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13. ABSTRACT (Maximum 200 Words)

A novel hierarchical biomaterial capable of incorporating any biotinylated biomolecule has been created. Our strategy is to biotinylate one-dimensional electroactive polymers and use a bridging streptavidin protein on Langmuir-Blodgett (LB) organized films. The following copolymeric system which enables functionalization of other molecules and formation of good monolayers was employed. Biotinylated poly(3-methanolthiophene-co-3-undecylthiophene) (B-PMUT) demonstrated a significantly better isotherm implying superior molecular packing compared to poly(3-methanol thiophene-co-3-undecyl thiophene) (PMUT) on the LB air-water surface. The isotherm showed significant area expansion when streptavidin was injected below the B-PMUT monolayer in 0.1 mM NaH₂PO₄/0.1 M NaCl buffer (ph 6.8) subphase. We then incorporated biotinylated phycoerythrin (B-PE) into this novel biomaterial by binding the unoccupied biotin binding sites on the bound streptavidin (4 sites total). The pressure-area isotherm of the protein injected monolayer showed area expansion. A characteristic fluorescent emission peak at 576 nm was detected from the monolayer transferred onto a solid substrate. These observations demonstrated the function of B-PMUT in hierarchical monolayer assembly of molecules incorporating the biotin / streptavidin interaction.

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**BIOTINYLATED POLYTHIOPHENE COPOLYMER
- A NOVEL ELECTROACTIVE BIOMATERIAL
UTILIZING THE BIOTIN-STREPTAVIDIN INTERACTION**

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ABSTRACT

A novel hierarchical biomaterial capable of incorporating any biotinylated biomolecule has been created. Our strategy is to biotinylate one-dimensional electroactive polymers and use a bridging streptavidin protein on Langmuir-Blodgett (LB) organized films. The following copolymeric system which enables functionalization of other molecules and formation of good monolayers was employed. Biotinylated poly(3-methanolthiophene-co-3-undecylthiophene) (B-PMUT) demonstrated a significantly better isotherm implying superior molecular packing compared to poly(3-methanolthiophene-co-3-undecylthiophene) (PMUT) on the LB air-water surface. The isotherm showed significant area expansion when streptavidin was injected below the B-PMUT monolayer in 0.1mM NaH₂PO₄/0.1M NaCl buffer (pH 6.8) subphase. We then incorporated biotinylated phycoerythrin (B-PE) into this novel biomaterial by binding the unoccupied biotin binding sites on the bound streptavidin (4 sites total). The pressure-area isotherm of the protein injected monolayer showed area expansion. A characteristic fluorescent emission peak at 576nm was detected from the monolayer transferred onto a solid substrate. These observations demonstrated the function of B-PMUT in hierarchical monolayer assembly of molecules incorporating the biotin / streptavidin interaction.

INTRODUCTION

Polythiophene is one of the most important heteroaromatic electrically conducting polymers. It possesses good thermal and environmental stability and its ease in processibility makes it attractive for possible applications in electronics, sensors and nonlinear optics [1-4]. Although the aromatic rings are responsible for rigidity and strong intra- and interchain interactions, solubility can be obtained by substituting alkyl chains (longer than 4 carbons) on the polymer backbone [5].

Incorporation of biological molecules, which possess inherent intelligent properties, into well-defined, oriented assemblies is extremely important for potential bioelectronic, biomedical and biotechnological applications. Here, the polymeric material primarily serves to immobilize biological molecules but could potentially function in a signal transduction role as well. The Langmuir-Blodgett technique is advantageous for the fabrication of a hierarchical structure due to its ability to organize molecules into an ordered monolayer and subsequently to manipulate monomers into multilayer films toward a desired architecture. Recently, this technique has been used to prepare spatially oriented organized protein molecular assemblies [6].

Our approach has involved the highly specific recognition of biotin by streptavidin. The binding affinity of biotin to the tetrameric protein streptavidin is strong (10^{15} M) and once formed the complex is essentially irreversible [7]. It was demonstrated earlier that one can use the LB cassette approach to create ordered monolayers using biotinylated lipid which first binds to streptavidin [8,9]. This streptavidin can subsequently bind biotinylated phycoerythrin, a fluorescent protein. The advantages of using polymer material over lipid include the monolayer film stability and strength.

