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Peptides partitioning in an aqueous dextran–polyethylene glycol two-phase system

Alexander Zaslavsky, Nellie Gulyaeva, Boris Zaslavsky*

Analiza, Inc., 4440 Warrensville Center Road, Cleveland, OH 44128, USA

Abstract

Partitioning of glycine, lysine and aspartic acid and their oligopeptides in an aqueous dextran–polyethylene glycol two-phase system containing 0.15 *M* NaCl in 0.01 sodium phosphate buffer, pH 7.3 and 0.11 *M* sodium phosphate buffer, pH 7.3 was examined. Relative hydrophobicity of the amino acid residues and peptide bonds was estimated and expressed in equivalent numbers of methylene units. Analysis of a series of reversed di- and tripeptides in terms of relative hydrophobicity showed that the additivity principle does hold for the hydrophobicity of short peptides. The relative hydrophobicity of peptides is affected by the ionic composition of aqueous media as well as by the type of amino acid residues forming peptide bonds in a given peptide sequence.

Keywords: Partitioning; Aqueous two-phase systems; Peptides

1. Introduction

Understanding the relationships between the chemical structure of peptides and proteins and their physical and biological properties is important for biochemistry, pharmacology and medicine.

Analysis of quantitative structure–activity relationships (QSARs) is used in pharmacology for rational drug design. It is based on the assumption [1] that the chemical structure of a drug may be described quantitatively in terms of its physicochemical properties. It is also assumed [1–3] that physicochemical characteristics of the drug may be used as the indices of its relative biological potency and/or its interaction with the particular (even unknown) component of the biological system. One of the most important characteristics of a molecule is purported to be [1] its hydrophobicity. Hydrophobicity of a molecule represents the intensity of the molecule's interactions with an aqueous medium. It appears to affect distribution of a compound throughout the body organs and tissues and the interactions of the compound with its target (receptor) in the biological system.

Hydrophobicity is one of the leading factors in QSAR analysis for common drugs, i.e., it is related to biological activity and/or function of a solute. Any change in the molecule's conformation that affects the groups accessible to the solvent would affect the molecule's hydrophobicity. Therefore, hydrophobicity may be viewed as a factor representing its functionally active conformation. As suggested by Tanford [4], the distribution of a biological solute throughout bodily fluids and tissues is governed by the difference between the solute–solvent interactions in various phases or places in the body.

*Corresponding author. Tel.: +1-216-5864-595; fax: +1-216-5863-886.

E-mail address: bz@analiza.com (B. Zaslavsky)

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Finally, hydrophobicity is a very general descriptor of a structure that allows one to compare biological compounds with totally different structures and functions (peptides, proteins, RNA, DNA, etc.) on the same universal scale. Measurements of hydrophobicity of biological solutes, however, represent a very serious challenge.

Hydrophobic character of a typical organic solute is commonly measured by the partition coefficient of the solute in an octanol–water two-phase system, P_{ow}, (or by the more frequently used form, log P_{ow}). This measure is widely used as a structure descriptor for QSAR analysis in drug design [1]. It cannot be applied to labile biological solutes, however, due to their liability to denaturation or conformational changes induced by octanol or other organic solvents. The only experimental technique allowing one to quantify the relative hydrophobicity of biological solutes currently appears to be aqueous two-phase partitioning [5,6].

Aqueous two-phase systems arise in aqueous mixtures of different water-soluble polymers or a single polymer and a specific salt. When two certain polymers, e.g., dextran (Dex) and polyethylene glycol (PEG), are mixed in water above certain concentrations, the mixture separates into two immiscible aqueous phases. One phase is rich in one polymer and the other phase is rich in the other polymer. The aqueous solvent in both phases provides media suitable for biological products.

When a solute is introduced into such a system, it distributes between the two phases. Partitioning of a solute is characterized by the partition coefficient K defined as the ratio between the concentrations of the solute in the two phases. It was previously shown [5] that phase separation in aqueous polymer systems results from different effects of two polymers (or a single polymer and a salt) on the water structure. As a result, the solvent features of aqueous media in the coexisting phases are different as established by dielectric, solvatochromic, potentiometric and partition measurements reviewed in Refs. [5,7].

