Permeability Characteristics of Liposomes in a Net—Membranes of Dihexadecyl Phosphate with Polymerizable Gegenions

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ABSTRACT: The sonication of hydrated bilayers of choline methacrylate dihexadecyl phosphate (1) produces small vesicles (90-nm diameter) with a lipid-phase transition at 37 °C. The vesicles are readily photopolymerized by irradiation of the 209-nm absorption band. Both the unpolymerized and the polymerized vesicles of 1 entrap the water-soluble marker [^{3}H]glucose. The light-induced polymerization increased the temperature of the lipid-phase transition and decreased the membrane permeability to [^{3}H]glucose by a factor of 4.6. The absorption spectra and phase-transition behavior indicate that the hydrophobic chromophore at one end of the water-soluble monomeric gegenion, choline methacrylate, penetrates into the bilayer of dihexadecyl phosphates. Unsymmetrical membrane vesicles of dihexadecyl phosphate (DHP) and choline methacrylate on either the vesicle exterior or interior were prepared. Unpolymerized and polymerized unsymmetrical vesicles successfully entrapped [^{3}H]glucose. The [^{3}H]glucose permeability of outside membranes was reduced by a factor of 3.4 by polymerization but was not altered significantly for the inside membranes. This suggests that the vesicle permeability is primarily controlled by the outside leaflet of the bilayer of DHP membranes.

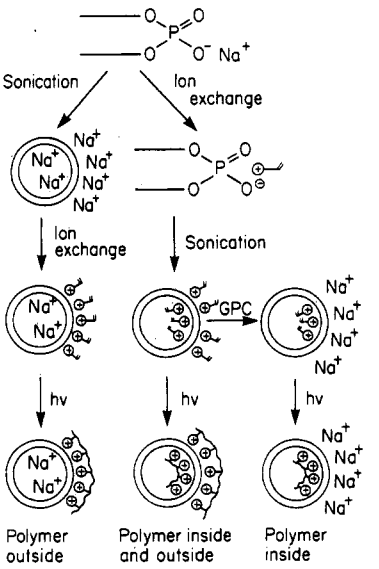
Liposomes or vesicles, i.e. closed spherical lipid bilayer structures, have become a useful model for biochemical and biophysical investigations during the last two decades.[1,2] Vesicles are also of interest for their ability to encapsulate reagents, for the separation of charges, and for the effect of the organized bilayer on chemical reactions. The introduction of readily available synthetic amphiphiles[3] produced an enormous variety of new membrane-forming compounds.[4] Within a short time several groups demonstrated that vesicles could be stabilized by covalent linking of lipid membranes within the bilayer, the formation of "polymerized vesicles".[5]

Some biomembranes are stabilized by a polymer coat that supports the membrane. The architecture of biomembranes is highly unsymmetric, and supporting biopolymers usually occur only at one side of the double layer. This is illustrated by the spectrin net, attached to the inner red cell membrane surface, or the murein coat, surrounding the outer membrane surface of bacteria as a component of the cell wall.

Attempts to mimic this natural process of membrane stabilization by coating a bilayer membrane with polymers are described in the literature. Polymers have been fixed to vesicle membranes by ionic interactions[6-11] via hydrophobic anchor groups[12] or by polymerization of charged, water-soluble monomers at membrane surfaces. These ionic monomers were fixed to charged lipid molecules either via salt formation[13,14] or as counterions.[15-19] Stabilization of monolayers from cationic amphiphiles at air–water interfaces by polyion complex formation was described recently.[20] In principle, the association of monomers with charged lipid vesicles, and their polymerization in place, allows for the formation of membranes in which the hydrophobic region of the bilayer is not directly altered, yet the vesicle is protected by the polymer coat.

The electrostatic association of monomers with the vesicle surface provides a means of preparing unsymmetrical polymerized vesicles. Previous papers describe attempts to mimic the cytoskeleton by preparation of unsymmetrical polymer-coated vesicle membranes.[18,19] These vesicles are based on the exchange of counterions at the outer surface of preformed small unilamellar vesicles according to Scheme I.

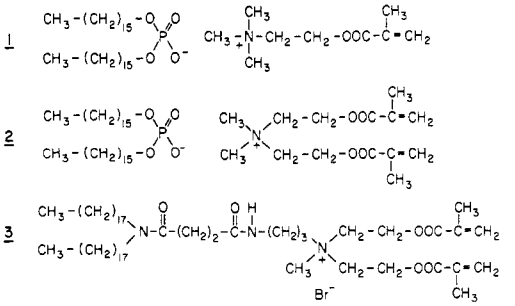
Scheme I



Thus, polymerizable counterions can be fixed and polymerized only at one side of the bilayer. We present here a kinetic analysis of the permeability properties of glucose across these monomeric and polymerized unsymmetrical bilayers. These lipid vesicle properties are compared with those of vesicles from sodium dihexadecyl phosphate (NaDHP) and dihexadecylphosphoric acid (DHP).[21-26]

Experimental Section

Compounds. The following dihexadecyl salts, with either a monofunctional choline methacrylate counterion 1 or a bifunctional counterion 2, were used for this study.



