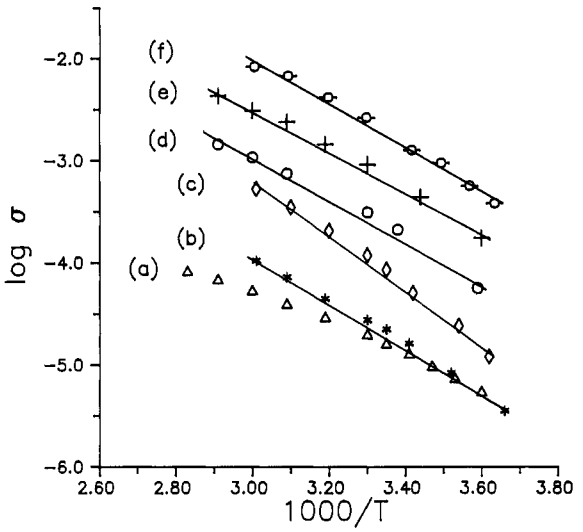


Figure 5. log σ vs $1000/T$ for (a) $MEEP_8LiI$, (b) $MEEP_4LiI_8$, (c) $MEEP_2LiI_5$, (d) $MEEP_{0.67}I$, (e) $MEEP_4NaI_9$, and (f) $MEEP_4NaI_{11}$.

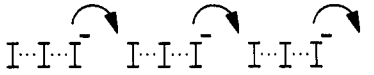
complexes with high glass transition temperatures.

Arrhenius plots for a range of MEEP-polyiodide complexes are shown in Figure 5. Activation energies derived from these plots are listed in Table V. For many complexes the Vogel-Tamman-Fulcher (VTF) equation

$$\sigma = AT^{-1/2} \exp\{-B/(T - T_0)\}$$

where T_0 is related to T_g, can be employed to predict the curved-line response observed in an Arrhenius plot of conductivity data.[34] The curvature of the response in these complexes is most evident in the case of the simple iodide complexes $MEEP_xMI$. Higher polyiodides show very shallow curvature and can be as well described as an Arrhenius-type straight-line response.

The nature of the charge carriers in these complexes has not been directly determined; however, alkali-metal cations are not required to produce high conductivities. Whether a cationic species produced by disproportionation of iodine is involved in conduction remains unknown. The high concentrations of iodine (1-10 M) in these complexes, along with the known stability of polyiodides, make these complexes likely candidates for an ion-hopping mechanism involving iodide transfer between polyiodides:



This process might show greater dependence on iodine concentration than on the glass transition temperature of the complex and would be expected to exhibit the Arrhenius behavior observed. Gileadi et al.[35] have examined equivalent conductivity and transference numbers of R_4NBr in Br_2-containing solutions and implicated an ion-hopping mechanism involving a polybromide species when $[Br_2]$ exceeds 1 M. A similar mechanism has also been suggested to account for conduction in iodine.[36,37]

Acknowledgment. This research was supported by DOE Grant DE-FG02-85ER45220, and the facilities were provided by the Northwestern Materials Research Center sponsored by National Science Foundation Grant No. DMR 881571. M.M.L. acknowledges support by an NSF Postdoctoral Fellowship.

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Chemisorbed Phospholipid Monolayers on Gold: Well-Defined and Stable Phospholipid Surfaces for Cell Adhesion Studies[1]

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The cell adhesion properties of chemisorbed phospholipid monolayers (CPMs) on gold, prepared from thiol- and disulfide-bearing phosphatidylcholines, have been defined. In the presence of a nonadhesive protein, albumin, adhesion of Chinese hamster ovary (CHO) cells to each CPM surface investigated was minimal; in the presence of fibronectin and vitronectin, substantial adhesion was observed. The ability of CPMs to support adhesion of CHO cells was essentially independent of the molecular structure of the phosphatidylcholine that was employed. Inhibition studies, carried out with GRGDS and with a monoclonal antibody that binds to the fibronectin receptor, provide strong evidence that fibronectin-mediated cell adhesion to CPMs involves a natural ligand-receptor recognition in which the RGD moiety plays a central role. Chemisorbed phospholipid monolayers are proposed as novel surfaces from which to append adhesion-promoting ligands and to investigate, *quantitatively*, their interaction with cell surfaces.

