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Short Communication

Molecular mass of proteins and their partitioning in aqueous two-phase systems

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Abstract

Twenty proteins with molecular masses between 10^4 and 10^6 were partitioned in aqueous dextran–poly(ethylene glycol) two-phase systems with either 0.1 *M* KCl or 0.1 *M* KCl plus 3 *M* NaCl. The partition coefficient of all proteins were higher in the system with 0.1 *M* KCl plus 3 *M* NaCl than in the system with 0.1 *M* KCl alone. The ratios between the partition coefficients in the two systems were strongly correlated with the molecular masses of the proteins. This fact may be used for analytical as well as preparative purposes.

1. Introduction

When aqueous solutions of two polymers are mixed, two immiscible phases are formed. Cells, cell organelles, proteins and nucleic acids can be introduced into such systems without being damaged, and will assume a characteristic distribution between the two phases and/or their interface, depending on the properties of the phase system and of the partitioned substance. Aqueous two-phase partitioning can be used for a variety of analytical and preparative purposes in biochemistry, cell biology and biotechnology (for reviews, see refs. 1–4).

In aqueous dextran–poly(ethylene glycol) two-phase systems, proteins partition between the two phases. Their partition coefficients depend on net charge, surface hydrophobicity and specific interactions with the polymers ("affinity partitioning"). The influences of these parameters on protein partitioning have been studied in great detail (for reviews, see refs. 5 and 6). The influence of the molecular mass of proteins has been less well characterized. It has been a general finding that a protein tends to partition more one-sided, the larger its molecular mass. However, there are many exceptions to the rule, and no obvious correlation between molecular mass and partition coefficient has been established so far [5–8].

In the present communication, I describe a strong correlation between protein molecular mass and the ratio between the partition coefficient in phase systems with different salt concentrations.

2. Materials and methods

2.1. Materials

The following proteins were purchased from the indicated sources: alcohol dehydrogenase from baker's yeast (Sigma A 7011), rabbit muscle aldolase (Sigma A 7145), equine apoferritin (Sigma A 3641), bovine α-chymotrypsin (Sigma

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C 7762), bovine catalase (Sigma C 3155), chicken conalbumin (Sigma C 0755), equine cytochrome *c* (Sigma C 7752), equine ferritin (Sigma F 4503), porcine heart fumarase (Sigma F 1757), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (Sigma G 2267), bovine haemoglobin (Sigma H 2500), bovine β-lactoglobulin (Sigma L 0130), whale myoglobin (Sigma M 0380), chicken ovalbumin (Sigma A 2512), porcine pepsin (Boehringer-Mannheim), bovine ribonuclease A (Sigma R 4875), bovine serum albumin (Boehringer-Mannheim), bovine thyroglobulin (Sigma T 1001), bovine trypsin (Sigma T 8003), bovine pancreas trypsin inhibitor (Sigma A 1153) and soybean trypsin inhibitor (Boehringer-Mannheim). Dextran T500 (lot No. FA 13748) was from Pharmacia. Poly(ethylene glycol) 6000 was from Merck. All other chemicals were of analytical grade.

Before use, the proteins were dialysed extensively against a suitable buffer.

2.2. Two-phase systems and partitioning

Proteins were partitioned in 1 ml dextran T500–poly(ethylene glycol) 6000 two-phase systems, containing 6.25% (w/v) of each of the polymers, 6 m*M* Tris, 2 m*M* K_2HPO_4, pH 8.1, 10% (v/v) glycerol and salts as indicated below. Each phase system contained 1–5 mg protein. Partitioning was performed at 0–4°C. Each phase system was vortex mixed for 15 s, and centrifuged for 1 min at 10 000 *g* in order to accelerate separation of the bulk phases. The partition coefficients were calculated as the ratios between the absorbances at 280 nm of diluted aliquots of the top and the bottom phases. Each partition coefficient given below is the geometric mean of at least four determinations.

Phase diagrams were constructed by the cloud point method [9]. The concentrations of KCl plus NaCl in the phases were determined by measuring the conductivity of diluted aliquots of the phases and comparison to a standard curve.

