Journal of Chromatography, 513 (1990) 137–143
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 496

Correlation of protein partitioning in aqueous polymer two-phase systems

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(First received June 22nd, 1989; revised manuscript received March 1st, 1990)

ABSTRACT

A correlation is presented for the partitioning of proteins in aqueous two-phase systems. The correlation relates the natural logarithm of the protein partition coefficient to the polyethylene glycol (PEG) concentration difference between the phases, $(w_1'' - w_1')$, by the equation, $\ln K = A(w_1'' - w_1') + b(w_1'' - w_1')^2$. This relationship was shown to fit protein partitioning data obtained from eight two-phase systems at pH 7.0, four consisting of PEG 8000–Dextran T-500–water systems and four consisting of PEG 3400–potassium phosphate–water systems. Seven different proteins, ribonuclease, lysozyme, trypsin, rhodanese, transferrin, hexokinase and invertase, with a molecular weight range of 10 000–270 000, were utilized in this work. Each of the proteins partitioned with different values of the empirical constants A and b.

INTRODUCTION

Aqueous polymer two-phase systems such as those composed of polyethylene glycol (PEG)–dextran–water and PEG–potassium phosphate–water provide both a gentle and economical means for the purification of biological materials[1,2]. In order to facilitate their use, a simple method is needed for correlation of partition coefficients. Such a correlation would provide the groundwork necessary for the selection of an appropriate system for separation, and the eventual scale up of the purification process.

The partition coefficient, K, has been postulated as a function of many parameters, including electrical charge, hydrophobicity, biospecificity, size and conformation[1]. Much work has been done to elucidate the effects of pH[3], the influence of salts[4,5], hydrophobicity[6,7] and biomolecule and polymer molecular weight[8,9] on protein partitioning. Several theoretical models have been proposed for the thermodynamic behavior of protein partitioning in aqueous polymer two-phase systems. Walter *et al.*[2] and Albertsson *et al.*[9] have shown that the lattice model of Flory[10] and Huggins[11] could be used to qualitatively predict protein partitioning. Baskir *et al.*[12]

have modified the theory of Scheutjens and Fleer[13,14], while King *et al.*[15] extended the model of Edmond and Ogston[16] to take into account electrostatic charge. However, a simple correlation still has not resulted. Diamond and Hsu[17] presented a linear semilogarithmic relationship for correlating biomolecule partitioning, with respect to the PEG concentration difference between the phases, based on Flory–Huggins solution thermodynamics. The relationship proved to be adequate for dipeptide and low-molecular-weight protein partitioning. However, the correlation was not appropriate for high-molecular-weight proteins, which apparently exhibited partitioning with non-linear dependence on the PEG concentration difference. In this paper, the correlation of Diamond and Hsu is generalized to include a quadratic dependence on PEG concentration difference for high-molecular-weight protein partitioning. The generalized expression is shown to be applicable to both PEG–dextran–water and PEG–potassium phosphate–water two-phase systems for seven proteins.

MATERIALS AND METHODS

Materials

Dextran T-500 (Lot 06905) was obtained from Pharmacia (Piscataway, NJ, U.S.A.). PEGs of molecular weights 3400 (Lot 00304 EV) and 8000 (Lot 02316 EV) were purchased from Aldrich (Milwaukee, WI, U.S.A.).

Ribonuclease (bovine pancreas), trypsin, lysozyme (chicken egg), rhodanese (bovine liver), hexokinase (bakers yeast), invertase (bakers yeast) and transferrin (human) were obtained from Sigma (St. Louis, MO, U.S.A.).

