GENERAL RULE OF PARTITION BEHAVIOUR OF CELLS AND SOLUBLE SUBSTANCES IN AQUEOUS TWO-PHASE POLYMERIC SYSTEMS

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Received 3 July 1978

1. Introduction

Partition in aqueous two-phase polymeric systems is widely used for separation and purification of different biological materials [1]. Recently, we have reported the possibility of using the partition technique as a method for providing quantitative information on the amphiphilic surface properties of the solutes being partitioned [2]. It was shown that the phases of the system differ in the relative hydrophobicity and that this difference is affected by the ionic composition of the system [2].

It is known [1] that cells and other biological particles when partitioned in the commonly used two-phase dextran–polyethylene glycol system are distributed between the top phase of the system and the interface. Recently, we have reported a new ficoll-400–dextran-40 phase system [3] the advantage of which consists in the fact that cells are distributed between the interface and the bottom phase of the system or between the top phase and the interface depending on the ionic composition of the system.

In this communication we examine the influence of the ionic composition of the system on human red cells partition more closely. The partition of human serum albumin, gamma-globulin and a number of sodium alkyl sulfates is also studied and a general rule of partition behaviour of cells and soluble substances in the system in the presence of NaCl and Na-phosphate buffer is established.

2. Materials and methods

2.1. *Materials*

Dextran-40, mol. wt 40 000 (Ferak, West Berlin), ficoll-400 (Pharmacia, Sweden), human serum albumin (ICN, USA) and human gamma-globulin (Central Institute of Haematology and Blood Transfusion, USSR) were used. Sodium alkyl sulfates R_nOSO_3Na ($R_n = C_nH_{2n+1}$, $n = 6, 8, 12$) were prepared as in [4]. All other chemicals were of analytical grade.

Erythrocytes were obtained from blood drawn shortly before use from different donors. Before use the cells were washed 3 times with the Na-phosphate-buffered isotonic saline (pH 7.4) and resuspended in this buffer or in a 0.11 M Na-phosphate buffer (pH 7.4). Osmolarity of both buffers was 310 imOsm as measured with a Knauer osmometer.

2.2. *Phase systems and partition experiments*

The phase systems were prepared as in [1–3]. They contained 14% (w/w) ficoll-400, 10% (w/w) dextran-40 and the amounts of NaCl and Na-phosphate buffer as in the legend to fig.1. The phases were allowed to settle at room temperature (20–23°C) the time for separation was 24 h.

Cells, 7×10^7–7×10^8, in an appropriate buffer solution were delivered into each system. The quantity of cells in the top or bottom phases was either measured with a ZBI Coulter Counter or determined by measuring the $A_{535\ nm}$ of the erythrocytes lysate

in a Gilford spectrophotometer as in [3].

Cell partition was characterized by the partition coefficient, K_{cell}, calculated as the ratio of the quantity of cells in the top phase to the quantity of cells attached to the interface or as the ratio of the quantity of cells at the interface to that of cells in the bottom phase, depending on the partition behaviour in each system. Partition experiments were performed 7 times and the data in fig.1 represent mean values for all these experiments.

The partition coefficient, K, for soluble substances was calculated as the ratio of the sample concentration in the top phase to the sample concentration in the bottom phase [1]. These series of experiments were performed 2–3 times, the values of the partition coefficients were obtained graphically according to [5]. Concentrations of sodium alkyl sulphates were measured by the technique in [6] and the protein concentrations were determined as in [7]. The deviation from the mean K-value did not exceed 3% for all the soluble substances studied.

