Investigation of Amino Acid Partitioning in Aqueous Two-Phase Systems Containing Polyethylene Glycol and Inorganic Salts

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The partitioning behavior of three amino acids, L-tryptophan, L-phenylalanine, and L-tyrosine, has been studied in aqueous two-phase systems of polyethylene glycol (PEG6000) + salts + H_2O at 298.15 K. The salts used were magnesium sulfate, sodium sulfate, and ammonium sulfate. The effects of tie line length, salt, and side chain structure on the partition coefficients of amino acids have been studied. The results showed that increasing amino acid hydrophobicity and tie line length led to a corresponding increase of the partition coefficients. In addition, we also showed that the partition coefficients of the amino acids in the systems containing Na_2SO_4 are also significantly greater than the other two salts. The experimental data are correlated using a modified virial-type model. Comparisons between model and experimental data reveal a good agreement.

1. Introduction

Liquid−liquid extraction using aqueous two-phase systems (ATPS) is a powerful technique for the separation and purification of biological particles.[1,2] These two-phase systems can be made from aqueous solutions of two water-soluble polymers or a polymer and a salt. The most common ATPS used for the separation of biomolecules are polyethylene glycol (PEG)/dextran or PEG/salt systems because of low-cost, rapid mass transfer, and rapid phase equilibration. The processes scale-up easily, can be used as a continuous process, and are more economical than other separation techniques. Some aqueous two-phase systems have been designed for scale-up of downstream processes for biomaterial separation.[3–5] There is also a large literature base on the partitioning behavior of amino acids or low molecular mass peptides in ATPS.[6–8] Partitioning of the amino acids glycine, lysine, and aspartic acid was studied in ATPS of PEG8000/dextran by Zaslavsky et al.[6] The partitioning behavior of the amino acids serine, alanine, valine, isoleucine, and phenylalanine was reported in ATPS composed of volatile salts for the first time by Van Berlo et al.[8] In other research, the partitioning behavior of some amino acids such as cysteine, phenylalanine, methionine, lysine, glycine, and glutamic acid have been also studied in ATPS composed of polyethylene glycol and potassium phosphate salts by some research groups.[9–12] Recently, as an alternative approach, Salabat et al.[13] have invertigated the partitioning behavior of the amino acids alanine, valine, and lucine in aqueous two-phase systems formed by polypropylene glycol and some inorganic salts.

In this research, for the first time, the partitioning behavior of three amino acids with polar and nonpolar side chains, L-tryptophan, L-phenylalanine, and L-tyrosine, in the aqueous two-phase system of PEG6000 and $MgSO_4$, Na_2SO_4, or $(NH_4)_2SO_4$ at 298.15 K has been studied. The effects of tie line length, salt, and side chain structure on the partition coefficients of the amino acids have been studied in detail. The results may be used to predict the separation of some proteins whose main surface exposed amino acid residues are these three amino acids. The experimental data on amino acid partitioning were correlated using an excess Gibbs energy virial-type model modified to account for Coulombic interactions.

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2. Experimental Section

2.1. Chemicals. Polyethylene glycol, of molecular weight 6000 $g \cdot mol^{-1}$, magnesium sulfate heptahydrate (GR, min 99.5 %), ammonium sulfate (GR, min 99.5 %), and sodium sulfate (GR, min 99 %) were obtained from Merck. L-Tryptophan [(99.0 to 101.0) %], L-phenylalanine (> 99 %), and L-tyrosine (> 99 %) were also purchased from Merck. All chemicals were used without further purification.

2.2. Preparation of Phase Systems. The experiments were performed in 15 mL plastic bottles with tightly closed lids. Aqueous two-phase systems were prepared from solid PEG and different salts containing magnesium sulfate, sodium sulfate, or ammonium sulfate and different amino acids in pure water. The total weight of these components for each sample was about 10 g. The initial amounts of the amino acids were $4.0 \cdot 10^{-4}$ g for L-tryptophan, $5.0 \cdot 10^{-4}$ g for L-tyrosine, and $4.0 \cdot 10^{-3}$ g for L-phenylalanine. The mixtures were shaken for about 30 min and then placed in a thermostatic water bath at 298.15 K for at least 12 h to ensure complete equilibration, as indicated by the absence of turbidity in each phase. After equilibration of the systems, samples of approximately 3 mL from the upper and lower phases were carefully removed for analysis using syringes.

