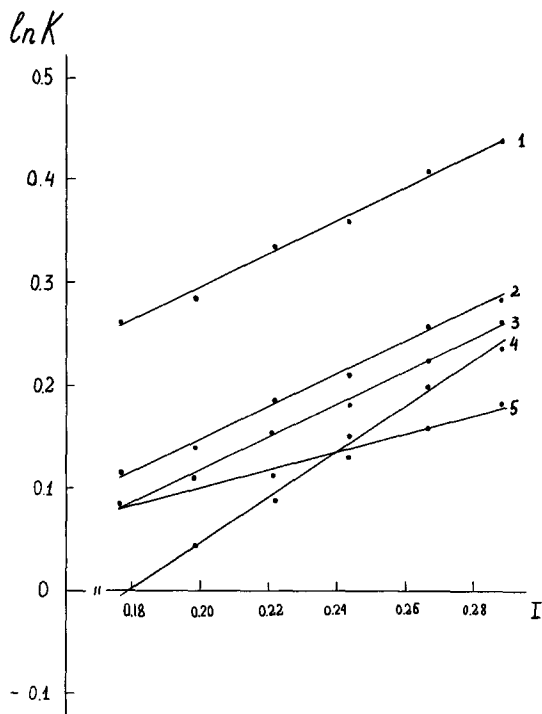where I is the ionic strength and A and B are constants.

Fig. 1. Logarithm of the partition coefficient (K) as a function of ionic strength (I) in the ficoll–dextran phase system. Polymer composition of the system: 12.5 % (w/w) ficoll, 10.8 % (w/w) dextran. Amounts of sodium chloride and sodium phosphate buffer (pH 7.4) in the systems can be calculated from the equations $C_{NaCl} = (0.288 - I)/0.75$ and $C_{buffer} = 0.11 - 0.67 \cdot C_{NaCl}$ where C_{NaCl} and C_{buffer} are the sodium chloride and sodium phosphate buffer concentrations, respectively, and I is the ionic strength in the phase system. 1 = DNP-2-amino-n-octoic acid; 2 = DNP-glutamine; 3 = DNP-serine; 4 = DNP-glutamic acid; 5 = α-DNP-lysine.

The physical meaning of A and B was discussed earlier[7–9] and it has shown that B reflects the effect of the ionic strength and/or ionic composition on the transfer of a given compound ionogenic group between the system phases[11]. A represents the relative hydrophobicity of the substance under study at zero ionic strength in the medium[7–9,11].

A least-squares treatment of the experimental data according to the above equation led to the A and B values for the solutes listed in Table I. It should be noted that the B value for all of the DNP-amino acids with apolar side-chains was found to be the same within experimental error and it was averaged as indicated in Table I.

TABLE I

CHARACTERISTICS OF THE PARTITION BEHAVIOUR OF DINITROPHENYLATED AMINO ACIDS IN FICOLL–DEXTRAN PHASE SYSTEM

Logarithm of the partition coefficient (K) depends on the ionic strength of the system (I) according to the equation $\ln K = A + BI$ (for details see text). the correlation coefficient exceeded 0.993 for all of the substances examined.

α-DNP-derivative	A★	B (kg/mole)★★
Gly	−0.190	1.624 ± 0.010
Ala	−0.152	1.624 ± 0.010
Val	−0.125	1.624 ± 0.010
Leu	−0.102	1.624 ± 0.010
Ile	−0.086	1.624 ± 0.010
Phe	0.001	1.624 ± 0.010
Thr	−0.170	1.624 ± 0.010
Ser	−0.203	1.624 ± 0.010
Pro	−0.181	1.624 ± 0.010
Met	−0.107	1.624 ± 0.010
Gln	−0.181	1.624 ± 0.010
Asn	−0.203	1.624 ± 0.010
NVal	−0.107	1.624 ± 0.010
NLeu	−0.087	1.624 ± 0.010
NAO	−0.019	1.624 ± 0.010
Trp	0.275	1.624 ± 0.010
Glu·	−0.411	2.277 ± 0.116
Asp	−0.355	1.866 ± 0.061
Arg	−0.089	1.267 ± 0.097
Lys	−0.062	0.829 ± 0.040

★ Mean values; standard deviation 0.027 in all instances.
★★ Mean values ± standard deviation.

In order to calculate the difference in the relative hydrophobicities between the two phases of the system used, the effect of a CH_2 group on the $\ln K$ value was established by comparison of the A values for the DNP derivatives of Gly, Ala, NVal, NLeu and NAO. The A values are plotted in Fig. 2 as a function of the number of carbon atoms in the aliphatic side-chain. The slope of the observed linear relationship is a measure of the effect of a CH_2 group on the $\ln K$ value. It can be seen from Fig. 2 that the effect of adding an aliphatic CH_2 group to the side-chain increases the $\ln K$ value by 0.027 logarithmic units.
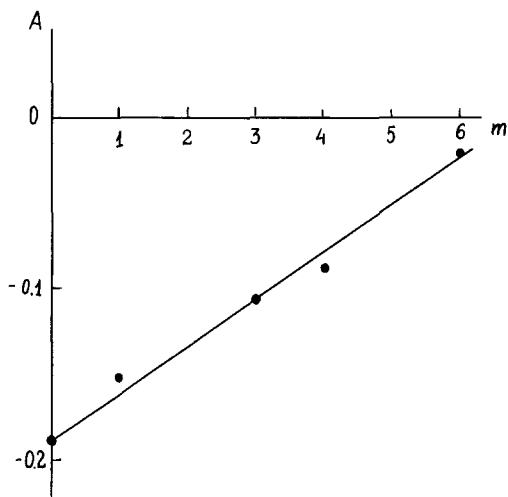
Fig. 2. A as a function of the aliphatic side-chain length (m) of the dinitrophenylated amino acids: glycine, alanine, norvaline, norleucine and 2-amino-n-octoic acid.