The basic rules of solute partitioning in aqueous two-phase systems were shown [5,8] to be similar to those in water–organic solvent systems. The differences between the properties of the two phases in aqueous polymer systems are very small relative to those observed in water–organic solvent systems, as should be expected for a pair of solvents of the same (aqueous) nature. The small difference between the solvent features of the phases in aqueous two-phase systems actually enhances sensitivity of solute partitioning toward modifications in the solute structure.

Since log P is a Gibbs energy transfer function, it is an additive–constitutive function of molecular structure. This means that an introduction of a substituent group into an organic compound should change its hydrophobicity (log P) by a fixed increment specific to the substituent group. The fragmental constant method of Hansch and Leo [1] is based on the additivity principle. This method allows one to calculate and predict hydrophobicity values for compounds for which a partition coefficient cannot be readily determined experimentally. The opportunity to use the additivity principle dramatically increases efficiency of QSAR analysis and rational drug design. Therefore it is important to examine if the additivity principle is valid for peptide structures.

It has been shown previously that QSAR analysis may be applied to opioid peptides [9] using only relative hydrophobicity as a structural descriptor for peptides. The possibility of using QSARs for rational design of peptide structure with a required biological potency depends, however, on the applicability of the additivity principle. The goal of the present work was to explore whether the relative hydrophobicity of a peptide as measured by the aqueous two-phase partitioning technique may be viewed as an additive–constitutive property.

2. Materials and methods

2.1. Polymers

Dextran-80 (relative molecular mass ~78 000 by light scattering, lot 95H0023) and PEG with relative molecular mass about 8000 (PEG-8000, lot 85H0654) were purchased from Sigma, St. Louis, MO, USA.

2.2. Peptides, amino acids and reagents

Dinitrophenylated amino acids *N*-2,4-DNP-Gly, *N*-2,4-DNP-L-Ala, *N*-2,4-DNP-DL-norvaline, *N*-2,4-DNP-DL-norleucine and *N*-2,4-DNP-DL-α-amino-*n*-

caprylic acid, glycine, DL-aspartic acid and DL-lysine hydrochloride were obtained from Sigma. Glycylaspartic acid (Gly–Asp), aspartylglycine (Asp–Gly), lysylglycine (Lys–Gly), glycyllysine (Gly–Lys) and homooligopeptides ($n=2$ to 6) of glycine, aspartic acid and lysine were purchased from Sigma. Glycylglutamic acid (Gly–Glu), glutamylglycine (Glu–Gly) and tripeptides glycyllysylglycine (Gly–Lys–Gly), glycylglycyllysine (Gly–Gly–Lys) and lysylglycylglycine (Lys–Gly–Gly) were obtained from Bachem Bioscience (King of Prussia, PA, USA). *o*-Phthaldialdehyde (OPA) reagent solution (incomplete) and 2-mercaptoethanol were obtained from Sigma. The 96-well black microplates with clear bottoms manufactured by Corning were purchased from VWR Scientific Products (West Chester, PA, USA). All inorganic salts and reagents used were of analytical reagent grade.

2.3. Phase systems

A mixture of polymers was prepared by dispensing appropriate amounts of the aqueous stock ca. 35% (w/w) Dex-78 solution and 40.00% (w/w) PEG-8000 solution into 1.2-ml microtubes using a Hamilton MICROLAB 2200 single-probe robotic sample processor. Appropriate amounts of 0.44 *M* Na-phosphate buffer (PB), pH 7.3 or 0.6 *M* NaCl in 0.04 *M* Na-phosphate buffer, pH 7.3 were added so as to give the required ionic composition (ionic strength) and polymer composition of 6.00% (w/w) PEG-8000 and 12.18% (w/w) Dex-78. Aqueous polymer two-phase systems prepared had the same polymer composition and two different salt compositions of 0.15 *M* NaCl in 0.01 *M* Na-phosphate buffer, pH 7.3 and 0.11 *M* Na-phosphate buffer, pH 7.3.