Here, we have chosen the copolymer system of 3-undecylthiophene and 3-methanolthiophene, with modification of the hydroxyl group of methanol by biotin in order to extend the cassette approach. Our goal is to combine the conductivity

properties of the copolymer with the inherent flexibility of the biotin / streptavidin binding system. In the present study, we present the method of synthesis of the novel biotinylated copolymer and demonstrate the self-assembly of the cassette system using the fluorescent properties of biotinylated phycoerythrin.

EXPERIMENTAL

Polymer Synthesis

Synthesis of poly(3-undecylthiophene-co-3-methanalthiophene) (PMUT)

Synthetic grade anhydrous ferric chloride (Aldrich), 0.09 mol, was dried under vacuum at 100°C prior to reaction. Then nitrogen was introduced along with 100 ml of dry chloroform (Aldrich). 0.02 mol of 3-undecylthiophene (TCI America) and 0.01 mol of 3-methanalthiophene (Aldrich) in 10 ml of chloroform was added dropwise under vigorous stirring. The reaction mixture was allowed to stir for two days till the reaction was complete. The reactant solution was precipitated into 500 ml methanol (Aldrich). The product was then cleansed with methanol in a Soxhlet extractor for two days.

Synthesis of biotinylated poly(3-undecylthiophene-co-3-methanalthiophene) (B-PMUT)

A solution of 0.01 mol biotin (Biomedica), 0.011 mol N,N-dicyclo-hexylcarbodiimide (Aldrich), 0.011 mol poly(3-undecylthiophene-co-3-methanalthiophene) and 0.001 mol 4-pyrrolidinopyridine (Aldrich) in 50 ml dichloromethane (Aldrich) was stirred at room temperature until esterification was complete. The N,N-dicycoundecyl urea was filtered and the filtrate was washed with water (3x50 ml), 5% acetic acid solution (3x50 ml) and again with water (3x50 ml), dried (MgSO₄) and solvent was evaporated in rotary evaporator under reduced pressure to give the biotinylated copolymer.

LB monolayer formation

All monolayer studies were carried out on a Lauda MGW Filmwaag trough with a surface area of approximately 930cm². In the case of pressure-area isotherms of BMUT and B-PMUT, 0.5 mM chloroform solution was spread onto the purified MilliQ water subphase. For the measurement of pressure-area isotherms following streptavidin injection under the B-PMUT monolayer, the subphase was composed of an aqueous solution of 0.1mM NaH₂PO₄ and 0.1M NaCl, at pH 6.8. Streptavidin (0.1mg in 5ml of the buffered subphase) was injected under the spread film and left to incubate for two hours at 30°C, and subsequently biotinylated phycoerythrin was introduced under the polymer and streptavidin layer. The streptavidin and biotinylated phycoerythrin were purchased from Biomedica Co. and used as received. Compression was then carried out at a speed of 2 mm²/min until collapse of the film was observed. For transfer studies, the polymer was spread, followed by streptavidin introduction and incubation in the expanded state for two hours, subsequently followed by B-PE introduction and incubation for two hours and then compressed to an annealing surface pressure of approximately 15 mN/m for deposition. Monolayer films were then transferred onto glass solid supports for fluorescence spectroscopy.

RESULTS AND DISCUSSION

Materials Synthesis and Characterization

The synthesis of biotinylated copolymer involves two steps, the synthesis of copolymer of 3-undecylthiophene and 3-methanalthiophene and attachment of biotin in the second step as shown in Figure 1.