Understanding the structural and functional basis of the adhesive interactions that occur between cell surfaces and the extracellular matrix represents one of the most difficult problems facing chemists and biologists. While extensive investigations have been carried out with fibronectin (Fn) and other cell attachment proteins, the precise interactions that occur between a cell surface and the extracellular matrix remain to be elucidated. The discovery that the Arg-Gly-Asp (RGD) sequence represents a key unit within the fibronectin molecule and other adhesive proteins

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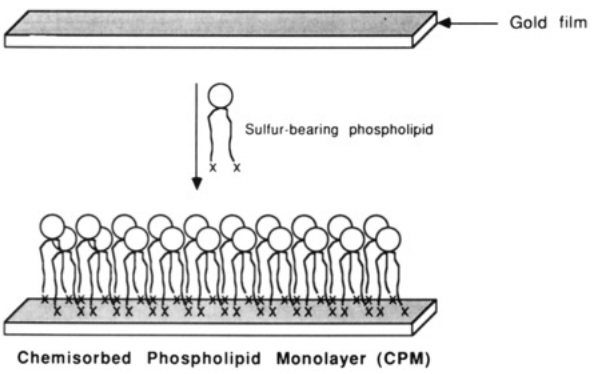
Scheme I



provides, for the first time, the possibility of examining cellular adhesion at a level that is sufficient to define the importance of *ligand density and distribution on the attachment process*.[3-13]

While Sepharose and polystyrene "surfaces" have proven useful in studying, qualitatively, the adhesion characteristics of certain nonadhesive peptides,[14] their structural complexity precludes a quantitative assessment of peptide–cell binding. In particular, the immobilization of RGD units to Sepharose (a three-dimensional swollen gel) is likely to result in a significant population of ligands that are buried within the gel and are inaccessible to cell surface receptors. In addition, the microenvironment that surrounds each pendant RGD group is likely to be nonuniform. Similar ambiguities exist when amorphous hydrocarbon polymer "surfaces" are employed as supports. The surface morphology of polyethylene film, for example, has been likened to that of a "surface of a can of worms".[15]

We have recently begun a program that is aimed at creating *well-defined surfaces that are inherently nonadhesive toward cells* and that can be used to probe the cell-binding characteristics of pendant ligands. One specific approach that we have taken is to construct chemisorbed phospholipid monolayers (CPMs, Scheme I) on gold.[16,17] Such assemblies, which are analogous to Langmuir–Blodgett (LB) films[18] and to covalently bound lipid films,[19] represent molecularly smooth and stable phospholipid surfaces. In contrast to LB films, however, CPMs can be constructed without the use of a film balance and are very stable; e.g., they can withstand the presence of a variety of organic solvents. While our earlier work established that densely packed and macroscopically uniform CPMs could be prepared from phosphatidylcholine molecules bearing thiol or disulfide moieties, their cell adhesion properties were not investigated.

The primary objective of the work described herein was to evaluate the feasibility of using CPMs as supports for cell adhesion studies. In particular, this study was aimed at answering three specific questions: (i) Are CPMs, by themselves, inherently adhesive or nonadhesive toward cells? (ii) Are the cell adhesion properties of CPMs sensitive to the precise structure of the phosphatidylcholine that is used? (iii) Do CPMs adsorb adhesive promoting proteins such as fibronectin, and does the latter promote adhesion of cells to such surfaces by an RGD-mediated pathway? This last question was of interest to us for two reasons. First, if fibronectin induces the adhesion of cells to CPMs *via an RGD mechanism*, this would infer that the CPM surface can support a natural cell adhesion process (i.e., specific biochemical recognition). If cell attachment on fibronectin-coated CPM surfaces occurs by some other mechanism (e.g., nonspecific denaturation/adsorption phenomenon), this would indicate the unsuitability of CPM surfaces for biological investigations. Second, if CPM surfaces do not permit the binding of adhesion-promoting proteins, then they might constitute a class of *novel biomaterials* that could have immediate practical importance for the fabrication of nonthrombogenic surfaces for medical devices.

Experimental Section

Materials. Unless stated otherwise, all reagents and chemicals were obtained from commercial sources and used without further purification. House-deionized water was purified by use of a Milli-Q filtering system containing one carbon and two ion-exchange stages. Chloroform and methanol used for chromatography were reagent grade. Methanol (Burdick & Jackson Solvents Co.) used for monolayer formation was purified by distillation over 4-Å molecular sieves (8–12 mesh). 4-(Dimethylamino)pyridine was recrystallized once from toluene prior to use. Chloroform used for phospholipid synthesis was distilled over phosphorus pentoxide.

Cell media (α-MEM), fetal calf serum, 1 M HEPES solution, and antibodies were supplied by Gibco. Adhesion inhibiting peptide GRGDS was a gift from the UpJohn Co. Isotope ^{35}S-Translabel (1000 Ci/mM) was from ICN laboratories. Bovine albumin fraction V (BSA) was obtained from Miles. Bovine plasma fibronectin was prepared as previously described.[20] Vitronectin was prepared from human plasma as described,[21] except that the FPLC ion exchange chromatography was performed on a Mono Q column (Pharmacia) with a 20 mM Tris–saline (pH 7.6) buffer in the absence of urea.

