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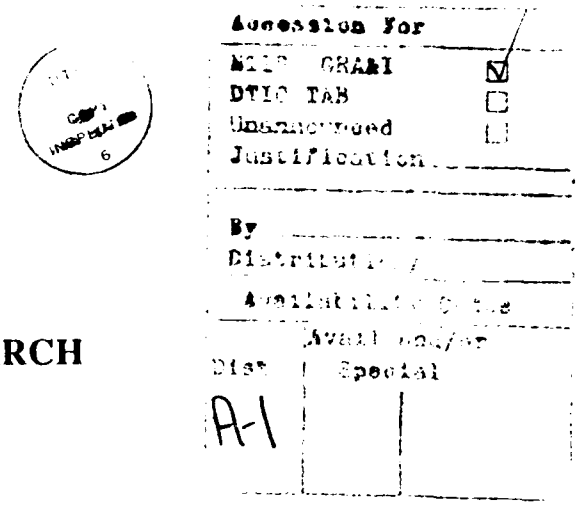

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by

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STREPTAVIDIN-PHYCOERYTHRIN CONJUGATED PROTEINS BOUND TO BIOTIN ON LANGMUIR-BLODGETT FILMS OF BIOTINYLATED LIPID MONOLAYERS

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ABSTRACT

Studies involving the specific and non-specific surface recognition of biotin on biotinylated LB lipid monolayers by streptavidin and avidin conjugated phycoerythrin are presented. Both streptavidin and avidin conjugates were injected under the monolayer and found to preferentially adsorb to the biotinylated monolayers at the air-water interface. Pressure-area isotherms displayed a biotin-streptavidin/avidin complex dependent increase in surface pressure at expanded areas indicating protein adsorption. The binding of protein was confirmed by transferring the monolayer films to solid supports and measuring the characteristic intense phycoerythrin fluorescence at 576 nm. The effect of protein charge, monolayer packing density and structure, and activation of the tetramer proteins towards specific and non-specific binding are discussed. These results suggest a novel and general methodology for the two-dimensional ordering of protein monolayers with potential bioelectronic, optical and protein structure research applications.

INTRODUCTION

The Langmuir-Blodgett technique has been used extensively in the past as a method to simultaneously orient and couple various organic surfactant materials to electronic and optical substrates for an extensive number of molecular device applications. Recently, there has been a great interest in extending this methodology to incorporate biological materials into these assemblies. The end purpose is to elicit the desired inherent, intelligent materials properties which nature has evolved and fine-tune them to serve in many biomedical research and biotechnology applications. In addition, such integrated assemblies should simultaneously provide unique biomimetic or simple environments for the study of protein structure. One such example may include crystallographic TEM structure studies on ordered two-dimensional protein crystals where the two-dimensional or large three-dimensional protein crystals are difficult to obtain [1].

This research, therefore, involves the development of a novel methodology which incorporates and couples a photodynamic, water soluble protein, phycoerythrin, into biomimetic monolayer films via the Langmuir-Blodgett technique. Phycoerythrin is the outer most phycobiliprotein of the phycobilisome "Light Harvesting System" found in red algae [2,3]. These are highly pigmented proteins that exist as well organized geometrical discs or rods, which in this arrangement, serve to extend the narrow visible range absorption of chlorophyll to longer wavelengths by channeling ambient light through a nonradioactive energy transfer process to Photosystem II with very high quantum efficiencies [4]. The chromophores which give rise to the intense color of these phycobiliproteins are based on tetrapyrrole functionalities.

Phycoerythrin is highly and characteristically fluorescent, with a very large Stoke's shift of 81 nm (495 nm excitation and 576 nm emission), which is approximately 2.7 times that of fluorescein [5]. In addition, the time resolved fluorescence properties and molecular environment of the chromophores in these proteins are reasonably well understood [6,7]. These properties in conjunction with the protein's stability and ability to function efficiently in low light level situations suggests promising new biomedical research, biotechnology and biosensor applications.