2.3. Analysis of Samples. The liquid−liquid equilibrium data from our previous work[14] was used for the preparation of the samples. Therefore, the concentrations of the PEG and salts are known for any selected tie lines. The amino acid concentrations were determined by a double beam Perkin-Elmer Lambda 15 UV visible spectrophotometer at 275.6 nm for L-tryptophan, 255.2 nm for L-phenylalanine, and 272.4 nm for L-tyrosine. None of the salts have absorbance, but PEG shows a little absorbance. To correct the PEG absorbance in the measure-

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ments, samples were diluted two to four times, and the average amounts of PEG in the top and bottom phases were also used as blanks. A calibration curve for absorbance versus amino acid concentration, with a regression coefficient of 0.9995, was prepared for determination of the amino acid concentration. The relative uncertainty of the experimental results for the amino acid concentration by this method is about 2 %.

3. Thermodynamic Framework

The excess Gibbs energy model used to correlate the experimental data obtained in this work is based on the model proposed by Edmond and Ogston,[15] which was recently employed by Haraguchi et al.[16] to model data of the insulin partition coefficient in an ATPS. In this model, the short-range term of the excess Gibbs energy is given by

$$\frac{1000}{n_w M_w}\frac{G^{E,SR}}{RT} = \sum_i \sum_j A_{ij} m_i m_j \quad (1)$$

where M_w is the molecular mass of water (in g·mol^{-1}); n_w is number of moles of water; A_{ij} is the second virial coefficient related to species i and j; and m_i is the molality of species i.

The long-range term is a modified Debye−Hückel term written as

$$\frac{1000}{n_w M_w}\frac{G^{E,LR}}{RT} = -\frac{4A_\gamma I}{b}\ln(1 + b\sqrt{I}) \quad (2)$$

where b is a constant equal to 1.2 kg$^{1/2}$·mol$^{-1/2}$ and A_γ for water at 298.15 K is 0.3914 kg$^{1/2}$·mol$^{-1/2}$. The ionic strength I is calculated as

$$I = \frac{1}{2}\sum_i m_i Z_i^2 \quad (3)$$

where Z_i is the charge of ion i.

The partition coefficient is calculated for the infinite dilution limit. Defining the partition coefficient as

$$K = \frac{C_a^{top}}{C_a^{bottom}} \quad (4)$$

wherein the subscript a stands for amino acid. Using the equilibrium criterion, this relation can be written as

$$K = \frac{\gamma_a^{bottom}}{\gamma_a^{top}} \quad (5)$$

where γ is the activity coefficient. Within the infinite dilution limit

$$K = \frac{\gamma_a^{\infty,bottom}}{\gamma_a^{\infty,top}} \quad (6)$$

The activity coefficient of the amino acid can be straightforwardly obtained from the excess Gibbs energy model through the activity coefficients evaluated from the thermodynamic expression

$$\ln \gamma_a = \frac{1}{RT}\left(\frac{\partial G^E}{\partial n_a}\right)_{T,P,n_{i\neq a}} \quad (7)$$

Using the above expression, the activity coefficient of salt (γ_s) and polymer (γ_p) can also be obtained.

Table 1. Equilibrium Composition (M/mol·L^{-1}) and Partition Coefficients of L-Tryptophan in Aqueous Two-Phase Systems of PEG 6000 + Salts + H_2O at 298.15 K

	$M\cdot10^4$			
TLL	upper phase	lower phase	K_{exptl}	K_{theor}
		$MgSO_4$		
25.1	3.04	1.29	2.36	1.94
29.4	3.07	1.25	2.46	2.14
38.1	3.49	1.13	3.08	2.80
45.9	3.74	0.96	3.90	3.93
		Na_2SO_4		
23.9	3.29	1.13	2.91	2.23
35.7	3.48	0.89	3.93	3.74
38.7	3.76	0.78	4.80	4.38
49.2	2.59	0.38	6.87	9.45
		$(NH_4)_2SO_4$		
28.7	3.32	1.13	2.50	1.65
41.2	3.64	1.08	3.37	2.48
51.3	3.61	0.87	4.15	4.14
58.0	3.74	0.76	4.90	5.76