2.4. Partition experiments

Acetate salts of peptides were prepared by titration with glacial acetic acid in certain cases indicated below. Sodium salts of DNP-amino acids were prepared by titration with NaOH. Solutions of amino acids and peptides (or their Na-salts or acetate salts) were prepared in water at the concentration of about 1 to 5 mg/ml. A varied amount (30, 60, 90, 150 and 200 μl) of a given peptide (amino acid) solution and the corresponding amount (170, 140, 110, 50 and 0 μl) of water were added to a polymer mixture. The system was vigorously vortex mixed and centrifuged for 30 min at approximately 3000 rpm (1160 *g*) to speed the phase settling. Aliquots of about 10 μl from the top and bottom phases were withdrawn in duplicate and combined with 300 μl of OPA reagent solution (complete) in microplate wells. OPA reagent solution (complete) was made by the addition of 0.2 ml of 2-mercaptoethanol to 100 ml of OPA reagent solution (incomplete) immediately prior to use. Samples were allowed to incubate for 2 min with moderate shaking at room temperature and the fluorescence was determined using a Bio-Tek FL500 fluorescence plate reader with a 360 nm, 40 nm bandwidth excitation filter and a 460 nm, 40 nm bandwidth emission filter, with the sensitivity setting varied from 38 to 60. In all instances the correspondingly diluted pure phases were used as blank solutions.

The partition coefficient, *K*, is defined as the ratio of the sample concentration (or fluorescence) in the PEG-rich (upper) phase to the sample concentration in the dextran-rich (bottom) phase. The *K* value for each solute was determined as the slope of the concentration in the upper 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 a given ionic composition of the system. The deviation from the average *K* value did not exceed 2% for any of the substances examined.

3. Results and discussion

3.1. Calibration of the aqueous dextran–PEG two-phase system

Sodium salts of DNP-derivatives of amino acids (Gly, Ala, nor-Val, nor-Leu and α-amino-*n*-caprylic acid) were partitioned in the system at two different salt compositions as described above. The results obtained are plotted in Fig. 1 as logarithms of the partition coefficient, *K*, values vs. length of the aliphatic side-chain expressed in terms of equivalent number of CH_2 groups (see below).

Partitioning of a homologous series of monofunc-

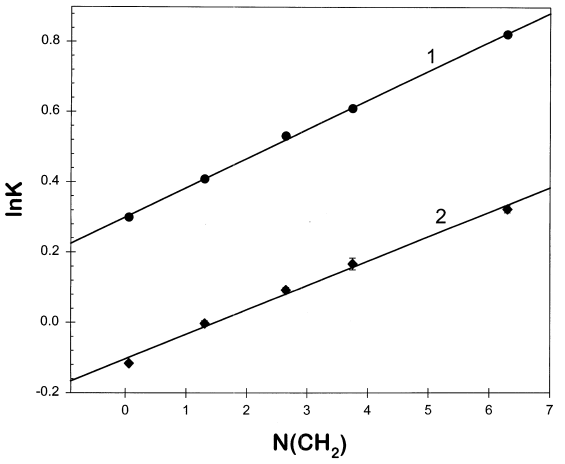

Fig. 1. Logarithm of the partition coefficient, ln *K*, value for sodium salts of DNP-amino acids (Gly, Ala, nor-Val, nor-Leu and α-amino-*n*-caprylic acid) in the aqueous Dex–PEG two-phase system as a function of the length of the aliphatic side-chain expressed in terms of equivalent number of CH_2 units. Salt composition of the Dex–PEG system: 1–0.11 *M* sodium phosphate buffer, pH 7.3; 2–0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.3.

tional aliphatic compounds in aqueous polymer two-phase systems is described as in [5]:

$$\ln K = A + E \cdot N_C \quad (1)$$

where K is the solute partition coefficient; N_C is the number of carbon atoms in the aliphatic alkyl chain of the partitioned solute molecule; A and E are constants. The coefficient E in Eq. (1) represents an average ln K increment per CH_2 group. Coefficient E amounts to 0.070 ± 0.003 in the system containing 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.3 and to 0.083 ± 0.002 in the system containing 0.11 *M* sodium phosphate buffer, pH 7.3.