Phycoerythrin, however, is a large, bulky, water-soluble protein which alone will not form monolayer films using the Langmuir-Blodgett technique. Therefore, the present approach involved utilization of the well known biotin-avidin or streptavidin complex. Avidin and streptavidin are tetramer proteins (four binding sites) which have a high specificity for binding biotin functionalities. The binding affinity of biotin to these

tetramer proteins is well known (10^{15} M) and once formed the complex is essentially irreversible [8-10] with a stability comparable to that of a covalent bond.

In this study, avidin conjugated phycoerythrin (Av-PE) and streptavidin conjugated phycoerythrin (Str-PE) monolayer assemblies were prepared by injecting the conjugated protein system underneath a biotinylated phospholipid monolayer. The binding of the protein system to the monolayer was confirmed through pressure-area isotherms and fluorescence spectroscopy.

MATERIALS AND METHODS

The tetramer protein conjugated phycoerythrin materials and unconjugated phycoerythrin, Av-PE, Str-PE, and PE, were all purchased from Biomeda Corporation (Foster City, California) and used as received. The biotinylated phospholipid, N-(biotinoyl) dipalmitoyl-L-α-phosphatidylethanolamine, triethylammonium salt (B-DPPE) was purchased from Molecular Probes (Eugene, Oregon). The control phospholipid, L-α-dipalmitoyl phosphatidylethanolamine (DPPE), was obtained from Avanti-Polar-Lipids (Pelham, Alabama).

All monolayer studies were performed on Lauda MGW Filmwaag troughs which had a surface area of approximately 930 cm^2. The subphase was composed of an aqueous solution of 0.1 mM sodium phosphate, 0.1 M NaCl, at pH 6.8. Film preparation involved the spreading of the lipid from a 0.5 mM chloroform solution and the injection of 0.1 mg of the protein in 5 ml of the buffered subphase under the spread film. The film was then incubated for two hours at 30° C in the expanded state to allow sufficient time for the protein to adsorb onto the monolayer film. Pressure-area isotherms were then measured by compressing the film at a speed of approximately 2 mm^2/min until collapse of the film was observed. For transfer studies, the monolayer was compressed to an annealing surface pressure of approximately 15 mN/m prior to deposition. The monolayer films were then transferred onto glass slides for fluorescence spectroscopy.

RESULTS AND DISCUSSION

To establish adsorption of the proteins onto the biotinylated phospholipid monolayers, pressure-area isotherms of protein injected monolayers were compared to that of the pure phospholipid. Figure 1 gives the structure of the biotinylated phospholipid (B-DPPE) and a comparison of the pressure-area isotherms of the pure B-DPPE and the three protein injected B-DPPE monolayers (PE, Av-PE, and Str-PE) after a two hour incubation period. A significant increase in surface pressure of the lipid in the gas expanded phase was observed for both the Av-PE and Str-PE injected monolayers. However, it is interesting to note that all four isotherms gave a relatively steep, overlapping, slope after 15 mN/m until collapse of the film was observed. Extrapolation of this slope gave an area of approximately 100 $Å^2$ for each of the monolayer films.

The Av-PE and Str-PE monolayers at higher surface pressure are not expanding, thus suggesting that these bulky protein systems responsible for the initial high surface pressure may be "swinging" down into the aqueous subphase upon compression of the lipid molecules. A schematic illustrating the possible spatial organization and reorientation of these materials during monolayer compression is provided in figure 2. In addition, injection of the unconjugated phycoerythrin caused little change in the isotherm in comparison to the pure B-DPPE. This control system provides strong evidence that the biotin binding sites of the avidin and streptavidin tetramer proteins are directly involved in the adsorption process through biotin lipid tetramer protein complex formation.

Monolayer samples were deposited onto solid glass supports using the vertical dipping technique, at an annealing surface pressure of 15 mN/m. Deposition proceeded with facility resulting in transfer ratios of approximately 100 to 150 %. Fluorescence spectroscopy was then used to probe the presence of phycoerythrin in the transferred monolayer assemblies. A schematic of the fluorescence instrumentation is given in figure 3. All measurements were carried out by scanning the emission from 515 nm to 670 nm, while exciting the samples with 496 nm light from an Argon Ion laser.

