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INTERACTION BETWEEN MEMBRANES AND AMMONIUM SALTS WITH DIFFERENT ALKYL CHAIN LENGTH

MARIAN PODOLAK

Institute of Physics, Opole University, Oleska 48, 45-052 Opole, Poland

The paper contains a review and analysis of the results of the studies concerning the interactions between cationic, quaternary ammonium salts (AS) and biological and model membranes. Special attention was paid to the role of the alkyl chain length of the compounds studied. The analysis proves a destructive effect of ammonium salts on all of the membranes studied. The AS destructive activity increases monotonically with their alkyl chain length but shows a tendency to stabilize for the compounds containing 14 and 16 carbon atoms in the chain.

INTRODUCTION

Quaternary ammonium salts (AS) constitute a broad class of the metabolites commonly occurring in nature with more than a hundred examples in literature reported (Anthoni, Christophersen, Hougaard & Nielsen, 1991). These compounds are also synthesized in laboratory (Witek & Grobelny, 1978; Witek, Oświęcimska, Ptaszkowska, Bakuniak, Górska & Łaszcz, 1978). AS are thought to play an important role in the adaptation of organisms to stressful and changeable environmental conditions. The AS biological activity and their molecular mechanisms are the subject of different science center studies (Rucka, Oświęcimska & Witek, 1983; Lindstedt, Allenmark, Thompson & Edebo, 1990; Devinsky, Masarova, Lacko & Mlynarcik, 1991; Podolak, Kocherginsky, Osak, Przestalski & Witek, 1992; Przestalski & Kuczera 1992; Podolak, Waga & Przestalski, 1994; Podolak, Man, Waga & Przestalski, 1996). A lot of these compounds have got the bactericidal, algicidal and fungicidal properties (Rucka *et al.*, 1983). Most of them belong to the amphiphilic substances. One edge of the long AS molecule contains a hydrophilic polar head group, but the residual part of the molecule composes the hydrophobic alkyl chain. The introduction of the AS with living organisms occurs, as one can suppose, through the membranes.

The structural core of membranes compose the polar lipid bilayers. Polar lipids, like most of the AS, are amphiphilic compounds and therfore the AS molecules can, with relative ease, build into the membrane structures and modify their properties.

This paper summarizes the results of the studies on interaction between a selected group of AS and biological and model membranes.

THE STUDIED COMPOUNDS CHARACTERISTIC

AS discussed in the paper (eight compounds) were synthesized in prof. Witek`s laboratory at the Institute of Polymer Technology of the Technical University of Wrocław, Poland. Six of them compose the AS V*n* group of the general formula,

$$(CH_3)_3N^+ - CH_2COOC_nH_{2n+1}\ Cl^-$$

where n=6, 8, 10, 12, 14 or 16.

Two compounds of the chemical formulae,

$$(CH_3)_2N^+(C_{12}H_{25})\text{-}CH_2COOCH_2Cl\ Cl^- \qquad \text{(AS VA)}$$

and

$$(CH_3)_3N^+ - CH_2COOCH_2Cl\ Cl^- \qquad \text{(AS VB)},$$

compose another group of AS. The compounds belonging to the AS Vn group have the same polar head group, but differ in hydrophobic alkyl chain length.

The AS VA and AS VB compounds have also the same polar head group (other than AS V*n* compounds), but AS VA contains a 12-carbon alkyl chain, while AS VB is devoid of such a chain and does not have the hydrophobic properties. All of

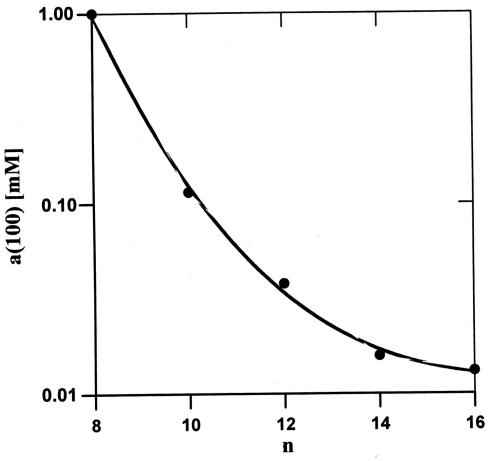

Fig.1 Dependence of the AS Vn concentration a(100) causing 100% hemolysis of the pig blood erythrocytes on the number (n) of carbon atoms in their alkyl chains (Kleszczyńska *et al.*, 1990).