Partition experiments

The PEG 8000–Dextran T-500–water systems at 4°C and the PEG 3400–potassium phosphate–water systems at 20°C were prepared as described earlier[17–19] and had the following phase compositions (in %, w/w):

(1) Bottom phase: 8.83% dextran–1.63% PEG, top phase: 0.86% dextran–4.91% PEG

(2) Bottom phase: 14.11% dextran–0.73% PEG, top phase: 0.11% dextran–7.03% PEG

(3) Bottom phase: 18.73% dextran–0.43% PEG, top phase: 0.03% dextran–9.27% PEG

(4) Bottom phase: 21.61% dextran–0.30% PEG, top phase: 0.01% dextran–10.83% PEG

(5) Bottom phase: 14.06% phosphate–4.23% PEG, top-phase: 8.19% phosphate–15.96% PEG

(6) Bottom phase: 15.46% phosphate–2.54% PEG, top phase: 7.01% phosphate–19.16% PEG

(7) Bottom phase: 17.41% phosphate–1.30% PEG, top phase: 5.56% phosphate–23.90% PEG

(8) Bottom phase: 19.41% phosphate–0.78% PEG, top phase: 4.55% phosphate–28.15% PEG

The PEG–dextran phase diagram data were taken from Diamond and Hsu[17], while the composition of systems 5–8 of the PEG–potassium phosphate phase diagram were obtained from Albertsson[1].

Partition experiments were performed as previously described[17], in which the PEG–dextran–water systems were maintained at pH 7.0 with 0.01 *m* potassium phosphate buffer. The potassium phosphate in the PEG–potassium phosphate–water systems consisted of a mono- to dibasic weight ratio of 0.55, thus maintaining at pH 7.0. A 10-ml volume of phase system was poured into 15 ml polypropylene centrifuge tubes. A 10-mg amount of protein was added, and the tubes tightly sealed. The protein was then dissolved by gently mixing the contents of the centrifuge tube with a vortex mixer. The phases of the PEG–dextran system were allowed to settle for 24 h at 4.0 ± 0.1°C in a temperature controlled refrigerator, while the PEG–phosphate systems were permitted to settle for 24 h at 20.0 ± 0.5°C in the laboratory environment. A pasteur pipet was used to collect the top phase, while the lower phase was drained from the tube by piercing a hole at its bottom.

Protein concentration was determined by diluting the phase aliquot with water, and measuring absorbance at 280 nm *versus* an equally diluted phase blank. The partition coefficient, K, was defined as protein concentration in the top phase divided by that in the bottom. Two or more partition experiments were performed for a protein in a particular system, and the resulting partition coefficients did not differ by more than 5%. The K values used in this work represent the average value of the measured partition coefficients.

RESULTS AND DISCUSSION

It has been demonstrated that for low-molecular-weight proteins and small molecules such as dipeptides, the natural logarithm of the partition coefficient may be correlated with the PEG concentration difference in a system composed of PEG, dextran and water[17]. Zaslavsky *et al.*[20], using dinitrophenylated amino acids, have also shown that the Gibbs free energy of transfer of a CH_2 group varied linearly with the PEG concentration difference between the phases for a PEG–dextran–water system. However, they did not provide a theoretical basis for their plot. The linear relationship between ln K and the PEG concentration difference, as derived from Flory–Huggins polymer solution thermodynamics, was found to be[17]:

$$\ln K = A(w_1'' - w_1') \tag{1}$$

where w is weight percent, the subscript 1 refers to PEG, and the double and single prime superscripts represent the top and bottom phase, respectively. The slope, A, is a function of biomolecule and polymer molecular weight, and the interaction of the biomolecule with the polymers and water. The above relationship was not applicable to high-molecular-weight proteins which tended to deviate from eqn. 1. Introducing a second degree term into eqn. 1 yields the following relationship:

$$\ln K = A(w_1'' - w_1') + b(w_1'' - w_1')^2 \tag{2}$$

Although empirical in nature, the coefficient b, like A, may reflect the interaction of the protein with the polymers and water. Eqn. 2 may be simplified by dividing through by $w_1'' - w_1'$ to yield the linear relationship:

$$\frac{\ln K}{w_1'' - w_1'} = A + b(w_1'' - w_1') \tag{3}$$

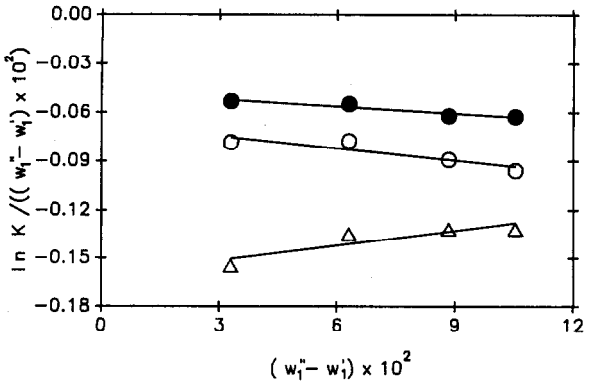

Fig. 1. Correlation of low-molecular-weight protein partitioning data in the PEG 8000–Dextran T-500–water system according to eqn. 3. ○ = Ribonuclease; ● = trypsin; △ = lysozyme.

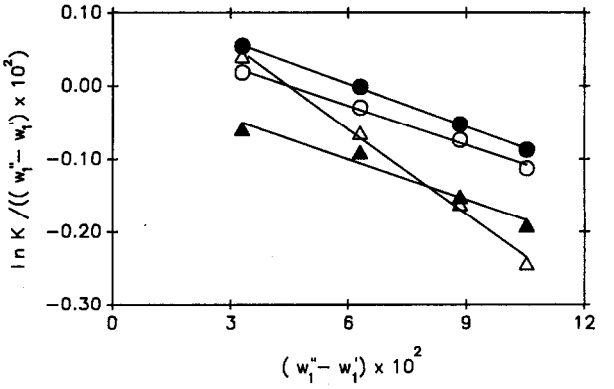

Fig. 2. Correlation of high-molecular-weight protein partitioning data in the PEG 8000–Dextran T-500–water system according to eqn. 3. ○ = Rhodanese; ● = hexokinase; △ = invertase; ▲ = transferrin.

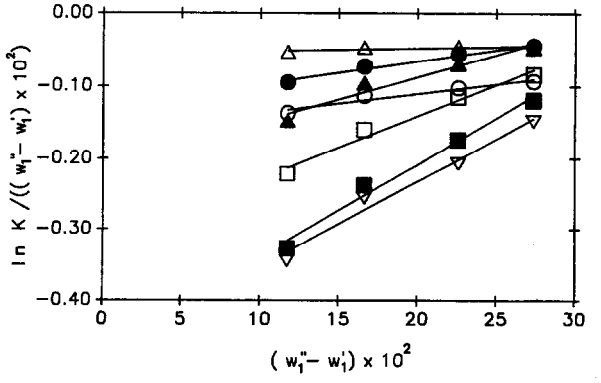

Fig. 3. Correlation of protein partitioning data in the PEG 3400–potassium phosphate–water system according to eqn. 3. ○ = Ribonuclease; ● = trypsin; △ = lysozyme; ▲ = rhodanese; □ = hexokinase; ■ = invertase; ▽ = transferrin.

TABLE I

THE CORRELATION CONSTANTS, A AND B, OF EQN. 3 FOR PROTEIN PARTITIONING IN THE PEG 8000–DEXTRAN T-500–WATER AND PEG 3400–POTASSIUM PHOSPHATE–WATER PHASE SYSTEMS

Protein	*Molecular weight*	*Isoelectric pH*	*PEG 8000–Dextran T-500*[a]		*PEG 3400–potassium phosphate*	
			A	*b*	*A*	*b*
Ribonuclease	12 600	9.3	− 6.7	− 30	−16.4	30
Lysozyme	13 900	11.0	−16.1	30	− 5.6	4
Trypsin	23 200	8.7	− 4.8	− 20	−12.9	30
Rhodanese	37 570	4.7	8.0	−180	−21.1	60
Transferrin	77 000	5.9	1.1	−190	−47.3	120
Hexokinase	102 000	4.7	11.9	−190	−31.5	90
Invertase	270 000	4.0	17.3	− 39	−46.9	130

[a] System contains 0.01 *m* potassium phosphate buffer, pH 7.0.