The coefficient A represents the total contribution of a polar moiety of the solute molecule. In the present case coefficient A amounts to -0.103 ± 0.012 in the presence of 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.3, and to $+0.0300 \pm 0.005$ in the system containing 0.11 *M* sodium phosphate buffer, pH 7.3.

The coefficient E is related to the free energy of transfer of a CH_2 group from one to the other phase in a given two-phase system, $\Delta G(CH_2)$:

$$\Delta G(CH_2) = -RT \cdot E \quad (2)$$

It should be particularly noted that the methylene group increment, E, into the ln K value is independent of the nature of the aliphatic solutes being partitioned [5], and hence E or alternatively $\Delta G(CH_2)$ may be used as a measure of the difference between the affinities of the two phases for a CH_2 group, i.e., difference between the hydrophobic character of the two phases [5–7].

It has been demonstrated by various techniques (reviewed in Refs. [5,7]) that partitioning of a solute in an aqueous polymer two-phase system is governed by the difference between the intensity of the solute–solvent interactions in the two phases. Hence the partition coefficient of a solute in such a system represents the free energy of transfer of the solute between two aqueous media of different solvent properties and therefore may be used as a measure of the relative hydrophobicity of the solute [5,6].

The ratio expressed as

$$\Delta G(\text{solute})_{tr} / \Delta G(CH_2) = n(CH_2) \quad (3)$$

or

$$\ln K/E = n(CH_2) \quad (3a)$$

has been defined as the equivalent quantity of methylene units and suggested [5–7] to be used as a measure of the relative hydrophobicity of a solute (or a moiety). A positive value of $n(CH_2)$ means that a given solute is hydrophobic and its relative hydophobicity is equal to that of an n number of methylene units. A negative value of $n(CH_2)$ means that the solute is hydrophilic and its relative hydrophobicity is the reverse of that of an n number of CH_2 units.

3.2. Relative hydrophobicity of homooligopeptides

Partition coefficient values for homooligopeptides of aspartic acid, lysine and glycine in the form of acetate salts and as free peptides in the aqueous two-phase systems employed at two different ionic compositions are plotted in Figs. 2 and 3 vs. number of residues in the corresponding peptides. The relative hydrophobicity values $N(CH_2)$ for the peptides were found to be linear functions of the number of residues (over a range of $n = 1$ to 6) in the corresponding peptides described as:

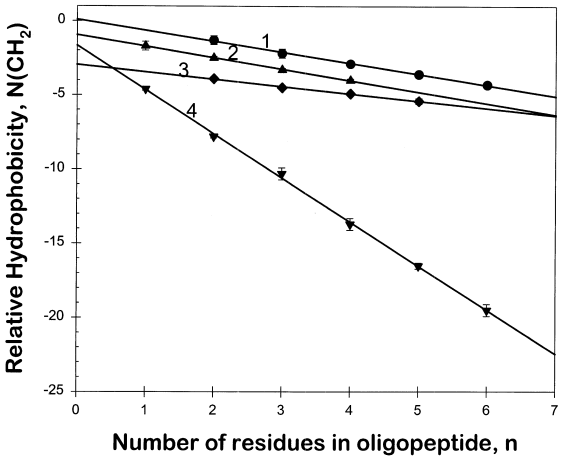

Fig. 2. Relative hydrophobicity of homooligopeptides $(Gly)_n$-acetate (1); $(Gly)_n$ (2); $(Lys)_n$ (3); and $(Asp)_n$ (4) in the presence of 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.3 as a function of the number of residues in the peptide.

$$N(CH_2)_{jn} = a_j + b_j \cdot n \quad (4)$$

where $N(CH_2)_{jn}$ is the relative hydrophobicity of a peptide with number n of amino acid residues of jth type; a_j is a constant; and b_j is the slope representing the total contribution of an nth residue and newly formed peptide bond into the relative hydrophobicity of a given type of peptide. The a_j and b_j values for the homooligopeptides examined here are given in Table 1.