The molecular masses, isoelectric points and the numbers of subunits of the proteins were those given in biochemistry textbooks or in refs. 10–13.

3. Results

A number of proteins were partitioned in aqueous dextran–poly(ethylene glycol) two-phase systems with 0.1 *M* KCl; with 0.1 *M* KCl plus 0.9 *M* NaCl; with 0.1 *M* KCl plus 3 *M* NaCl; and with 0.22 *M* KCl plus 0.26 *M* Li_2SO_4. Fig. 1 shows double-logarithmic plots of the partition coefficients *versus* the molecular masses. In the case of oligomeric proteins, the molecular masses of the oligomers were used. It is seen that the proteins tended to partition more one-sided, the larger their molecular mass, but the data points are quite scattered, particularly in the molecular mass range 10^4–10^5. For all tested

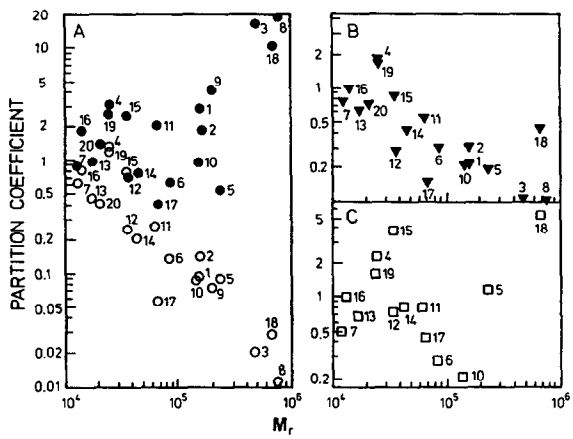

Fig. 1. Partition coefficients of proteins in phase systems with (A) 0.1 *M* KCl (○) and 0.1 *M* KCl + 3 *M* NaCl (●); (B) 0.1 *M* KCl plus 0.9 *M* NaCl (▼); (C) 0.22 *M* KCl plus 0.26 *M* Li_2SO_4 (□). The partition coefficients are plotted double-logarithmically *versus* their molecular masses. The partitioned proteins were: 1 = alcohol dehydrogenase (tetramer, M_r 150 000); 2 = aldolase (tetramer, M_r 156 000); 3 = apoferritin (24-mer, M_r 460 000–490 000); 4 = α-chymotrypsin (M_r 24 300); 5 = catalase (tetramer, M_r 230 000); 6 = conalbumin (M_r 84 000); 7 = cytochrome *c* (M_r 12 400); 8 = ferritin (24-mer, M_r 700 000–800 000); 9 = fumarase (tetramer, M_r 194 000); 10 = glyceraldehyde-3-phosphate dehydrogenase (tetramer, M_r 142 800); 11 = haemoglobin (tetramer, M_r 62 000); 12 = β-lactoglobulin (dimer, M_r 35 000); 13 = myoglobin (M_r 17 000); 14 = ovalbumin (M_r 43 000); 15 = pepsin (M_r 34 700); 16 = ribonuclease A (M_r 13 700); 17 = serum albumin (M_r 66 300); 18 = thyroglobulin (dimer, M_r 669 000); 19 = trypsin (M_r 24 000); 20 = soybean trypsin inhibitor (M_r 20 100). Partitioning of a few of the proteins was not performed in the systems with 0.1 *M* KCl plus 0.9 *M* NaCl and with 0.22 *M* KCl plus 0.26 *M* Li_2SO_4.

proteins, the partition coefficient in systems with KCl plus NaCl increased with the total salt concentration. The direction of the changes of the partition coefficients when going from systems with 0.1 *M* KCl plus 0.9 *M* NaCl to systems with 0.22 *M* KCl plus 0.26 *M* Li_2SO_4 (which have identical ionic strengths) is that expected from a protein net charge-dependent influence of the identity of the salts [5,14], considering the p*I* values and the pH of the phase systems (an increase for negatively charged proteins, a decrease for positively charged proteins).

