Amphiphilic Derivatives of Betaine Esters as Modifiers of Macrovesicular BLM

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Abstract. A series of amphiphilic derivatives of betaine esters (V-*n*), with the chemical structure $(CH_3)_3N^+COOC_nH_{2n+1}Cl^-$ (n = 10, 12, 14 or 16) were studied with respect to their effects on the electrical properties of lecithin macrovesicular membranes. Normalized resistance and breakdown voltage were found to depend on the V-*n* concentration in the membrane and on the alkyl chain length (n). Resistance decreases up to about 10^4 ohm . cm^2 and breakdown voltage decreases by 111 mV were detected in the V-*n*: lecithin molar ratio range measured (0.005—0.05). Maximal decrease in breakdown voltage was observed for V-14. These findings together with the featured anionic selectivity suggest that, due to the interaction of V-*n* with phospholipids, hydrophilic pores are formed in the lipid bilayers. This assumption is supported by the results obtained by electron paramagnetic resonance (EPR) measurements which showed no collective changes in bilayer dynamics or ordering. In particular, rotational correlation times and order parameters of the spin probe molecules dissolved in the membrane did not change in the concentration range tested. Since a large number of defects in the membrane can be expected to influence the collective ordering and dynamics, this observation also suggest that the number of pores formed is small.

Key words: Amphiphiles — Macrovesicular BLM — Ion transport — Ion channels — Breakdown voltage

Introduction

Amphiphilic ammonium salts are known to possess strong biological activity

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(Witek et al. 1978 a, b; Rucka et al. 1983) and to influence certain processes in model biological membranes (Grupe et al. 1977; Bangham and Lea 1978; Sarapuk et al. 1984; 1985; Przestalski et al. 1983; Frischleder et al. 1984). In particular they modify ion transport processes through liposome phospholipid membranes by increasing the rate of ion permeation (Gabrielska et al. 1979; 1981; Kuczera et al. 1983; 1985 a). However, the molecular mechanism of action of these compounds and the nature of the induced membrane structure alteration which determines the mechanism of transport are not yet completely known.

Thus it seemed advisable to conduct further studies using large-area bilayer lipid membranes such as macrovesicular lecithin membranes. Bilayer lipid membranes (BLMs) are widely used as model biological membranes (for a review see Tien 1974). The process of electrical breakdown of BLMs has been extensively studied (Abidor et al. 1979; Dimitrov and Jain 1984). A variety of biologically active compounds were found to modify the electrical and transport properties of BLMs (see e.g. Cherny et al. 1982; Simonova et al. 1986; Janas et al. 1986; 1987). In this paper transport of Na^+ and Cl^- ions across macrospherical membranes modified with amphiphilic derivatives of a series of betaine esters (V-*n*) with different chain lengths was studied by membrane electrical resistivity, breakdown voltage, and electron paramagnetic resonance (EPR) measurements.

Materials and Methods

Chemicals

The amphiphilic derivatives of betaine esters with the chemical structure $(CH_3)_3N^+—CH_2—COO—C_nH_{2n+1}Cl^-$ where $n = 10$, 12, 14 or 16 (V-*n*) were synthesized in our laboratory. The elemental analysis and spectral data confirmed the chemical structure shown above.

Lecithin was extracted from egg yolk according to Singleton et al. (1965) and then purified on a silica acid (Mallincrodt, St. Louis, USA) column, so that a single spot was detected on Silica Gel TLP plates (Merck, Darmstadt, FRG) using chloroform/methanol/water (65 : 25 : 4, by vol.) and chloroform/methanol/acetic acid/water (50 : 38 : 8 : 4, by vol.).