DISCUSSION

The unitary free energy of transfer, ΔG_{tr}, of a solute from one to the other phase of a given phase system is related to the partition coefficient, K, by the equation $\Delta G_{tr} = RT \ln K$. Contributions of amino acid side-chains to the unitary free energies of transfer (Δg_{tr}) can be calculated by assuming additivity of the free energy of solvent interactions and using glycine as a reference, as described by Nozaki and Tanford[3].

The range of values of the contributions for the apolar amino acid side-chains as reported in the literature 3–6 is approximately 1000–2500 cal/mole. It can be seen from the data in Table I, however, that the free energy of transfer of the Ile side-chain does not exceed 70 cal/mole, compared with 2300–2900 cal/mole as found by Nozaki and Tanford and other workers[3-6]. The only reasonable explanation of the observed disagreement seems to be related to the fact that the free energy of transfer of a CH_2 group depends on the phase system used in a given study[12]. In order to take account of the probable effect of this dependence we have attempted to estimate the relative hydrophobicity of the amino acid side-chains not in terms of Δg_{tr} but in terms of the equivalent number of CH_2 groups, n, as proposed earlier[7-9,11]. The number n is obviously related to the Δg_{tr} value according to equation

$$n_i = \Delta g_{tr} / \Delta g_{tr_{CH_2}}$$

where $\Delta g_{tr_{CH_2}}$ is the free energy of transfer of a CH_2 group in a given phase system and i denotes the amino acid in question. A positive value of n means that the relative hydrophobicity of a given moiety is equal to that produced by n CH_2 groups, and a negative value of n means that the moiety is hydrophilic and its hydrophobicity is the reverse of that produced by n CH_2 groups. The n values calculated from the data in Table I are presented in Table II together with those calculated from the literature data[3-6]. It can be seen that the data are in good agreement. The only exception

TABLE II

RELATIVE HYDROPHOBICITIES OF AMINO ACID SIDE-CHAINS EXPRESSED IN TERMS OF EQUIVALENT NUMBER OF CH$_2$ GROUPS AT ZERO IONIC STRENGTH IN THE MEDIUM

Side-chain of	Relative hydrophobicity ($n = \Delta g_{tr_i} / \Delta g_{tr_{CH_2}}$)									
	Ficoll→ dextran[★]	Ethanol, dioxane→ water[★★]	Hexane→ water[★★★]	Water surface→ bulk water[§]	Isoamyl alcohol→ water[§§]	Hexanol→ water[§§]	Octanol→ water[§§]	CHCl$_3$→ water[§§]	CCl$_4$→ water[§§]	Dibutyl ether→ water[§§]
Trp	17.22[§§§§]	5.77								
Phe	7.06[§§§§]	4.24	3.60	3.64						
NAO	6.33									
Lys	4.74									
NLeu	3.82	4.41	3.90	3.84	4.01	3.98	3.96		4.04	3.89
Leu	3.30	3.06	3.83	3.53	3.61	3.69	4.20		3.92	3.91
Ile	3.85									
Arg	3.74									
Met	3.07	2.21								
NVal	3.07				2.75	2.87	2.89	3.01	2.99	3.23
Val	2.41	2.55	2.43	2.44	2.49	2.66	3.04	3.09	3.00	3.30
Ala	1.41	0.85	−0.61	0.31	0.77	0.81	0.81	1.02	1.07	1.02
Thr	0.74	0.68	−0.06							
Pro	0.33		0.36	1.53						
Gln	0.33									
Gly	0	0	0	0	0	0	0	0	0	0
Ser	−0.48	−0.51	−1.11	−0.61						
Asn	−0.48									
Asp	−6.11									
Glu	−8.19									

★ This work, $\Delta g_{tr_{CH_2}} = 16 \pm 1$ cal/mole.

★★ The data published in ref. 3 were recalculated as indicated in the text using $\Delta g_{tr_{CH_2}} = 589 \pm 83$ cal/mole, as found from the results reported in ref. 3 for Gly, Ala, NLeu, Val, Leu and Thr.

★★★ The data were taken from ref. 4 and recalculated as above using $\Delta g_{tr_{CH_2}} = 692 \pm 60$ cal/mole, as found from the data in ref. 4 for Gly, Val, Leu and Phe.