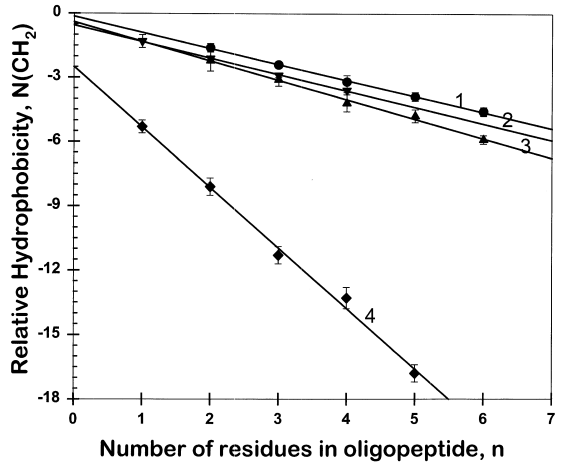

Fig. 3. Relative hydrophobicity of homooligopeptides $(Gly)_n$-acetate (1); $(Gly)_n$ (2); $(Asp)_n$ (3); and $(Lys)_n$ (4) in the presence of 0.11 *M* sodium phosphate buffer, pH 7.3 as a function of the number of residues in the peptide.

It follows from these data that, on average, an acetate salt of an oligo-glycine is more hydrophobic than its free form by 1.0 ± 0.4 equivalent CH_2 units in the presence of 0.15 *M* NaCl in 0.01 *M* Na-PB, pH 7.3, and by only 0.4 ± 0.1 equivalent CH_2 units in 0.11 *M* Na-PB, pH 7.3.

The contribution of a peptide bond formed by two identical amino acid residues to the relative hydrophobicity of a peptide may be estimated as the difference between the relative hydrophobicity of a n-oligo-peptide and the total relative hydrophobicity of a separate amino acid and $(n-1)$-oligo-peptide described as:

$$x_{j-j} = a_j + n \cdot b_j - [a_j + (n-1) \cdot b_j] - (a_j + b_j) = -a_j \quad (5)$$

The contributions of peptide bonds formed by different amino acid residues estimated with Eq. (5) are presented in Table 2.

The contribution of a peptide bond formed by two glycyl residues estimated with Eq. (5) amounts to $+0.9\pm0.3$ equivalent CH_2 units in the presence of 0.15 *M* NaCl in 0.01 *M* Na-PB, pH 7.3, and to $+0.6\pm0.1$ equivalent CH_2 units in 0.11 *M* Na-PB, pH 7.3, i.e., it seems to be independent of the ionic composition of the aqueous media over the range explored.

In contrast, the peptide bonds formed by addition of the charged residues Lys or Asp contribute differently to the hydrophobicity of the peptide depending on the composition of the buffer. The contribution of a peptide bond formed by two aspartyl residues estimated with Eq. (5) amounts to $+1.7\pm0.2$ equivalent CH_2 units in the presence of 0.15 *M* NaCl in 0.01 *M* Na-PB, pH 7.3, and to $+0.4\pm0.2$ equivalent CH_2 units in 0.11 *M* Na-PB, pH 7.3. The contribution of a peptide bond formed by two lysyl residues amounts to $+3.0\pm0.1$ equivalent CH_2 units in the presence of 0.15 *M* NaCl in 0.01 *M* Na-PB, pH 7.3, and to $+2.5\pm0.4$ equivalent CH_2 units in 0.11 *M* Na-PB, pH 7.3.

Thus, the results obtained here indicate that (a) the contribution of peptide bonds formed by different amino acid residues to the relative hydrophobicity of a peptide depends on the residues forming the bond,

Table 1
Values of constants a_j and b_j for the homooligopeptides studied

Amino acid	0.15 *M* NaCl in 0.01 *M* Na-PB, pH 7.3		0.11 *M* Na-PB, pH 7.3	
	a_j	b_j	a_j	b_j
Gly	−0.85±0.27	−0.75±0.09	−0.55±0.05	−0.77±0.02
Gly-acetate salt	0.10±0.10	−0.74±0.02	−0.14±0.06	−0.75±0.02
Asp	−1.67±0.17	−2.97±0.04	−0.40±0.17	−0.91±0.04
Lys	−2.96±0.10	−0.49±0.03	−2.50±0.38	−2.82±0.11
Asp side-chain	−0.82±0.44	−2.22±0.13	0.15±0.22	−0.16±0.06
β-COOH	−1.82±0.54	−3.22±0.23	−0.85±0.32	−1.16±0.16
Lys side-chain	−2.11±0.37	0.26±0.12	−1.95±0.43	−2.07±0.13
NH_2 group	−6.11±0.77	−3.74±0.52	−4.95±0.83	−6.07±0.44

(b) for the peptides examined this contribution is positive in all the cases, and (c) when the bond is formed by residues with an ionic side chain, its contribution may vary with the ionic composition of the aqueous media.