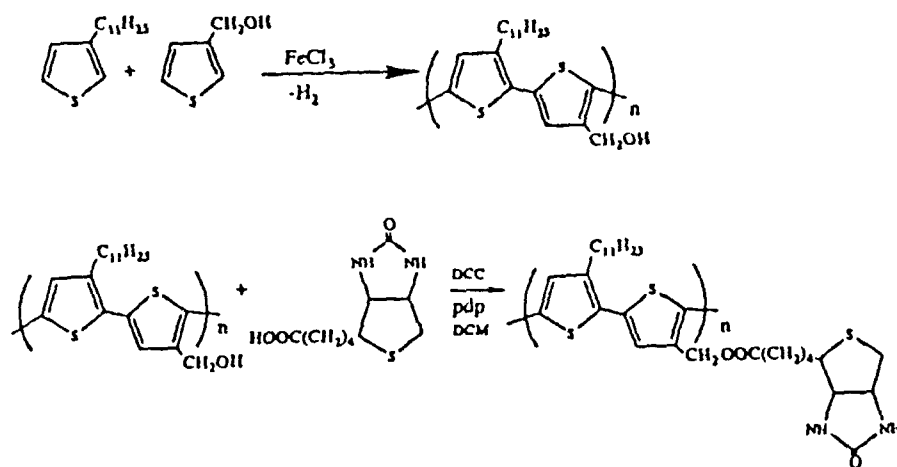


Figure 1. The synthesis schematic of PMUT and B-PMUT.

Infrared measurements of PMUT and B-PMUT were carried out on KBr discs and are shown in Figure 2. Both PMUT and B-PMUT showed a principal absorption peak at 780 cm^{-1} due to the C-H out-of-plane vibration of the 2,5-disubstituted thiophene and a distinct peak around 810 cm^{-1} due to the C-H out-of-plane vibration of the 2,3,5-trisubstituted thiophene [10]. B-PMUT exhibited new characteristic peaks at 1678 cm^{-1} due to ester linkage and a sharp peak at 3400 cm^{-1} from N-H stretching. Meanwhile, the broad O-H absorption peak at 3400 cm^{-1} shown in PMUT disappeared in B-PMUT.

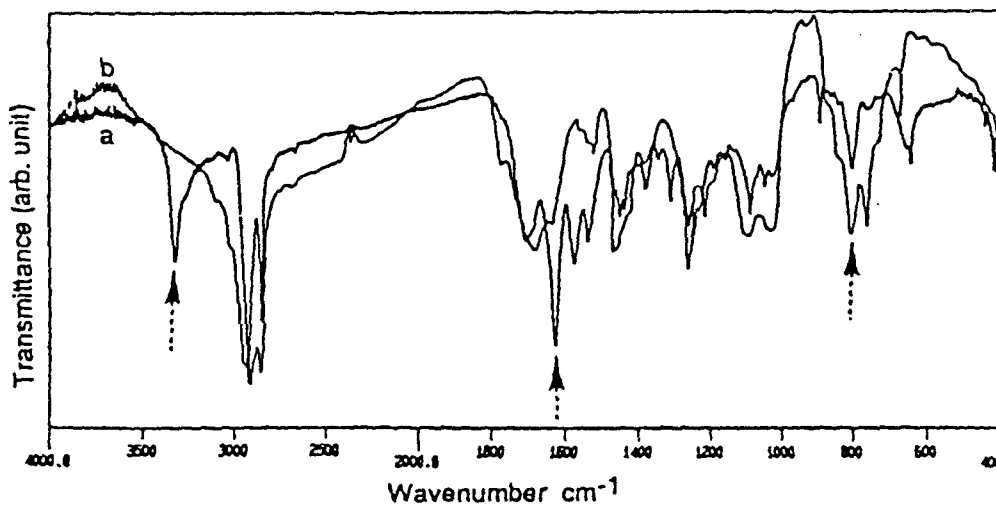


Figure 2. FT-IR spectra of (a). PMUT (b). B-PMUT.

Visible spectra were measured in chloroform and are shown in Figure 3. Both the copolymers showed a broad λ_{max} around 400-450 nm with absorption increasing from 600 nm indicating the presence of an extended π -conjugation along the polymer backbone. B-PMUT showed a blue shift due to the interruption of π -conjugation by the introduction of biotin.

