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Bovine serum albumin partitioning in an aqueous two-phase system: Effect of pH and sodium chloride concentration

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Abstract

The partitioning of bovine serum albumin (BSA) in a polyethylene glycol 3350 (8% w/w)–dextran 37 500 (6% w/w)–0.05 *M* phosphate aqueous two-phase was investigated at different pHs, at varying concentrations of sodium chloride at 20°C. The effect of NaCl concentration on the partition coefficient of BSA was studied for the PEG–dx systems with initial pH values of 4.2, 5.0, 7.0, 9.0, and 9.8. The NaCl concentrations in the phase systems with constant pH value were 0.06, 0.1, 0.2, 0.3, and 0.34 *M*. It was observed that the BSA partition coefficient decreased at concentrations smaller than 0.2 *M* NaCl and increased at concentrations greater than 0.2 *M* NaCl for all systems with initial pHs of 4.2, 5.0, 7.0, 9.0, and 9.8. It was also seen that the partition coefficient of BSA decreased as the pH of the aqueous two-phase systems increased at any NaCl salt concentration studied.

Keywords: Partitioning; Aqueous two-phase systems; pH; Bovine serum albumin; Sodium chloride

1. Introduction

The extraction of biomolecules using aqueous two-phase systems is important since it allows the separation and purification of these substances in biocompatible surroundings. To form the two aqueous phases, aqueous solutions of two polymers, usually polyethylene glycol (PEG) and dextran, are required.

The general properties of aqueous two-phase systems have been studied by several researchers, see for instance Refs. [1,2] and the references cited therein. However, the mechanism governing the partition of biological materials is still not well understood. The observed partition coefficient is a result of van der Waals, hydrophobic, hydrogen bond, and ionic interactions of the biomolecules with the surrounding phase. Therefore, the partition coefficient is influenced by many factors, including the concentrations and molecular weights of PEG and dextran, type and concentration of added salts, temperature and pH.

A change in pH and addition of salts are often used to alter the partitioning of a biomolecule. The partition coefficient K of a biomolecule varies exponentially with the electrochemical potential difference between the phases and the net charge of the partitioned biomolecule [1], and therefore can be postulated as

$$\ln K = \ln K_0 + \frac{FZ\,\Delta\Phi}{RT} \quad (1)$$

where K_0 is the value of this coefficient in the absence of an electrochemical potential, F is the Faraday constant, Z is the charge on the biomolecule, T is temperature, and $\Delta\Phi$ is the potential created by the salt in the system.

Since pH determines the net electrical charge on biomolecules, it can play an important role in effecting the partitioning. A plot of ln K versus the

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charge Z should be a straight line whose slope is proportional to $\Delta\Phi$. However, a change in pH may also affect $\Delta\Phi$, the composition of the phases, the interaction between the ions of the salt and the protein, and the interactions between the polymers and the protein. Therefore, deviations from the linear behaviour are common [3].

As it has been observed by some researchers [1,4], many salts in the PEG–dextran two-phase system are slightly partitioned into the dextran-rich bottom phase. This results in an electrostatic potential difference between the two phases even though each phase is electrically neutral. Reitherman et al. [5], Brooks et al. [6], and Zaslavsky et al. [7] measured the interfacial potential differences for systems containing different inorganic salts. The measured potential differences were in the range of 1 to 3 mV for the PEG–dx systems studied. As a result of such a potential, negatively charged substances are preferentially partitioned into the top phase and positively charged substances into the bottom phase.

It appears likely that the specific interactions between the salts and proteins, in addition to the potential difference created by the salt, are responsible for the effect of different salts on protein partitioning. Also, different salts affect the water structure and hydrophobic interactions differently, and as salt concentration increases, the partition coefficient of a biomolecule with a large hydrophobic region or surface in its structure will change due to its interaction with the surrounding phases [8].

