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A Correlation for Predicting Partition Coefficients in Aqueous Two-Phase Systems

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Abstract

Partition coefficients of amino acids and peptides are examined in poly(ethylene glycol)/magnesium sulfate systems. A correlation is proposed which relates the logarithm of the partition coefficient to the solute hydrophobicity and to the concentration difference between the phases of one of the phase-forming components. Partitioning data for several neutral amino acids were used to calculate individual amino acid residue hydrophobicities. These hydrophobicities were then used successfully to predict the partition coefficients of peptides in the same phase systems. The predicted and observed partition coefficients are compared for several peptides composed of two to four amino acid residues.

INTRODUCTION

Aqueous two-phase systems occur when two mutually incompatible components, such as poly(ethylene glycol) (PEG) and dextran, or PEG and certain salts, are dissolved together in water. As shown by Albertsson (*1*), at concentrations above those defined by a phase boundary, two liquid phases will form, with each phase tending to be enriched by one of the incompatible components. The term "aqueous" is appropriate since over 80 wt% of each phase is composed of water. A solute added to such a phase system often partitions unequally between the phases, and the solute partition coefficient, *K*, is defined as its upper phase concentration divided by its lower phase concentration. Many types of solues, including small organic molecules (*2–4*), salts (*5, 6*), peptides (*7*), and proteins (*8–10*), have been shown to partition in aqueous two-phase systems. Since such

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systems are composed primarily of water, they provide a gentle environment for the fractionation of biomaterials (*1, 11–14*). A detailed review of the properties and practical uses of aqueous two-phase systems for the recovery of proteins has recently been provided by Huddleston and Lyddiatt (*15*).

Numerous studies have focused on the general prediction of partition coefficients in aqueous two-phase systems. Partition coefficients are thought to depend on several factors such as solute hydrophobicity (*7, 16*), molecular weight (*17*), temperature (*1*), pH (*18–20*), solute charge (*13*), and the presence of additional salts (*21, 22*). Models and correlations which incorporate some of these effects have been developed to predict partition coefficients in aqueous two-phase systems. These correlations include recent derivations using the Flory–Huggins lattice model (*23, 24*), a modified lattice model (*25*), UNIQUAC (*26, 27*), an extension of an osmotic pressure virial expansion (*28*), Hill solution theory (*29*), and a statistical mechanical model (*30*). The models have provided insights into polymer solution behavior and partitioning, but many have limited use due to the difficulty of obtaining values for the model parameters. For example, data for the aqueous behavior of pure solutes are often required but unavailable. Ideally, since a pure sample of the solute may not be available, a predictive model could be developed incorporating the concept of group contribution, widely used in correlating solubility and biological activity (*31–34*). With the knowledge of amino acid partitioning behavior, one may be able to predict peptide and ultimately protein partitioning. Similar principles have been applied successfully to predict HPLC retention times of peptides from the contributions of amino acids (*35–37*). We recently (*16*) developed a model to predict partition coefficients of large molecules in aqueous two-phase systems from the partitioning data of smaller constitutent compounds. This work extends that model for additional amino acids and peptides.

MATHEMATICAL MODEL

Zaslavsky et al. (*38–41*) studied the effect of the number of methylene groups on an aliphatic chain on the solute molecule on its partition coefficient. In all systems studied, a linear relationship existed between the logarithm of the partition coefficient and the number of methylene groups. Hydrophobic properties of aqueous two-phase systems were therefore quantified by the free energy to transfer a methylene group ($\text{—CH}_2\text{—}$) between the phases, Δg^{CH_2} (*42*). The introduction of a hydrophobicity scale in this fashion permits comparison of different phase systems.