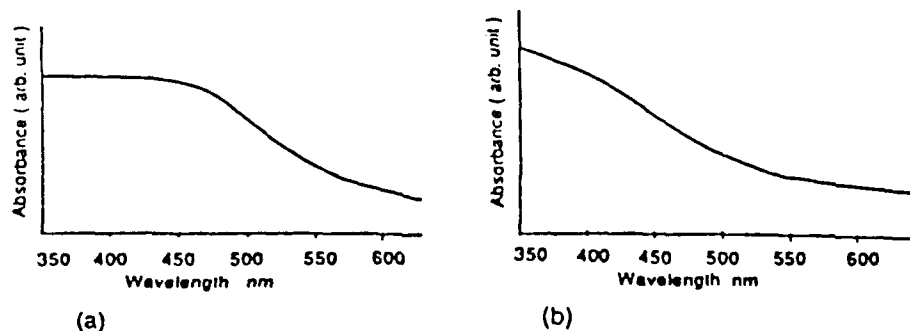


Figure 3. Visible spectra of (a). PMUT (b). B-PMUT.

In order to evaluate the effectiveness of biotinylation of PMUT in the functions of LB formation and subsequent bindings with streptavidin and B-PE, a series of pressure-area isotherm measurements were performed. The isotherms of the PMUT, B-PMUT, streptavidin injected B-PMUT monolayers and B-PE injected streptavidin/B-PMUT monolayers are given in Figures 4 and 5.

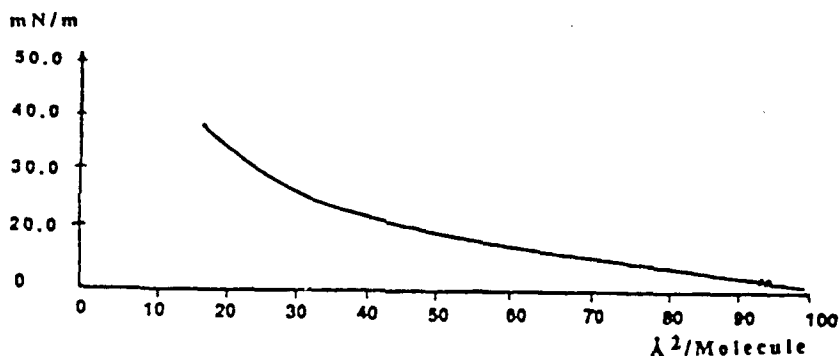


Figure 4. The pressure-area isotherm of PMUT on MilliQ water at 30°C.

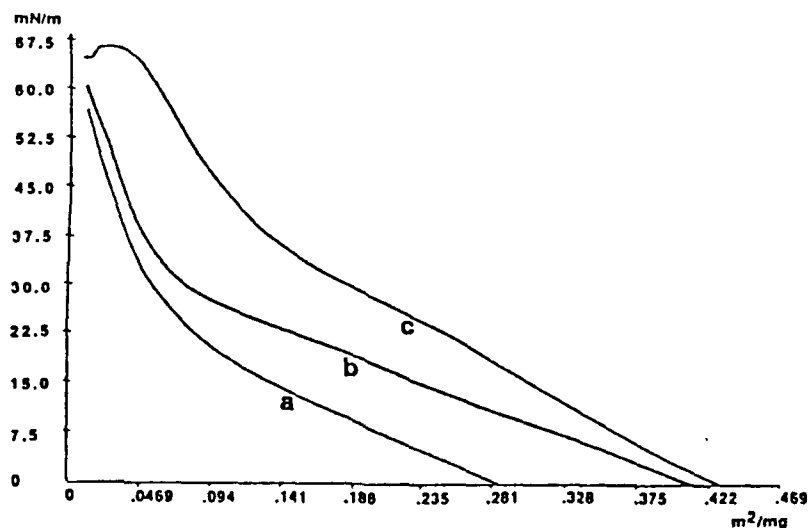


Figure 5. The pressure-area isotherms of (a). B-PMUT (b). B-PMUT / Streptavidin (c). B-PMUT / Streptavidin / B-PE.

Biotinylated copolymer (B-PMUT) demonstrated a significantly better isotherm than copolymer (PMUT) implying superior packing compared to the copolymer on the air-water interface. This significant improvement in the formation of a B-PMUT monolayer suggests that the biotinylation enhanced the LB film formation by contributing hydrophilicity to the copolymer molecule.

