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Polymerization in black lipid membranes

Influence on ion transport

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Abstract. A variety of different lipids containing dienoyl groups in the side chains were tested for membrane formation using the planar lipid bilayer approach. One of these lipids formed stable bilayers which could be polymerized using UV-illumination. The influence of the polymerization was studied in monolayers, lipid vesicles and planar bilayers. The stability of the lipid bilayer membranes was increased by polymerization. Thus, the lifetime of the membranes increased from about 1 h to 4–5 h or longer. Furthermore, the specific conductance of unmodified membranes and of carrier-mediated transport is reduced. The transport of lipophilic ions was investigated as a function of polymerization using the charge-pulse method. The absorption of dipicrylamine (DPA^-) is not affected. The translocation of this compound and of tetraphenylborate ($B(Ph)_4^-$) showed a strong decrease with polymerization time. The influence of polymerization on the membrane structure may be explained on the basis of a strong viscosity increase in the lipid bilayer membrane.

Key words: Black lipid membrane, carrier, charge pulse relaxation, lipophilic ions, polymerizable lipid

Introduction

Lipid bilayers and lipid vesicles have been extensively used in recent years as models for biological membranes. The great disadvantage of all these systems is their limited stability, especially in the presence of membrane active surfactants. Polymerized lipid systems have a much higher stability (for a review see: Fendler (1984)). Subsequently, polymerized liposomes were investigated as potential stable model systems for drug application and compartmentation (Bader et al. 1984). In all cases investigated so far, the polymerization of the lipids used for formation of monolayers and vesicles led to long-term stability of these structures and to a reduced permeability of the vesicles (Bader et al. 1985). On the other hand it could be shown that reconstituted enzymes retained their activity after polymerization (Wagner et al. 1981; Pabst et al. 1983).

The stability of planar lipid bilayer membranes could be a severe problem. Whereas the polymerization of lipids in vesicles has been investigated in detail, only one study has been published so far in which the successful polymerization of lipids in lipid bilayer membranes has been reported (Benz et al. 1982). In this study it was shown that the stability of the membranes was, in fact, increased after polymerization. However, because of the lateral contraction of the lipid molecules during polymerization the membranes became leaky, presumably because of the formation of holes (Benz et al. 1982). In this study a number of polymerizable lipids containing one or two dienoyl side chains were tested for their ability to form lipid bilayer membranes and, if possible the properties of these lipid bilayers were measured (compare Table 1). One of the lipids containing one dienoyl- and one C_{18}-side chain formed stable bilayers (see Table 1). The effect of the polymerization on the permeability properties of these membranes were studied in detail. Polymerization, in general, led to reduced permeability of the membranes. Furthermore, stability and lifetime of the membranes was found to be drastically increased.

Materials and methods

Dienoyl lipids

The dienoyl lipids used in this study are shown in Table 1. Compounds (2), (3) and (4) were prepared as described by Büschl (1984).

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Synthesis of the dienoyl-lipid (1)

Compound (1) was synthesized as described (Elbert 1984) starting from octadeca-2,4-dienoic acid via esterification of the acid chloride with N,N-dimethylethanolamine (see below). Subsequently the ternary amino group was transferred to a quaternary ammonium group by quaternization with octadecylbromide.

Esterification of the octadeca-2,4-dienoic acid with N,N-dimethylethanolamine: Octadeca-2,4-dienoic acid (3.7 g, 13.2 mmol) was stirred with a 4-fold excess of oxalyl chloride (6.70 g, 52.8 mmol) for 20 h at 20 °C. Then the reaction product was distilled at 0.2 mm Hg and 145 °C. The yield was 1.55 g (5.2 mmol, 52%) of fatty acid chloride. N,N-dimethylethanolamine (0.48 g, 5.4 mmol) was dissolved in 30 ml of dry ether. A solution of 1.55 g (5.2 mmol) of the fatty acid chloride in 20 ml dry ether was added dropwise at a temperature of 20 °C to the N,N-dimethylethanolamine. The reaction mixture was stirred for 1 h. The precipitate was filtered, dissolved in 50 ml chloroform and washed with 0.1 *M* NaOH and water. After removal of the residual water with Na_2SO_4 the organic solvent was evaporated. The crude reaction product was used for the quaternization without further purification.

Quaternization with octadecylbromide: The reaction product of the esterification (1.82 g, 5.2 mmol) and 3.5 g (10.5 mmol) octadecylbromide were dissolved in 50 ml acetone and were kept at 40 °C for 2 days. Then the reaction mixture was transferred to an ice bath. The precipitate was filtered and purified by chromatography across a silica gel column using $CHCl_3/CH_3OH$/aqueous NH_3 (65 : 15 : 1) as eluate to give the pure compound (1) (yield: 2 g (2.9 mmol, 56%), m.p.: 85 °C). The elemental analysis gave C: 70.14%, H: 11.47%, and N: 2.04% which agrees well with the values calculated from the formula $C_{40}H_{78}BrNO_2$ (MW 684.97): C: 70.08%, H: 11.52%, and N: 1.65%.

Monolayer and liposome experiments

Monolayer isotherms were measured on a computer controlled filmbalance similar to that described by Albrecht (1983). In the cover of the filmbalance a low pressure mercury lamp was installed for polymerization of the lipids. Compounds were spread from dilute (0.5 mg/ml) solutions in chloroform on the surface of pure unbuffered water. For polymerization, the monolayer was kept at a surface pressure of 10 mN/m and irradiated with UV-light. During polymerization experiments the whole monolayer trough was flushed with nitrogen.