Zaslavsky et al. noted that in a system with a given composition of components, the partition coefficients of a homologous series of compounds vary linearly:

$$RT \ln K = RTC + \Delta g^{\mathrm{CH_2}} n \tag{1}$$

where R is the gas constant, T is the absolute temperature, C is a parameter which depends on the particular series of homologous compounds, and n the number of $-\mathrm{CH_2}-$ groups. A change in the composition of the phases (i.e., the location of the solution on the phase diagram) alters the values of C and $\Delta g^{\mathrm{CH_2}} n$. The value of RTC normalizes the partition coefficients for the particular homologous series in that phase system. Thus, RTC is related to the hydrophobicity of the remaining part of the solute (i.e., the fragment without $-\mathrm{CH_2}-$ groups).

We have recently shown *(16)* that the value of RTC may be divided into two parts:

$$RTC = RTc + \Delta g^{\mathrm{R}} \tag{2}$$

where Δg^{R} is the theoretical free energy of transfer for the fragment of the solute not containing $-\mathrm{CH_2}-$ groups, and therefore depends on the solute. RTc is that portion independent of the solute. The values of the parameters in Eqs. (1) and (2) are valid only for a specific phase system composition. Preferably, the partition coefficient could be expressed in terms independent of the composition.

Several previous studies *(1, 4, 23, 28)* have noted that to a first approximation, the logarithm of the partition coefficient is proportional to the tie line length from the phase diagram. The tie line length is proportional to the concentration difference between the phases of one of the phase-forming components:

$$\ln K = k \Delta w_2 \tag{3}$$

Here, Δw_2 is the concentration (wt%/wt%) difference between the phases of the component which has enriched the upper phase (by convention, Component 2). k is a proportionality constant which is independent of the phase system composition.

Equations (1), (2), and (3) suggested that a general relationship might be found for the partition coefficient in aqueous two-phase systems, and the following was defined *(16)*:

$$\Delta f \Delta w_2 = \Delta g^{\mathrm{R}} + \Delta g^{\mathrm{CH_2}} n \tag{4}$$

such that Δf depends on the solute and the phase system, but not on the concentrations of the components in each phase. In a system having a specific concentration (Δw_2 constant), Δf is proportional to the theoretical free energy of transfer of the solute, and thus is interpreted as being a relative measure of the solute hydrophobicity.

The parameter c may also be related to the concentration difference between the phases:

$$\alpha_P \Delta w_2 = RTc \tag{5}$$

The phase constant, α_P, depends on the phase system but is independent of the concentrations of the phase-forming components. From Eqs. (1), (4), and (5):

$$RT \ln K = (\alpha_P + \Delta f)\Delta w_2 \tag{6}$$

Since the relative solute hydrophobicity is proportional to the free energy of transfer, its value has additive-constitutive properties. The total relative hydrophobicity (Δf_m) of a large molecule composed of m constituent molecules united by condensation reactions is equal to the sum of the relative hydrophobicities of the constituent molecules:

$$\Delta f = \Delta f_m = \sum_{i=1}^{m} \Delta f_i - (m - 1)\Delta f_c - \Delta f^* \tag{7}$$

Here, Δf_i is the relative hydrophobicity of the ith constituent molecule, Δf_c is the effective relative hydrophobicity lost in the solute by each condensation, and Δf^* is the total free energy (in terms of relative hydrophobicity) lost by interactions among constituent molecules. Equation (7) assumes that each condensation is accompanied by an identical loss of hydrophobicity, which may not always be the case. In a large peptide or protein, the interaction term may dominate the calculation of hydrophobicity. However, if the peptide is sufficiently short, the interaction term may be assumed to be negligible, as it will be throughout this paper.

Before Eqs. (6) and (7) may be used to calculate hydrophobicities (and then partition coefficients), values are required for the phase constant (α_P) and the effective relative hydrophobicity lost by each condensation (Δf_c). For the PEG/$MgSO_4$ system at 25°C, the phase constant has been calculated to be -2310 cal/mol, while the value of Δf_c is 480 cal/mol (*16*).

In the study reported here, several amino acids were selected for partitioning in PEG/$MgSO_4$ aqueous two-phase systems. These two-phase systems are mildly buffering, and therefore the pH remains constant for different concentrations of components and solutes. Moreover, the systems are ideal for the study of amino acids, since the pH of these solutions is in the range of 5.5 to 6.0, where many amino acids are essentially neutral. For this reason, one may be confident that any charge effects will be negligible in comparison to the effect due to solute hydrophobicity. An additional factor will likely be necessary to describe charge effects.