Fig. 2 shows a double-logarithmic plot of the *ratio* between the partition coefficients in the phase systems with 0.1 *M* KCl plus 3 *M* NaCl and 0.1 *M* KCl *versus* the molecular masses. It is seen that there was a strong correlation between

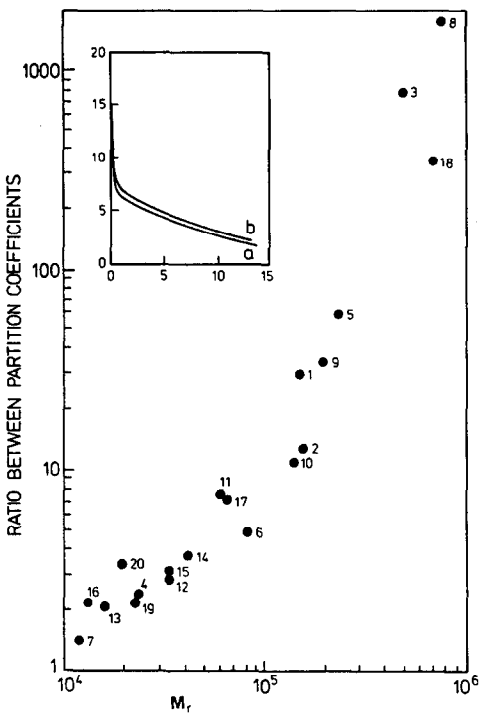

Fig. 2. Ratio between the partition coefficients of proteins in phase systems with 0.1 *M* KCl plus 3 *M* NaCl and 0.1 *M* KCl, plotted double-logarithmically *versus* their molecular masses. Numbering of partitioned proteins as in Fig. 1. The inset shows phase diagrams for systems with 0.1 *M* KCl (a) and 0.1 *M* KCl plus 3 *M* NaCl (b). The dextran T500 percentage is given on the abcissa, and the poly(ethylene glycol) 6000 percentage on the ordinate.

this ratio and the molecular mass. The ratio increased from 1–2 for proteins with molecular mass $1 \cdot 10^4$–$2 \cdot 10^4$ to more than 1000 for the largest proteins tested, having molecular masses of $7 \cdot 10^5$–$8 \cdot 10^5$. There was a nearly linear relationship between the ratio and the molecular mass in the range 10^4–10^5, although the correlation was not linear over the entire molecular mass range. The linear correlation coefficient in that range was 0.886, considerably better than those found for the direct plots of the partition coefficients *versus* molecular masses (−0.735 and −0.365 for 0.1 *M* KCl and 0.1 *M* KCl plus 3 *M* NaCl, respectively). The ratio did not decrease below 1 for substances with molecular masses smaller than 10^4. For instance, the ratio was 1.15 for bovine pancreas trypsin inhibitor (M_r 6500) and 1.19 for the steroid triamcinolone acetonide (M_r 434). A similar correlation existed for the ratios between the partition coefficients in systems with 0.1 *M* KCl plus 1 *M* NaCl and with 0.1 *M* KCl (data not shown).

With 0.1 *M* KCl, the volume of the top phase constituted 64% of the total volume; with 0.1 *M* KCl plus 3 *M* NaCl, it constituted 44% of the total volume. The phase diagrams were different for systems with 0.1 *M* KCl and for systems with 0.1 *M* KCl plus 3 *M* NaCl. With a given polymer composition, the high salt concentration moves the system closer to critical point (Fig. 2). The partition coefficient of KCl plus NaCl was not measurably different from 1 in any of the phase systems.

4. Discussion

Formally, the partition coefficients of proteins in aqueous two-phase systems may be split into several terms [1,2]:

$$\ln K = \ln K_{el} + \ln K_{hphob} + \ln K_{hphil} + \ldots$$

where K_{el}, K_{hphob}, K_{hphil} etc. represent the contribution to the partition coefficient from electrostatic, hydrophobic, hydrophilic etc. interactions between the protein and the phases. The strategy that has been employed for characteriza-