Liposomes were prepared by sonication of a dispersion of lipid (1 mg/ml) in pure water with a Branson sonifier B 15 P (microtip, output power 20–25 W) for 5 min. The polymerization was carried out with a 150 W high pressure Xe-lamp (full spectrum). The degree of polymerization was followed with a Beckmann Acta IV UV/Vis spectrophotometer.

Black lipid membrane experiments

Black lipid membranes were formed from 1–2% (w/v) solutions in *n*-decane/chloroform 1:1 (v/v) in the case of the polymerizable amphiphiles and in *n*-decane alone in the case of dioleoyllecithin, dioleoylphosphatidyl-ethanolamine and diphytanoyllecithin. Chloroform was obtained from Merck (p.A. grade) and *n*-decane from Fluka (puriss. grade). Dioleoyllecithin, dioleoylphosphatidyl-ethanolamine and diphytanoyllecithin were purchased from Avanti Biochemicals (Birmingham, Alabama). Nonactin was from Fluka and puriss. grade. Dipicrylamine (DPA) and sodium-tetraphenylborate ($NaB(Ph)_4$) were from Merck.

The carrier and the lipophilic ions were added to the aqueous phase in the form of concentrated solutions in ethanol. The concentration of ethanol in the aqueous phase did not exceed 0.1% by volume.

The aqueous solutions were prepared using twice distilled water or water that has been purified by a Millipore purification system (Milli Q, Millipore Corp.). NaCl and KCl both were from Merck and p.A. grade. Concentrations of the salts were 1 M unless otherwise stated. Experiments were performed at 48–52 °C in Teflon chambers with two compartments separated by a thin wall with an aperture 1 mm in diameter. The stationary conductance was measured by applying a voltage of 10 mV to the membrane and measuring the current with a Keithley 610 C electrometer. For the measurements of the electrical capacity, rectangular voltage pulses of 25 mV were applied from a Hewlett Packard HP 8011 A pulse generator through Ag/AgCl-electrodes to the black membranes. The decay of the capacitive current was measured via the voltage drop across a resistor (5–20 kOhm) with a Gould OS 4040 digital storage oscilloscope. The membrane capacitance, C, was determined by integrating the exponential decay of the current with time according to the relation

$$C = I_0 \tau / V_0 , \quad (1)$$

where I_0 is the current extrapolated to zero time, τ is the decay time of the current and V_0 is the applied voltage. The specific membrane capacitance, C_M, was obtained by correcting for the stray capacitances

and by dividing the capacitance, C, by the actual area of the membrane. In control experiments the setup was checked using equivalent circuits simulating the bilayer membrane system. The results agreed within 1%.

The charge pulse measurements were carried out as described previously (Benz et al. 1976). In brief: one Ag/AgCl electrode was connected to the pulse generator (Philips PM 5712) through a fast diode (reverse voltage resistance $> 10^{11}$ Ohm) and the other Ag/AgCl electrode grounded. A resistance of 10^6 Ohm was introduced between the two electrodes in order to receive a defined RC-time of the membrane. The voltage between these two electrodes was measured by a fast voltage amplifier with a high input resistance on the basis of a Burr Brown operational amplifier and a digital storage oscilloscope (Nicolet Explorer III). The time resolution of the system was better than 500 ns. The exponential decays were analysed with a HP 9825 calculator and a HP 9862 plotter (Benz et al. 1983). The voltage relaxations in the charge pulse experiments could be described in all cases by the sum of two relaxation processes.

In order to reach partition equilibrium for the adsorption of lipophilic ions to the bilayers, all kinetic measurements were carried out at least 30 min after the membrane had completely turned black (Benz et al. 1976). For the polymerization experiments the membranes were irradiated with a low-pressure mercury lamp (Quarzlampen Gesellschaft Hanau) through the quartz window in the teflon chamber (full spectrum, intensity at the membrane surface 0.5 mW cm^{-2}).

Theory

Nonactin mediated K^+-transport

The treatment of carrier mediated ion transport under stationary conditions has been given in detail in previous publications (Stark and Benz 1971; Benz and Stark 1975). The model assumes 1:1 stoichiometry between carrier molecule and ion. Furthermore, it is based on the assumption that the complexation reaction between carrier S (total aqueous concentration c_0) and ion M^+ (aqueous concentration c_M) takes place at the membrane solution interface with the on-rate k_R and the off-rate k_D. Complex MS^+ and free carrier S cross the membrane with the rate constants k_{MS} and k_S, respectively. It was shown that the rate limiting step in the nonactin-mediated ion transport is the movement of the charged molecules across the membranes, which means that the carrier-ion system is in the "equilibrium" domain (Stark and Benz 1971; Benz and Stark 1975; Eisenman et al. 1975). The membrane conductance λ_0 in the limit of small voltages is given by the following equation (Benz 1978).

$$\lambda_0 = \frac{F^2 d}{2RT} \frac{k_{MS} K \gamma_{MS} c_M c_0}{(K c_M + 1)}, \tag{2}$$

where d is the membrane thickness, K is the stability constant of the complexes in the aqueous phase, γ_{MS} is the partition coefficient of the carrier-ion complex between membrane and aqueous phase, F is Faraday's constant, R is the gas constant and T is the absolute temperature.

Transport of lipophilic ions

The theory of the movement of lipophilic ions across lipid bilayer membranes has been given in full detail in previous publications (Ketterer et al. 1971; Benz et al. 1976; Benz and Conti 1981). Here we will only summarize the basic assumptions and list the simplified equations which allow the calculation of the transport parameters from the experimental results. It is assumed that the lipophilic ions are adsorbed to deep free energy minima on both sides of the membrane with a total concentration of N_t per unit surface. For symmetry reasons (equal solutions and symmetric bilayer) it is assumed that at zero membrane potential the lipophilic ions are equally distributed between the two membrane-solution interfaces and cross the intermediate free energy barrier with the same rate constant, k_i, in either direction. Under these conditions the shape of the barrier has no influence on the characteristics of the voltage relaxation measured in a charge pulse experiment as long as the initial membrane potential perturbation is much smaller than 25 mV (Benz and Zimmermann 1983). Finally we neglect the exchange of lipophilic ions between the membrane and the aqueous phase during a single relaxation measurement because it is rate limited by slow diffusion (Benz et al. 1976) and has characteristic times much longer than the passive RC time constants, τ_M, of the lipid bilayer membranes.