According to Scheme I, the monofunctional salt 1 was used to prepare vesicle membranes covered with linear polymer chains at the inner, outer, or both sides of the bilayer. The bifunctional

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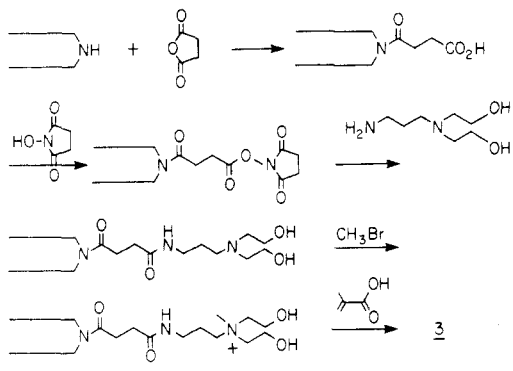
Scheme II

Covalent crosslinker 3 synthesis



ionic cross-linker **2** was used to create a polymer net only at the inner vesicle surface. The cross-linker lipid **3**, with two covalently bound polymerizable groups, was combined with lipid **1**. This bilipid mixture leads to a cross-linked polyelectrolyte net at the inner membrane surface, which is covalently bound to the membrane by hydrophobic anchor groups due to lipid **3**. This system is termed "artificial cytoskeleton" in this paper.

The preparation of these compounds is detailed in the previous paper,[19] and the preparation of cross-linker lipid **3** is outlined in Scheme II.

Lipids **1** and **2** were prepared by ion exchange with a cation-exchange resin (Merck Lewatit SP 1080). This resin was loaded with polymerizable cations by treatment with an excess of a 10% solution of the appropriate bromide salt in water. After column resin was loaded, the water was replaced by methanol by washing with excess methanol. A solution of NaDHP in methanol was passed through this column. The eluent fractions containing the lipid were collected, and the solvent was evaporated. The residue was recrystallized from acetone two times. The NMR spectrum indicated a 95–100% exchange of sodium ion against the organic cation.

Materials. Dihexadecyl phosphate (DHP) and Sephadex G 50 medium and PD 10 columns were purchased from Sigma. Tritiated glucose was obtained from New England Nuclear and had a specific activity of 33.1 Ci/mmol. The concentration of the stock solution was 1 mCi/mL (ethanol/water, 9/1). Water was purified by a Millipore Milli-Q system.

Preparation of Symmetrical Vesicles. The lipid was weighed into polycarbonate screw-cap vials, water was added, and the mixture was sonicated with a Heat Systems Cup Horn Sonicator for 15 min at a temperature above the phase transition of the lipid (energy output 60 W). The final lipid concentration was 10 mg/mL for DHP and 20 mg/mL for the DHP salts.

Preparation of Unsymmetrical Vesicles. (a) Monomer and Polymer Only at the Outer Membrane Surface. A glass column (17 × 1.5 cm) was filled with a cation-exchange resin (Merck Lewatit SP 1080). This resin was loaded with choline methacrylate cations by treatment with an excess of 10% solution of the appropriate bromide salt in water. The excess salt was then removed by washing the resin with water. Five-milliliter vesicles from NaDHP (20 mg/mL) were passed through this column by a peristaltic pump at a flow rate of 0.5 mL/min. The main vesicle fraction, indicated by UV absorption at 254 nm, was collected and divided into two samples. One of them was polymerized by UV irradiation immediately.

(b) Monomer and Polymer at the Inner Membrane Surface. Symmetric vesicles from compounds **1** and **2** (20 mg/mL) were prepared as described above. The polymerizable counterions were exchanged only at the outer membrane surface against sodium ions by two successive gel permeation chromatographies (GPC), using PD 10 columns (5 × 1.5 cm). The first GPC used a 20 mM NaCl solution as an eluent. The excess NaCl was removed from these vesicles by a second GPC, which was carried out immediately, with water as an eluent. UV spectroscopy indicated 35% of the polymerizable counterions were recovered after the second GPC. The vesicle fraction from the second GPC was divided, and one sample was polymerized by UV-irradiation immediately.

(c) Preparation of an Artificial Cytoskeleton. Vesicles from a mixture of 90 mol % of lipid **1** and 10 mol % of lipid **3** were prepared. The counterions at the outer surface were removed as described in (b). The resulting system has the covalent cross-linker **3** on both sides of the membrane but the polymerizable counterions from **1** only inside. After copolymerization of the two lipids at the inner membrane surface, the "artificial cytoskeleton" is formed.

Polymerization of Vesicles. (a) Azo Initiator. The vesicle solution (20 mg/mL lipid) was mixed with an aliquot of a 10 mM 4,4′-azobis(4-cyanovaleric acid) (ACVA) solution in water, to give a final concentration of initiator of 5 mol %. The solution was flushed with argon for 5 min and then sonicated briefly at 55 °C to speed the diffusion of initiator into the vesicles (which did not cause a decrease in monomer absorption). Polymerization of the mixture was accomplished by heating the sample for 2 h at 70 °C. The polymerized vesicle solution showed a strongly increased turbidity.

(b) UV Irradiation. The vesicle solution was transferred to a 1-cm quartz cuvette with Teflon stopper and flushed with argon for 5 min. This sample was irradiated at 4 °C with a low-pressure mercury lamp (Pen-Ray) at a distance of 2 cm for 1.2 h. During irradiation the solution was stirred magnetically.

The total conversion of the monomer was indicated by decrease in the absorption maximum at 209 nm.