General Methods. ^{1}H NMR and IR spectra were recorded on JEOL FX 90 Q (or Bruker 500 MHz) and Mattson Sirus 100 spectrometers, respectively. Chemical shifts are reported relative to tetramethylsilane. Specific procedures that were used for chromatographic separations, contact angle and ellipsometry measurements, spontaneous monolayer formation, and gold evaporation were similar to those previously described.[17] Samples that were preared at Lehigh and sent to the University of North Carolina for biological surface studies were sent by overnight mail. Lipid-coated slides were fully immersed in pure methanol and sealed in glass vials for shipment. The specific diameters of these vials were chosen such that only the edges of the gold-coated slides were in contact with the glass walls of the vial. 1,2-Bis[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine (**3**) was synthesized by using previously described procedures.[22] Mass

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spectral analyses were performed at the Midwest Center for Mass Spectrometry (University of Nebraska).

Chemical Synthesis Methods. 16-Bromohexadecanoic Acid. 16-Hexadecanolyde (5.4 g, 21.1 mmol) was added to a mixture of 48% hydrobromic acid (7 mL) and glacial acetic acid (70 mL) in a 250-mL round-bottomed flask, equipped with a Teflon-coated magnetic stirring bar and reflux condenser and refluxed for 4 h. After the product mixture cooled to room temperature, 50 mL of water was added, and the crude 16-bromohexadecanoic acid (precipitate) was isolated by filtration. The isolated yield obtained after drying [12 h, 23 °C (0.05 mmHg)] was 6.76 g (95%): mp 68–71 °C (mp 68.5–69.0 °C).[23]

16-Mercaptohexadecanoic Acid. 16-Bromohexadecanoic acid (3.00 g, 8.98 mmol) was dissolved in ethanol (32 mL) in a two-necked 100-mL round-bottomed flask equipped with a Teflon-coated magnetic stirring bar and reflux condenser. After the solution was warmed in order to dissolve all of the bromo acid, thiourea (0.684 g, 8.98 mmol) was added, and the mixture refluxed for 4 h. Sodium hydroxide (2.55 g 63.7 mmol) dissolved in warm 80% ethanol (14 mL) was then added, and the mixture allowed to reflux for an additional 48 h. After cooling to ca. 50 °C, 18 mL of 4 M HCl was slowly added with stirring, resulting in the formation of a white precipitate. The precipitate was triturated with 3 × 50 mL of diethyl ether, and the ether-insoluble portion discarded. The combined etheral solution was washed sequentially with 1.0 M HCl (2 × 50 mL) and water (3 × 50 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. Recrystallization from ethanol/water (5/3 v/v) afforded 1.75 g (68%) of 16-mercaptohexadecanoic acid having mp 63–65 °C (mp 66–67 °C);[24] ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.28 (m, 22 H), 1.62 (m, 4 H), 2.35 (t, 2 H), 2.53 (q, 2 H).

16-(2-Pyridyldithio)hexadecanoic Acid. 16-Mercaptohexadecanoic acid (0.695 g, 2.41 mmol) was dissolved in ethyl acetate (10 mL) in a 50-mL round-bottomed flask equipped with a Teflon-coated magnetic stirring bar and nitrogen gas inlet. A solution of 2,2'-dipyridyl disulfide (0.531 g, 2.41 mmol) plus 0.0014 g (9.9 μmol) of boron trifluoride etherate, dissolved in 10 mL of ethyl acetate, was then added to the mercaptohexadecanoic acid solution and stirred under a nitrogen atmosphere for 20 h. The solution was then concentrated under reduced pressure. Chromatographic purification of the residue on a silica gel column (25 g, 2.5-cm diameter), eluting with chloroform, afforded after drying [12 h, 23 °C (0.05 mmHg)], 0.826 g (86%) of 16-(2-pyridyldithio)hexadecanoic acid as a colorless solid having mp 53–55 °C; R_f 0.23 [silica, $CHCl_3/CH_3OH$ (20:1, v/v)]; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.28 (br s, 26 H), 2.35 (t, 2 H), 2.80 (t, 2 H), 7.1 (m, 1 H), 7.7 (m, 2 H), 8.5 (d, 1 H); IR (KBr) $\nu_{C=O}$ 1700, ν_{CH-S} 1575, ν_{CH_2-S} 1400–1500 cm^{-1}. Anal. Calcd for $C_{21}H_{35}NO_2S_2$: C, 63.44; H, 8.88; S, 16.10. Found: C, 64.13; H, 9.21; S, 15.72.