In a charge pulse experiment the system is in equilibrium at times $t < 0$ and the membrane capacitance is charged instantaneously at $t = 0$ to an initial voltage V_0. The decay of membrane voltage with time, $V(t)$, is given by two exponential relaxations:

$$V(t) = V_0(a_1 \exp(-\tau_1 t) + a_2 \exp(-\tau_2 t)), \tag{3}$$

where a_1, $a_2 = 1 - a_1$, τ_1 and τ_2 are known functions of k_i, N_t and τ_m (Benz and Conti 1981; Benz and Zimmermann 1983). The inverse relations are given

Table 1. Properties of black lipid membranes from polymerizable lipids

No.	Compound	Temp. [°C]	Life-time	Cond. [$S\,cm^{-2}$]	Cap. [$\mu F\,cm^{-2}$]
–	Diphytanoyllecithin	25	2–4 h	10^{-8}	0.29 ± 0.03
(1)	$CH_3-(CH_2)_{12}-CH=CH-CH=CH-COO-CH_2-CH_2-N^+(CH_3)(CH_3-(CH_2)_{17})(CH_3)\ Br^-$	50	ca. 1 h	$10^{-5}-10^{-6}$	0.52 ± 0.08
(2)	$[CH_3-(CH_2)_{12}-CH=CH-CH=CH-COO-CH_2-CH_2-N^+(CH_3)_2-CH_2-CH_2-OOC-CH=CH-CH=CH-(CH_2)_{12}-CH_3]\ 2Br^-$	40	1–5 min	10^{-5}	–
(2) / Cholesterol 1:1 mixture		40	5–10 min	$10^{-4}-10^{-5}$	–
(3)	$CH_3-(CH_2)_{10}-CH=CH-CH=CH-COO-CH_2-CH_2-N^+(H)(CH_2-CH_2-SO_3^-)-CH_2-CH_2-OOC-CH=CH-CH=CH-(CH_2)_{10}-CH_3$	10–50	no formation of black lipid membranes		
(4)	$CH_3-(CH_2)_{12}-CH=CH-CH=CH-COO-CH_2-CH_2-N^+(H)(CH_2-CH_2-SO_3^-)-CH_2-CH_2-OOC-CH=CH-CH=CH-(CH_2)_{12}-CH_3$	10–50	no formation of black lipid membranes		

by:

$$k_i = (a_1 \tau_2 + a_2 \tau_1)/2 \quad (4)$$

$$N_t = 2RTC_M(\tau_1 + \tau_2 - 2k_i - \tau_1\tau_2/k_i)\,Fk_i \quad (5)$$

$$\tau_m = 2k_i/\tau_1\tau_2\,, \quad (6)$$

where R is the gas constant, T is the absolute temperature and F is the Faraday constant.

Results

Polymerization in monolayers and liposomes

The dienoyl-lipids used for polymerization studies in black lipid membranes are given in Table 1.

From the four monomers investigated only the mixed chain lipid containing one polymerizable unit (compound (1)) was able to form BLMs stable enough for further investigations. This lipid was thus characterized in monolayers and in liposomes. If forms stable monolayers at the gas-water interface which can easily be polymerized by irradiation with UV-light (254 nm). Polymerization in monolayers can be followed via the change in area at constant surface pressure (see Fig. 1). After polymerization the monolayer exhibits enhanced stability. It shows a higher collapse pressure compared to the monomeric monolayer.

The polymerization reaction is schematically described in the following reaction scheme (Scheme 1). The polymer backbone shows a 1.4-*trans*-poly(butadiene) structure as described for the corresponding monolayer polymer of the long chain dienoic acid (Schupp 1981).

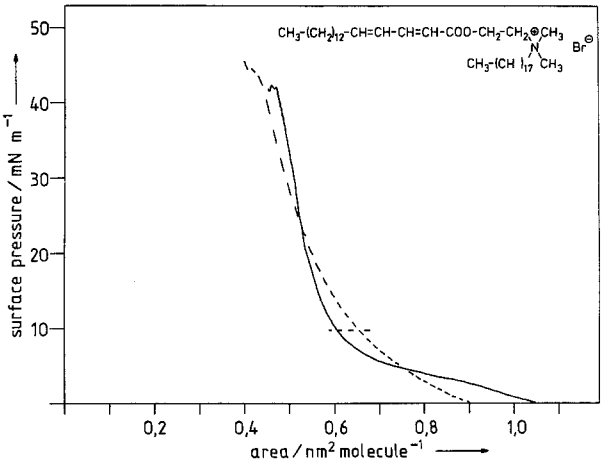
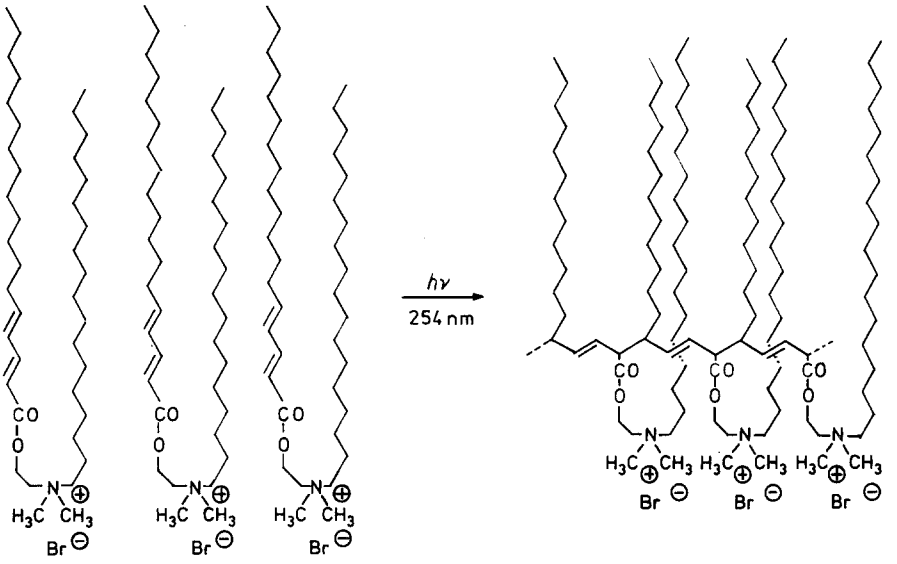