The relative hydrophobic character of the side-chain of aspartic acid can be estimated as the difference between the b_j values for Asp and Gly presented in Table 1. The relative hydrophobicity of the aspartyl side-chain clearly depends on the salt composition of the media and amounts to -2.2 ± 0.1 equivalent CH_2 units in the presence of 0.15 *M* NaCl in 0.01 *M* Na-PB, pH 7.3, and -0.2 ± 0.1 equivalent CH_2 units in the presence of 0.11 *M* Na-PB, pH 7.3. The effect of the salt composition and/or ionic strength is as might be expected, since an increase in the ionic strength of the media should hinder dissociation of the carboxylic hydrogen, and reduce its affinity for the aqueous media. The relative hydrophobicity of the lysine side-chain depends on the salt composition of the media also. It amounts to $+0.3\pm0.1$ equivalent CH_2 units in the presence of 0.15 *M* NaCl in 0.01 *M* Na-PB, pH 7.3, and -2.1 ± 0.1 equivalent CH_2 units in the presence of 0.11 *M* Na-PB, pH 7.3. That means that the relative hydrophobicity of NH_2 group, in contrast to that of carboxylic group, is increased in 0.15 *M* NaCl in 0.01 *M* Na-PB, pH 7.3.

The data obtained for homooligopeptides imply that the additivity principle can be applied to the relative hydrophobicity of these peptides. Additional experiments with hetero-di- and tripeptides were performed to verify this conclusion.

3.3. Relative hydrophobicity of di- and tripeptides

The partition coefficient values for the di- and tripeptides studied are given in Table 3. As an illustration of the approach used here, reversed dipeptides Gly–Lys and Lys–Gly, were subjected to partitioning at the two different salt compositions. For this discussion, data for the system containing 0.15 *M* NaCl in 0.01 *M* PB, pH 7.3 are presented, but the relationship also holds true when the buffer is 0.11 *M* PB, pH 7.3. It was found that the relative hydrophobicities of Gly–Lys and Lys–Gly are

Table 2
Contributions of different peptide bonds expressed in terms of the equivalent number of methylene units, $N(CH_2)$, to relative hydrophobicity of peptides at indicated salt composition

Bond between amino acid residues	$N(CH_2)$	
	0.15 *M* NaCl in 0.01 *M* PB, pH 7.3	0.11 *M* PB, pH 7.3
Gly and Gly	0.9±0.3	0.6±0.1
Lys and Lys	3.0±0.1	2.5±0.4
Asp and Asp	1.7±0.2	0.4±0.2
Gly and Lys	1.7±0.6	1.0±0.6
Gly and Asp	0.7±0.2	−0.5±0.7

Table 3
Partition coefficients for di- and tri-peptides in the aqueous Dex–PEG two-phase system at different salt compositions

Peptide	K^a	ln K^a	K^b	ln K^b
Gly–Lys	0.789±0.011	−0.237±0.014	0.627±0.013	−0.467±0.021
Lys–Gly	0.789±0.008	−0.237±0.010	0.629±0.003	−0.464±0.005
Gly–Asp	0.683±0.008	−0.381±0.012	0.771±0.005	−0.260±0.006
Asp–Gly	0.680±0.005	−0.386±0.007	0.778±0.014	−0.251±0.018
Glu–Gly	0.700±0.006	−0.357±0.009	0.813±0.001	−0.207±0.001
Gly–Glu	0.697±0.007	−0.360±0.010	0.820±0.003	−0.198±0.004
Gly–Gly–Lys	0.761±0.007	−0.273±0.009	0.612±0.010	−0.491±0.016
Lys–Gly–Gly	0.757±0.006	−0.278±0.008	0.615±0.005	−0.486±0.008
Gly–Lys–Gly	0.751±0.003	−0.286±0.004	0.614±0.007	−0.488±0.011