1,2-Bis[16-(2-pyridyldithio)hexadecanoyl]-*sn*-glycerol-3-phosphocholine. A mixture of 0.482 g (1.22 mmol) of 16-(2-pyridyldithio)hexadecanoic acid, 0.139 g (0.305 mmol) of *sn*-glycero-3-phosphorylcholine ($CdCl_2$ complex), 0.073 g (0.610 mmol) of 4-(dimethylamino)pyridine, and 0.251 g (1.22 mmol) of dicyclohexylcarbodiimide was suspended in 2.5 mL of freshly distilled chloroform and stirred under nitrogen in the dark for 48 h. After removal of solvent in vacuo, the residue was dissolved in a minimal volume of chloroform and applied to a silica gel column (10 g, 2.0-cm diameter). Sequential elution with $CHCl_3$, $CHCl_3/CH_3OH$ (65/35), and $CHCl_3/CH_3OH/H_2O$ (65/25/4) afforded, after drying [12 h, 22 °C (0.05 mmHg)] 0.181 g (58%) of 1,2-bis[16-(2-pyridyldithio)hexadecanoyl]-*sn*-glycero-3-phosphocholine as a colorless wax: R_f 0.22 [silica, $CHCl_3/CH_3OH/H_2O$ (65/25/4, v/v/v)]; IR (KBr) $\nu N(CH_3)_3$ 950, 1050, 1100 cm^{-1}; 1H NMR ($CDCl_3$, 500 MHz) δ 1.25 (br s, 44 H), 1.6 (m, 8 H), 2.25 (m, 4 H), 2.75 (t, 4 H), 3.3–3.4 (br s, 9 H), 3.85 (m, 4 H), 4.1 (br m, 2 H), 4.35 (m, 2 H), 5.7 (s, 2 H), 7.0 (m, 2 H), 7.6–7.7 (m, 4 H), 8.45 (d, 2 H).

1,2-Bis(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (1). 1,2-Bis[16-(2-pyridyldithio)hexadecanoyl]-*sn*-glycero-3-phosphocholine (0.030 g, 29.6 μmol) was dissolved in 2.5 mL of absolute ethanol. After addition of 0.183 g (1.18 mmol) of dithiothreitol, the resulting solution was allowed to stir under a nitrogen atmosphere, in the dark, for 48 h. Chromatographic purification, using procedures similar to those previously described (Samuel, 1985), afforded a 91% (0.021 g) isolated yield of 1, having a ^{1}H NMR spectrum and R_f value identical with those of an authentic sample.

1-Palmitoyl-2-(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (2). Procedures that were used to prepare 2 were exactly analogous to those described for the preparation of 1, except that monopalmitoyl lecithin was used as the starting lipid. The isolated yield obtained for 1-palmitoyl-2-[16-(2-pyridyldithio)hexadecanoyl]-*sn*-glycero-3-phosphocholine was 57%; R_f 0.28 [silica, $CHCl_3/CH_3OH/H_2O$ (65/25/4, v/v/v)]; IR (KBr) $\nu N(CH_3)_3$ 950, 1050, 1100 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, 3 H), 1.3 (br s, 48 H), 1.6–1.7 (m, 6 H), 2.3 (m, 4 H), 2.8 (t, 2 H), 3.4 (br s, 9 H), 3.9–4.0 (m, 4 H), 4.1 (m, 2 H), 4.4 (m, 2 H), 5.7 (br s, 1 H), 7.1 (m, 1 H), 7.6–7.75 (m, 2 H), 8.48 (d, 1 H). The isolated yield of 2, after the deprotection step, was 91%; R_f 0.4 [silica, $CHCl_3/CH_3OH/H_2O$ (65/25/4, v/v/v)]; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 0.9 (m, 3 H), 1.2–1.4 (br s, 46 H), 1.45–1.7 (m, 6 H), 2.2–2.4 (t, 4 H), 2.5–2.8 (m, 2 H), 3.4 (br s, 9 H), 3.5–4.0 (m, 6 H), 4.4 (br s, 2 H), 5.2 (br s, 1 H); FAB mass spectrum calcd for $C_{40}H_{80}NO_8PS$ 765.5342, found m/e 766.5424 (M + H).

Biological Studies. Protein Adsorption to CPMs. Adhesive polyester film (Falcon no. 3073) was used to isolate round areas (7.5-mm diameter) on the gold surfaces and on tissue culture treated plastic surfaces. ^{125}I-labeled fibronectin was added in different concentrations to the preformed wells on the gold surfaces as a 50-μL drop. Samples were incubated for 1 h at 37 °C in Petri dishes used in order to prevent the evaporation of the applied samples. The drops of material were then aspirated, and residual protein adsorption sites on the gold were blocked by incubation with 3% BSA (50 μL/well) in PBS for 1 h at 37 °C. The unabsorbed material was then extensively washed with α-MEM + 1% BSA + 20 mM HEPES (pH 7.3). Adsorbed proteins were extracted with 1 M NaOH (50 μL/well for 1 h at 20 °C; the efficiency of the extraction was ca. 80–90%) and counted for radioactivity. Adsorption data are presented as an amount of the adsorbed ^{125}I-Fn per square unit of the surface.