Table 2. Equilibrium Composition (M/mol·L^{-1}) and Partition Coefficients of L-Phenylalanine in Aqueous Two-Phase Systems of PEG 6000 + Salts + H_2O at 298.15 K

	$M\cdot10^3$			
TLL	upper phase	lower phase	K_{exptl}	K_{theor}
		$MgSO_4$		
25.1	2.63	3.26	0.81	1.06
29.4	2.69	3.02	0.89	1.02
38.1	2.95	3.11	0.95	1.00
45.9	3.21	3.13	1.02	1.02
		Na_2SO_4		
23.9	2.87	1.45	1.98	1.53
35.7	2.93	1.35	2.17	1.96
38.7	3.07	1.30	2.36	2.15
49.2	2.91	1.18	2.47	2.53
		$(NH_4)_2SO_4$		
28.7	3.17	3.15	1.01	1.12
41.2	3.18	3.11	1.02	1.16
51.3	3.67	3.22	1.14	1.15
58.0	3.97	3.08	1.29	1.18

4. Results and Discussion

First, it has to be noted that the influence of the dissolved amino acids on the phase behavior of the aqueous two-phase systems can be neglected. Analysis of the polymer and salt for the phase system of PEG + $MgSO_4$ + L-phenylalanine + water confirms this. Because of this, the polymer and salt concentrations were not measured for these systems, and all were interpolated from the corresponding systems without amino acids. The compositions and tie line length of PEG 6000 + salt + H_2O two-phase systems at 298.15 K, used in this work, were taken from our previous work.[14]

4.1. Effect of TLL and Side Chain of Amino Acids on Partitioning. Tables 1 to 3 show the equilibrium composition of the amino acids as molarity (mol·L^{-1}) in the top and bottom phases for different tie line lengths at 298.15 K.

The tie line length (TLL) is calculated by the following equation

$$TLL = \sqrt{(w_s^{top} - w_s^{bottom})^2 + (w_p^{top} - w_p^{bottom})^2} \quad (8)$$

where w_s and w_p are the weight percents of the salt and PEG in the top or bottom phase. In fact, TLL shows the difference between polymer and salt concentrations in the top and bottom phases. In other words, with increasing TLL, polymer concentration in the top phase and salt concentration in the bottom phase increases. Figures 1 to 3 show the dependence of partition coefficients for all amino acids on the TLL for various salts.

Table 3. Equilibrium Composition (M/mol·L^{-1}) and Partition Coefficients of L-Tyrosine in Aqueous Two-Phase Systems of PEG 6000 + Salts + H_2O at 298.15 K

	$M \cdot 10^4$			
TLL	upper phase	lower phase	K_{exptl}	K_{theor}
		$MgSO_4$		
25.1	3.27	2.53	1.29	1.61
29.4	3.20	2.72	1.18	1.74
38.1	3.45	2.56	1.35	2.15
45.9	3.60	2.48	1.45	2.75
		Na_2SO_4		
23.9	3.45	2.54	1.36	1.33
35.7	3.62	2.36	1.53	1.57
38.7	3.73	2.37	1.58	1.67
49.2	4.21	2.13	1.98	1.89
		$(NH_4)_2SO_4$		
28.7	3.76	3.05	1.23	1.27
41.2	4.04	2.88	1.40	1.51
51.3	4.25	2.78	1.48	1.85
58.0	4.85	2.72	1.78	2.10

An important property influencing the partition coefficient of amino acids is hydrophobicity of the amino acids. Nozaki and Tanford[17] calculated a hydrophobicity scale for amino acids based on the free energy of transfer of amino acid side chains from an organic solvent to water. On this scale, they reported L-tryptophan, L-phenylalanine, and L-tyrosine as 3400, 2500, and 2300, respectively. They concluded that the larger the hydrophobicity of an amino acid, the larger the affinity for the more hydrophobic PEG-rich phase, and consequently, the larger the partition coefficient. Experimental studies on partitioning of amino acids in polymer−salt aqueous two-phase systems have proven the validity of such a statement.[7]

