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The LB Films of Dansyl Chloride Labeled Octadecylamine and Its Fluorescence Lifetime

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Abstract: Octadecylamine was derivatized with dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride) in order to simplify and understand the LB films of fluorescent probe labeling proteins. Its monolayer and multilayers in the absence and presence of stearic acid were deposited by LB technique. Fluorescence spectra and lifetimes of the fluorescent products were studied to elucidate the microenvironment of molecules in the LB films.

Keywords: LB film, fluorescent probe labeling, fluorescent spectra and lifetime.

Introduction

Fluorescent probe labeling [1,2], microchip and LB (Langmuir-Blodgett) films of DNA and proteins[3] are important in molecular electronic and biotechnological applications such as information processing and molecular recognition. As an imitated and simplified model of above complex cases, here we prepare the LB films of dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride, denoted as DNS) labeled octadecyl amine (AM). A molecule based naphthalene sulfonate is well known fluorescent probes which react with primary or secondary amino group to form highly fluorescent derivative[4,5]. The DNS is a covalently bound probe reacting its sulfonyl chloride group with an amino group. The LB films of DNS-derivatized octadecylamine in the absence and presence of stearic acid (SA) were deposited on solid substrates. Their steady-state fluorescent spectra and fluorescent lifetime were studied.

Experimental Section

DNS was purchased from Fluka Chemie AG. It was used as received. Other reagents were all analytical grade. (1) Dansylation: A mixture of 3 mg of octadecylamine, an aliquot of acetone solution containing 30 mg of dansyl chloride, and 5 mg of sodium bicarbonate (4 g/L) was placed in a tightly closed 25 mL volumetric flask, shaken and dipped into a water bath with exclusion of light. The reaction continued for 1 hour at 55 ^{0}C. After cooling to room temperature in the dark, the dansylated product was extracted

with ~5 mL chloroform. The organic layer was treated with anhydrous sodium sulfate to remove water. (2) Deposition of LB films: Spreading solution is DNS labeled amine (DNS-AM) and DNS labeled amine in stearic acid (DNS-AM/SA) (1:9). The trough used was a domestic product. An aliquot of the sample solution taken up by a micro syringe was spread on the aqua subphase in the trough. DNS-AM monolayer was transferred at 25 mN/m, while DNS-AM/SA was transferred at 40 mN/m. (3) Fluorescence measurement: Fluorescence emission spectra was taken on a Shimadzu model RF-5000 spectrofluorimeter. The excitation was at ~360 nm and the emission was monitored at 400~600 nm. The fluorescence lifetimes were measured with an Applied Photophysics model SP-70 nanosecond spectrofluorimeter by the method of time-correlated single photon counting. The excitation wavelength was 337 nm and the monitoring wavelength was ~ 490 nm.

Results and Discussion

The surface pressure-molecular area (π–A) curves for DNS-AM and DNS-AM/SA show that solid condensed film is formed. The collapse pressure for DNS-AM and DNS-AM/SA (1:9) is 31 and 56 mN/m respectively. As the consequence of labeling, the amphiphilicity of the resultant octadecylamine derivatives is lowered in some extent, so the collapse pressure for pure DNS-AM is lower than that of corresponding mixed films. However, the value of 31 mN/m is not low comparing to 18 mN/m of DLA[6]. The images of LB monolayers of the labeled molecules alone are still even under electron microscopy. The π-A isotherms of DNS-AM and DNS-AM/SA showed in **Figure 1** are unusual with an inflection shape, being similar to that reported in DDHA study[7]. This is suggested that the behavior was associated with a change in the configuration of DDHA on the surface which is accompanied by a tendency toward aggregation. The surface area per molecule is ~0.27 nm^2 for DNS-AM/SA (1:9) and is 0.56 nm^2 for DNS-AM. In the former case the stearic acid contributes the main part in the molecular area of ANS-AM/SA system, and in the latter case the area represents ANS-AM itself. Estimated by using bond lengths[7], the molecular area of the dansyl moiety in the flat configuration is about 0.75 nm^2 and the cross-sectional area of group standing vertically on the surface is assumed to be on the order of 0.5 nm^2. So it means that in our deposited DNS-AM monolayer the head group of labelled molecules is in a tilted orientation.