Cell Adhesion Assays. Wild type Chinese hamster ovary (CHO) suspension culture cells were grown as previously described.[25] CHO cells have been extensively studied in terms of their adhesion behavior, and they represent a useful cell type for these studies. For adhesion assays, exponentially growing cells (10^5–10^6 cells/mL) were labeled overnight with ^{35}S-Translabel (2–5 μCi/mL), washed in α-MEM + 1% BSA + 20 mM HEPES, and then used in the assays. Radiolabeled cells were suspended in α-MEM + 1% BSA + 20 mM HEPES pH 7.3 ((1–5) × 10^5 cells/mL) and allowed to attach to the surfaces in some experiments for 1 h in a 37 °C incubator. Thereafter, unattached cells were removed by washing with α-MEM + 1% BSA + 20 mM HEPES pH 7.3 at 37 °C, and residual attached cells lysed with 2% SDS, transferred to scintillation fluid, and counted in a scintillation counter. Results of adhesion assays are expressed either as a percentage of the total number of cells or as a percentage of positive control (see figure legends). The number of cells in suspension was measured with a particle counter (ElectroZone Celloscope). All of the data in the absorption studies and adhesion assays represent triplicate independent measurements. Bars indicate the standard error.

Results and Discussion

Sulfur-Bearing Phospholipids. Three phospholipids that have been chosen for the present study are 1,2-bis(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (1, Chart I), 1-palmitoyl-2-(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (2), and 1,2-bis[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine (3). On the basis of analogy to simple thiol-bearing alkanes,[26] lipid 1

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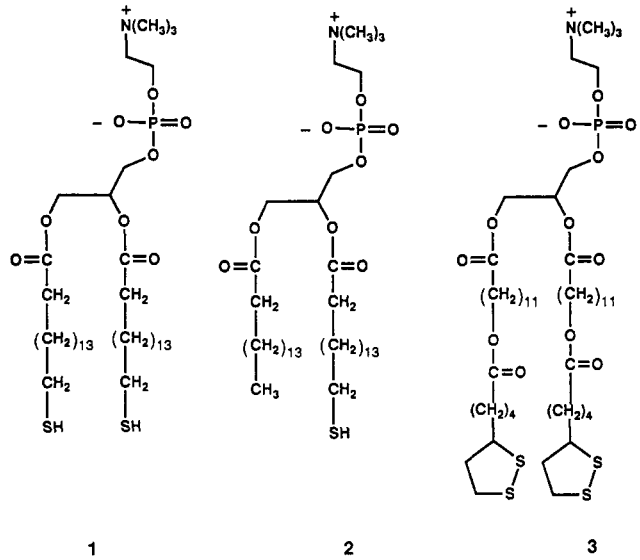
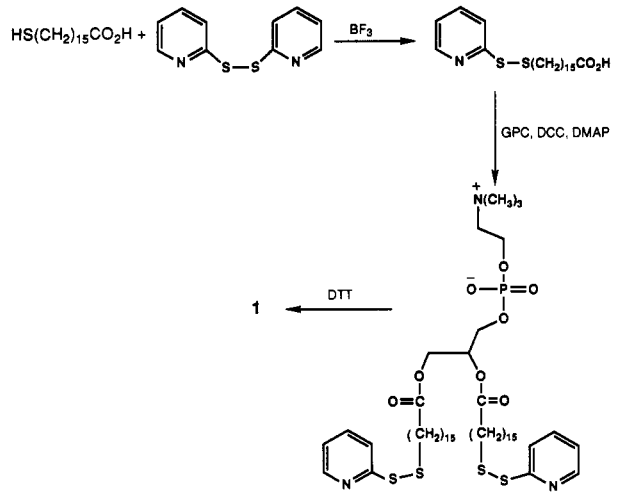
Chart I



1 2 3

Scheme II



was expected to form very tightly packed monolayers in which the alkyl chains are in an all-anti conformation. Space-filling molecular models revealed that a densely packed monolayer of **3**, having an all-anti conformation with each sulfur atom chemisorbed onto the gold surface, should exhibit a significant tilt, due to the placement of the disulfide moieties. With lipid **2**, having only one thiol group on the *sn*-2 chain, one might expect a "looser" and more conformationally flexible membrane surface. Exactly how such variations in phospholipid structure might affect the adhesive characteristics of the resulting CPMs, however, cannot be predicted with certainly.

We have previously described the chemical synthesis of phospholipids **1**, **2**, and **3**.[22,27] In this paper, we include an improved method for the synthesis of **1** and **2** based on an approach that is outlined in Schemes II and III. In brief, protection of the mercapto group of 16-mercaptohexadecanoic acid with 2,2′-dipyridyl disulfide afforded the corresponding 2-pyridyldithio acid. Subsequent esterification with the cadmium chloride complex of *sn*-glycero-3-phosphocholine, followed by reduction with dithiothreitol provided ready access to **1** (Scheme II).

