

Effect of Dissolved CO₂ on Protein Solubilization in Reverse Micelle

Hai Fei ZHANG, Bu Xing HAN*, Jie LU, Guan Ying YANG, Hai Ke YAN

Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080

Abstract: Effect of dissolved CO₂ on bovine serum albumin (BSA) solubilization in the reverse micelles of sodium bis (2-ethylhexyl) sulfosuccinate (AOT) in 2,2,4-trimethylpentane (iso-octane) has been studied at 308.2 K. It was found that BSA can be precipitated completely by CO₂ while the AOT and water remain in the iso-octane continuous phase. This opens up a possibility for recovery of pure protein solids directly from reverse micellar solutions.

Keywords: Compressed CO₂, protein, precipitation, reverse micelle.

Surfactants can form reverse micelles in apolar solvents at suitable conditions. The inner polar cores of the reverse micelles can not only host significant amount of water, but also solubilize polar solutes of different sizes, such as proteins¹. Reverse micelles have many applications in biotechnology. Different methods have been used to control protein solubilization in reverse micelles in extraction and reextraction processes, such as changing the ionic strength and/or pH of the aqueous phases contacting with the organic solvent continuous phase², and forming gas hydrates in the reverse micelles at lower temperatures³.

The purity of CO₂ was 99.995% supplied by Beijing Analytical Instrument Factory. The iso-octane was A. R. grade produced by Beijing Chemical Plant. The crystalline BSA was supplied by Sigma with a purity of >98%. The surfactant AOT, 99% pure, was also purchased from Sigma.

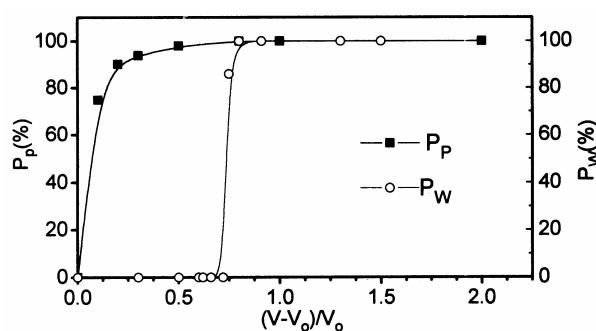
The preparation of the solution was done by dissolving AOT in iso-octane. Desired amount of aqueous solution of BSA of suitable concentration was then added to the AOT-iso-octane solution. CO₂ was dissolved into the solution in the cell at suitable pressure. The concentration of the BSA in the solution was determined by an UV-Vis spectrophotometer (Beijing Instrument Company, Model UT-1201) and used the absorption band at 278 nm. The temperature-controlled high-pressure UV cell was the same as that used previously⁴.

Figure 1 shows the dependence of the percentage of precipitated BSA(P_p) on the volume expansion of the solution caused by the dissolved gases at 308.2 K. The concentration of BSA in the gas-free solution was 1.0 mg/ml. The molar water-to-AOT ratios W_o ([H₂O]/[AOT]) in the system was 20. V_o and V are respectively the volume of gas-free solution and that of the expanded solution, and P_p is defined as following:

$$P_p = 100\% \times (m_o - m) / m_o = 100\% \times (C_o V_o - CV) / C_o V_o \quad (1)$$

where m_0 stands for the mass of BSA in the gas-free solution, m is the mass of BSA in the solution after expansion. C_0 and C denote the concentration of BSA in the gas-free solution and that in the expanded solution, respectively.

Figure 1. Dependence of percentage of precipitated BSA (P_p) and water (P_w) on the volume expansion of the solution.



The BSA solubilized begins to precipitate at very low $(V-V_0)/V_0$, and is precipitated completely when $(V-V_0)/V_0$ is higher than 0.5, as can be known from **Figure 1**. Precipitation of water from the reverse micelles induced by the dissolved gas was measured using a high pressure optical cell in which the volume of the precipitated water and that of the solution can be seen quantitatively and clearly. The dependence of the percentage of precipitated water (P_w) on the $(V-V_0)/V_0$ determined is also shown in **Figure 1**. From the data in **Figure 1** it can be known that all of the solubilized BSA is precipitated before the water in the reverse micelles begin to come out. Our UV measurements showed that precipitation of AOT will not occur if $(V-V_0)/V_0$ is lower than 3. Thus BSA precipitation may be mainly caused by variation of the interaction between BSA and the polar head of AOT. Some of the CO_2 molecules in the water pools of the reverse micelles are ionized, which shields the electrostatic interaction between the BSA and the head groups of AOT and the BSA is precipitated.

Acknowledgments

The authors are grateful to the National Natural Science Foundation of China for the financial support (29725308, 29633020).

References

1. L. Malde, P. Walde, G. Zampieri, E. Battistel, Q. Peng, E. Trotta, M. Maestro, P. L. Luisi, *Colloids and Surfaces*, **1988**, 30, 193.
2. D. G. Hayes, *Biotechnol. and Bioeng.* **1997**, 53, 583.
3. K. Nagahama, H. Noritomi, A. Koyama, *Fluid Phase Equilibria*, **1996**, 116, 126.
4. J. Lu, B.X. Han, H. K. Yan, *Ber. Bunsenges. Phys. Chem.*, **1998**, 102, 695.

Received 20 August 1998