Eqn. 3 was tested by partitioning seven proteins in two types of aqueous two-phase systems. Figs. 1 and 2 represent the partitioning data for three low-molecular-weight proteins and four high-molecular-weight proteins, respectively. The system utilized was PEG 8000–Dextran T-500–water at 4°C. Similarly, in Fig. 3, the same seven proteins have been partitioned in the system composed of PEG 3400–potassium phosphate–water at 20°C. The values of A and b for each of the proteins in the two systems as obtained by unweighted least squares fitting of eqn. 3, along with protein molecular weight and isoelectric pH, are presented in Table I. It should be pointed out that the A values for ribonuclease, lysozyme, and trypsin were found to be −9.0, −13.4 and −6.1, respectively, when correlated according to eqn. 1[17]. These values differ slightly from those reported in Table I due to the equation of regression.

Examination of Fig. 1 reveals that, although the regression lines are not perfectly horizontal, the second term of eqn. 3 appears to be small compared to the first term (the intercept, A) for the low-molecular-weight proteins. Since the low-molecular-weight proteins in the PEG–dextran system partitioned according to eqn. 1[17], then b will be small, and the plot of eqn. 3 will approximate a horizontal line.

In Fig. 2, the linear relationship of eqn. 3 was tested in the PEG–dextran system for the high-molecular-weight proteins, rhodanese, hexokinase, invertase and transferrin. The linearity obtained for each of the proteins suggests that the addition of the second degree term was appropriate.

The partitioning data for the seven proteins in the PEG–phosphate system was correlated according to eqn. 3 and is presented in Fig. 3. The linear relationships for all of the proteins verify the applicability of eqn. 3 to protein partitioning in the PEG–salt system. Since the PEG–salt systems are more economical than PEG–dextran two-phase systems for protein purification[2], the results should be useful for the selection of the proper phase system.

Although eqn. 3 must at present be considered empirical, a trend is observed when the A values for the proteins are compared with their molecular weights. The A term was found to be both a function of protein molecular weight and the interaction

of the protein with the polymers and water. Examination of the PEG–dextran data in Table I reveals that as protein molecular weight is increased, the A value shows an increasing trend. A similar trend is observed for the PEG–phosphate data where A, in general, becomes more negative as protein molecular weight is increased. However, a definite relationship can not be established between protein partitioning and molecular weight unless the protein–polymer and protein–water interaction can be measured. In addition to these interactions, the protein tertiary structure must be known at each tie of the phase diagram since the interactions will change with the three-dimensional structure of the protein. It also has been shown that the primary structure of a protein has an effect on partitioning behavior in PEG–phosphate system[19].

It should be pointed out that eqn. 3 is applicable over a region of the phase diagram where partitioning experiments are most frequently performed. When using eqn. 3, the question arises as to what happens when $w_1'' - w_1' = 0$, *i.e.*, the plait point is reached, or when $w_1'' - w_1'$ becomes very large, *i.e.*, far removed from the plait point. At the plait point, the composition of the two phases is identical, and the partition coefficient is unity. Therefore, $\ln K/(w_1'' - w_1') = 0/0$, and is undefined. However, according to eqn. 3, it can be seen that $\ln K/(w_1'' - w_1')$ approaches A as $w_1'' - w_1'$ approaches zero. To verify whether this limit is true, partition experiments must be performed close to the plait point. At present, no such data exists in the literature due to the sensitivity of the two-phase systems near the plait point, and the closeness of the K values to unity. Similarly, when $w_1'' - w_1'$ becomes large, $\ln K/(w_1'' - w_1')$ should large. However, partitioning far removed from the plait point is difficult to perform due to the high viscosity of the phases, limited solubility of the protein at high polymer concentrations, and the problem of denaturation and precipitation at the interface.

CONCLUSIONS

Based on the relationship of eqn. 3, an empirical equation has been presented for correlating protein partitioning in PEG–dextran–water and PEG–potassium phosphate–water two-phase systems at pH 7.0. The relationship was found to apply to the seven proteins partitioned in the above two-phase systems. Future work involves applying the equation to other aqueous two-phase systems, and varying environmental parameters such as temperature, pH and ionic composition.

ACKNOWLEDGEMENT

This research was supported by the National Science Foundation Grant Nos. EET-8708839 and CBT-8702912.

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