[a] 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.3.
[b] 0.11 *M* sodium phosphate buffer, pH 7.3.

identical and amount to -3.4 ± 0.1 equivalent CH_2 units. Extending the chain by addition of a glycine residue generates the tripeptides Gly–Gly–Lys, Lys–Gly–Gly and Gly–Lys–Gly. From Table 1, the newly added glycine residue contributes -0.75 ± 0.09 equivalent CH_2 units to the relative hydrophobicity of the peptide, making the calculated value -4.2 ± 0.2. To check if this estimate is right, the relative hydrophobicities of tripeptides Gly–Gly–Lys, Lys–Gly–Gly and Gly–Lys–Gly were measured. The experimental values obtained for the relative hydrophobicity of all these tripeptides amount to -4.0 ± 0.2 equivalent CH_2 units. The relative hydrophobicity calculated from the estimates for Gly, Lys, and for the peptide bonds formed by Gly and Gly, and Gly and Lys amounts to -4.1 ± 0.9 equivalent CH_2 units. This unambiguously validates the additivity principle, and suggests that data from a series of homooligopeptides can be extrapolated to allow estimates of the relative hydrophobicity for a heterologous oligopeptide.

Alternatively, the formation of Gly–Gly–Lys or Lys–Gly–Gly can be considered as the addition of a Lys residue to the dipeptide Gly–Gly. The relative hydrophobicity of Gly–Gly is -2.4 ± 0.2 equivalent CH_2 units. From Table 1, the addition of a Lys residue contributes only -0.49 ± 0.03 to oligo-lysine, which results in a prediction for the tripeptide of -2.9 ± 0.2 equivalent CH_2 units. This is clearly different from the measured value of -4.0. The discrepancy could be ascribed to a difference in the relative hydrophobicity of the peptide bonds formed between different residues or to interactions of the side chain with neighboring groups. Additional factors such as amino acid sequence may come into play for longer peptides. Only by extending our studies to oligopeptides of varied composition can we assess these possibilities. It can be concluded that additional studies of different oligopeptides are necessary to test the applicability of the additivity principle.

The relative hydrophobicities of Gly–Asp and Asp–Gly are identical at a given ionic composition and amount to -5.5 ± 0.1 equivalent CH_2 units in the presence of 0.15 *M* NaCl in 0.01 *M* Na-PB, pH 7.3. Comparison of this value with that for Asp–Asp (-7.6 ± 0.2 equivalent CH_2 units) results in the relative hydrophobicity of the side-chain of Asp residue of -2.1 ± 0.3 equivalent CH_2 units that agrees well with the one presented in Table 1 (-2.2 ± 0.1 equivalent CH_2 units). Comparison of the same value with that for Gly–Gly (-2.4 ± 0.1 equivalent CH_2 unit), however, results in the estimate of the relative hydrophobicity for the side-chain of Asp residue of -3.1 ± 0.2 equivalent CH_2 units. These results seem to imply that the relative hydrophobicity of the peptide bond between Asp and Asp and Asp and Gly residues is similar, i.e., it is governed by the residue with the ionic side-chain (Asp).

In systems prepared with 0.11 *M* Na-PB, pH 7.3, the relative hydrophobicities of Gly–Asp and Asp–Gly are identical and amount to -3.1 ± 0.2 equivalent CH_2 units. Comparison of this value with that for Asp–Asp (-2.2 ± 0.3 equivalent CH_2 units) results in the relative hydrophobicity of the side-chain of Asp residue of $+0.9\pm0.5$ equivalent CH_2 units that disagrees completely with the one presented in Table 1 (-0.2 ± 0.1 equivalent CH_2 units).