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Scheme III

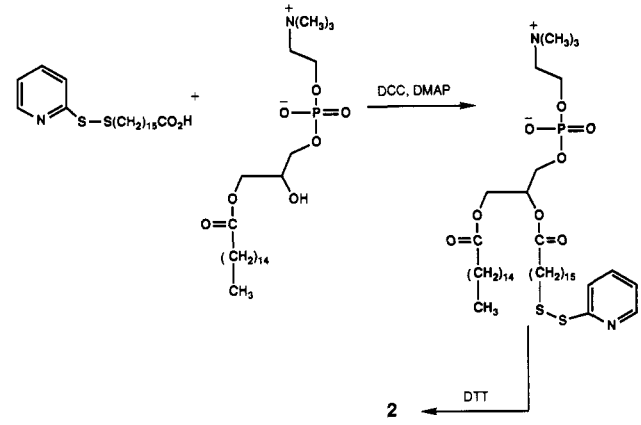

Table I. Wettability and Film Thickness of Chemisorbed Phospholipid Monolayers

phospholipid	advancing contact angle[a] for water, deg	thickness,[b] Å
1	19 ± 3	25 ± 2
2	46 ± 2	28 ± 1
3	20 ± 1	22 ± 2

[a] Advancing contact angles were measured in 50–70% humidity. [b] The refractive index used in all cases was 1.50. The uniformity of each CPM, as judged by contact angle and ellipsometry measurements, was good; standard deviations determined from at least six measurements made at different locations along the surface, were normally less than 2° and 2 Å, respectively.

Analogous esterification of 1-palmitoyl-*sn*-glycero-3-phosphocholine and reduction with dithiothreitol afforded **2** (Scheme III). The principal advantages of this new synthesis over our earlier procedures are (i) the avoidance of the noxious ethyl ethanethiosulfinate protecting reagent, (ii) a simpler chromatographic separation of the 2-pyridyldithio acid from the starting thiol acid, and (iii) the higher yields obtained of the phospholipid product upon deprotection.

Spontaneous Assembly of Phospholipid Monolayers on Gold. Experimental procedures that we have used to prepare CPMs in this study were similar to those that we have previously published.[17] In essence, a glass microscope slide that has been freshly coated with a thin layer of gold (ca. 1000 Å) is immersed in a 1 mM methanolic solution of the desired phospholipid for 24 h. The slide is then removed from the solution, rinsed with methanol, and allowed to air dry for ca. 10 min prior to ellipsometry and contact angle measurements. Advancing contact angles and film thicknesses that were recorded for monolayers of **1**, **2**, and **3** on gold are summarized in Table I. It should be noted that while these lipid films may be regarded as "smooth" at the molecular level, the surfaces are not perfectly flat. Specifically, their macroscopic surface topography has a likeness to a "rolling hills" type of morphology. This can best be visualized by scanning tunneling microscopy[28] and by scanning electron microscopy.[29] Nonetheless, compared with Sepharose, polyethylene, and polystyrene, they can be regarded as true surfaces, where a *well-defined phase-boundary exists.*

Cell Adhesion. Chinese hamster ovary (CHO) cells were chosen for this investigation because they represent an extensively studied, clonable cell line that grows well

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Table II. Adhesion of CHO Cells to Chemisorbed Phospholipid Monolayers[a]

protein	cell adhesion (% of total cells)			
	untreated gold	1 on gold	2 on gold	3 on gold
albumin (10 mg/mL)	1.2 ± 0.3	1.2 ± 0.4	8.1 ± 2.5	4.0 ± 1.1
fibronectin (1 μg/mL)	91.7 ± 6.5	93 ± 4.3	94.4 ± 3.4	82.3 ± 8.8
vitronectin (5 μg/mL)	100 ± 2.5	75 ± 5.2	86.3 ± 4.1	77.6 ± 5.1

[a] Cells were allowed to adhere to untreated gold surfaces or chemisorbed phospholipid monolayers that were pretreated with the indicated proteins for 1 h at 37 °C, and subsequently blocked with 3% bovine albumin. The cell adhesion experiment was performed at 37 °C in α-MEM + 1% albumin + 20 mM HEPES.

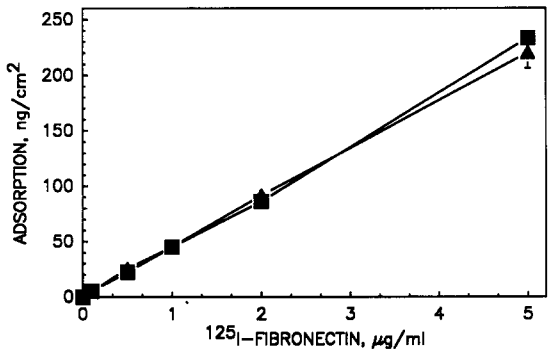

Figure 1. Adsorption of ^{125}I to (▲) gold and (■) 3-modified gold surfaces. Purified fibronectin was labeled with ^{125}I as described in the methods section. Protein adsorption to gold or CPMs of lipid 3 was permitted for 1 h at 37 °C. Thereafter, the samples were thoroughly washed with buffer, and the absorbed proteins stripped from the surfaces with 1 M NaOH. Both the adsorbed protein and the protein remaining in solution were analyzed in a γ counter. Ordinate, ng of Fn/cm² of surface area; abscissa, solution concentration of Fn (μg/mL).