[^{3}H]Glucose Release. Sample Preparation and Gel Permeation Chromatography (GPC). (a) Symmetrical Vesicles. The procedure used is similar to that reported by Dorn et al.[27] [^{3}H]Glucose stock solution (1.5 mL) was pipetted into a glass vial, and the solvent was evaporated by a stream of nitrogen. A 2-mL vesicle suspension (20 mg/mL) was added and sonicated for 1 h at 50 °C in an ultrasonic bath to speed the diffusion of the marker into the vesicles. The sample prepared from the polymerizable lipid **1** was divided, and one part was polymerized by UV irradiation or a water-soluble azo initiator.

(b) Vesicles from DHP. [^{3}H]Glucose (1.5 mCi), 16 mg of DHP, and 1.6 mL of water were mixed in a polycarbonate tube and sonicated in the cuphorn sonicator for 15 min at 70 °C and for 15 min at 80 °C (70-W output).

(c) Unsymmetrical Vesicles from Lipids 1 or 2. [^{3}H]Glucose (750 μCi) was added to 1050-μL polymeric vesicle solution and heated for 1 h at 60 °C without sonication, to maintain the unsymmetrical structure. Monomeric samples were treated by the same procedure, which in this case resulted in a symmetric distribution of the different counterions across the membranes.

GPC with Symmetrical Vesicles. The symmetrical-vesicle samples were chromatographed with a Sephadex G 50M column (Pharmacia, 1 × 35 cm) at 20 °C to separate the vesicles containing entrapped [^{3}H]glucose from free [^{3}H]glucose. A low-intensity UV monitor (254 nm) was used to monitor the fractions. A 700-μL sample of vesicles was applied to the column by a syringe connected to a three-way valve. A peristaltic pump was used to obtain a constant flow rate of 0.5 mL/min. Fractions of 1 mL were collected. The vesicles were recovered between 10 and 18 mL, and the free glucose was recovered between 20 and 40 mL. Two-milliliters of the vesicle fraction was used for dialysis.

GPC of Unsymmetrical Vesicles. Vesicles with polymer only at one side of the membrane were chromatographed with a Sephadex G 25M column (PD 10, Pharmacia); 1 mL of the vesicle solution was applied, and fractions of 1 mL were collected, using water as eluent. The vesicle fraction with the entrapped glucose appeared between 3 and 5 mL. The first part of this fraction, eluted between 3 and 4 mL, was used for dialysis. Neumann et al.[28] have shown that this procedure results in a good separation of vesicles from free [^{3}H]glucose.

Scintillation Counting. Aliquots (25 μL) of each fraction were pipetted into 20-mL screw-cap vials containing 10-mL scintillation cocktail and counted for 2 min in a Packard Tri-Carb liquid scintillation spectrometer (Model 3380). The total activity of the vesicle solutions was determined as follows: A 25-μL aliquot was diluted to 25 mL, and aliquots of 25 and 50 μL were counted. The average value was taken for the total activity.

Dialysis. The vesicle fraction (1 or 2 mL) was placed in a dialysis bag (MW cutoff 10 000) and dialyzed against 200 mL of water. At different time intervals aliquots (between 0.1 and 1 mL) were taken, and the activity of the released [^{3}H]glucose was

Table I
Estimated Diameters and Permeability to [^{3}H]Glucose of Symmetric and Unsymmetric, Monomeric, and Polymerized Vesicle Membranes at 24 °C

compd	polymerizable counterion: inside	polymerizable counterion: outside	mono	poly	effective diameter (nm) determined at an angle of: 45°	90°	135°	$10^{-5}k_1$, s	$10^{-10}P_1$, cm s^{-1}	entrap, %
1	×	×	×		132	89	73	7.7	11.5	1.9
1	×	×		× (UV)	147	73	65	2.2	2.7	3.6
1	×	×		× (azo)	307	136	96	...	...	
1		×	×		93	83	73	3.9	5.3	
1		×		× (UV)	92	80	72	1.2	1.6	
1	×		×		57	52	46	2.5	2.1	
1	×			× (UV)	102	68	57	2.4	2.7	
2	×		×		67	60	51	3.2	3.2	
2	×			× (UV)	102	83	75	2.4	3.4	
1 + 3 (10%)	×		×		100	76	61	2.7	3.4	
1 + 3 (10%)	×			× (UV)	94	76	64	2.3	2.9	
NaDHP					76	68	65	3.5	3.9	0.5
DHP					197	168	167	15.2	42	0.7

determined by scintillation counting (50-min counting time). For each aliquot, the same amount of water was added. The 100% value was determined by releasing the contents of the dialysis bag. This value agreed well with the original activity applied to the bag.

Glucose Permeability. A quantitative picture of the permeability of the membranes to glucose was obtained from the fraction of [^{3}H]glucose released from the dialysis bag over time. The permeability data were analyzed by the procedure of Johnson and Bangham.[2,29] This analysis assumes that the fast initial rate is due to the outer bilayer of the vesicles and that the permeability of the dialysis bag is fast and can be neglected compared to the membrane permeability. The vesicles did not affect the dialysis rate of the free [^{3}H]glucose out of the bag.

The analysis leads to the following equation

$$\ln\left[\frac{NV_0}{V_i + V_0} - N_t\right] = \ln\frac{NV_0}{V_i + V_0} - k_1\frac{V_0 + V_i}{V_0}t \qquad (1)$$

where $k_1 = (A_1/V_1)P_1$, A_1 = surface area of the inside membranes, V_1 = internal volume of the vesicles, P_1 = permeability of the membrane, N = counts in membranes, V_0 = volume of the dialysate, V_i = volume inside the dialysis bag, and N_t = counts in dialysate at $t = t'$. A plot of eq 1 as a function of time should result in a straight line whose slope, $k_1[V_0 + V_i/V_0]$, is related to the size and permeability of the membranes. Evaluation of the vesicle size by light scattering or electron microscopy allows the calculation of the permeability, P_1.