Albertson [1] reported the effect of NaCl concentration on the partition coefficient of ovalbumin in an aqueous two-phase system of PEG 4000 (8% w/w)–dx 500 (8% w/w)–0.5 m*M* Na phosphate (pH = 6.9) at 20°C. The ovalbumin partition coefficient has been observed to decrease at concentrations smaller than 0.023 *M* NaCl and to increase at concentrations greater than 0.023 *M* NaCl in this system. Walter et al. [2] gave the partition coefficients of five proteins (phycocyanin, barely albumin, phycoerythrin, ceruloplasmin and serum albumin) in an aqueous two-phase system of PEG 8000 (4% w/w)–dx 500 (7% w/w)–10 m*M* KPB (pH = 6.8) at 20°C. The minimum K values for all proteins were obtained in this specific aqueous two-phase system containing NaCl salt with concentrations in the range 0.1–0.2 M.

In our work we use BSA as a model protein to examine the effects of pH and concentration of NaCl salt concentration on partitioning in aqueous PEG–dx two-phase systems.

2. Experimental

2.1. Materials

Dextran 37 500 (Lot # 44H0568), and PEG 3350 (Lot # 44H0122) and bovine serum albumin (Lot # 57H1090) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Crystallized and lyophilized BSA was used without purification to prepare a stock solution, by mass, with an accurately known concentration (10%). Concentrations of PEG and dextran stock solutions were 25% polymer by mass. Different stock solutions of K_2HPO_4 and KH_2PO_4 were used to prepare the aqueous two-phase systems at different pHs.

2.2. Partition coefficient measurements

Aqueous two-phase partitioning experiments were performed at 20 °C by weighing and mixing the phase forming polymer stock solutions with stock solutions of BSA, buffer, and salts. Distilled water was then added to obtain the final mass (10 g). The systems were well mixed by a vortex mixer and left in a water bath (Memmert, Germany) overnight. Samples were carefully withdrawn from the top phase and to take samples from the bottom phase the bottom of the tubes were pinched. For the determination of BSA concentration, a sample withdrawn from each phase was diluted with a known amount of distilled water and its ultraviolet absorbance was measured in a dual-beam spectrophotometer (Hach DR/4000 UV–VIS, Loveland, Colorada, USA) at 280 nm. An identically diluted solution of the corresponding phase from a system containing no BSA was used as a blank.

3. Results and discussion

Bovine serum albumin were partitioned in the 25 aqueous two-phase systems, in which all of them

Table 1
Partition coefficients of BSA in PEG 3350 (8% w/w)–dx 37 500 (6% w/w)–water system at 20°C at different pHs and NaCl molarities

NaCl pH	0.06 M	0.1 M	0.2 M	0.3 M	0.34 M
4.2	0.058	0,062	0,040	0,045	0,064
5.0	0.046	0.038	0.031	0.037	0.049
7.0	0.038	0.030	0.022	0.031	0.036
9.0	0.030	0.027	0.020	0.026	0.030
9.8	0.028	0.021	0.018	0.023	0.024

differ in pH and NaCl salt concentration (Table 1). The systems were prepared at pHs of 4.2, 5.0, 7.0, 9.0, and 9.8, with different salt concentrations of 0.06, 0.1, 0.2, 0.30 and 0.34 M.

Fig. 1 shows partition coefficients as a function of pH at constant salt concentrations. We have seen from this figure that, as BSA became more negative (pI=4.82) by increasing the pH of the systems at constant salt concentration, the partition coefficient decreased. From this observation we may conclude that the electrochemical potential difference which drives polyanions to the top phase in PEG–dx systems is not the dominant factor in the partitioning of BSA in the systems we have studied.

To confirm this conclusion we have also studied the partitioning of negatively charged BSA at pH=7 at different phosphate concentrations. Table 2 shows our results. We have observed that an increase in phosphate concentration caused a decrease in the partition coefficient of negatively charged BSA at this pH. If the electrochemical potential effect had been dominant, the BSA partition coefficient should have increased significantly on adding potassium phosphate. This suggests that factors other than electrical charge play an important role in BSA partitioning.