Fig. 1. Monolayer isotherm of compound (1) before —— and after ---- polymerization. $T = 20$ °C. The polymerization was carried out at 10 mN/m by irradiation with UV-light for 5 min. (Intensity: 2 mW/cm²)

Lipid (1) can also be polymerized in liposomes. The polyreaction can be followed by UV-spectroscopy via the decrease in the absorbance band of the dienoyl group at 261 nm. This is shown in Fig. 2. The polyreaction is complete within at least 15 min.

After the polymerization the liposomes also exhibit enhanced stability and their aqueous dispersions remain stable for several weeks. In contrast to



Scheme 1. Reaction scheme for the UV polymerization of the dienoyl-lipid (1)

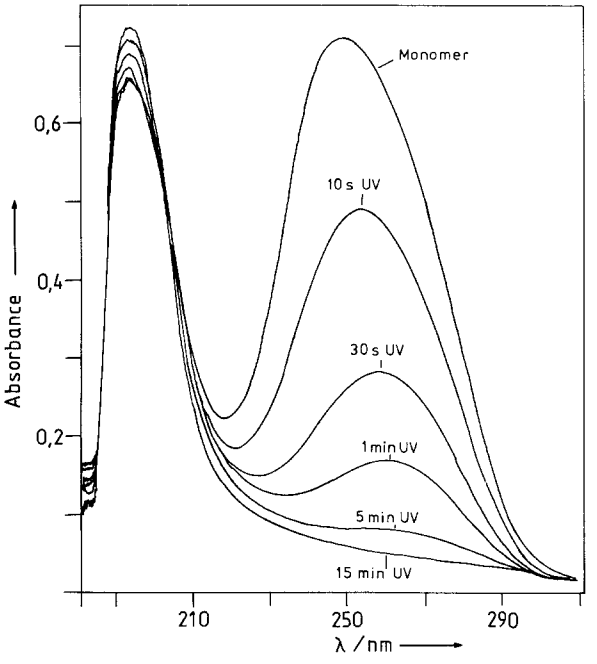


Fig. 2. Polymerization of the dienoyllipid (1) in liposomes as followed by UV-spectroscopy

this, monomeric vesicles precipitate within a few days.

Lipid bilayer experiments

Conductivity and capacitance. Membranes from the non-polymerized (monomeric) lipid (1) remained stable for about 1 h (at 50 °C) (compare Table 1). The specific conductance was in the range 10^{-5} to 10^{-6} $S\,cm^{-2}$, whereas the capacitance was 0.52 $\pm$ 0.08 $\mu F\,cm^{-2}$ (mean of 25 membranes). When the black membranes were irradiated with UV-light, they usually became stable for longer times (several hours). Capacitance did not change during UV-irradiation, whereas the conductivity decreased by a factor of about 5 within 10 min as shown in Fig. 3.

Both the increase in lifetime and the decrease in conductance of the BLMs on UV-irradiation suggests that the UV-induced polymerization of compound (1) results in an increase of membrane stability.

Influence of polymerization on nonactin mediated carrier transport. In order to get more detailed information about the change of membrane structure and its kinetics during polymerization, the effect on the carrier-mediated conductance of membranes from lipid (1) was studied. Because of the sensitivity of valinomycin towards UV-irradiation, nonactin, which is UV-stable, was used in these studies. The UV-stability of nonactin was checked with bilayer membranes from lecithin where no effect of UV-light on nonactin conductance was observed. The membranes from the dienoyl lipid (1) had a conductance of

$$120 \pm 50\ \mu S\,cm^{-2}$$

in a solution of 100 mM KCl and 1 μM Nonactin (mean of 10 membranes). UV-irradiation led to a strong decrease of the nonactin induced membrane conductance. The effect of polymerization is shown in Fig. 4. The nonactin mediated K^+-transport decreased rapidly to less than 10% of the initial value.

