Available online at www.sciencedirect.com
ScienceDirect
Journal of Colloid and Interface Science 316 (2007) 238–249
JOURNAL OF Colloid and Interface Science
www.elsevier.com/locate/jcis

# Interaction of *N*-nitrosodiethylamine/bovine serum albumin complexes with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine monolayers at the air–water interface

D.E. Valencia-Rivera [a], A. Básaca-Loya [a], M.G. Burboa [a,b], L.E. Gutiérrez-Millán [a,b], R.D. Cadena-Nava [c], J. Ruiz-García [c], M.A. Valdez [a,d,*]

[a] *Departamento de Investigación en Polímeros y Materiales, Universidad de Sonora, Rosales y Transversal, 83000 Hermosillo, Sonora, México*
[b] *Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora, Rosales y Transversal, 83000 Hermosillo, Sonora, México*
[c] *Instituto de Física, Universidad Autónoma de San Luis Potosí, 78000 San Luis Potosí, SLP, México*
[d] *Departamento de Física, Universidad de Sonora, Rosales y Transversal, 83000 Hermosillo, Sonora, México*

Received 2 May 2007; accepted 30 July 2007

Available online 25 September 2007

**Abstract**

We report the effect of *N*-nitrosodiethylamine (NDA) on the interaction between bovine serum albumin (BSA) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine monolayers (DPPC) at the air–water interface. We prepared aqueous solutions of NDA/BSA complexes maintaining a constant concentration of BSA of $1.49 \times 10^{-9}$ M and using NDA concentrations to obtain 2000, 4000, 6000, 12,500, and 25,000 NDA/BSA molar ratios. The hysteresis area and the compressional modulus of the compression–expansion cycles performed at different times were dependent on the NDA concentration. The cycles performed demonstrate the stability of the new phase of DPPC/BSA and DPPC/NDA/BSA monolayers. This was achieved probably because the BSA concentration used was lower than the one needed for BSA to inhibit the return of DPPC molecules to the interface. Results of the compressional modulus at the onset of the new phase, obtained around 17 mN/m, 15 min and 1, 3, 5, and 12 h after DPPC deposition, indicated that the $3.0 \times 10^{-6}$ M NDA concentration produced a more rigid film, probably due to the higher $\alpha$-helix content of BSA. AFM images were obtained for DPPC/BSA and two DPPC/NDA/BSA complexes. Our images show that 12,500 NDA/BSA molecules were mostly adsorbed in the liquid condensed phase. However, BSA molecules were distributed more homogeneously.


*Keywords:* DPPC monolayers; BSA; *N*-nitrosodiethylamine; AFM; Compressional modulus

## 1. Introduction

Proteins are known to be adsorbed spontaneously at the air–water interface due to their dualistic hydrophilic–hydrophobic behavior. This thermodynamically favorable process has been used extensively in several applications, such as emulsions, foams, and thin films [1]. At short times, protein adsorption at interfaces is mainly controlled by a diffusion process [2]; however, at longer times, it has been demonstrated that adsorbed protein molecules have to jump surface pressure and electrostatic energy barriers in order to penetrate the interface [1,3]. Adsorption of proteins at the air–water interface is generally accepted to occur in three main steps [4,5]: first, diffusion of the proteins to the interface, resulting in a surface pressure increase; second, adsorption, probably followed by unfolding and penetration processes; and third, the rearrangement of the protein molecules. A multilayer building mechanism can occur at longer times, depending, among other factors, on the protein concentration of the solution [6,7]. A lag time and an induction period are observed at short adsorption times, usually at low protein concentrations and under some pH conditions. This induction period, attributed to the time needed for unfolding of protein molecules upon adsorption, was analyzed by Cho and Cornec [8]. Also, Wei et al. [9] measured the kinetics of surface tension for five model proteins and found a strong correlation

* Corresponding author. Fax: +662 2592109.
*E-mail address:* mvaldez@correo.fisica.uson.mx (M.A. Valdez).

0021-9797/$ – see front matter 
doi:10.1016/j.jcis.2007.07.079

between the rate of the surface tension decrement and the conformation of the proteins.

The adsorption of proteins by lipid monolayers at the air–water interface has been extensively investigated with the Langmuir balance by measuring the pressure increment of the lipid–protein monolayers at long times [10,11]. Also, the compression–expansion cycles performed in the isotherms of lipids [12] and phospholipids [4] have been useful to analyze monolayer stability. Broniatowski et al. [13] associate the low stability of monolayers with the systematic shift toward smaller areas in subsequent cycles. Brehmer and Tauer [14] conclude that the background of the hysteresis cycles is the asymmetric behavior of aggregation and disaggregation rates at the same surface pressure; the larger the hysteresis, the more intensive the self-organization process of the Langmuir film is. Recently, Merzlyakov et al. [15] have pointed out that the hysteresis area of peptides adsorbed by lipid monolayers indicates strong peptide–peptide interactions.