Inelastic Light Scattering. A sample of the vesicle solution (100 μL) was diluted to 3 mL with water and then filtered through a Nucleopore filter (0.45 μm) directly into the measuring cell. The measurements were performed with a Brookhaven light-scattering goniometer equipped with a helium–neon laser. The data were analyzed with a Brookhaven Instruments digital correlator BI-2030. The scattered light was observed at angles of 45, 90, and 135°.

Differential Scanning Calorimetry (DSC). The samples were measured in a Hart Scientific differential scanning calorimeter, Model 701, at a scan rate of 20 °C/h for both heating and cooling. Hydrated bilayers were prepared by mixing the lipid with water to obtain a final concentration of 10 mg/mL and heating the sample above the phase transition for 2 h. In addition, this mixture was vortexed several times. Sonicated vesicles were prepared as described above for symmetrical vesicles (20 mg/mL).

Transmission Electron Microscopy. The vesicle solution (2–5 mg/mL) was placed for 15 s on a Formvar-covered copper grid, and then the drop was sucked off with filter paper. The staining solution (2% phosphotungstic acid, pH 7.1) was placed on the grid for 30 s and then removed with filter paper. The grid was observed with a JEOL 100 S electron microscope.

Results and Discussion

Vesicle Formation. Milky white lipid bilayers were prepared by hydration of compounds **1**, **2**, **3**, or their mixtures with Milli-Q water. The bilayers (10–20 mg/mL) were sonicated under argon in a cuphorn at temperatures above the lipid-phase transition (see below) for sufficient time to form opalescent suspensions. Inelastic light-scattering measurements at 90° indicated that the effective diameter of the dispersed particles produced by the sonication varied from 50 to 170 nm depending on the lipid (see Table I). The differing effective diameters measured at 45° and 135° from that measured at 90° reflect the polydispersity of the vesicle diameters. Vesicle samples were observed under negative stain by transmission electron microscopy. A micrograph (Figure 1a) from a sample of vesicles of **1** shows circular images 50–100 nm in diameter, which are ascribed to the unpolymerized vesicles.

The absorption spectrum of vesicles of **1** has a maximum at 209 nm (ϵ 6 × 10^3). The absorption data are consistent with the empirical rules of Woodward for the absorption of α-methyl-substituted α,β-unsaturated esters, which predicts the maxima to be at 209 nm in ethanol.[30] The absorption at 209 nm for the choline methacrylate ion associated with dialkyl phosphate vesicles indicates that the chromophore is in a less polar environment than water. This observation can be reasonably accommodated by a model (Figure 2) for the vesicles of **1** where the positive choline end of the gegenion is in the water interface of the phosphate vesicles and the polymerizable methacryloyl chromophore extends into the bilayer rather than away from it as implied by the representation in Scheme I.

A model for the vesicles of **1** where the gegenion penetrates into the bilayer leads to an expectation of bilayer disordering on going from vesicles of sodium dihexadecyl phosphate (NaDHP) to choline methacrylate dihexadecyl phosphate (**1**). This prediction is confirmed by the phase behavior of each of these membranes. Differential scanning calorimetry measurements of NaDHP bilayer membranes (Figure 3a) show a sharp transition at 64 °C, whereas the bilayer membranes of **1** have a significantly lower phase-transition temperature (40 °C, Figure 3b). A similar depression of the transition temperature was found for dimyristoylphosphatic acid bilayers when the gegenion was changed from sodium to choline methacrylate.[19]

Vesicle Polymerization. Both photo and thermal initiation of vesicle polymerization were evaluated for vesicles of **1**. If the vesicles (10 mg/mL) are polymerized by UV irradiation at 4 °C (below the lipid-phase transition), the resulting vesicles are spherical with little sign of aggregation (see electron micrograph in Figure 1b). The vesicle size determined by inelastic light scattering (Table I) is not significantly altered by the photopolymerization.