The effect of polymerization on nonactin mediated K^+-transport may be discussed on the basis of Eq. (2). The only two constants which could depend on membrane properties are the translocation rate constant k_{MS} and the partition coefficient γ_{MS} for the carrier-ion complexes. We cannot completely exclude the possibility that γ_{MS} is dependent on the

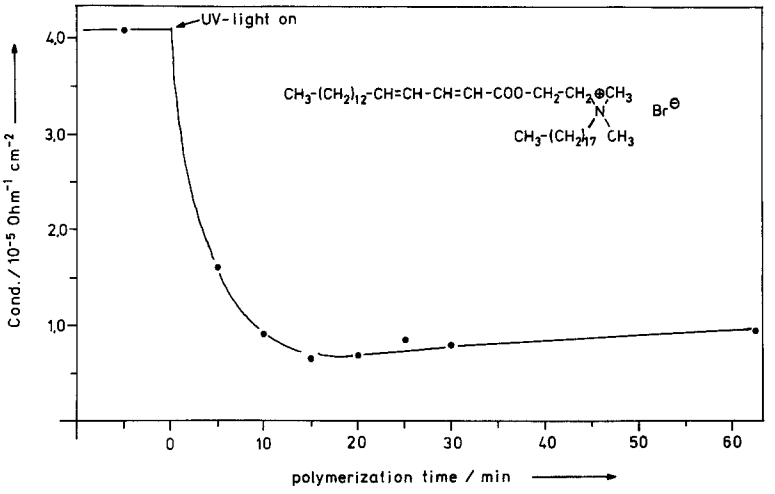


Fig. 3. Effect of lipid polymerization on membrane conductivity. The lipid was compound (1). The aqueous phase contained 100 m*M* KCl. $T = 50\,°C$. UV-light intensity was 0.5 mW/cm^2

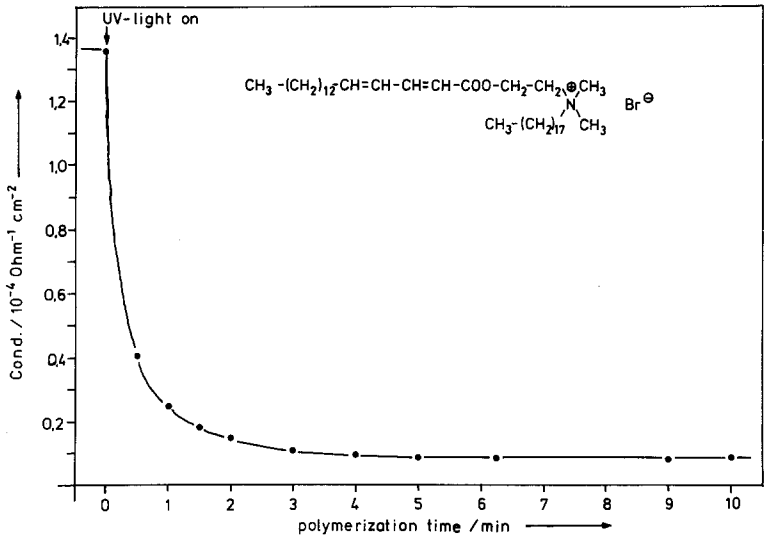


Fig. 4. Effect of lipid polymerization on carrier-mediated conductivity in BLMs formed dienoyllipid (1). The aqueous phase contained 100 m*M* KCl and 1 µ*M* Nonactin. $T = 50\,°C$. The UV-light intensity was 0.5 mW/cm^2

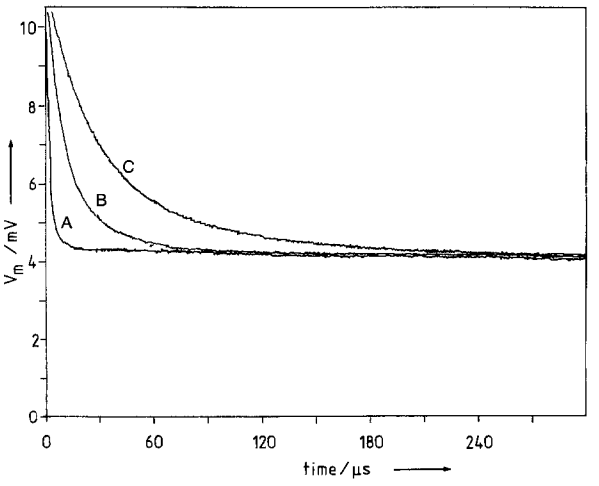


Fig. 5. Records of voltage relaxations following charge pulses applied to a membrane from dienoyllipid (1). The aqueous phase contained 1 *M* NaCl and 3×10^{-8} M DPA^-; $T = 50\,°C$. Traces (*A*) to (*C*) represent measurements at 0 min (*A*), 1 min (*B*) and 10 min UV-illumination (*C*). Shown are the first 300 µs of records of 800 µs total length. The start of the UV-illumination was 30 min after formation of the membrane. The UV-light intensity was 0.5 mW/cm^2

polymerization of the membrane forming dienoyl lipids. However, we consider this possibility as rather unlikely. The decrease of the conductance is most probably caused by an increased viscosity of the membrane, thus decreasing the translocation rate constant k_{MS}.

Influence of polymerization on transport of lipophilic ions. From the results of the experiments with the nonactin-K^+ system it can be concluded that polymerization has a strong influence on the translocation of the ion-carrier complexes through the membranes. To establish that the decrease of the translocation rate constant is the only reason for the conductance decrease, experiments were performed in the presence of lipophilic ions as probes for membrane structure. The transport of these probes is described by two parameters, the rate constant k_i and the total concentration N_t of lipophilic ions. Both can be calculated from a single charge pulse experiment.

Record 1 of Fig. 5 shows a charge pulse experiment taken from a membrane from the dienoyl lipid (1) 30 min after the membrane had turned completely black. The DPA concentration was $3 \cdot 10^{-8}$ M. The decay of the voltage with time could be fitted to two exponential processes with time constants of approximately 2 μs and 1 ms for the fast and the slow process, respectively. Illumination of the membrane with UV-light had a strong influence on the time constant of the fast process. Record 2 of Fig. 5 shows a charge pulse experiment taken 1 min after the start of the illumination while record 3 was taken 9 min later (in total 10 min after the start of the UV-light). The relaxation time constant of the fast process increased from 2 μs to 11 μs at 1 min and to 33 μs after 10 min illumination. The parameters of the slow process and the amplitude of the fast voltage relaxation were not influenced by the polymerization of the lipid.