§ The data were taken from ref. 5 and recalculated as above using $\Delta g_{tr_{CH_2}} = 640 \pm 50$ cal/mole, as found from the results reported in ref. 5 for Gly, Val, Leu and Phe.

§§ The data were taken from ref. 6 and recalculated as above using the following $\Delta g_{tr_{CH_2}}$ obtained from the results given in ref. 6 for the derivatives of Gly, Ala, NVal and NLeu: water–isoamyl alcohol, −573 ± 30; water–hexanol, −587 ± 24; water–octanol, −602 ± 20; water–CHCl$_3$, −731 ± 17; water–CCl$_4$, −784 ± 8; water–dibutyl ether, −631 ± 34 cal/mole.

§§§§ The n values obtained in this work for the Trp and Phe side-chains seem to be incorrect (for explanation, see text).

appears to be the n-values obtained by us for the Trp and Phe derivatives. The observed overestimated relative hydrophobicity of these amino acid side-chains can be attributed to the probable effect of the dinitrophenyl moiety on interactions of these side-chains with water.

Our data make it possible to evaluate the relative hydrophobicity of the ε-amino group in lysine by comparison of the n value for DNP-NLeu with that for α-DNP-Lys. It appears that the relative hydrophobicity of the ε-amino group at zero ionic strength in the medium corresponds to that produced by 0.93 CH_2 groups. The hydrophobic character of the side-chain amide group under the same conditions corresponds to -1.5 CH_2 groups and that of the side-chain carboxyl group to -6.6 CH_2 groups. The relative hydrophobicity of an aliphatic OH group can be estimated by comparison of the n values for DNP-Ser and DNP-Thr with that for DNP-Ala, and it appears to correspond to $ca.$ -1.44 CH_2 groups, compared with -1.19 CH_2 groups established by Nozaki and Tanford[3]. Unlike their results[3], however, our data indicate that the methionyl sulphur atom has no effect on the hydrophobic character of the side-chain, within experimental error.

The data on the relative hydrophobicities of the amino acid side-chains listed in Table II represent a hydrophobicity scale for the side-chains in the absence of salts in the medium. The data given in Table I, however, indicate that in contrast to the apolar side-chains, the hydrophobic character of which similarly depends on the ionic strength and/or ionic composition of the medium (the B values are equal), the relative hydrophobicities of the ionogenic side-chains depend on the ionic strength differently. In particular, the ln K value for α-DNP-Lys does not depend on the ionic strength as greatly as that for DNP-Arg or DNP-Gly. It can be seen from the B values in Table I that the relative hydrophobicity of the side-chain carboxyl group depends on the ionic strength differently to that of the α-carboxyl group. The effect of the ionic strength and/or ionic composition of the medium seems to be related to the position of a carboxyl group in the side-chain. Therefore, the hydrophobicity scale for amino acid side-chains depends on the ionic composition and ionic strength of the medium. In particular, in the presence of 0.15 M sodium chloride in 0.01 M sodium phosphate buffer (pH 7.4), the relative hydrophobicities of the side-chains of Lys, Arg, Asp and Glu correspond to -0.45, 1.41, -4.53 and -3.92 CH_2 groups, respectively.

It seems that the effect of the ionic strength and/or ionic composition of the medium on the relative hydrophobicity of amino acid side-chains may be of fundamental importance as a factor affecting protein conformation and function *in vivo*.

REFERENCES*

1 C. Tanford, *Science*, 200 (1978) 1012–1018.
2 J.-L. Ochoa, *Biochemie*, 60 (1978) 1–15.
3 Y. Nozaki and C. Tanford, *J. Biol. Chem.*, 246 (1971) 2211–2217.
4 J. H. Fendler, F. Nome and J. Nagyvary, *J. Mol. Evol.*, 6 (1975) 215–232.
5 H. B. Bull and K. Breese, *Arch. Biochem. Biophys.*, 161 (1974) 665–670.
6 P. K. Nandi, *Int. J. Peptide Protein Res.*, 8 (1976) 253–264.
7 B. Yu. Zaslavsky, L. M. Miheeva, N. M. Mestechkina, V. M. Pogorelov and S. V. Rogozhin, *FEBS Lett.*, 94 (1978) 77–80.

* *Editor's note:* see also V. Pliška, M. Schmidt and J. L. Fauchère, *J. Chromatogr.*, 216 (1981) 79.

8 B. Yu. Zaslavsky, N. M. Mestechkina and S. V. Rogozhin, *Biochim. Biophys. Acta*, 579 (1979) 463–465.

9 B. Yu. Zaslavsky, L. M. Miheeva and S. V. Rogozhin, *Biochim. Biophys. Acta*, 588 (1979) 89–101.

10 W. A. Schroeder and J. Le Gotte, *J. Amer. Chem. Soc.*, 75 (1953) 4612–4615.

11 B. Yu. Zaslavsky, L. M. Miheeva, S. V. Rogozhin, L. V. Borsova and G. I. Kosinez, *Biochim. Biophys. Acta*, 597 (1980) 53–63.

12 J. Gelles and M. H. Klapper, *Biochim. Biophys. Acta*, 533 (1978) 465–477.