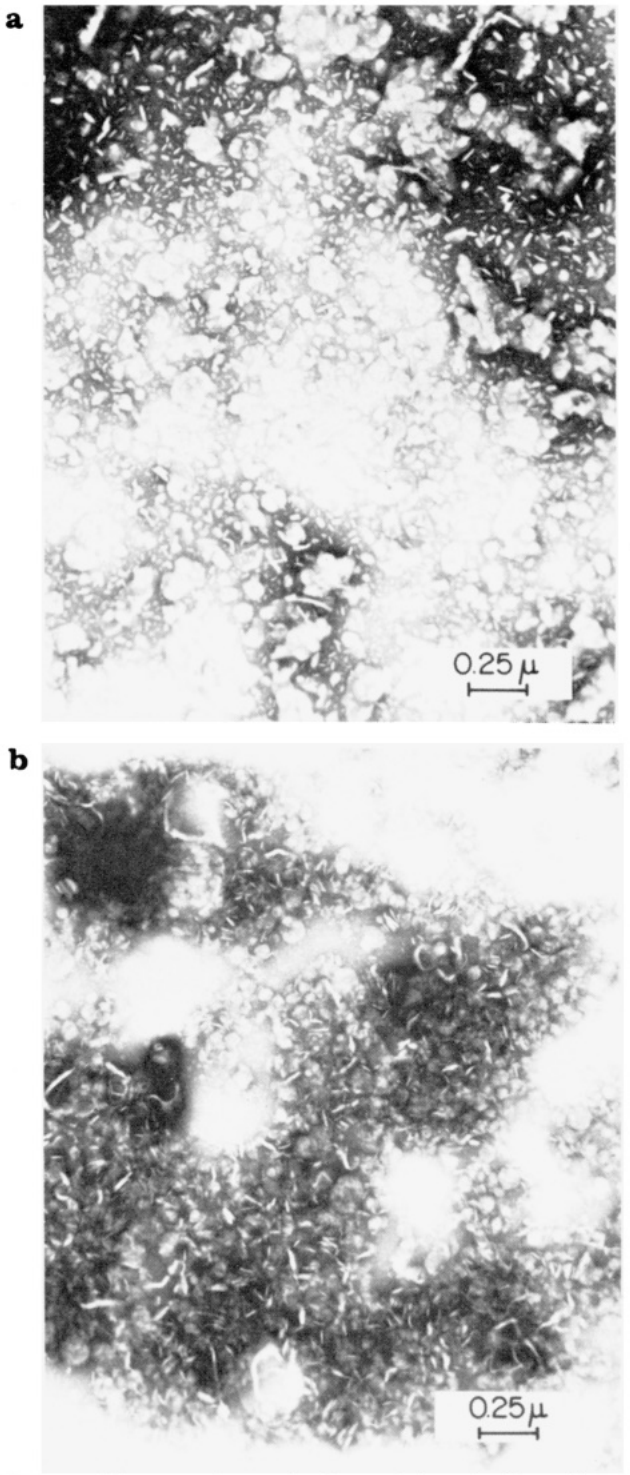


Figure 1. Electron micrograph of negatively stained membrane vesicles of 1 (a) before and (b) after photopolymerization.

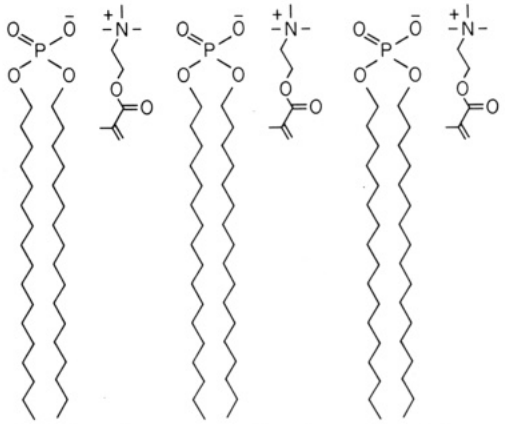


Figure 2. Schematic representation of a portion of the membrane of choline methacrylate dihexydecyl phosphate, 1.

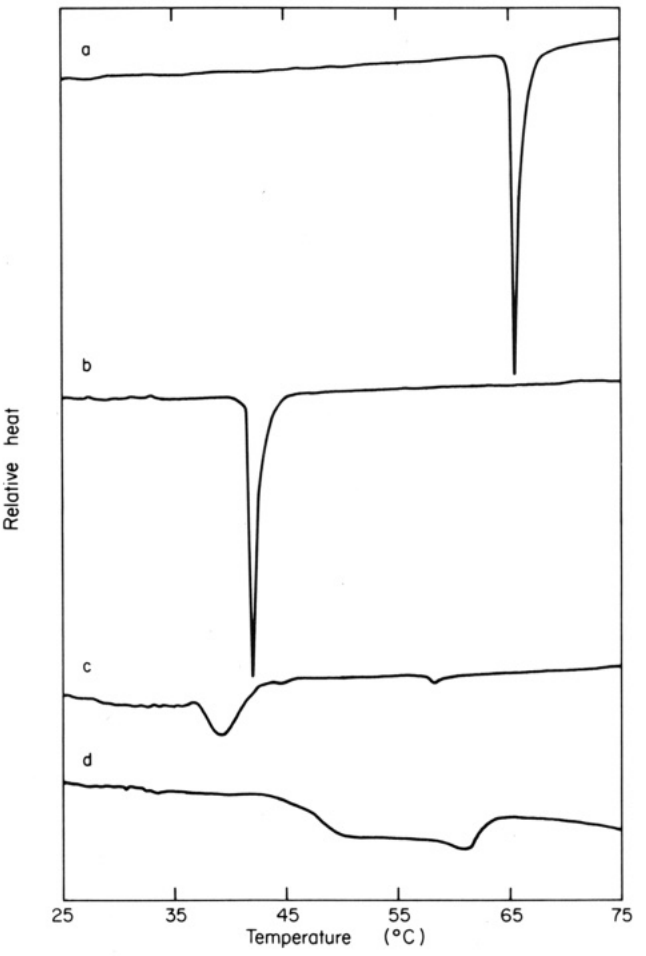


Figure 3. Differential scanning calorimetry heating curves: (a) hydrated bilayers from NaDHP; (b) hydrated bilayers of lipid 1; (c) sonicated vesicles of monomeric lipid 1; (d) sonicated vesicles of polymerized lipid 1.

The photoconversion was greater than 95% as determined by the loss of the 209-nm absorption.