Table 2. Analysis of the experiment illustrated in Fig. 5 according to Eqs. (2)–(5). The bathing solutions contained 1 *M* NaCl and 3×10^{-8} M DPA^-; $T = 50$ °C. The membrane was formed from dienoyllipid (1) dissolved in $CHCl_3$/*n*-decane (ratio 1:1). $C_M = 520$ nF/cm^2. The time given in the first column is the time after switching on UV-illumination

Time [min]	τ_1 [μs]	τ_2 [ms]	a_1	k_i [10^3 s^{-1}]	N_t [pmol cm^{-2}]
0	2.0	0.98	0.64	84.0	1.2
0.5	6.0	1.1	0.63	31.0	1.0
1.0	11.0	1.2	0.64	17.0	1.0
1.5	17.0	1.0	0.62	12.0	0.90
2.0	21.0	1.3	0.62	9.3	0.90
5.0	31.0	1.4	0.63	6.2	0.91
10.0	33.0	1.2	0.62	6.0	0.85
40.0	35.0	0.85	0.57	6.5	0.66

The analysis of the data given in Fig. 5 on the basis of Eqs. (3)–(5), allowed a quantitative description of the effect of the polymerization on DPA^--transport. The results are summarized in Table 2.

The UV-illumination caused a decrease of k_i by a factor of about 15 during the first 10 min. Further illumination for another 30 min did not lead to an additional decrease in k_i. N_t was not affected by the UV-illumination. This result indicated that DPA^- is not destroyed by UV-light and that the adsorption of DPA^- is not changed during the polymerization of the membrane lipid. Control experiments were performed with membranes from dioleoyl phosphatidylcholine/*n*-decane and diphytanoyl phosphatidylcholine/*n*-decane in order to determine whether it is the polymerization or the UV-illumination which causes the decrease of k_i in the experiments with the dienoyl lipid.

In fact, UV-illumination had virtually no influence on DPA^--transport in the control experiments. Figure 6 shows the time course of k_i during the experiment given in Table 1 and in a control experiment with a membrane from dioleoyl phosphatidylcholine (DOPC)/*n*-decane. k_i increases in the control experiment by less than a factor of two, which presumably has nothing to do with the UV-light but is associated with the decrease of the membrane thickness with age (Benz and Janko 1976; Benz and Gisin 1978).

The decay of k_i after the start of the UV-illumination of the membranes from the dienoyl lipid was approximately exponential. The time constant of the experiment given in Fig. 6 is about 45 s. This time constant was dependent on the light intensity. A reduction of the light intensity by a factor of two

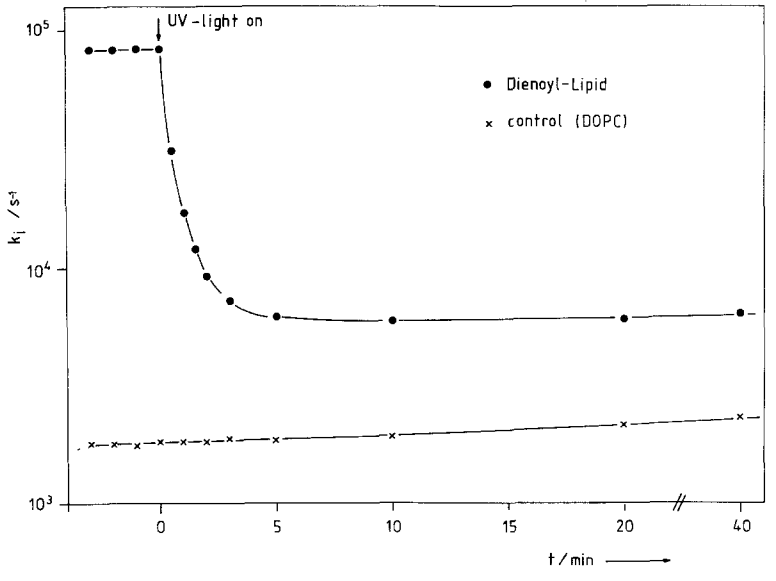


Fig. 6. Time course of the translocation rate constant k_i of DPA^- in a membrane from dienoyllipid (1) and in a control experiment as a function of the duration of the UV-illumination. The control membrane was formed from DOPC/*n*-decane. The aqueous phase contained, in both cases, 1 *M* NaCl and 3×10^{-8} M DPA; $T = 50$ °C

led to an increase of the time constant to about 2 min.

The reproducibility of the time constant at a given light intensity was usually below ± 30% from the mean value. The same was true for the value of k_i before and after long time UV-irradiation. (k_i = 81,000 ± 4,800 s^{-1} and k_i = 5,800 ± 800 s^{-1}, respectively.) k_i after long time UV-irradiation was always around 6,000 s^{-1} irrespective of the light intensity. This means that polymerization in the membrane always resulted in the same final membrane structure independent of the polymerization speed.