tion of a protein by aqueous two-phase partitioning consists in changing the composition of the phase system in a way believed to alter only one type of interaction between the protein and the phases. To the extent that each type of interaction can be varied independently of the others, the properties of the protein can be assessed from the variations of its partition coefficient in response to the changes in the composition of the phase system. This principle has for instance been employed for assessing (1) protein surface hydrophobicity, by measuring the change of partition coefficient of the protein in response to substitution of poly(ethylene glycol) with poly(ethylene glycol) esterified with fatty acids; (2) protein net charge, by measuring the change of partition coefficient of the protein in response to changes of the identity of the salts in the system (for instance, substituting NaCl with Li_2SO_4), which influences the interfacial potential [5]. Thus, it is the *ratio* between the partition coefficients at different partitioning conditions that is useful.

In the present investigation, the *ratio* between the partition coefficients of proteins at two different salt concentrations (0.1 *M* KCl plus 3 *M* NaCl and 0.1 *M* KCl) was shown to be correlated with their molecular masses. In contrast, there was no correlation between the absolute values of the partition coefficients and the molecular masses. The contribution from protein net charge was expected to be the same in systems with 0.1 *M* KCl plus 3 *M* NaCl and with 0.1 *M* KCl, and this was verified by finding that the partition coefficients of serum albumin and ovalbumin varied identically with pH in the two systems (data not shown). Thus, the contribution to the partition coefficient from protein net charge was removed by taking the ratio between the partition coefficients. But there was a lack of correlation of the molecular masses and the absolute values of the partition coefficients in systems with KCl plus Li_2SO_4 as well as in systems with only KCl and NaCl; partitioning in the former is almost insensitive to protein net charge [14]. It should also be noted that a lack of correlation between the molecular masses and the partition coefficients at the isoelectric points were previously reported for a group of haemo- and non-haemoproteins [8]. Therefore, the lack of correlation between the molecular masses and the absolute values of the partition coefficients is not only due to the contribution from protein net charge, and charge-*in*dependent contributions to the partition coefficient, except that from protein size, are also removed by taking the ratio between the partition coefficients at the two salt concentrations.

An increase of protein partition coefficients with increasing NaCl concentrations was first reported by Albertsson [1]. However, the physico-chemical basis of this salt effect is not understood. Generally, the effect of salts on the interactions between the polymer components is expected to have a pronounced effect on the composition of the phases, which may in turn influence protein partition coefficients; in addition, the presence of salts can influence the protein partitioning by modifying the intermolecular forces between the protein and the polymer coils [6]. In agreement with previous findings [1], the increase of the total salt concentration from 0.1 to 3.1 *M* was found here to change the composition of the phases. A number of models (based on the so-called osmotic virial-expansion approaches and lattice model approaches) have been developed recently to predict protein partitioning by physico-chemical calculations. These models do predict that changes of phase composition will influence protein partition coefficients in a way increasing with protein size, but they do not predict an elimination of other contributions (like interactions between polymer and protein) to the partition coefficient by taking the ratio between the partition coefficient at different phase compositions [4,6,15–17]. The most promising approach in the present context seems to be the scaling-thermodynamic formulation of Abbott *et al.* [18–21]. This theory emphasizes physical exclusion between polymer and protein as well as weak attractive forces between polymer and protein. Considering phases of individually dispersed polymers (as opposed to entangled polymer network), this theory was able to account qualitatively for the correlation between protein size

and the *ratio* between the partition coefficients in systems with poly(ethylene glycol)s of different molecular masses [22,23], in the absence of changes in the polymer concentrations of the phases. Although the effect of using poly(ethylene glycol)s of different molecular masses was considerably smaller than the salt effect described here, it seems possible that the scaling-thermodynamic theory may be developed to account for the present observation.

The finding reported here may be quite useful for an estimation of molecular masses of proteins. It can be employed to proteins in crude mixtures, provided that an assay for the protein is available. Aqueous two-phase partitionings are performed under non-denaturing conditions, so the method can also be employed to oligomeric proteins, provided that the oligomeric structure does not change with the salt concentration. The method is extremely rapid, since the partitioning can be performed in a few minutes. Sequential partitionings at different salt concentrations may be useful for preparative fractionations of proteins on the basis of molecular mass.

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