MATERIALS AND METHODS

Poly(ethylene glycol) (PEG), molecular weight 8000 (Lot 49F-0383), and all peptides used in the partitioning experiments were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. Magnesium sulfate ($MgSO_4 \cdot 7H_2O$) (Lot 746031) was obtained from Fisher Scientific Co., Pittsburgh, Pennsylvania, USA. Five 10.0 mL solutions of $MgSO_4$ were prepared for partitioning experiments as previously described (*16*).

Various solutes (in 10–50 mg quantities) were added to each of these five phase systems. After equilibration, the phases were carefully separated with glass Pastuer pipets. Liquid chromatography was employed to determine the solute concentration in each phase. The partition coefficients for small molecules were found to be independent of solute concentration for the range of dilute solutions prepared.

The HPLC system was comprised of a Waters pumps model 510, with a Gilson model 231 sample injector and Hewlett-Packard 3392A integrator. The peptides were analyzed by the HPLC with a Whatman 5 μm Partisphere C_8 column and a Waters UV detector model 481. Since the amino acids studied had poor UV absorbances, they were analyzed by a derivitization method (*43*) using a Waters 5 μm Resolve Radial-Pak C_8 column and a Gilson model 121 fluorometer. The standard error of the mean from the analyses did not exceed 7% for the analytical techniques.

RESULTS AND DISCUSSION

To use Eqs. (6) and (7) to predict partition coefficients of peptides, the hydrophobicities of amino acids must first be calculated by partitioning neutral amino acids in an appropriate aqueous two-phase system. Figure 1 shows the partition coefficients of several amino acids in five PEG/$MgSO_4$ systems. Using the slope of each set of partitioning data, Eq. (6) may be used to calculate the hydrophobicity of each amino acid. Table 1 lists these calculated hydrophobicities, along with other values calculated previously (*16*). The isoelectric points for these amino acids are also shown in Table 1, and most are in the range of the PEG/$MgSO_4$ systems (5.5–6.0). The calculated amino acid hydrophobicities may be compared to other amino acid hydrophobicity scales determined independently. For example, No-

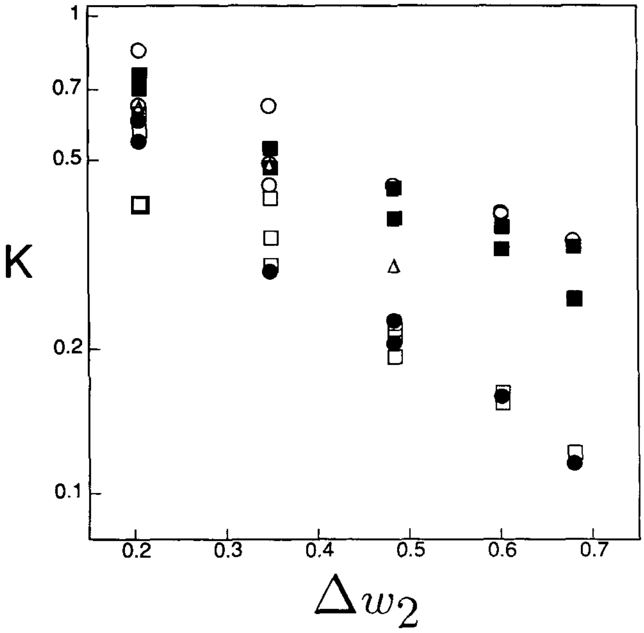

FIG. 1. Measured partition coefficients (K) of amino acids versus the PEG concentration difference between the phases (Δw_2) in the PEG/$MgSO_4$ aqueous two-phase system at 25°C: valine (■), serine (●), proline (△), methionine (○), asparagine (□).