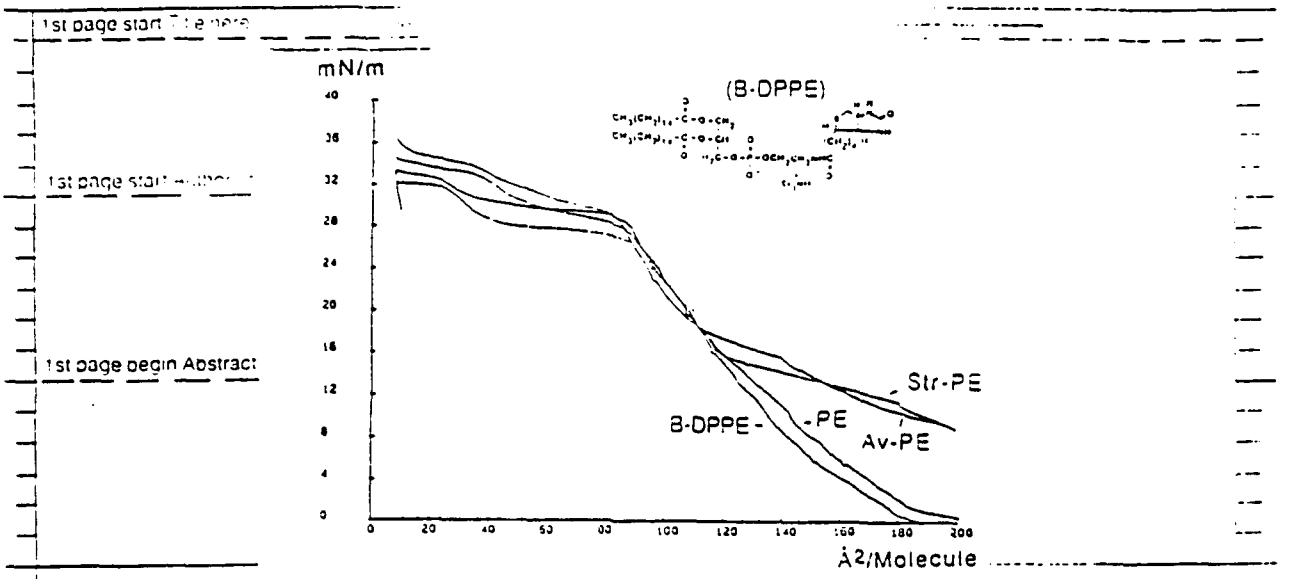

Figure 1. Structure of the biotinylated phospholipid (B-DPPE) and pressure-area isotherms of B-DPPE and protein injected B-DPPE (PE, Av-PE and Str-PE).

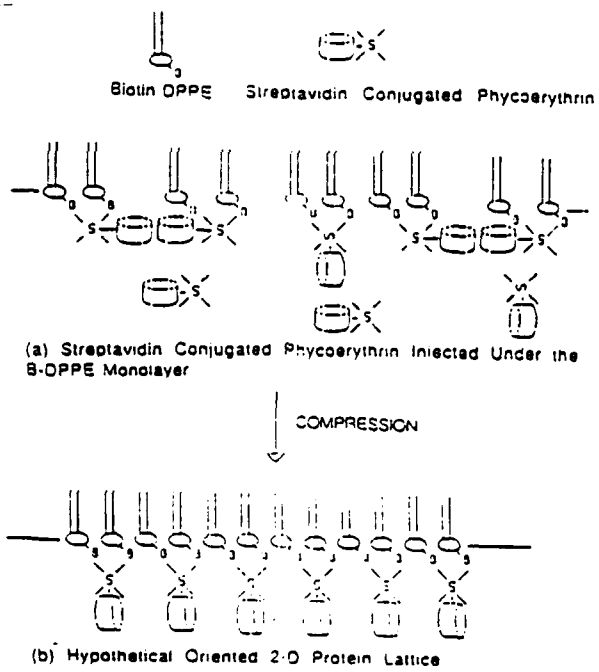

Figure 2. Idealized schematic of the two-dimensional ordering of derivatized protein monolayers onto a biotinylated lipid LB film.