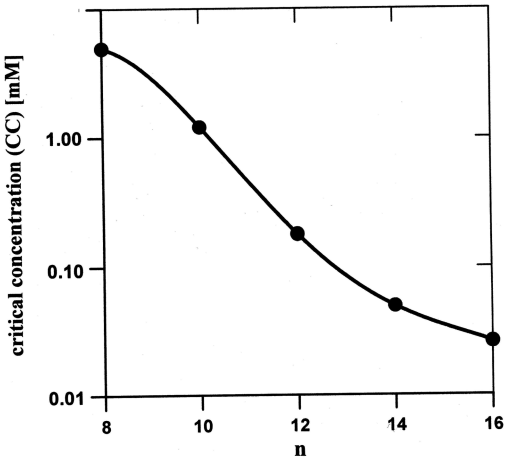

Fig.2 Dependence of the AS Vn critical concentration (CC), at which the lifetime of BLM does not exceed 5 minutes, on the number (n) of carbon atoms in their alkyl chains (Kleszczyńska *et al.*, 1990).

the AS molecules studied contain positively charged nitrogen ion in the head group and all but one (AS VB) contain single, saturated alkyl chain with different number of the carbon atoms.

INTERACTION OF THE AS WITH BIOLOGICAL AND MODEL MEMBRANES

Biological membranes discussed in the paper are represented by pig erythrocytes and *Sphaerotilus natans* bacteria cell membranes. Model membranes have the shape of liposome, bilayer lipid membranes (BLM), formed from egg yolk lecithine (EYL), and filters with lauric fatty acid ester impregnated (FI).

Rucka *et al.* (1983) states that AS V*n* (where *n*=10, 12, 14 and 16) cause, among others, the inhibition of *Sphaerotilus natans* bacteria growth. The AS V*n* activity in this process depends on the number of carbon atoms (*n*) in the compound molecule alkyl chains and on their concentration (Table 1).

As can be seen in Table 1, the AS V*n* inhibition activity increases with n value and it reaches maximum for AS V14 compound. Furthermore, the AS V*n* activity depends very strongly on their concentration. Twice as large increase in the compound concentration causes, on average, the inhibition of the bacteria growth rate by a factor of three. The compounds studied, added to the pig blood erythrocytes dispersion in buffer solution induce their hemolysis (Kleszczyńska, Sarapuk, Przestalski & Witek,1986; Kleszczyńska, Sarapuk, Przestalski & Kilian, 1990). Fig. 1 shows the dependence of the AS V*n* concentration causing

Table 1. Inhibition of *Sphaerotilus natans* bacteria growth (%) by AS V*n* where *n* = 10, 12, 14 and 16 (Rucka *et al.*, 1983)

AS	compound concentration (mg/dm^3) (mM)	growth inhibition (%)
V 10	100 0.34	19.5
V 12	100 0.31	21.5
V 14	100 0.29	30.1
V 16	100 0.26	22.3
V 10	200 0.68	60.5
V 12	200 0.62	60.8
V 14	200 0.58	72.5
V 16	200 0.52	70.1

Table 2. The AS V critical micelle (CMC) and lytic (LC) concentrations (Podolak *et al.*, 1988; Różycka-Roszak *et al.*, 1988)

AS V	CMC [mM]	LC [mM]
V 8	-[a]	41±0.5
V 10	18± 2	2.4 ±0.1
V 12	5.5 ±0.5	0.25± 0.05
V 14	1.9 ±0.1	0.074 ± 0.001
V 16	0.33± 0.02	0.07± 0.0005
V A	3.8 ±0.2	0.41± 0.05
V B	-[b]	no lysis