TABLE 1
Relative Amino Acid Hydrophobicities Calculated in the PEG/$MgSO_4$ Aqueous Two-Phase System at 25°C

Amino acid	Calculated hydrophobicity (cal/mol)	pI
gly	0	5.97
asn	470	5.41
ser	480	5.68
ala	670[a]	6.02
pro	970	6.30
val	1220	5.97
met	1310	5.75
leu	1760[a]	5.98
phe	2400[a]	5.48
tyr	2490[a]	5.65
trp	3340[a]	5.88

[a] From Reference *16*.

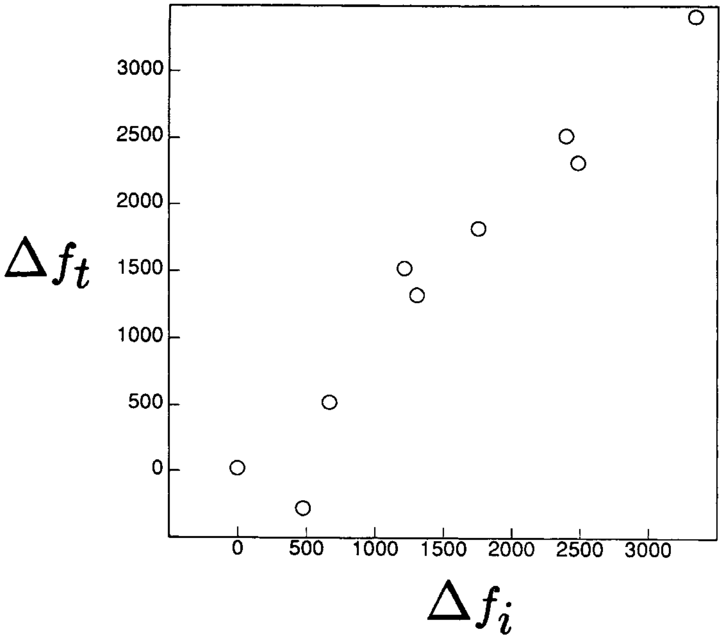

FIG. 2. Comparison of relative amino acid hydrophobicity (Δf_i, cal/mol) with hydrophobicity scale (Δf_t, cal/mol) of Nozaki and Tanford (*44*).

zaki and Tanford calculated a hydrophobicity scale using mixed-solvent solubilities of amino acids (*44*). Figure 2 compares this scale with the amino acid hydrophobicities calculated from partitioning in aqueous two-phase systems, and the two scales are in general agreement.

The isoelectric points of asparagine, phenylalanine, proline, and alanine are most distant from the approximate pH of the two-phase systems studied. Thus, these amino acids are slightly charged in these solutions. Any charge effects resulting from the small differences between the p*I* values of these amino acids and the pH of the systems might account for any small difference between the two hydrophobicity scales. However, an effect of charge probably does not account for the more significant difference observed between the scales for the amino acid serine.

Figures 3–6 show the results of partitioning several peptides in the PEG/$MgSO_4$ two-phase system. Using Eq. (7), the predicted partition coefficients were calculated from the individual amino acid hydrophobicities shown in Table 1. The value of the relative hydrophobicity lost by the condensation reaction, Δf_c, was taken to be 480 cal/mol (*16*), while the interaction term, Δf^*, was assumed to be negligible. Values for the "observed" hydrophobicities for these peptides were calculated by performing

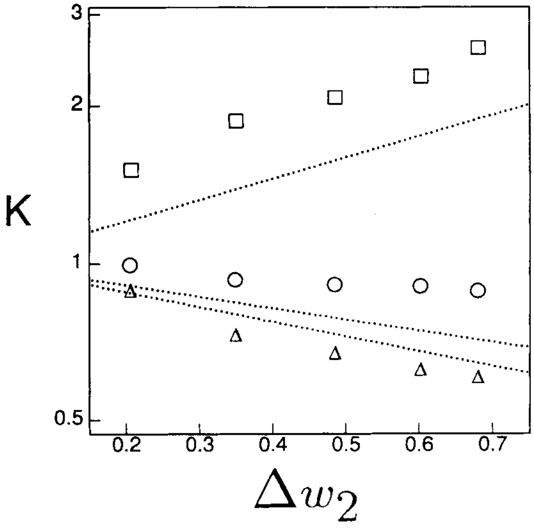

FIG. 3. Measured partition coefficients (K) of glycine-containing dipeptides versus the PEG concentration difference between the phases (Δw_2) in the PEG/MgSO$_4$ aqueous two-phase system at 25°C: gly-trp, (□), gly-tyr (○), gly-phe (△).