A comparison of the fluorescence spectra of a B-DPPE monolayer with Str-PE, a B-DPPE monolayer with PE and a monolayer of DPPE (not biotinylated) with Str-PE is given in figure 4. As shown, the two controls give no fluorescence signal, while the B-DPPE Str-PE sample exhibits a strong emission at 576 nm which corresponds to the fluorescence spectrum of the native phycoerythrin protein. In each of the two controls, one component of the biotin-avidin or streptavidin complex was missing. The fact that no fluorescence was observed with these samples is direct evidence that this complexation plays the major role in the adsorption of protein to the monolayer. Similar results were obtained with clean glass slides which were vertically raised out of the protein containing subphase, without the lipid monolayer, to rule out any spontaneous protein adsorption onto the bare glass surface.

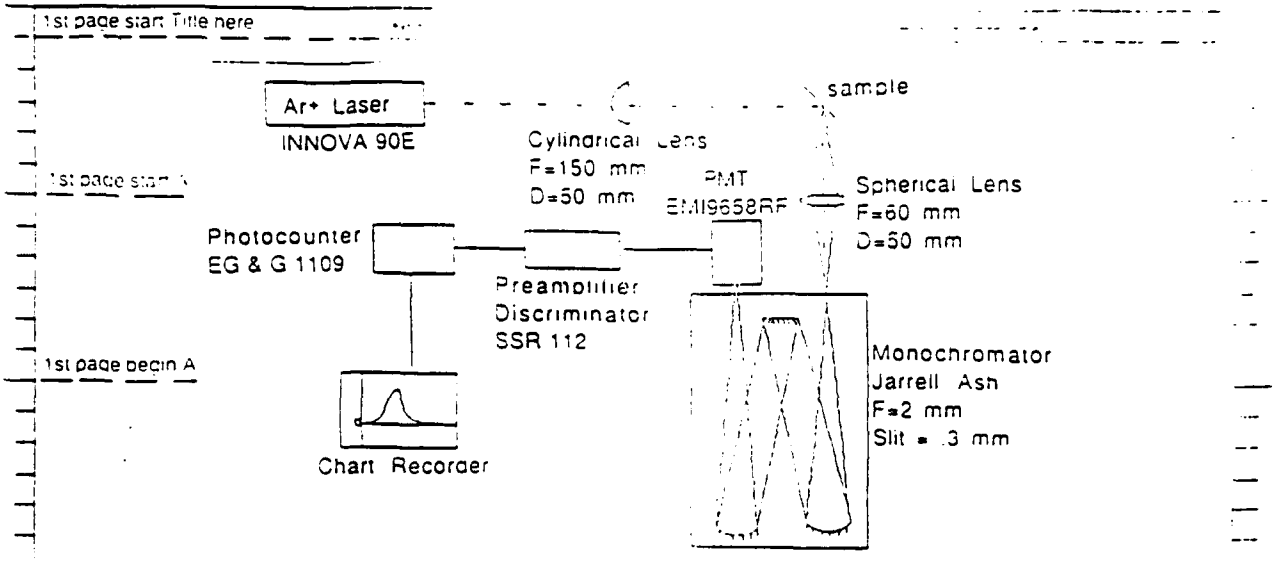

Figure 3. Schematic diagram of fluorescence set-up.

