

## The Effect of pH Difference Between Two Phases on the Partition of Lysozyme in Aqueous Two-Phase System

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**Abstract:** In the investigation of effect of KSCN on the partitioning of lysozyme in PEG2000/ammonium sulfate aqueous two-phase system, it was found that the KSCN could alter the pH difference between the two phases, and thus affect the partition of lysozyme. The relationship between partition coefficients of lysozyme and pH differences between two phases was discussed.

**Keywords:** Aqueous two-phase, polyethylene glycol 2000, lysozyme.

Aqueous two-phase systems (ATPS) are formed when two aqueous solutions of hydrophilic polymers or a polymer and a salt are mixed at high concentration. For the charged proteins, Albertsson has developed the classical model for the electrochemical partitioning in ATPS:

$$\ln K_p = \ln K_0 + (z_p F / RT) \Delta \phi \quad (1)$$

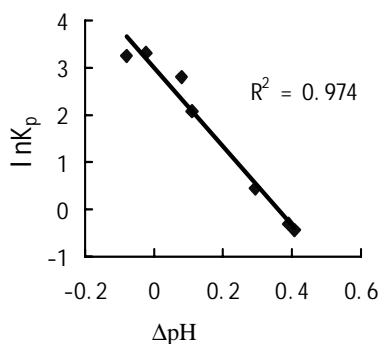
Here  $K_p$  denotes the partition coefficient of proteins,  $\ln K_0$  includes all the other factors affecting protein partitioning, and  $(z_p F / RT) \Delta \phi$  is the electrostatic term as a product of protein surface charge  $z_p$  and  $\Delta \phi$ , the electrostatic potential difference between the two phase. However, it has been pointed out that  $\Delta \phi$  in the equation should be the pure electrostatic potential difference<sup>2</sup>, which differs from the potential difference measured by two Ag/AgCl electrodes, and some discrepancy have been observed<sup>3</sup>.

The addition of some salt can alter the partition coefficients of charged proteins in ATPS<sup>1</sup>. Previous studies of salt effect on protein partitioning have focused on changes in the potential differences, *i.e.*  $\Delta \phi$  in Eq. (1), and the investigations were mostly confined to the polymer/polymer systems. In this study, we investigated the effect of potassium thiocyanate on the partitioning of lysozyme in the polyethylene glycol (PEG) 2000/ammonium sulfate system. The partition coefficient ( $K_p$ ) of lysozyme could be enhanced by potassium thiocyanate near neutral pH. It was found that the enhancement of partition coefficient of lysozyme is related to the effect of potassium thiocyanate on the pH difference ( $\Delta \text{pH}$ ) between the two phases. The linear relationship between  $\ln K_p$  and  $\Delta \text{pH}$  was observed.

The stock solutions of PEG 2000 (30% w/w),  $(\text{NH}_4)_2\text{SO}_4$  (30% w/w), 6 mol/L KSCN and 10 mg/mL lysozyme were prepared respectively by dissolving certain amount of reagents in distilled water. The concentrations of lysozyme were determined with Bradford method<sup>4</sup> and the partition coefficient was defined as ratio of the absorbances of top phase and bottom phase at 595 nm. The  $\Delta\text{pH}$  equals pH of the top phase ( $\text{pH}_t$ ) minus that of the bottom phase ( $\text{pH}_b$ ).

At near neutral pH, lysozyme ( $\text{pI}=11.0$ ) has positive charge, potassium thiocyanate can increase the partition coefficient of lysozyme in PEG 2000/ammonium sulfate system. In our experiments, it was found that the addition of potassium thiocyanate in PEG2000/ammonium sulfate could alter the pH difference between the top and bottom phase. We correlated natural logarithm of the partition coefficient ( $\ln K_p$ ) of lysozyme and the  $\Delta\text{pH}$ , a linear relationship was obtained (**Figure 1**). The  $\Delta\text{pH}$  represents the distribution of protons between the two phases. The charged substance is nothing but protonating or deprotonating, so  $\Delta\text{pH}$  can be employed as the measurement of electrostatic driving force for partitioning of the charged proteins.

**Figure 1.** The relationship between  $\ln K_p$  and  $\Delta\text{pH}$



### Acknowledgments

This work is supported by State Key Project of Fundamental Research (G1998061302) and the National Natural Science Foundation of China (Grant NO. 29771028, 29801004)

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Received 25 November 1999

Revised 27 January 2000