Differential scanning calorimetry of lipid bilayers is sensitive to size of the vesicle because of the lipid chain disorder induced in small unilamellar vesicles by sonication. Consequently, it is important to compare DSC measurements on preparations of the same morphology if possible. The measurements on unpolymerized membranes of 1 were done with extended bilayers because the lipid-phase transitions of extended bilayers are normally sharper and more pronounced than those of small sonicated vesicles. However, to determine the phase transition of membranes of poly-1, it was necessary to use small sonicated vesicles because the polymerization of extended bilayers resulted in precipitation. Consequently, the DSC of monomeric sonicated vesicles of 1 was measured as well as of the polymerized vesicles. The monomeric vesicles show a broad phase transition at 37 °C (Figure 3c). After polymerization of the vesicles of 1, two broad overlapping peaks are found at about 48 and 57 °C (Figure 3d). The elevation of the phase-transition temperature indicates that the bilayer is stabilized by the reaction and suggests a model of the membrane where the cationic polymer penetrates less into the bilayer than the monomeric choline methacrylate ion. The presence of two transitions indi-

cates two distinct populations of lipid arrays. One interesting possible explanation, which will require further exploration, is that one transition is due to the inside half of the bilayer and the other to the outside half of the bilayer of poly-1 vesicles. This could be brought about by the vesicle radius of curvature and the effect of curvature on the polymer–DHP interaction.

Thermal-initiated polymerization of vesicles of 1 with water-soluble 4,4′-azobis(4-cyanovaleric acid) (ACVA) occurs above the phase-transition temperature of the membranes. The monomer absorption at 209 nm decreased to less than 5% of the starting value after 2 h at 70 °C. The thermally polymerized vesicle solutions show a large turbidity increase, even at low lipid concentrations (3 mg/mL). These vesicles have almost doubled in diameter upon polymerization as shown by inelastic light scattering (Table I). The probable cause of the size increase is aggregation of the small unpolymerized vesicles. Electron micrographs indicate the presence of aggregates of flat, disk-like particles after polymerization with ACVA. These polymerized vesicles do not pass through a gel permeation column, and therefore they were not pursued further in this study.

All unsymmetrical vesicle membranes were prepared by the ion-exchange methods described in the Experimental Section. Photopolymerization with UV radiation was employed to yield unsymmetrical polymerized vesicles. Preparation of the linear polymer from vesicles with the choline methacrylate ion only outside the vesicle did not alter the vesicle size (see Table I). Polymerization of vesicles with the polymerizable gegenion only on the inside of the vesicles resulted in a slight increase in diameter (Table I).

Attempts to polymerize vesicles prepared from the bifunctional lipid 2 (cross-linker–gegenion) were not successful. These vesicles precipitated and formed gels during UV irradiation and thermally initiated polymerizations (concentration range, 2–20 mg/mL). An attempt to minimize intervesicle reaction was unsuccessful with lipid mixtures prepared from 95/5 mol % lipid 1/2. The vesicles precipitated upon UV irradiation (20 mg/mL). Intervesicle polymerization was prevented by exchange of the bifunctional counterions at the outer vesicle surface for sodium ions. It is then possible to polymerize the remaining bifunctional counterions at the inner membrane surface and presumably form a polymer shell on the inside of the vesicle even at lipid concentrations of 20 mg/mL. The formation of a cross-linked polymer at the inner membrane surface slightly increases the diameter of the vesicles (Table I).

Entrapment of [^{3}H]Glucose. The vesicle dispersions effectively entrap water-soluble compounds such as [^{3}H]glucose. The monomeric and photopolymerized vesicles behave in a typical fashion for lipid vesicles on gel permeation chromatography. The column profile for the separation of polymerized vesicles of 1, with polymer chains on both sides of the membrane, is shown in Figure 4. The values of activity and the turbidity of the vesicle fractions correlate well, as indicated by the UV monitor. There is good separation between vesicles, which contain the entrapped marker, and the free glucose. The amount of entrapped [^{3}H]glucose is 3.6%. The column profiles for monomeric vesicles and that of NaDHP (0.5% entrapped marker) look similar. The lower entrapment efficiency in the case of NaDHP vesicles is probably due to their smaller size (Table I). Some authors have reported that NaDHP vesicles display a disklike shape.[21,23]

Vesicles from the nonionized DHP showed an unusual column profile (Figure 5). The turbid fraction, which was

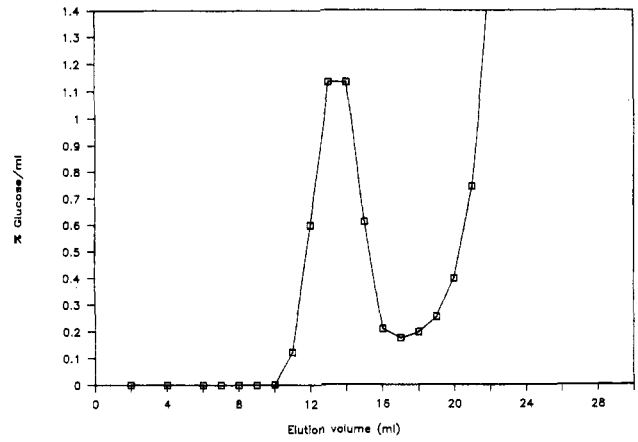


Figure 4. Column profile for the separation of polymerized vesicles of 1 with entrapped [^{3}H]glucose from free [^{3}H]glucose on Sephadex G 50 column.