Another advantage of the B-PMUT includes increased stability of the monolayer during transfer and efficient deposition onto solid supports. It was found that a constant surface pressure of 15 mN/m was maintained over a period of 15 minutes with a transfer ratio of approximately 65%. B-PMUT was observed to possess fairly strong mechanical properties as shown by the formation of an elastic fiber-like string when the collapsed monolayer film was drawn up using a tellon-coated tip.

The isotherm shown in Figure 5 showed area expansions when streptavidin and B-PE were injected below the B-PMUT monolayer indicating the occurrence of effective binding between the biotin and streptavidin and subsequently biotinylated PE with streptavidin. This change supports the original goal of the biotinylation of this polymer, which was to employ the biotin-streptavidin complexation for subsequent immobilization of any biotinylated macromolecular assembly into LB films.

The monolayer films were transferred to hydrophobic solid glass supports using the horizontal dipping technique at an annealing pressure of 15 mN/m. The presence of the phycoerythrin is probed by its intense and characteristic fluorescence. Measurements were carried out by exciting the samples with 496 nm light from an Argon ion laser and scanning from 510 to 670 nm [8]. The fluorescence spectra of B-PMUT with streptavidin, and B-PMUT and streptavidin with B-PE, are shown in Figure 6. As shown, only the B-PMUT/streptavidin/B-PE monolayer gives a strong emission at 576 nm which corresponds to the fluorescence spectrum of the native phycoerythrin [9]. These results along with other controls provide convincing evidence that the protein has adsorbed to the B-PMUT / streptavidin monolayer via the bridging biotin / streptavidin interaction.

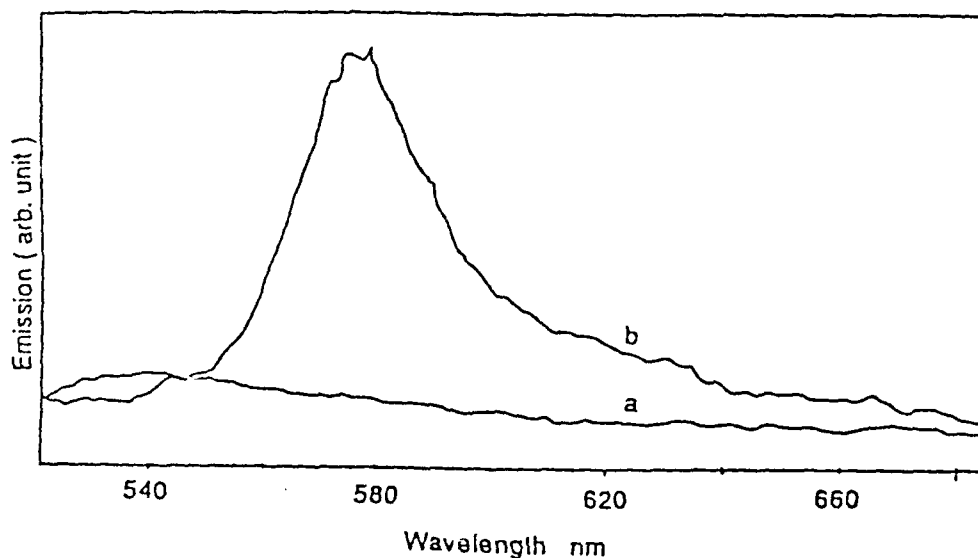


Figure 6. Fluorescence spectra of (a). B-PMUT / Streptavidin
(b). B-PMUT / Streptavidin / B-PE.

CONCLUSION

We have shown that biotinylation of thiophene copolymer enabled formation of superior LB films at the air-water interface by contributing a flexible spacer group and enhancing the hydrophilicity of the copolymeric molecule. In addition, through the biotin / streptavidin complexation, hierarchical structure fabrication containing protein was demonstrated. These results suggest that this novel copolymer is a promising material for potential device applications in which any biotinylated macromolecule may be attached to the polymer monolayer via the bridging biotin / streptavidin interaction system.

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