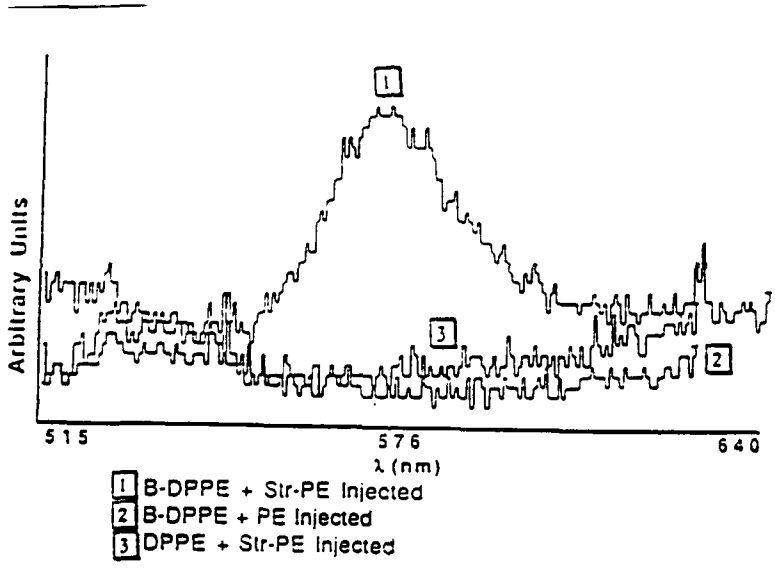

Figure 4. Fluorescence spectra of protein adsorbed LB films.

A question is, however, raised as to whether the protein adsorption to the monolayer proceeds entirely through the specific biotin-streptavidin or avidin mechanism or also includes non-specific binding mechanisms. To address this issue similar studies were carried out with the Av-PE injected monolayer protein system. The structure of avidin is post translationally modified by surface carbohydrate residues and known to complex to the biotin lipid monolayer by both specific and non-specific (carbohydrate and electrostatic) binding mechanisms [11]. Streptavidin however, is not chemically modified by carbohydrate, and binds by what appears to be only the specific mechanism [12].

The spectra in figure 5 summarize and support these binding mechanisms as a direct comparison is made of the fluorescence of B-DPPE monolayers with the three phycobiliprotein systems, Av-PE, Str-PE and PE. As shown, the Av-PE injected monolayer gives a strong emission at 576 nm, which indicates binding of the protein to the monolayer. This emission however, is observed to be considerably stronger in comparison to that of the Str-PE monolayer. Such a difference in intensity would be expected if both specific and non-specific binding of the avidin conjugated protein system to the monolayer was occurring.

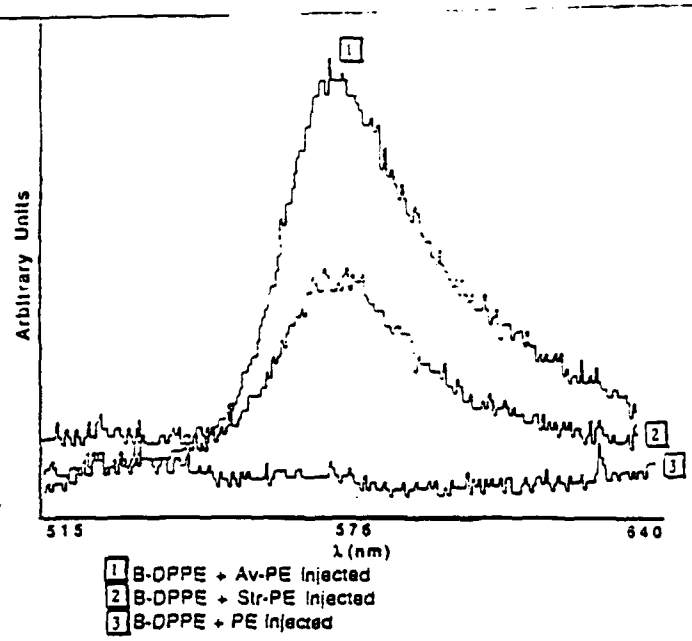

Figure 5. Fluorescence spectra of protein adsorbed LB films.

CONCLUSION

These results demonstrate for the first time the formation of monomolecular films of the photodynamic phycobiliprotein, phycoerythrin. It was shown that avidin and streptavidin conjugated phycoerythrin, when injected under a monolayer, preferentially adsorb to only biotinylated monolayers at the air-water interface. Fluorescence measurements of transferred monolayers confirm the presence of phycoerythrin and provide evidence that the avidin conjugated system may bind by both specific and non-specific mechanisms, while the streptavidin systems bind via only a specific mechanism. This work establishes a potential general technique for the two-dimensional ordering, in a monolayer form, of any biomolecular system which may be derivatized with biotin or avidin/streptavidin. In addition, the multiple biotin binding sites of the tetramer proteins will allow for the incorporation of various interacting biomolecules in an organized two-dimensional hierarchy. Such versatility should lead to the design and fabrication of a tremendous variety of biological supramolecular assemblies.

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