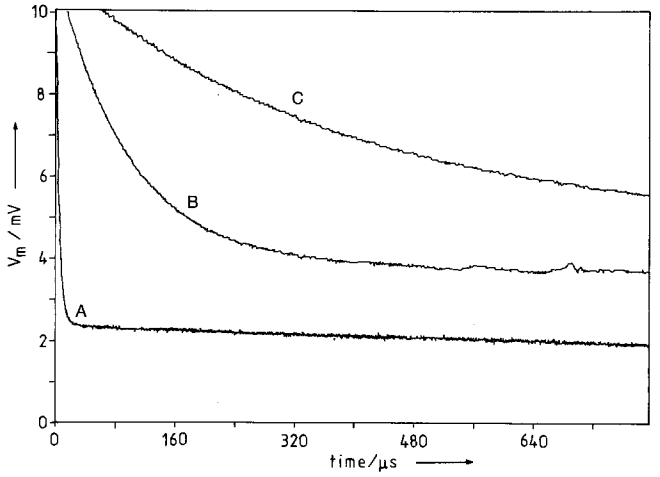


Fig. 7. Records of voltage relaxations following charge pulses applied to a membrane from dienoyllipid (1). The aqueous phase contained 1 *M* NaCl and 3×10^{-7} M $B(Ph)_4^-$. Traces (*A*) to (*C*) represent experiments at 0 min (*A*), 30 s (*B*) and 1 min UV-illumination (*C*). Shown are the first 800 µs of records of 800 µs (*A*), 2 ms (*B*) and 4 ms (*C*). The start of the UV-illumination was 30 min after formation of the membrane. The UV-light intensity was 0.5 mW/cm^2

The influence of polymerization on the membrane structure was also investigated using tetraphenylborate ($B(Ph)_4^-$) as a molecular probe. This lipophilic ion has a similar partition coefficient to DPA^-, but its translocation rate constant is usually 10 to 40 times smaller (Benz et al. 1976; Pickar and Benz 1977). Figure 7 shows charge pulse experiments performed with a membrane from dienoyl lipid (1) in the presence of $3 \cdot 10^{-7}$ M $B(Ph)_4^-$.

Record 1 corresponds to a control experiment taken immediately before the start of the UV-illumination. Records 2 and 3 were taken 30 s and 1 min later, respectively. The UV-illumination again had a strong influence on the voltage relaxation following a charge pulse. Surprisingly, both relaxation processes were affected in contrast to the experiments with DPA, in which only the fast process was changed.

Table 3 shows the results of the analysis of the charge pulse experiments shown in Fig. 7. The UV-illumination caused a decrease of k_i by a factor of 250 and a decrease of N_t by a factor of about 40. The further change of k_i and N_t could not be investigated because it was impossible to separate the two relaxation processes at 20 min and at 40 min UV-illumination.

However, it is obvious from the data given in Table 2 that k_i was already saturated after 10 min which means that the further decrease must be rather small. The strong decrease of N_t was unex-

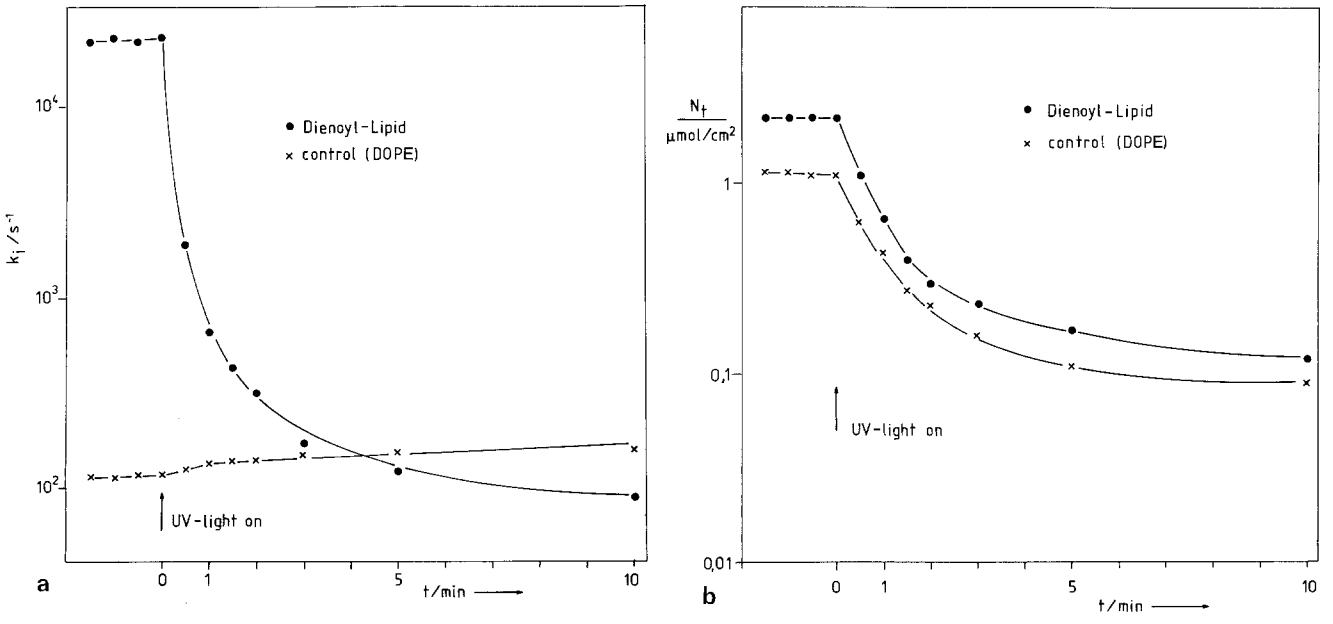


Fig. 8 a and b. Time course of the translocation rate constant k_i **a** and N_t **b** of $B(Ph)_4^-$ in a membrane from dienoyllipid (1) and in a control experiment as a function of UV-illumination time. The control membrane was formed from DOPE/*n*-decane. The aqueous phase contained, in both cases, 1 *M* NaCl and 3×10^{-7} M $B(Ph)_4^-$; T = 50 °C