BLM Measurements

Macrovesicular bilayer lipid membranes were formed according to the technique of Schagina et al. (1976) on a Teflon capillary tube in unbuffered aqueous solutions of 0.1 mol/l and 0.2 mol/l NaCl inside and outside the membrane, respectively (see also Janas et al. 1986). Lecithin or lecithin/V-*n* mixtures used for membrane formation were dissolved in *n*-decane/butanol (2 : 1, v/v) to obtain a concentration of 5 mg lipid per ml solvent, and molar concentration ratios of V-*n*: lecithin of 0.005, 0.02, and 0.05. The organic solvents were passed through an Aluminium Oxide (Merck, Darmstadt, FRG) column to remove impurities. The experimental set-up used for electrical measurements of macrovesicular BLMs is shown in Fig. 1. Ag/AgCl electrodes were used to apply an external voltage and to detect the electrical potentials. Electrometers with internal resistance larger than $10^{12}\,\Omega$ and

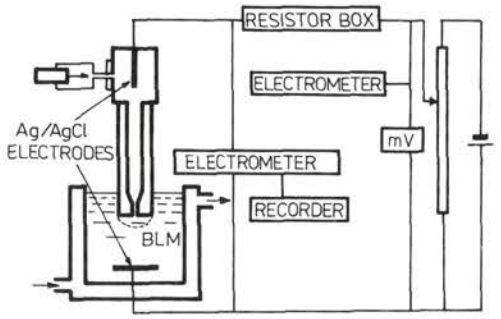

Fig. 1. The experimental set-up employed for electrical measurements of macrovesicular BLMs. For details see text.

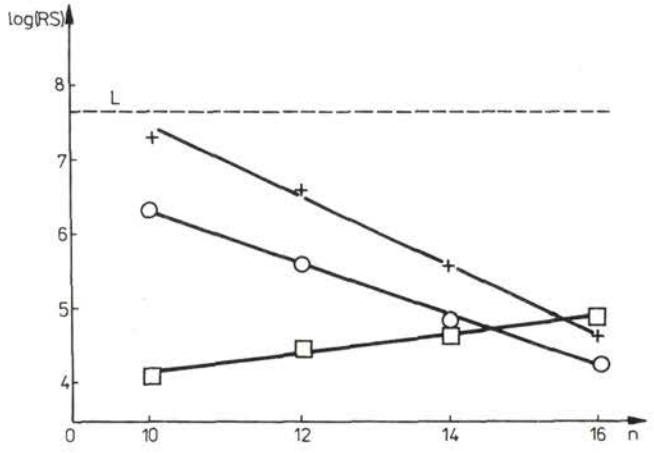

Fig. 2. Logarithm of normalized resistance of macrovesicular lecithin BLMs modified by V-*n* compounds vs. the number (*n*) of carbon atoms in the alkyl chain. V-*n*: lecithin molar ratios: 0.005 (+), 0.02 (○) and 0.05 (□). *R*-membrane electrical resistance, *S*-membrane area. The dashed line (*L*) represents the normalized resistance of the unmodified lecithin bilayer. The points represent mean values of measurements in at least 8 different bilayers.

an accuracy of 0.1 mV were used to measure the voltage drop across the membrane and the external resistance. The area of the membrane was determined by optical measurement with an accuracy of 0.06 mm^2. The temperature was kept at (25 ± 0.2)°C controlled by water circulating from an external bath. To obtain the values of breakdown voltage, the applied voltage was slowly increased at the rate of 10 mVs^{-1} until membrane rupture occurred. The electrical resistance of the membrane was calculated from current-voltage characteristics. The ionic transference numbers were calculated from diffusion potentials (Janas et al. 1986).