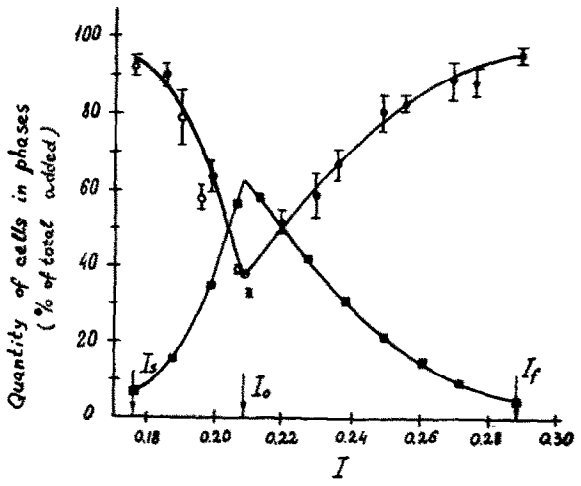

Fig.1. Distribution of human erythrocytes as a function of ionic strength (I) in the system 14% (w/w) ficoll-400, 10% (w/w) dextran-40. The quantity of cells is expressed as % total quantity in the system: (–○–) in the top phase, (–●–) in the bottom phase, (–■–) at the interface. At $I < I_0$ the cells are absent from the bottom phase, and at $I > I_0$ the cells are absent from the top phase. Salt composition at I_s = 0.176 M is 0.15 M NaCl + 0.01 M Na-phosphate buffer (pH 7.4), and at I_f = 0.288 M the composition is 0.11 M Na-phosphate buffer (pH 7.4).

3. Results and discussion

Close study of the dependence of human red cell partition on the ionic composition of the system shows that an increase in the Na-phosphate buffer concentration with a concomitant decrease in the NaCl concentration (to keep the overall salt concentration isotonic) results in a gradual transfer of cells from the top phase to the interface and then into the bottom phase. At the same time the ionic strength increases from 0.18 to 0.29 M. The partition dependence of red cells on the ionic strength of the system is shown in fig.1. The maximum (minimum) corresponds to the ionic strength, I_0, at which the largest portion of the total quantity of cells is concentrated at the interface.

The relationship shown in fig.1 may be described by the characteristic function, K^*_{cell}, expressed as follows:

$$-\ln K^*_{cell} = A + B \cdot I \qquad (1)$$

where $A = -B \cdot I_0$

$$B = \frac{\alpha \cdot \ln(K_{cell,\,o}/K_{cell,\,f}) + \beta \cdot \ln(K_{cell,\,s}/K_{cell,\,o})}{\beta \cdot I_s + \alpha \cdot I_f - (\beta + \alpha) \cdot I_o}$$

$$\alpha = \sqrt{[\ln^2(K_{cell,\,s}/K_{cell,\,o}) + (I_s - I_o)^2]}$$

and

$$\beta = \sqrt{[\ln^2(K_{cell,\,o}/K_{cell,\,f}) + (I_f - I_o)^2]},$$

where I is the ionic strength, I_o, I_s and I_f are the ionic strength values corresponding to the maximum (minimum) in fig.1, to the starting point, i.e., 0.176 M, and to the final point, i.e., 0.288 M, respectively; and $K_{cell,\,o}$, $K_{cell,\,s}$ and $K_{cell,\,f}$ are the partition coefficient values at ionic strengths I_o, I_s and I_f, respectively.

In order to find out if the relationship established between the $\ln K_{cell}$ and ionic strength is a specific feature of the ficoll–dextran system studied here, we have surveyed the literature data [8–12] on the effect of ionic composition on the partition of dif-

ferent cells in the dextran-500–poly(ethylene glycol)-6000 two-phase system. We have found that if the experimental data on the relationship between the partition coefficient, K_{cell}, and the ionic composition of the dextran–polyethylene glycol system are plotted as $\ln K_{cell}$ against I, these data transform into linear relationships given in fig.2. It is not necessary to use the characteristic function (1) in this case since cells are distributed in the dextran–polyethylene glycol system just between the top phase and the interface.

It should be noted that preliminary data obtained by us on the partition behaviour of erythrocytes of different origin, human platelets and leucocytes and influenza virus in the ficoll–dextran system indicate that the relationship given in fig.1 and expressed by equation (1) represents the general rule for biological particle partition dependence on ionic strength, in the presence of NaCl and Na-phosphate buffer. The $K_{cell,\,o}$ and I_o values are specific for a given type of cell.