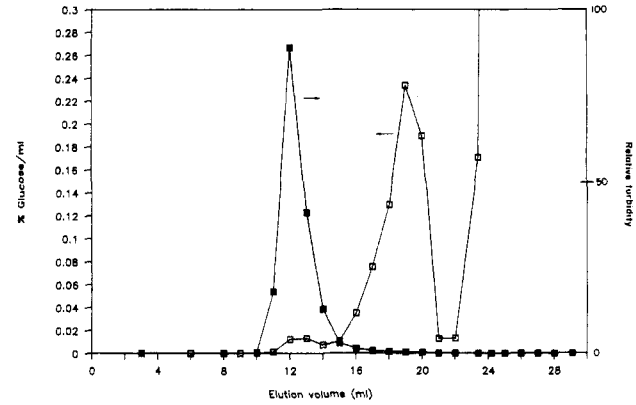


Figure 5. Column profile for the separation of DHP vesicles with entrapped [^{3}H]glucose from free [^{3}H]glucose on Sephadex with G 25 column: (a) □, percent [^{3}H]glucose/mL vs elution volume; (b) ■, relative turbidity vs elution volume as detected by UV monitor.

eluted in the void volume of the column, contained only 0.03% of the marker. This fraction was followed by a second one, containing 0.7% of the marker, but showing much less turbidity. Because the release behavior of this second fraction is significantly slower than that of free glucose, it must be a second vesicle fraction, which is different in size of shape. The nature of the first fraction has not been determined.

Permeability. Membrane permeability of vesicle bilayers can be conveniently measured with nonionic molecules such as [^{3}H]glucose, because it has a reasonable permeability through the membrane. The measurements may be made in a matter of several hours, since the value is $\sim 10^{-10}$ cm/s for glucose through phosphatidylcholine bilayers.[2] A nonionic marker is necessary to evaluate the permeability of the charged vesicles with associated gegenions (monomer or polymer) used in this study, since charged species would alter the vesicle. The glucose permeabilities for some other monomeric and polymerized vesicle systems have been reported[27,28,31] and serve as a basis of comparison of the data presented in this paper.

In all cases the release behavior of the vesicles showed an initial rate followed by a second, slower rate after a few hours. A typical release curve for glucose in symmetrical monomeric and polymeric vesicles from 1 is shown in Figure 6. The same data are used to obtain the logarithmic plot (according to eq 1) shown in Figure 7. These figures show that the formation of polymer chains from 1 bound ionically to the bilayer surfaces alters the mem-

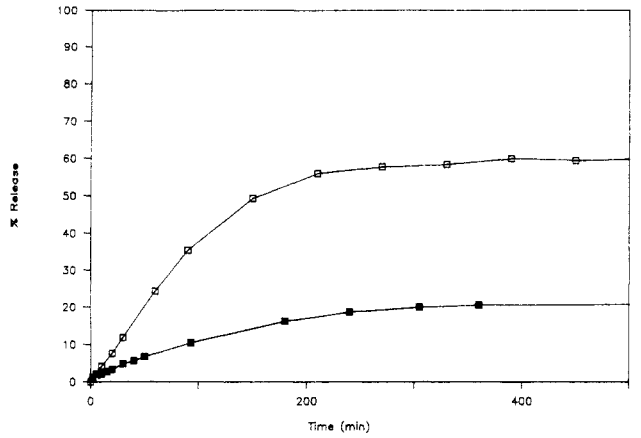


Figure 6. Percent release of [^{3}H]glucose vs time at 24 °C for vesicles of monomeric 1 (□) and polymerized 1 (■).

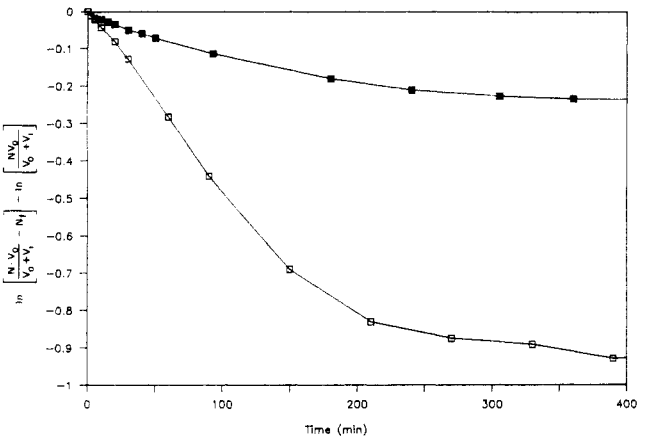


Figure 7. Semilogarithmic plot for data from Figure 6, plotted according to eq 1: monomeric 1, □; polymerized 1, ■.

brane in a manner to decrease the permeation rate for glucose. The reduction was a factor of 4.6 for poly-1, which is comparable to the effect found by Dorn et al.[27] for the polymerization of α-methacryloyl groups covalently bound to ammonium lipids. The permeability of monomeric 1 is significantly higher than that of NaDHP, and the polymerization results in a decrease to a permeability somewhat less than NaDHP vesicles. This observation is consistent with Figure 2 and suggests that the main effect of the polymerization is to remove the gegenion from the bilayer and allow the DHP^- to form a more tightly sealed membrane. If this explanation is correct, the chief barrier to permeability is the bilayer and not the polymer coat. Hence, this polymerized vesicle behaves like an erythrocyte, where the polymer controls the physical stability and shape and the bilayer controls the permeability.