Comparison of the same value with that for Gly–Gly (-2.1 ± 0.1 equivalent CH_2 units) results in the estimate of the relative hydrophobicity for the side-chain of Asp residue of -1.0 ± 0.3 equivalent CH_2 units, again clearly out of range. The relative hydrophobicity of the bond formed between Asp and Gly residues in 0.11 *M* Na-PB, pH 7.3 estimated based on the additivity principle amounts to -0.5 ± 0.7 equivalent CH_2 units. The relative hydrophobicity of the peptide bond between Asp and Asp ($+0.4\pm0.2$ equivalent CH_2 units) seems to be within the experimental error of the calculated estimate, although the agreement is far from perfect. It seems unavoidable to conclude that at this ionic composition the relative hydrophobicity of the peptide bond formed by Asp and Gly residues is governed neither by Asp residue nor by Gly residue alone. Examination of longer chain heterooligopeptides may reveal a relationship between amino acid composition (and/or sequence) and relative hydrophobicity at this ionic composition.

Relative hydrophobicities of dipeptides Gly–Glu and Glu–Gly are again identical at a given ionic composition and amount to -5.1 ± 0.2 equivalent CH_2 units in the presence of 0.15 *M* NaCl in 0.01 *M* Na-PB, pH 7.3, and to -2.4 ± 0.1 equivalent CH_2 units in the presence of 0.11 *M* Na-PB, pH 7.3. Comparison of these values with those for Gly–Asp and Asp–Gly in the two buffer systems (-5.5 ± 0.1 and -3.1 ± 0.2 equivalent CH_2 units) indicates that on average the dipeptides with Glu residue are more hydrophobic than those with Asp residue by 0.6 ± 0.2 equivalent CH_2 units. This result agrees well with the fact that the side-chain of Glu residue contains one extra CH_2 group compared with that of Asp residue. It also implies that the relative hydrophobicity of the peptide bond formed by Gly and Glu residues is close to that formed by Gly and Asp residues.

The finding that the partition coefficients for reversed dipeptides are similar in the aqueous Dex–PEG system differs from the data reported in the aqueous PEG-3400–potassium phosphate buffer (pH 7.0) system [10] and in the PEG-6000–sodium sulfate system [11]. Partitioning of dipeptides with reversed sequence differs significantly in the aqueous PEG–salt systems. The reason may be that the PEG–salt systems are more sensitive than the two-polymer systems toward small differences in the properties of ionic groups, e.g., N- and C-terminal groups. If this is true, then partitioning of ionic solutes in both two-polymer and single polymer–salt systems should be studied for better characterization of the solutes. This issue obviously requires further examination. The results reported by Diamond et al. [10] on partitioning of oligo-glycines in the PEG–salt system indicate that the additivity principle holds for the peptides having from three to six residues. The data reported by Bringmann et al. [12] for reversed dipeptides Trp–Phe and Phe–Trp in the aqueous Dex–PEG system, however, indicate that there is essentially no difference between the partition coefficients of these peptides under the same conditions. For example, as reported in [12] in the presence of 0.04 *M* NaCl at pH 7.25 to 7.5 *K* values are 1.23 ± 0.02 for Trp–Phe and 1.26 ± 0.02 for Phe–Trp, and in the presence of 0.04 *M* Na_2SO_4 at pH 8.6 to 8.9 *K* values are 1.54 ± 0.02 for Phe–Trp and 1.55 ± 0.02 for Trp–Phe. These results [12] agree with the data obtained in this work.

The data obtained so far indicate that the additivity principle does hold for relative hydrophobicity of small model peptides. However, more extensive studies are necessary to test the validity of this principle for calculation and prediction of the relative hydrophobicity of long conformationally flexible heterooligopeptides. The relative hydrophobicity of short peptides may be viewed as an additive–constitutive property of the peptide molecule. However, the effects of ionic composition of aqueous media must be taken into account. In addition, the influence of the type of amino acid residues forming peptide bonds upon the relative hydrophobicities of the bonds in a given peptide sequence must be considered. Deviation from the additivity principle may be indicative of conformational changes in a peptide. Many experimental studies are clearly necessary before the hydrophobicity of a hypothetical peptide structure may be calculated and its biological potency predicted based on QSAR analysis for a limited number of bioactive peptides. This goal, however, seems to be achievable.

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