Table 2
Effect of phosphate buffer on BSA partition coefficient (8%PEG–6%dx, pH=7)

Phosphate concentration (M)	Partition coefficient of BSA
0.0	0.047
0.05	0.043
0.10	0.041

In Fig. 2 the dependence of the BSA partition on the concentration of NaCl at constant pH values of 4.2, 5.0, 7.0, 9.0, and 9.8 is shown. The partition coefficient of the BSA decreases with increasing NaCl concentration in the small salt concentrations (up to 0.2 M NaCl concentration) as has been observed with other proteins also [1,9]. In this range of salt concentration, interactions of the protein molecules with the ions and the dextran molecules in the dextran-rich phase increased. Above 0.2 M salt concentration, higher partition coefficients of BSA at different pHs have been observed.

Zaslavsky et al. [10] studied the partition behaviour of a number of ionic and non-ionic substances in the polyethylene glycol–dextran system. The conclusion they have made is the partition of ionic amphiphiles depends on the relative hydrophobicity of the compounds as well as their charge.

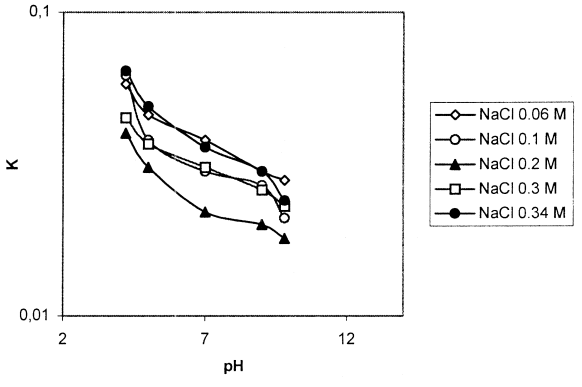

Fig. 1. Effect of pH on the partitioning of BSA in PEG 3350 (8% w/w)–dx 37 500 (6% w/w)–water system at 20°C.

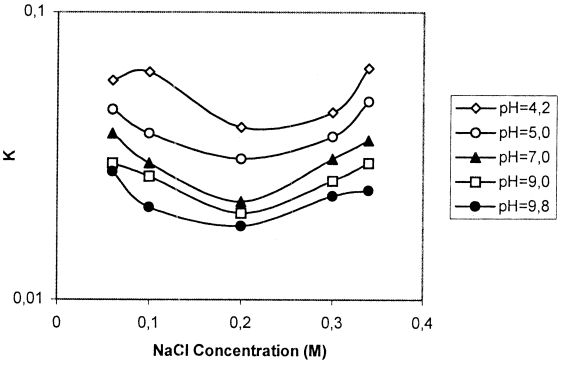

Fig. 2. Effect of NaCl concentration on the partitioning of BSA in PEG 3350 (8% w/w)–dx 37 500 (6% w/w)–water system at 20°C.

It is shown that at salt concentrations up to 0.1 *M* NaCl the charged solute partition is determined by its charge as well as its relative hydrophobicity, in the presence of 0.1–0.2 *M* NaCl the substance distribution is highly dependent on its charge and slightly on its lipophilicity. At the salt concentrations above 0.2 *M* the solute partition is determined just by its hydrophobic character and seems to be totally independent of its charge.

Franco et al. [11] analyzed the effect of NaCl to PEG–dx systems in terms of the hydrophobicity difference between the phases and their ability to promote hydrophobic interactions between the protein surface and PEG molecules.

From our study, we could make a similar conclusion for partitioning in systems containing salt with concentrations higher than 0.2 *M*. Our results suggest that at salt concentrations above 0.2 *M* NaCl, PEG, being more hydrophobic in nature, tends to strongly interact with the non-polar regions of BSA more and more.

Acknowledgements

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