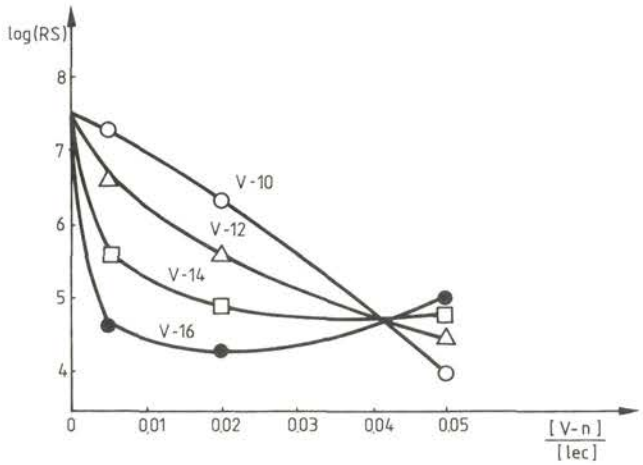

Fig. 3. Logarithm of normalized resistance of macrovesicular lecithin BLMs modified by V-10 (○), V-12 (△), V-14 (□) or V-16 (●) vs. V-*n*: lecithin molar ratio. *R*-membrane electrical resistance, *S*-membrane area. The points represent mean values of measurements in at least 8 different bilayers.

EPR Measurements

The amphiphilic spin probe nitroxide derivative of palmitic acid methyl ester (MeFASL (10,3)) was used. Liposomes were prepared from egg lecithin as follows: to an ethanol solution of 0.01 mmol/cm^3 egg yolk lecithin (final concentration) the chloride salt of decylbetaine ester (V-10) or the chloride salt of hexadecylbetaine ester (V-16) were added to the same final concentrations as in BLM measurements. The solution was dried on a rotating evaporator, so that a thin layer of the mixture was obtained on the walls of the glass tube, and then water or phosphate buffer was supplemented. The dispersion was sonicated for 10 min and centrifuged for 10 min at 10,000 × *g*. The liposome dispersion was incubated for 30 min with 5×10^{-9} mol of MeFASL (10,3) added previously to the glass tube in ethanol solution and subsequently evaporator dried. Measurements were performed on a Varian E-9 (Sunnyvalle, USA) spectrometer in glass capillaries of 1 mm diameter at 4 °C and 25 °C, both immediately after the preparation of the sample and 48 hours later.

Results

The areas of the vesicular membranes formed from lecithin and V-*n* compounds were 30—35 mm^2. The ionic transference numbers (t) were almost independent of the chain length of the betaine esters studied, and a (t_{Cl^-}/t_{Na^+}) ratio of about 2.2 was obtained at 25 °C for a relative concentration of 0.02. The experimental error (evaluated from measurements in at least 8 different bilayers) was less than 15 %.

Figs. 2 and 3 show the relationships between the logarithm of normalized resistance ($R \cdot S$) and the number of carbon atoms in the alkyl chain, and the

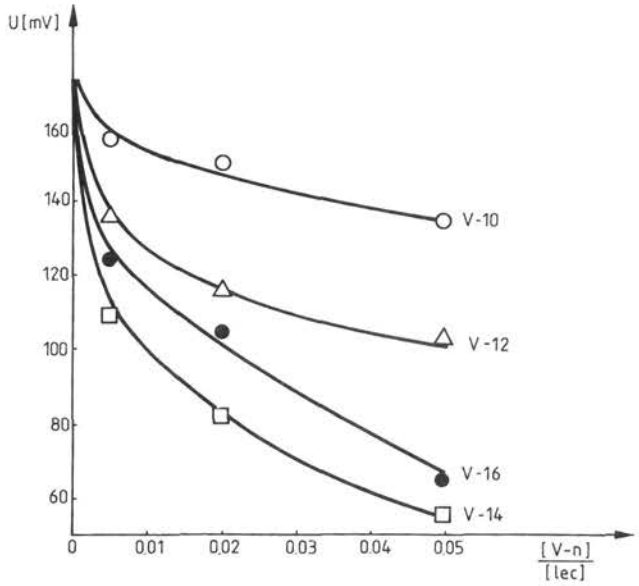

Fig. 4. Breakdown voltage (U) of macrovesicular lecithin BLMs modified by V-10 (○), V-12 (△), V-14 (□) or V-16 (●) vs. V-n: lecithin molar ratio. The points represent mean values of measurements in at least 8 different bilayers.