No significant difference was found between solution spectra in the absence or presence of stearic acid for DNS labeled octadecylamine (**Figure 2**). It means that the diluent stearic acid has no substantial perturbation to the spectral properties of fluorescent derivatives of AM. It is reported that a fluorescent probe DNS is virtually no fluorescent in aqueous solution. However, when the dye is present in a nonpolar medium or when they interact with proteins, their quantum yield increases and the fluorescence emission maximum shifts to shorter wavelengths. The emission bands of films fabricated have a small blue shift with respect to correspondent solution bands. This likely implies that the fluorescent probe experience more hydrophobic microenvironment in ordered LB films than in the random state in solution.

The lifetime of DNS has been reported to be 14 ns[8], but no detail information was

cited. Our lifetime results for solutions and films are collected in **Table 1**. The data for DNS-AM show that both solution and film values are approximately 14 ns. The mono-exponential analysis is consistent with the bi-exponential analysis. The results of monolayers films coincide with that of multilayers film on quartz. The lifetimes of multilayers have a little shorter value than that of 3 layers. This led us envisage that interlayer aggregates have formed. H. Toshiharu *et al*.[9] in measuring the fluorescence lifetime of ANS (8-anilino-1-naphthalene sulfonic acid) in the human erythrocyte host membrane suspension obtained two types of lifetimes: τ_1=15 ns is at low concentration of ANS which bonded at protein and τ_2=8.4 ns at high ANS concentration. The DNS-AM is consistent with low ANS concentration case.

Figure 1 The π-A isotherms of DNS-AM (a) and DNS-AM/SA (b)

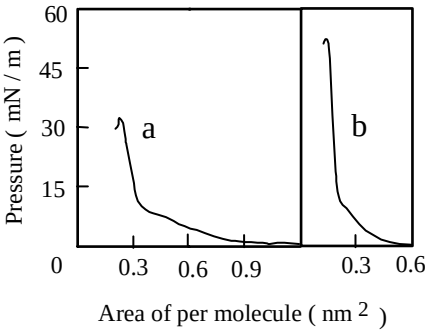
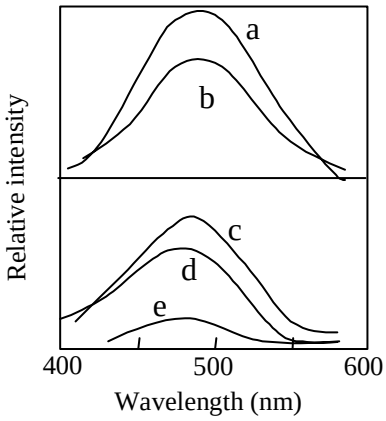


Figure 2 The fluorescence spectra of DNS-AM /SA and DNS-AM.



Top: DNS-AM/SA (a) and DNS-AM (b) in solution; bottom: DNS-AM/SA(c), 11 layers DNS-AM (d) and 3 layers DNS-AM (e) in LB film.

Table 1 Fluorescence lifetimes of labeled octadecylamine

Sample	Condition	τ /ns	χ^2
DNS-AM solution	$CHCl_3$	13.50	1.22
DNS-AM film	3 layers	14.02	1. 22
DNS-AM film	11 layers	14.36	1. 27
DNS-AM/SA solution	$CHCl_3$	13.20	1. 30
DNS-AM/SA film	3 layers	14.53	1. 21
DNS-AM/SA film	11 layers	14.97	1. 25

The results of the present work show that the introduction of fluorescent probe DNS into primary amines can make them accessible to spectral study. The ease of derivitization, successful deposition of monolayer and sensitive fluorimetric characterization promise a great potential utility of this labeling approach in structural and photophysical studies of LB films of molecules without suitable chromophores. It also seems that DNS is a convenient fluorescent probe to label amphiphilic amines,

because the derivatization procedure needs no special preparation and the π–A isotherm and transfer ratio of the LB monolayer are normal.

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