in suspension culture, and that readily adheres to substances coated with extracellular matrix proteins.[25,30,31] Specifically, we have examined the ability of CHO cells to form stable adhesions to glass slides coated with gold alone and with CPMs derived from 1–3. The adhesion assay that has been used in this study is similar to assays that have been widely used to study cell adhesion behavior. Adhesion experiments were carried out in the presence of bovine albumin or purified bovine fibronectin or vitronectin. The principal results that have been obtained are shown in Table II. In a medium containing only bovine albumin, the cells exhibited minimal adhesion to each of the surfaces examined. In experiments in which the purified cell adhesion factors fibronectin or vitronectin were included in the assay, the cells were able to adhere efficiently (>75%).

In separate experiments, the adsorption of fibronectin on CPMs derived from 3 and also on untreated gold surfaces was investigated via the use of a radiolabeled (^{125}I) form of the protein. As seen in Figure 1, both types of surfaces adsorb Fn at protein concentrations that are comparable to those used to prepare substrata for adhesion. In each case, the amount of protein that becomes adsorbed to the surface was found to be linearly related to the solution concentration. In preliminary studies, we have also found that ^{125}I-labeled albumin or vitronectin (data not shown) also show significant adsorption on both types of surfaces.

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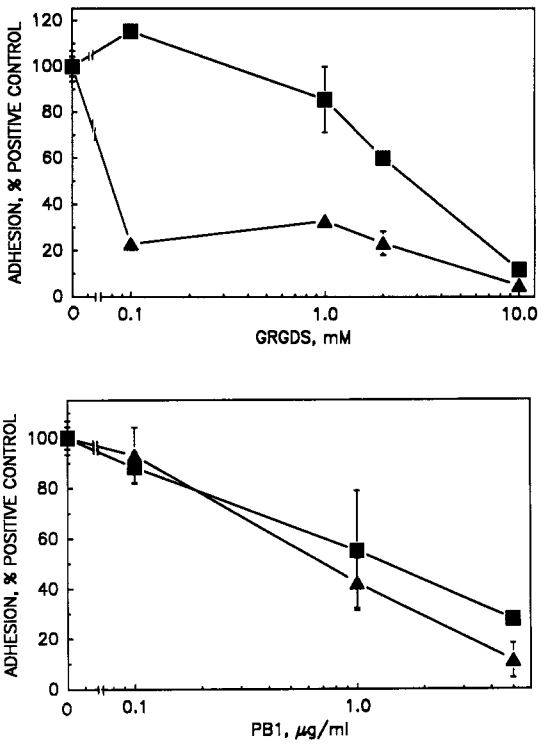

Figure 2. Top: inhibition of adhesion to CPMs of lipid 3 (■) and untreated gold (▲) by GRGDS. Surfaces were coated with Fn (1 μg/mL) and then blocked with BSA as described in the methods section. Cells were allowed to adhere to these surfaces in the presence or absence of various concentrations of GRGDS peptide for 1 h at 37 °C. The adherent cells were analyzed as described in the methods section. Ordinate, percent of control (untreated) adhesion; abscissa, concentration of GRGDS. Bottom: inhibition of adhesion to CPMs of lipid 3 (■) and untreated gold (▲) by PB1 antibody. CPMs coated with Fn were prepared as above. Cells were allowed to adhere to these surfaces in the presence or absence of various concentrations of PB1, an anti-fibronectin receptor monoclonal, for 1 h at 37 °C. Ordinate, percent of control (untreated) adhesion; abscissa, concentration of PB1 (μg/mL).

In an effort to establish whether or not the fibronectin-mediated binding of CHO cells to CPMs derived from 3 occurs via an RGD-mediated pathway, we have tested the ability of two types of reagents to block the adhesion process. Specifically, we have used a soluble peptide that contains the RGD sequence and which competitively inhibits interaction between cells and the RGD site in fibronectin. We have also used a monoclonal antibody that blocks the function of the cell surface receptor for fibronectin.[32] As seen in Figure 2, top, it is clear that GRGDS can fully inhibit the adhesion of CHO cells to a fibronectin-coated CPM and also to an untreated gold surface at concentrations that are approximately comparable to those used to block other RGD-mediated adhesion processes that have been reported.[13] Interestingly, the process of cell adhesion to fibronectin coated on gold is especially sensitive to inhibition of GRDGS. The exact reason for this is not clear at the present time. One possibility is that the fibronectin adopts a different conformation on gold surfaces than on the phospholipid modified gold. In Figure 2, bottom, it is also clear that PB1, a monoclonal antibody directed against the 140 KD cell surface receptor for fibronectin, can completely block adhesion to fibronectin-coated CPMs (or gold surfaces). Taken together, these results provide strong evidence that fibronectin induces the adhesion of CHO cells to such CPM surfaces entirely

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by interactions between the RGD site in fibronectin and its integrin-type cell surface receptor. They also imply that these phospholipid surfaces can support a natural cell adhesion process (i.e., specific biochemical recognition).