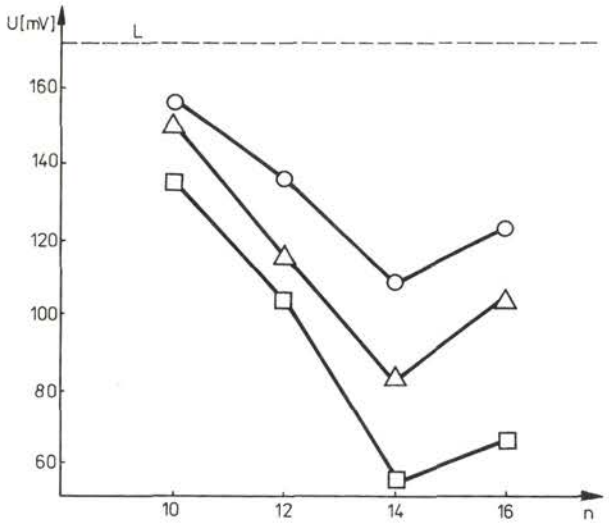

Fig. 5. Breakdown voltage (U) of macrovesicular lecithin BLMs modified by V-n vs. the number (n) of carbon atoms in the alkyl chain. V-n: lecithin molar ratios: 0.005 (○), 0.02 (△) and 0.05 (□). The dashed line (L) represents the normalized resistance of unmodified lecithin bilayer. The points represent mean values of measurements in at least 8 different bilayers.

molar concentration ratio of V-*n* compounds and lecithin respectively (*R* is the membrane resistance and *S* is the membrane area).

It may be noted that the presence of V-*n* molecules in the macrovesicular lecithin membrane decreases the membrane resistance in dependence on the alkyl chain length (*n*) for lower V-*n* concentrations, while a linear increase with *n* is observed for higher concentrations (0.05).

From the concentration dependence shown in Fig. 3 plotted for compounds with different alkyl chain lengths it is evident that for V-10 the membrane resistance decreases almost linearly with increasing concentration of the ester. A decrease by three orders of magnitude was observed for the concentration ratio of 0.05. For the other compounds a decrease in membrane resistance was observed at low V-*n* concentrations with weaker changes at higher concentrations of the same compounds. Almost no change was observed with V-14 in the molar ratio range of 0.015—0.05. For V-16 a rapid decrease by about three orders of magnitude was noted for the ratio of 0.005 and than a small increase in membrane resistance was observed at concentration ratios above 0.02.

Figs. 4 and 5 show the dependence of breakdown voltage on the molar concentration ratio of V-*n* in lecithin membranes and on the alkyl chain length. The efficiency of the modifiers in diminishing the membrane breakdown voltage increases with their concentration. The strongest change in the breakdown voltage was observed for V-14: it decreased the breakdown voltage from 166 mV to about 60 mV, while V-10 decreased it only from 166 mV to about 140 mV.

The EPR spectra show that neither the maximum hyperfine splitting of MeFASL (10,3) in the membrane, which reflects the ordering of hydrocarbon chains, nor the correlation time, which reflects the dynamics of the hydrocarbon chains, are influenced by the V-*n* compounds in the concentration range studied.

Discussion

It can be expected that amphiphilic derivatives of betaine esters, like other amphiphilic ammonium salts, are incorporated into phospholipid membranes with the alkyl chain in the hydrocarbon region and the hydrophilic head close to the polar part of the bilayer. We assume that betaine esters incorporated into membranes may cause defects in the lipid bilayer resulting in the formation of stable nonbilayer structures, as was observed for other single chain amphiphiles.

The results presented in Figs. 2 and 3 for Na^+ and Cl^- ion transport induced by an external electric field in the presence of amphiphilic compounds in the lecithin membrane showed that the normalized resistance of the membrane decreases by more than three orders of magnitude (up to about