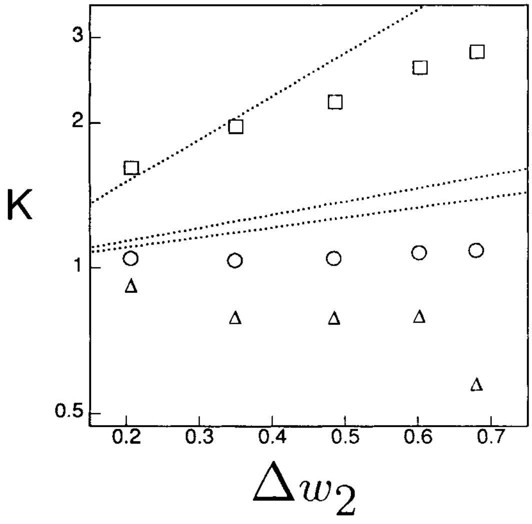

FIG. 4. Measured partition coefficients (K) of alanine-containing dipeptides versus the PEG concentration difference between the phases (Δw_2) in the PEG/MgSO$_4$ aqueous two-phase system at 25°C: ala-trp (□), ala-tyr (○), ala-phe (△).

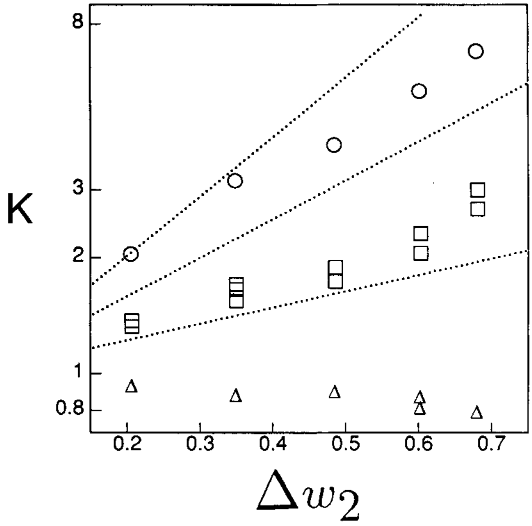

FIG. 5. Measured partition coefficients (K) of phenylalanine-containing dipeptides versus the PEG concentration difference between the phases (Δw_2) in the PEG/MgSO$_4$ aqueous two-phase system at 25°C: leu-phe (□), phe-tyr (○), pro-phe (△).

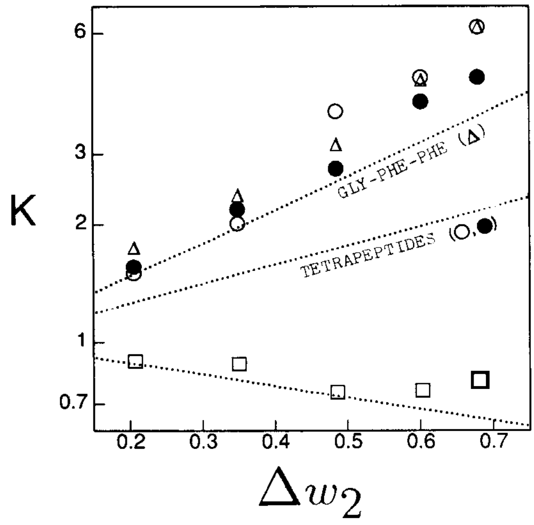

FIG. 6. Measured partition coefficients (K) of peptides versus the PEG concentration difference between the phases (Δw_2) in the PEG/MgSO$_4$ aqueous two-phase system at 25°C: gly-phe-ala (□), gly-phe-phe (△), phe-gly-gly-phe (●), phe-gly-phe-gly (○).