The formation of vesicles with monomer 1 on the vesicle exterior and NaDHP on the interior was accomplished by ion exchange of prepared vesicles as described earlier. The permeability of glucose through these membranes was a factor of 2 less than membranes of 1 on each side. Polymerization of the monomers at vesicle exterior reduced the vesicle permeability by a factor of 3.4 (Table I).

The unsymmetrical vesicle systems, obtained from compound 1 or 2, which bear the polyelectrolyte coat (linear or cross-linked) only at the inner membrane surface, show no significant difference in permeability compared with the corresponding monomeric systems. Even if the polymer chains of 1 are linked together and bound covalently to the inner membrane surface with 10 mol % of 3, the "artificial cytoskeleton", the change in permeability is very small.

These observations provide a new insight into the functioning of lipid bilayer in vesicles as permeability barriers. Note that the minimum permeability in all of these experiments is quite similar to that of the NaDHP vesicles. It is only when the choline methacrylate ion is associated with the exterior or both sides of the vesicle that the permeability is increased. This increase is negated when the gegenion is polymerized. As discussed above, this indicates that the choline methacrylate ion disturbs the bilayer packing in a manner that can be reversed upon polymerization. The curious observation is the lack of permeability effect when this gegenion (monomer or polymer) is only inside the vesicle. This suggests that the choline methacrylate does not interact strongly with the inside half of the bilayer. Since the DSC of vesicles of 1 (Figure 3c) does not indicate the presence of distinguishable populations of lipids, there is no clear evidence of a different type of interaction of the choline methacrylate with each side of the vesicle bilayer. This leaves the possibility that the chief barrier to glucose permeability is the outside leaflet of the bilayer and that the presence of DHP^- at that layer yields the minimum permeability of these membranes. Further experimentation will be necessary to test this hypothesis.

In summary, the absorption properties, permeability studies, and DSC measurements indicate that the choline methacrylate cation changes the properties of the DHP bilayer. The phase transition is shifted to lower temperature, and the permeability for glucose is increased by the replacement of sodium with cholinemethacrylate. This organic cation has a short, hydrophobic chromophore, which appears to insert into the hydrophobic region of the double layer. The positively charged ammonium groups are still in the aqueous phase, just as the negative charges of the dialkyl phosphate anions. The diffusion of the organic cation across the membrane is very slow because the hydrated ammonium group has little tendency to enter the membrane. The choline methacrylate structure is similar to that of the membrane impermeant, acetylcholine. Photopolymerization of this gegenion diminishes the perturbation of the bilayer, and the physical properties of the vesicles become more like those of the starting NaDHP membranes.

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Registry No. 1, 111254-66-1; 1 (homopolymer), 111244-29-2; 2, 111822-72-1; 2 (homopolymer), 111847-66-6; 3, 105317-34-8; (3)(1) (copolymer), 111847-67-7; NaDHP, 60285-46-3; glucose, 50-99-7.

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A Helical Poly(amino acid) Having Carbazole Side Chains: A Candidate for a Photoelectric Liquid Crystal. 3. A Conformational Study

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ABSTRACT: The conformation of a carbazole-substituted poly(α-amino acid), poly(N^{ϵ}-(carbazolylcarbonyl)-L-lysine) (PKL), has been studied in solid films and solutions by fourier transform infrared spectroscopy. In all samples investigated the main chain assumes the α-helical conformation. The side chains are strongly hydrogen bonded in the solid state, with approximately 20% unbounded groups. A side-chain conformation is proposed, where the major part of the side chains are stacked in three stacks almost parallel to the molecular axis. The average stack length exceeds 10 units and can in principle be infinite. The carbazole groups in the stacks are efficiently locked-in conformationally in a structure which probably favors effective energy transfer and photoconduction. In solution the hydrogen bonding of the side chains is disrupted.

Introduction

Carbazole-containing polymers have been extensively studied in recent years with respect to their photoelectrical, mainly photoconductive, properties.[1-3] These investigations have lead to the supposition that the spatial arrangement of the carbazole groups is decisive for the photoconductive properties.[4,5] It is thus desirable to correlate the conformation of these polymers with their photoconductive properties.

The charge carrier transport in photoconductive polymers and the related energy transfer of uncharged, excited electronic states (excitons), which may be studied by fluorescence techniques, are both π-orbital phenomena. Due to the inherent anisotropy of the π-orbital it is expected that the distance between neighboring carbazole groups as well as the angle between the plane normals will determine the orbital overlap and thus the interaction. The Förster theory of exciton diffusion requires a reciprocal sixth power dependence of the distance and a cosine square dependence of the angle.[4] For charge carrier transport one may speculate as to a similar dependence.

Also the macroscopic regularity of the spatial arrangement is important, since the charge carriers have to be transported through the entire sample in order to contribute to the conductivity. It is believed that structural defects act as traps for the carriers, immobilizing and eventually annihilating them,[5-7] leading to recombination.

In this article we present studies on the conformation of a carbazole-substituted polypeptide, poly(N^{ϵ}-(carbazolylcarbonyl)-L-lysine) (PKL). The chemical structure of this polymer enables the polypeptide backbone to form an α-helical structure, giving a rod-shaped macromolecule capable of forming liquid-crystalline (LC) solutions. Due to the helical backbone structure, the side-chain conformations are presumably within narrowly defined limits. Furthermore the LC properties are conducive for preparing samples with well-defined orientation and morphology. This polymer is thus suitable for conformational studies by polarized spectroscopy, since the results can be inter-

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