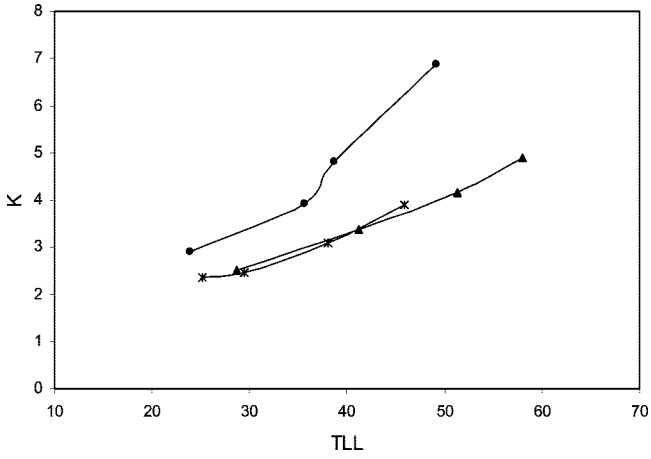

Figure 1. Effect of salt and tie line length on the partition coefficient of L-tryptophan at 298.15 K: ●, Na_2SO_4; *, $MgSO_4$; ▲, $(NH_4)_2SO_4$.

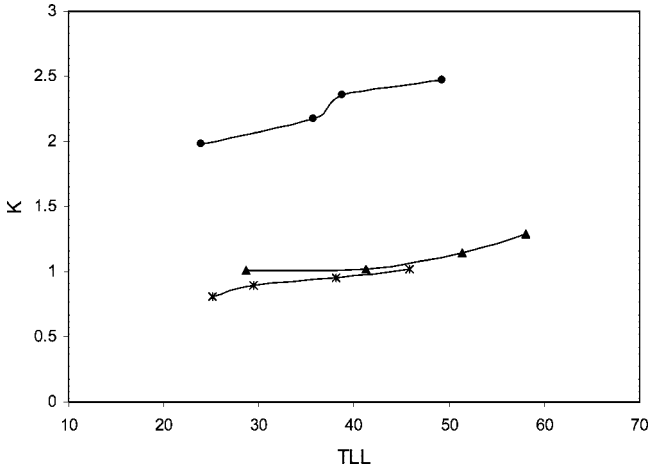

Figure 2. Effect of salt and tie line length on the partition coefficient of L-phenylalanine at 298.15 K: ●, Na_2SO_4; *, MgSO; ▲, $(NH_4)_2SO_4$.

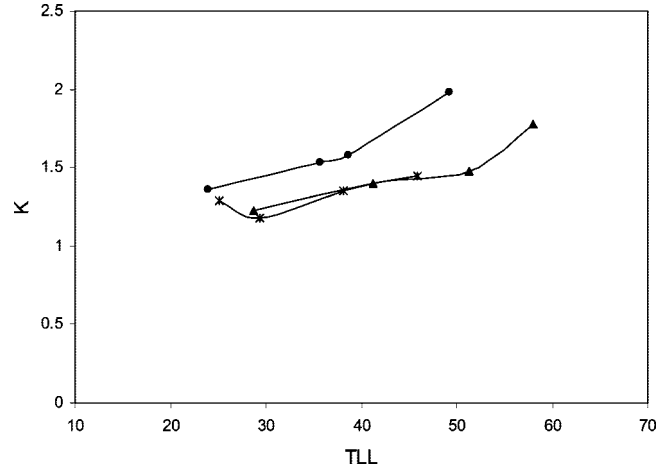

Figure 3. Effect of salt and tie line length on the partition coefficient of L-tyrosine at 298.15 K: ●, Na_2SO_4; *, $MgSO_4$; ▲, $(NH_4)_2SO_4$.

Table 4. Interaction Parameters A (kg·mol^{-1}) between Amino Acid/Salt (A_{sa}) and Amino Acid/Polymer (A_{pa}) in PEG 6000 + Salts + H_2O Systems at 298.15 K

	$MgSO_4$		$(NH_4)_2SO_4$		Na_2SO_4	
	A_{sa}	A_{pa}	A_{sa}	A_{pa}	A_{sa}	A_{pa}
L-tryptophan	0.740	−0.409	0.540	−0.950	0.005	−15.550
L-phenylalanine	0.627	9.194	−0.133	−3.140	0.538	−0.586
L-tyrosine	0.358	−2.950	0.173	−1.400	0.325	−0.892

The results in the present work, as we can see from Tables 1 to 3, also show the same dependence of amino acid partition coefficients on hydrophobicity. The hydrophobicity of the amino acids decreases as follows: L-tryptophan > L-phenylalanine > L-tyrosine. For all aqueous two-phase systems, L-tryptophan has a higher partition coefficient than the other two amino acids. However, for L-phenylalanine and L-tyrosine, the partition coefficients are similar, in agreement with the Nozaki and Tanford hydrophobicity scale.[17] On the other hand, as expected, the longer a tie line, the more PEG in the top phase and the greater increase in the partition coefficients. This increment in the partition coefficient of L-tryptophan with increasing TLL is also more than the two other amino acids (Figures 1 to 3).