Table 3. Analysis of the experiment shown in Fig. 7 according to Eqs. (2)–(5). The bathing solutions contained 1 *M* NaCl and 3×10^{-7} M $B(Ph)_4^-$; $T = 50$ °C. The membrane was formed from dienoyllipid (1) dissolved in $CHCl_3$/*n*-decane (ratio 1 : 1). $C_M = 520$ nF/cm^2. The time given in the first column is the time after switching on UV-illumination

Time [min]	τ_1 [μs]	τ_2 [ms]	a_1	k_i [10^3 s^{-1}]	N_t [pmol cm^{-2}]
0	4.5	4.7	0.79	23.0	2.3
0.5	94.0	16.0	0.65	1.9	1.1
1.0	360.0	23.0	0.54	0.66	0.65
1.5	680.0	22.0	0.43	0.43	0.40
2.0	1,000.0	21.0	0.43	0.32	0.30
3.0	2,200.0	25.0	0.35	0.17	0.23
5.0	3,200.0	24.0	0.30	0.12	0.17
10.0	4,400.0	19.0	0.30	0.087	0.12

pected from comparison with the DPA^- data. Therefore control experiments using the non-polymerizable lipid dioleoyl phosphatidylethanolamine (DOPE) were performed. The control experiments showed that UV-light had no influence on the translocation rate constant k_i but decreased the total concentration N_t of lipophilic ions adsorbed to the membranes, presumably because of the UV-sensitivity of $B(Ph)_4^-$.

Figure 8 demonstrates the effect of UV-illumination on $B(Ph)_4^-$-transport through the membranes from the polymerizable lipid and through the control membranes. Figure 8 A shows the strong effect of the polymerization on k_i and its absence in the control experiment. The decay of k_i with time can approximately be described by an exponential law. Its time constant is 30 s and is thus very close to that found for the effect on DPA^--translocation. The influence of UV-light on N_t is more or less the same in both experiments and the initial decay of N_t has a time constant of approximately 1 min. This result indicated that the polymerization had almost no effect on the $B(Ph)_4^-$ concentration in the membrane.

Discussion

In this publication we presented evidence, that the polymerization of the dienoyl lipid (1) is possible in planar lipid bilayer membranes. The polymerization of the lipid was monitored in different systems. Unmodified lipid bilayer membranes showed an increased lifetime and a decreased specific conductance as a consequence of the polymerization. The effect on the lifetime is easy to understand. The covalently linked fatty acid chains most likely decrease the number of defect structures which are responsible for the mechanical breakdown of the lipid bilayer (Benz et al. 1979), and thus increase the stability and lifetime of the membranes. The nature of these defect structures, however, is not yet known. The conductance of the unmodified lipid bilayer membranes is presumably also caused by these defect structures (Smith et al. 1985) and not by the passage of ions through the hydrocarbon core of the membranes. Consequently, the conductance of the unmodified membranes should also decrease during the polymerization as it does in fact.

The effect of the polymerization of the membrane forming dienoyl lipid (1) on the membrane structure was also studied using ionic probes. In previous publications it was shown that these probes, i.e. ion carriers and lipophilic ions, are sensitive for structural properties of artificial and biological membranes (Benz and Läuger 1977; Benz and Cros 1978; Benz and Gisin 1978; Benz and Conti 1981; Benz and Nonner 1981). These probe molecules sense in particular electrical properties of membranes like surface or dipolar potential but they are also sensitive to membrane thickness and membrane viscosity. Carrier-ion complexes like the nonactin-K^+ complex sense the electrical properties and the microviscosity of membranes. Thus, the polymerization of the membranes could in principle decrease both, the translocation of the complexes and their absorption to the membrane solution interface. However, this question cannot be resolved on the basis of stationary conductance data, although a change of the absorption seems to be rather unlikely if the effect of the polymerization on the transport of lipophilic ions is considered (see below). During polymerization, the specific conductance of the nonactin-K^+ system decreases approximately 10 fold to a value which is very close to the specific conductance of the unmodified membrane. Therefore we have to consider this factor as a lower limit for the change of the product $k_{MS}\gamma_{MS}$.

The experiments with the lipophilic ions allow a more quantitative description of the influence of the polymerization on the transport of the ionic probes through the membranes. In the case of DPA^- the translocation rate constant k_i is reduced, whereas the concentration of the lipophilic ions absorbed to the membrane stayed constant during polymerization. The latter result has two important implications. First of all, DPA^- itself is not UV-sensitive. Second the electrical potentials, i.e. surface and dipole potential of the membranes are not altered during the polymerization. The $B(Ph)_4^-$ absorption during the time course of the polymerization cannot be controlled because of the UV-sensitivity of this probe. Because of the control experiments and the DPA data we are very confident that the absorption of $B(Ph)_4^-$ is in principle also not influenced by the

polymerization of the lipid molecules within the membrane.

The translocation rate constants of DPA^- and $B(Ph)_4^-$ decrease by factors of 15 and 250, respectively, during polymerization. This finding indicated a somewhat different influence of the polymerized matrix on both probes, although the time course of the decreases is very similar (which is also valid for the other systems). The difference is easy to explain on the basis of the different structure of the two molecular probes. DPA^- is a long molecule with a length of about 1.4 nm and a diameter of about 0.8 nm, whereas $B(Ph)_4^-$ is spherical, with a diameter of about 1.1 nm as judged from space filling models. This means that that $B(Ph)_4^-$ could disturb the fatty acid chains a little more during its passage through the membrane. Polymerization would create a stronger influence on $B(Ph)_4^-$-transport, because of the larger sterical hindrance as compared with DPA^-.

In previous publications it was shown that polymerization of lipid molecules within monolayers and vesicles leads to increased stability of these model systems for biological membranes. Here it is shown that this concept is also applicable to the lipid bilayer approach. It could be shown that polymerization of lipid bilayer membranes leads to an increased stability and a decreased ion permeability.

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