Two of the parameters that characterize the rigidity or flexibility of lipid monolayers at the air–water interface are the compressional modulus $C_s^{-1}$ and its inverse, the two-dimensional compressibility $C_s$. Some authors [16,17] prefer the use of $C_s$ because of the ease of identifying the middle of the liquid expanded (LE)–liquid condensed (LC) phase coexistence; others use $C_s^{-1}$ to identify the onset of the LE–LC equilibrium phase transition [13,18,19]. In this work we use the compressional modulus to analyze the protein adsorption from the bulk into 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) monolayers at the air/water interface. Associated with the Langmuir and Langmuir–Blodgett techniques for studying films of proteins adsorbed in lipid monolayers are Brewster angle microscopy [4,20] (BAM) and atomic force microscopy [21] (AFM), which have been used to investigate, among other subjects, lipid–protein interactions and lipid–polysaccharide interactions [22].

Due to its biological interest, abundance, and low cost, bovine serum albumin (BSA) has been one of the most investigated proteins at interfaces [23,24] and interacting with lipids at the air–water interface [10,12,25]. BSA plays many roles in the mammalian circulatory system. One of its most significant functions is to transport solutes through the blood stream by a process that is still unclear but that probably depends on the nature of the ligand being carried [26].

In this direction, some researchers have been interested in understanding the interaction of BSA and human serum albumin (HSA) with different ligands [27–29]. In recent experiments [30], we have investigated the interactions between *N*-nitrosodiethylamine (NDA) and BSA at the air–water and chloroform–water interfaces using axisymmetric drop tensiometry, fluorescence, and circular dichroism. There, we demonstrated that different NDA concentrations change the BSA secondary structure and consequently the hydrophobic and elastic BSA properties in interfaces.

NDA is present in food, beverages, tobacco smoke, herbicides, pesticides, drinking water, and industrial pollution [31]. In food, it can be found in some types of cheese at a concentration of 0.5 μg/kg and in various fish at 1 to 147 μg/kg. In condensed tobacco smoke, whose carcinogenic effects on animals have been largely demonstrated [32], NDA was detected at concentrations of 1–28 ng/cigarette.

The interaction between NDA and proteins was investigated by Bemis et al. [33] in 1966 due to the former molecule's carcinogenic properties. Since then, very few studies concerning NDA–protein interactions have been published.

This research paper has resulted from investigating the effect that NDA has on BSA's capacity to penetrate DPPC monolayers at the air–water interface. We measured the hysteresis cycles and the compressional modulus of the isotherms at different protein adsorption times and NDA concentrations. An adsorption model was used to analyze the protein adsorption on the lipid monolayer [4]. Finally, we acquired AFM images of DPPC/BSA and DPPC/NDA/BSA films monolayers transferred directly onto cleaved mica at different times.

## 2. Experimental

BSA was obtained from BD Biosciences (99%, delipidized and globulin-free); DPPC was obtained from Avanti Polar Lipids (USA). Both were used without further treatment. NDA was obtained from TCI America (99%) and NaCl was purchased from Sigma (USA). Chloroform (HPLC grade) and ethyl alcohol (reactive grade) were obtained from Sigma-Aldrich (USA). Water used through the experiments was filtered with an Easy pure/Barnstead instrument with a resistivity of 18.3 MΩ cm.

DPPC was dissolved in chloroform (1 mg/ml) and the solution was spread on a water surface. As a subphase, we used BSA aqueous solutions ($1.49 \times 10^{-9}$ M) to which NDA was added at concentrations of $3.0 \times 10^{-6}$, $6.0 \times 10^{-6}$, $8.9 \times 10^{-6}$, $9 \times 10^{-5}$, and $3.7 \times 10^{-5}$ M to obtain a subphase solution corresponding to 2000/1, 4000/1, 6000/1, 12,500/1, and 25,000/1 NDA/BSA molar ratios, respectively.

A Langmuir balance (Nima Technologies, Ltd., Coventry, England, Langmuir–Blodgett trough, Model type 611), whose surface tension precision is 0.1 mN/m, was used to obtain DPPC isotherms modified with different NDA/BSA molar ratios in the aqueous subphase. The surface pressure $\pi = \gamma_0 - \gamma$, i.e., the surface tension difference of the clean surface and the covered surface, was measured using a Wilhelmy plate made of chromatography paper. The surface pressure and molecular area data were fed into a computer and recorded, using a barrier speed of 25 $cm^2$/min. Each DPPC sample (50 μl) was deposited on a clean water surface with a Hamilton microsyringe. Temperature was kept at 25 °C with the aid of a water circulator bath (Cole Palmer, 1268-24 USA) and stearic acid (Sigma, USA) isotherms were used as reference. All experiments were carried out inside a dust-free glass box.

Isotherms were performed at 15 min and 1, 3, 5, and 12 h after DPPC was deposited on the subphase containing the BSA and NDA/BSA aqueous solutions. After the surface was cleaned, DPPC was deposited on the subphase kept at a pressure lower than 0.1 mN/m. Isotherms were performed and repeated until the surface pressure differences were less than 1%. The average differences in the area were less than 4%.