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# Aggregation of the Adsorbed Proteins—in Solution or on the Surface?

Tong Wen XU*

**Abstract**: The adsorption of bovine serum albumin (BSA) on porous polyethylene (PE) membrane was studied based on adsorption and desorption measurements as well as FTIR analysis. A different mechanism was proposed which showed that a critical concentration existed in the adsorptional process. Below this concentration, the adsorption seems to be conducted in a normal side-on way; above this concentration, the adsorption is in an aggregation way.

**Keywords:** Bovine serum albumin, adsorption, desorption, FTIR.

## Introduction

Protein adsorption to solid surface, particularly to the membrane filters and the subsequent fouling of devices is a major obstacle to the application of the membrane technologies[1]. So study on adsorption mechanism of proteins is of great significance for treating with such fouling problems. To date, quite a few subjects have been developed on the interaction of serum proteins with synthetic surfaces such as polymeric membranes PE, PTFE, CA, PVDF, PS, respectively, in the literatures[1-3]. It is generally accepted that the adsorption conforms or conforms roughly to a Langmuir isotherm and the process is nearly irreversible in a way of side-on at low surface coverage and partly irreversible in a way of end-on at high surface coverage. However, it seems that no more attempts have been made to clarify the aggregation of proteins due to the adsorption process. Therefore, we try to investigate the aggregation of protein molecules during the adsorption process and clarify the contribution from aggregation on the surface or that in the solution based on the preliminary adsorption and desorption measurements as well as FTIR analysis.

## Experimental

Bovine Serum Albumin (BSA, Lot 113H0247,water content = 1.7%, molecular weight 69000, isoelectric point (IEP) = 4.9) was obtained from Sigma Chemical Co. PE membrane (H 2200) was provided from Asahi Kasei Co. (Japan) with a thickness 200 um, porosity 69% and specific area 18 $m^2 . g^{-1}$. All the chemicals were purchased from Wako Pure Chemical Industries Ltd (Japan). All solutions were prepared using Milli-Q water (Waters) and were sterilized using Sartorius Minisart NML disposable

* E-mail: twxu@ustc.edu.cn

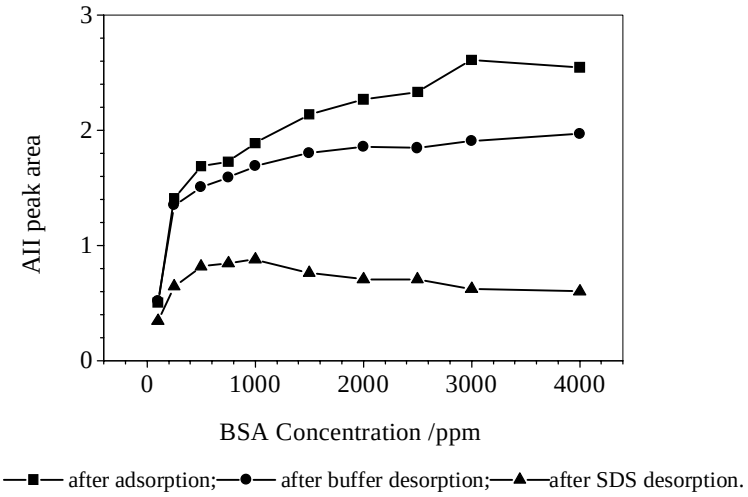
filter units. Adsorption and desorption experiments were conducted in a conventional batch-wise way in an incubate box (M-230F Taitec) at 25ºC. The desorptions were conducted in two ways: one is desorbed with pH 5 acetate buffer solution (buffer desorption) and another is desorbed with 1% sodium dodecyl sulfate (SDS desorption). The protein content in the supernatant was determined at 280 nm by UV adsorption (Shimadzu UV-160A) and the samples were frozen-dried for precise determination of the adsorbed amount by a Nic-Plan microscope FTIR instruments (with a Magna-IR System 560 and a Nicolet 0.25 MCT-A IR detector, Nicolet Instrument Co., Madsion, WI).