10^4 ohm . cm^2) in dependence on both the alkyl chain length and V-*n* concentration. Similar decreases in membrane resistance have been observed for membranes modified by some channel forming antibiotic ionophores (Vodyanoy et al. 1983; Hall et al. 1984). Moreover, the featured membrane anionic selectivity was observed. It might therefore be suggested that in lecithin BLM, modified by amphiphilic positively charged derivatives of betaine esters, hydrophilic channels facilitating ion transport are formed. This is confirmed by EPR measurements which showed no changes either in correlation time or in order parameter in the concentration range tested, since EPR detects only collective changes in the bilayer as a whole. Local defects like pores do not necessarily perturb the whole membrane structure. As suggested by de Gier (1979) and later by Weaver et al. (1984) and Smith et al. (1984) in a bilayer phospholipid membrane temporal pores may appear, even in the absence of any modifiers, as a result of thermal fluctuations. As described by Petrov (1981) the presence in the membrane of surfactant modifiers with a conic shape may stabilize toroidal pores. At very low concentrations of V-10 these molecules may exist as monomers in the membrane and, because of their large hydrophilic/lipophilic balance as compared with that of lecithin molecules, they may form defects in the membrane which facilitate ion transport across the membrane (Kuczera et al. 1985a). Higher concentrations of V-*n* may induce the formation of domains with large positive curvature, resulting in defects at the domain borders (Jain 1980), leading to the formation of pores. However, the defects seem to be so small in size, or the number of pores is so small, that they do not influence the overall membrane fluidity and therefore were not observed by EPR.

As shown in Fig. 3 the relation of normalized resistance to concentration for V-12 is similar to that for V-10. This relation however, is different for V-14 and particularly for V-16: after a rapid decrease at the concentration ratio of 0.005 the resistance slowly increases. It may be suggested that the increasing concentration of these compounds in the membrane is associated with an increase in the number of pores and in the amount of V-10 molecules at the pore surface. The former factor may result in a diminished resistance, but the latter one may act quite oppositely. An increased number of conically shaped positively charged molecules at the pore surface may result in narrowing of the pores and the increased electrical repulsion may cause a decay of the pores. The increase in resistance with increasing concentration of V-16 suggests that the aggregation of amphiphiles at the pore surface is of greater importance that the increase in the number of pores; this is also supported by EPR measurements. Probably, the occurrence of a larger number of pores would be associated with fluidity changes, and this was not observed by EPR. As V-14 does not change the membrane resistance over a wide concentration range, it may be concluded that the influence of this two factors is mutually abolished.

The results shown in Fig. 4 indicate that membrane breakdown voltage decreases with increasing concentrations of membrane modifiers. Comparing the dependences of membrane resistance (Fig. 3) and breakdown voltage on V-*n* concentration, qualitative similarities for low, and disimilarities for high concentrations are obvious. Presumably, lower modifier concentrations in the membrane associated with an increased voltage applied can result in a decreased domain or pore stability. The differences observed for higher concentrations may indicate that the decrease in breakdown voltage was due to a change in pore structure. More V-*n* molecules in the membrane may cause stronger repulsion interactions at the pore surface and decrease the surface tension (Kuczera et al. 1985b). This may facilitate the formation of pores of supercritical size. Fig. 5 shows that V-14 is most effective in lowering the membrane breakdown voltage. It also induces the strongest sulphate ion permeation across lecithin liposome membranes in the V-*n* series (Kuczera et al. 1985a). A rapid growth of the rate constant for ion permeation and the occurrence of a maximum at a certain alkyl chain length may indicate that membrane structure loosening is maximal for this chain length.

Summing up the results, it is worth paying attention to two practical aspects. First, small amounts of certain modifiers, e.g., residues of washing media or products of lipid oxidation, can cause pore formation within a membrane thus resulting in scattering of membrane resistance measurements. Second, our earlier studies (Gabrielska et al. 1979; 1981; Kuczera et al. 1983; 1985a) indicated generally good correlations between the results of measurements of ion transport across lecithin membranes and biological tests. Thus in view of practical uses of these compounds the existence of three ranges of V-*n* concentrations with respect to their activity is important. For modifier : lecithin ratios below 0.04, the activity decreases in the sequence: V-16 > V-14 > > V-12 > V-10, whereas for molar ratios higher than 0.04 the activity sequence is opposite (Fig. 3). Around the molar ratio of 0.04 the membrane resistivity is nearly the same for all the compounds studied.

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