TABLE 2
Predicted and Observed Relative Peptide Hydrophobicities in the PEG/MgSO$_4$ Aqueous Two-Phase System at 25°C

Peptide	Predicted hydrophobicity (cal/mol)	Observed hydrophobicity (cal/mol)
gly-trp	2860	3150
gly-tyr	2010	2190
gly-phe	1920	1830
ala-trp	3530	3240
ala-tyr	2680	2360
ala-phe	2590	1960
leu-phe	3680	3130
phe-tyr	4410	3970
pro-phe	2890	2110
gly-phe-ala	1920	2030
gly-phe-phe	3460	3830
phe-gly-gly-phe	2980	3630
phe-gly-phe-gly	2980	3860

a least-squares fit of the experimental data to Eq. (6). Table 2 compares these predicted and observed hydrophobicities.

Figure 3 depicts the partitioning behavior of three glycine-containing dipeptides, while Fig. 4 shows data for the analogous alanine-containing dipeptides. Figure 5 is for three different phenylalanine dipeptides, while Figure 6 shows the results for several larger peptides. As can be seen, the partition coefficients for the glycine-containing dipeptides are predicted well by the model. The substitution of an alanine residue for the glycine residue on any of these dipeptides should result in a more hydrophobic dipeptide, having, therefore, larger partition coefficients. Although the data indeed show this trend, partition coefficients predicted for each alanine dipeptide are greater than those observed. However, the order of increasing hydrophobicities is correctly predicted among the three.

The partition coefficients observed for the three dipeptides in Fig. 5 are smaller than the model predicts. As shown in Table 2, the predicted hydrophobicities for these three peptides are consistently 500–700 cal/mol greater than the observation. Nevertheless, the correlation is quite good at estimating the magnitude of the partition coefficients in the PEG/MgSO$_4$ two-phase system. Since the best predictions are for the least hydrophobic (i.e., glycine-containing) dipeptides, and the worst predictions are for peptides containing more hydrophobic residues, the interaction term may well be more significant for peptides having pairs of hydrophobic residues.

This same model may be applied to the prediction of partition coefficients of larger peptides, and these results are shown in Fig. 6. The partition coefficients of the tripeptide gly-phe-ala are predicted well by the model. The similar tripeptide gly-phe-phe is a much more hydrophobic peptide and, as predicted, the partition coefficients are well above unity. In comparison, the tetrapeptide containing two phenylalanine and two glycine residues was observed to have about the same partition coefficients as gly-phe-phe (and thus, the same observed hydrophobicity). These observations suggest two possibilities. Either the decrease in relative hydrophobicity associated with the first condensation is not equivalent to the net hydrophobicity lost by subsequent condensations, or the contribution from the two small hydrophilic glycine residues is negligible in the presence of two hydrophobic residues on this peptide. As predicted, the order of the residues does not have significant effect on the partition coefficient. That is, phe-gly-gly-phe has approximately the same partition coefficients as phe-gly-phe-gly.

These correlations have been developed and applied to neutral amino acids and peptides only, and therefore would be inappropriate to use for aqueous two-phase systems in which the solutes are charged (at other pH's). Presumably, relationships for a charge dependence may be incorporated into the hydrophobicity model to account for changes in the partition coefficient due to a change in pH.

CONCLUSIONS

The partition coefficients of peptides in the PEG/$MgSO_4$ aqueous two-phase system are correlated with the hydrophobicities of individual constituent amino acids, which have been calculated from their partitioning in systems in which they are uncharged. This method permits the *a priori* prediction of partition coefficients, which would be particularly valuable in cases where no pure sample of the solute is available. An additional term will be necessary to use the model in aqueous two-phase systems which have pH values causing the amino acids to carry a charge.

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