4.2. Effect of Salts on Partitioning. The effect of salt on the partition coefficients can be seen from Figures 1 to 3. The partition coefficients for Na_2SO_4 are significantly more than the systems containing the $(NH_4)_2SO_4$ and $MgSO_4$ salts. This trend is not compatible with the salting-out power of the salts,[18] which is $Mg^{2+} > Na^+ > NH_4^+$.

4.3. Correlation of Partition Coefficients and Composition with Experimental Data. The optimal values of the second virial coefficient between amino acids and other compounds (polymer and salt), A_{pa} and A_{sa}, were obtained by minimizing the following objective function

$$OF = \sqrt{\frac{\sum_n (K_i^{exptl} - K_i^{calcd})^2}{n}} \qquad (9)$$

The partition coefficients (K_i^{calcd}) were calculated according to eq 6, and the results for optimized virial coefficients are presented in Table 4. The calculated partition coefficients for different systems and different amino acids at 298.15 K are listed

in Tables 1 to 3. The calculated partition coefficients are reasonable and agree with the experimental values to within the experimental uncertainty of the measurement.

The model interaction parameters of polymer−polymer (A_{pp}), polymer−salt (A_{ps}), and salt−salt (A_{ss}) were estimated by minimizing the following objective function

$$OF = \sum_{p} \sum_{l} \sum_{i} (m_{p,l,i}^{\text{calcd}} - m_{p,l,i}^{\text{exptl}})^2 \quad (10)$$

where $m_{p,l,i}$ is the molality of component i in phase p for the lth tie line. The LLE data were correlated using eq 10 and the equilibrium condition

$$(m_i\gamma_i)^{\text{top}} = (m_i\gamma_i)^{\text{bot}} \quad (11)$$

When minimizing the objective function with this model, a single value was not obtained for the polymer/polymer interaction parameter, A_{pp}, and we found three different values of (601.1, 47.11, and 124.5) kg·mol^{-1}, for systems containing $MgSO_4$, Na_2SO_4, and $(NH_4)_2SO_4$, respectively. This may be because the concentrations of the components change over a wide range. The parameter A_{ps} was obtained as (87.52, 10.95, and 17.85) kg·mol^{-1} and the parameter A_{ss} as (3.92, 0.77, and 0.81) kg·mol^{-1} for $MgSO_4$, Na_2SO_4, and $(NH_4)_2SO_4$, respectively. The objective function (OF) for this calculation (eq 10) is about 0.023, and reasonable agreement between calculated and experimental compositions was obtained. It is important to mention that, because the polymer and salt concentrations in the top and bottom phase for this type of aqueous two-phase systems change considerably with TLL, we are not sure that the obtained parameters would be physically meaningful, and they are at best empirical parameters.

5. Conclusion

The partition coefficients of three amino acids L-tryptophan, L-thenylalanine, and L-tyrosine were obtained in aqueous two-phase systems of PEG + salts + H_2O at 298.15 K. The effect of the salts and tie line length on amino acid partitioning were also determined. It was verified that more hydrophobic amino acids had greater affinity for the more hydrophobic PEG-rich phase and, consequently, larger partition coefficients. Then, as expected, the longer a tie line, the greater the increase in partition coefficients. On the other hand, it was found that the effect of salts on the partition coefficients has a different trend to the salting-out power of the salts. The partition coefficients of the amino acids in the systems containing Na_2SO_4 were significantly greater than the other two salts. This reverse trend with respect to salting out power of the $MgSO_4$ may be because of complex formation between the amino acids and Mg^{2+} in the bottom phase, decreasing the partition coefficient. The experimental data on the phase diagram and partitioning behavior of the amino acids correlated reasonably well with a modified excess Gibbs energy virial-type model.

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