To determine whether the established rule governs the partition of soluble compounds as well as that of particles, we have studied the partition behaviour of a number of sodium alkyl sulfates and human serum proteins. The relationships between the

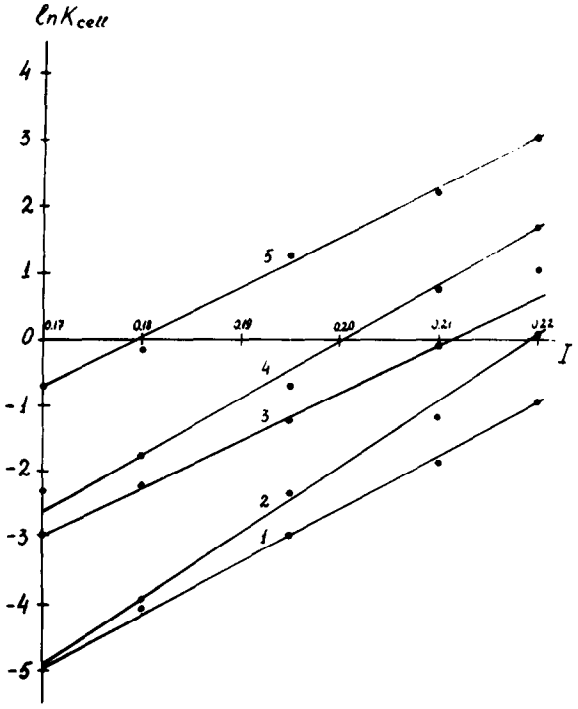

Fig.2. Logarithm of the partition coefficient ($\ln K_{cell}$) for erythrocytes from different species as a function of ionic strength (I) in the system 5% (w/w) dextran-500, 4% (w/w) poly(ethylene glycol)-6000. Data are calculated from [8–12]. Salt composition at I = 0.17 M is 0.15 M NaCl + 0.01 M Na-phosphate buffer (pH 6.8) and at I = 0.22 M is 0.11 M Na-phosphate buffer (pH 6.8). 1, sheep [8]; 2, human [8–10]; 3, dog [11]; 4, rat [12]; 5, mouse [12].

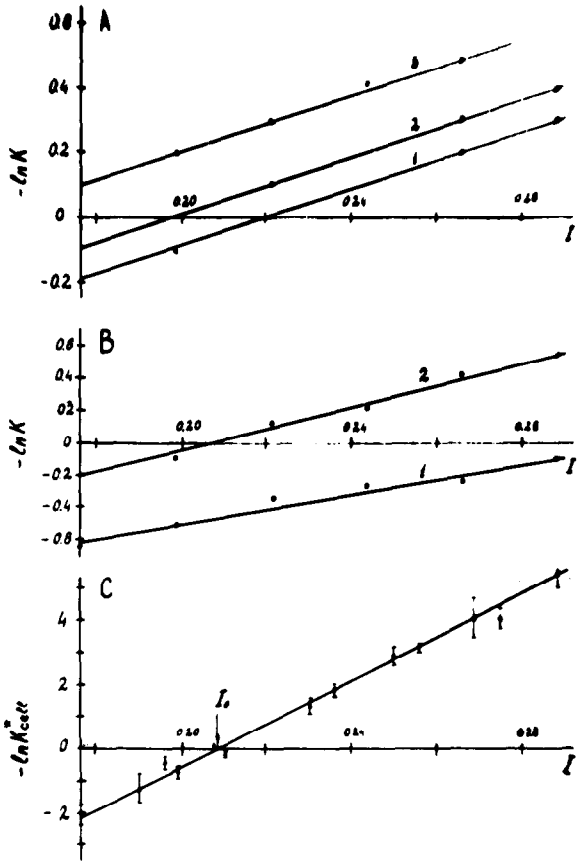

Fig.3. Logarithm of the partition coefficient ($\ln K$) in the ficoll-400–dextran-40 system as a function of ionic strength (I). Polymer and salt composition of the system are in the legend to fig.1. (A) Distribution of sodium alkyl sulfates. 1, R_6OSO_3Na; 2, R_8OSO_3Na; 3, $R_{12}OSO_3Na$. (B) Distribution of human proteins. 1, serum albumin; 2, gamma globulin. (C) Distribution of human red cells represented by the characteristic function ($\ln K^*_{cell}$) according to eq. (1) in the text.