Chemisorbed phospholipid monolayers, of the type described herein, should provide an attractive framework from which to covalently append adhesion-promoting ligands and to investigate, *quantitatively*, ligand–cell surface interactions, in the presence of nonadhesive proteins (e.g., albumin). Studies that are now in progress are being aimed at synthesizing derivatized CPMs for such use, where particular attention is being focused on the influence of ligand density and peptide sequence on the cell adhesion process.

Registry No. 1, 93404-44-5; 2, 116307-29-0; 3, 122862-88-8; GRGDS, 96426-21-0; Au, 7440-57-5; 16-bromohexadecanoic acid, 2536-35-8; 16-hexadecanolide, 109-29-5; 16-mercaptohexadecanoic acid, 69839-68-5; 2,2′-dipyridyl disulfide, 2127-03-9; 16-(2-pyridyldithio)hexadecanoic acid, 123265-49-6; 1,2-bis[16-(2-pyridydithio)hexadecanoyl)-*sn*-glycerol-3-phosphocholine, 123265-50-9; *sn*-glycero-3-phosphorylcholine, 28319-77-9; 1-palmitoyl-2-[16-(2-pyridyldithio)hexadecanoyl]-*sn*-glycero-3-phosphocholine, 123265-51-0; 1-palmitoyl-*sn*-glycero-3-phosphocholine, 12364-16-8.

Synthesis, Characterization, Theoretical Modeling, and Polymerization of New Fluorophore-Containing Derivatives of Thiophene and Pyrrole

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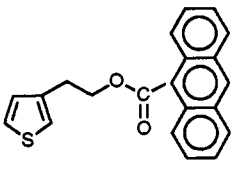
Two new derivatives of thiophene and pyrrole containing an anthracene model fluorophoric core were synthesized and characterized by NMR and IR spectroscopies, elemental analyses, voltammetry, and spectrofluorometry. Molecular orbital calculations were used to assess the conformational preferences and spin populations of these compounds. The cyclic voltammograms of these compounds contained signatures assignable to both the anthracene and the parent heterocyclic moieties. In this regard, these compounds represent good models for the concept of an electrophoric group discussed in our previous work. In the case of the thiophene derivative, the electrochemistry of the anthracene moiety preceded that of the thiophene ring. Open-shell calculations of the spin density within the unrestricted Hartree–Fock approximation revealed the electron density to be mostly delocalized onto the anthracene moiety. This coupled with unfavorable energetics inhibited polymerization of the thiophene derivative. On the other hand, polymerization of the pyrrole derivative was facile and readily probed by voltammetry. The electroactivity of the polymeric films was investigated with a ferrocene redox probe. In both cases, the fluorescence of the anthracene core was strongly quenched by the polymer backbone.

Introduction

A variety of pyrrole derivatives along with polymers derived from them have been reported in recent years.[1-29] Generally, derivatization begins with substitution at the N center followed by subsequent polymerization utilizing the α and α' positions of the pyrrole ring. An alternative strategy has utilized functionalization (e.g., acylation) of the pendant N–H groups at a polypyrrole surface.[22] A wide range of redox moieties have been incorporated via these two strategies including, for example, ferrocene,[22,25,28,29] $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridyl),[1-3,11,12,20,23] viologens,[5,12,13,17] macrocycles,[10,19,21,24,26,27] phenothiazine,[12,14] copper(II) bipyridyl,[20] $Cu(dpp)_2^+$ (dpp = 2,9-diphenyl-1,10-phenanthroline),[15] anthraquinone,[6] and organic nitroxides.[16] Much less chemistry has been reported for the thiophene system wherein derivatization has been carried out at the β position of the ring to yield either "self-doped" or soluble polymers and oligomers.[30]

In this article, we report the synthesis, characterization, and polymerization of new derivatives of thiophene and pyrrole (1 and 2) containing anthracene as a model fluorophoric core. Molecular orbital calculations were performed to assess the spin densities in the monomeric and



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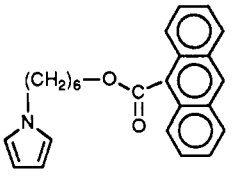

2

dimeric radical cation forms of 1 and 2. These, along with projections of the conformational preferences, aided in the

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