**Results and Discussion**

The adsorption isotherm at pH = 5.05 (IEP of BSA), which behaves the same Langmuir isotherm at low concentration as the most observations, was fitted to a Langmuir-type equation to give a saturated amount about 3.88 $mg \cdot m^{-2}$ for monolayer adsorption at pH = 5.

The amount adsorbed can also be easily obtained from spectrum of the adsorbed PE samples because there is nearly no absorbance of native PE around the amide bands. The absorbance intensity in amide II (AII) band region has been reported to be proportional to the amount of protein adsorbed[4] and it is believed not to be very sensitive to the conformation of the protein[1]. Our experimental results also confirm this fact. Therefore, the adsorbed amount of BSA can be quantitatively analyzed by amide II area in most cases. The effect of initial BSA concentration in solution on the AII peak area of the adsorbed PE, buffer-desorbed PE and SDS-desorbed PE are shown in **Figure 1**.

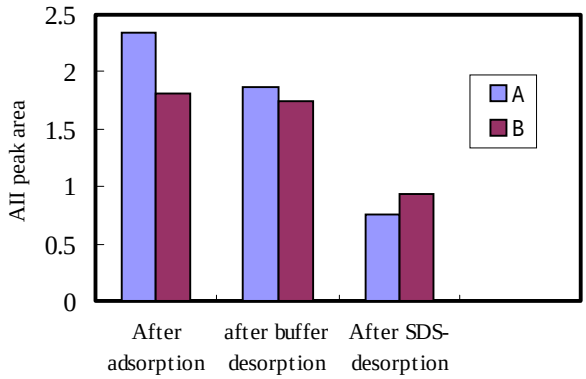
**Figure 1** Change of AII peak area with concentration at Ph = 5.05



It is reasonable to find that the peak area due to adsorption increases with initial concentration. After desorption in the same conditions of the adsorption, the area is decreased due to the release of loose-adsorbed BSA and it still increases with the concentration slightly. Interestingly, after SDS-desorption at 24 hr, the peak area keeps an increase trend at low concentration range (below 1000 ppm at this observation) and a decrease trend above this critical concentration. This phenomenon is not conformed to the available adsorption-desorption investigations. Since SDS shows no evidence for causing a decrease in amide II area in solid state[1,4,5], the more reasonable explanation may be that an increase in concentration will lead to formation of the clusters by aggregation of protein molecules[6]. As a consequence, the strength of intra-molecular and inter-molecular interaction within a cluster will be enhanced and the hydrophobic interaction between the adsorbed proteins and the surface attenuated.

In order to elucidate the essential differences between the solution aggregation or adsorbed aggregation on the surface, an additional experiment (case B) was performed. The condition for this experiment is completely the same as the 2000 ppm run (case A) in the concentration series. But the same amount of BSA as that in case A is divided into 6 equal parts and added 1 part into the adsorptional medium after per 4 hr-adsorption interval so that at initial adsorption stage, the concentration is only about 330 ppm. The result is compared with the previous 2000 ppm run and shown in **Figure 2**.

**Figure 2** Effect of the way that BSA is added on the adsorption




It is clearly seen that the adsorption is related to the way of addition of BSA according to the AII peak area of the samples. The amounts of BSA in the membrane after the adsorption and desorption in case B are less than those in case A; while amount of BSA after SDS desorption in case B is greater than that in case A. The possible explanation for this may be that, for case B, the adsorption is conducted in a side-on way, because the concentration is low at initial stage and hardly cause the aggregation of BSA molecules in solution. After the concentration increases, the additional adsorption can only be conducted on the basis of the previous adsorption equilibrium. The final adsorbed amount of BSA in case B is thus decreased due to the decrease in both the aggregation of BSA molecules and the available surface.

Correspondingly, the amount of BSA in case B is less than that in case A when the buffer extracts the superficial BSA molecules. But the residual BSA molecules after SDS-desorption in case B is greater than that in case A because the BSA molecules in the membrane which do not exist in a side-on way are extracted by SDS. This fact indicates that the aggregation of BSA molecules in the solution seems to play a decisive role on the adsorption process, *i.e.*, at high concentration, BSA molecules tend to aggregate into clusters and then adsorb on to solid surface.

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