logarithm of the partition coefficients of these compounds and the ionic strength of the system are plotted in fig.3 (A, B). It can be seen that the relationships between ln$K$ of the surfactants and ionic strength are linear and parallel to each other (fig.3A). The effect of ionic strength and alkyl chain length on the partition of sodium alkyl sulfates can be expressed as follows:

$$-\ln K = C + D \cdot I + E \cdot n \qquad (2)$$

where n is the number of CH_2 groups in the alkyl chain of the surfactant molecule, E represents the free energy of transfer of a CH_2 group between the phases, ΔG_{CH_2}, according to equation $\Delta G_{CH_2} = RT \cdot E$, as in [2]; the constant D reflects the influence of ionic strength on the free energy of transfer of the ionic $-OSO_3^-$ group between the phases. The significance of constant C is obscure at present. It should be emphasized that eq. (2) describes the partition of the surfactants in the system at ionic strengths varying from 0.18 to 0.29 M. It should also be noted that the free energy of a CH_2 group transfer between phases amounts to −29.6 cal. mol, i.e., is more than the value found for the same group in the dextran–polyethylene glycol system in the presence of 0.1 M NaCl, −21.3 cal. mol [2].

The distribution of human serum albumin and gamma-globulin are also characterized by the linear relationships between ln$K$ and I as shown in fig.3B. It seems reasonable to suggest that these relationships can be described as follows:

$$-\ln K = C_0 + [\sum_i (D_i \cdot c_i)] \cdot I + E \cdot m \qquad (3)$$

where D_i reflects the influence of ionic strength on the free energy of transfer of the i-th type of an ionic group, c_i is the amount of the i-th groups on the surface of the partitioned macromolecule, m is the total number of CH_2 groups on the macromolecule surface, E is similar to that in eq. (2), the significance of C_0 remains obscure.

The similarity of eq. (1) and eq. (3) seems to indicate that eq. (3) can be used to describe the partition of biological particles as well as that of solutes.

It thus appears that the dependence of the partition of biological particles and soluble substances in the two-phase polymeric system on the ionic strength (or composition) of the system under physiological conditions, in the presence of NaCl and Na-phosphate buffer, it governed by the general rule. We believe that this fact provides new possibilities for the study of the surface properties of cells and other biological particles as well as those of biological macromolecules.

References

[1] Albertsson, P. A. (1971) Partition of Cell Particles and Macromolecules, 2nd edn, Wiley, New York.
[2] Zaslavsky, B. Yu., Miheeva, L. M. and Rogozhin, S. V. (1978) Biochim. Biophys. Acta in press.
[3] Miheeva, L. M., Zaslavsky, B. Yu. and Rogozhin, S. V. (1978) Biochim. Biophys. Acta in press.
[4] Dreger, E. E., Keim, G. J., Miles, G. D., Shedlovsky, L. and Ross, J. (1944) Ind. Eng. Chem. 36, 610–617.
[5] Miheeva, L. M., Zaslavsky, B. Yu. and Rogozhin, S. V. (1976) Bioorg. Khim. 2, 1245–1251.
[6] Hayashi, K. (1975) Anal. Biochem. 67, 503–506.
[7] Bradford, M. M. (1976) Anal. Biochem. 72, 248–254.
[8] Albertsson, P. A. and Baird, G. D. (1962) Exp. Cell Res. 28, 296–322.
[9] Walter, H., Krob, E. J., Garza, R. and Ascher, G. S. (1969) Exp. Cell Res. 55, 57–63.
[10] Walter, H., Krob, E. J. and Garza, R. (1968) Biochim. Biophys. Acta 165, 507–514.
[11] Walter, H. and Selby, F. W. (1967) Biochim. Biophys. Acta 148, 517–524.
[12] Walter, H., Krob, E. J. and Brooks, D. E. (1976) Biochemistry 15, 2959–2964.