[a]No data available
[b]Does not form micelles

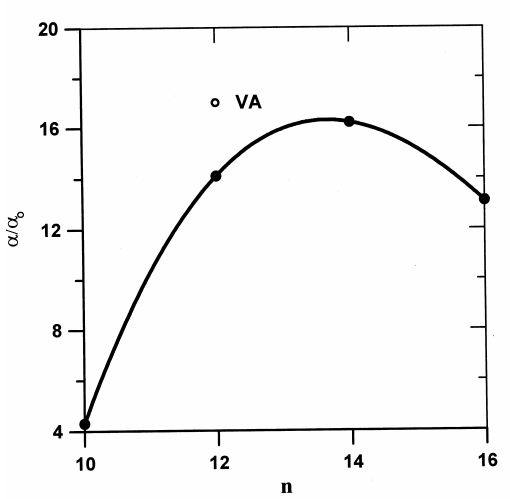

Fig.3 Dependence of the relative rate constant (α/α_0) of SO_4^{2-} ions permeation through EYL liposome membranes on the number (n) of carbon atoms in the AS Vn alkyl chain (α_0 - rate constant for membranes devoided of AS Vn admixtures). The AS Vn concentration was equal to 3mM (i.e. 1.5 in molar ratio to the EYL). The figure presents also relative rate constant of the AS VA compound (Kuczera *et al.*, 1985).

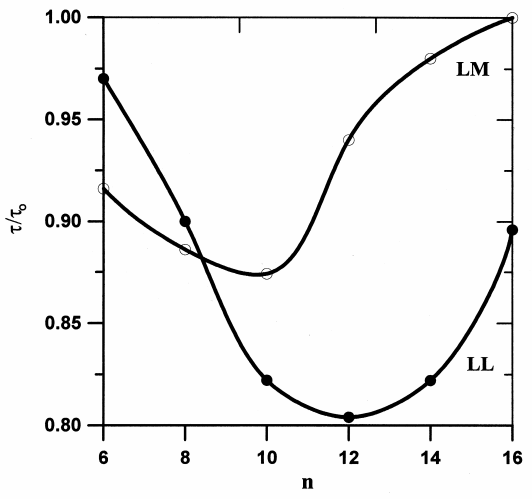

Fig.4 Dependence of the relative value parameter $\tau(\tau/\tau_k)$ of the spin probe 2-(14-carboxytetra-decyl)-2-ethyl-4,4-dimethyl-3-oxazo-lidinyloxyl (SYVA, USA) embedded in the liposomes LM formed from the EYL-AS Vn mixture (a), or LL formed from EYL, to which the AS Vn were added after liposomes forming (b), on number (n) of the carbon atoms in the AS Vn molecule alkyl chains. Parameter τ_k of the sample devoided of AS Vn was equal to 1.16ns. The measurements were performed at the temperature T = 20°C (Podolak *et al.*, 1987, 1994).

100% erythrocytes hemolysis (a 100) on the number (n) of carbon atoms in their alkyl chains.

As can be seen in Fig. 1, the AS Vn hemolytic activity increases monotonically with their alkyl chain length. Qualitatively similar destructive effect have the AS Vn on the bilayer lipid membranes (BLM) formed from egg yolk lecithine (EYL). These compounds, added to the BLM surrounding solution, shorten the membranes lifetime (Kleszczyńska *et al.*, 1990). Fig. 2 presents the dependence of the AS Vn critical concentration (CC) at which the lifetime of BLM does not exceed 5 minutes on the number (n) of carbon atoms in the alkyl chain of the compounds studied.

As shown in Fig. 2 the AS Vn destructive properties, in relation to BLM, increase monotonically with increasing alkyl chain length of their molecules. The chain length increase causes decreasing of the CC value.

Kuczera, Janas, Przestalski, Witek & Oświęcimska (1985) showed that in the presence of the AS Vn and AS VA compounds the rate of SO_4^{2-} ions permeation through EYL liposome membranes increases. The concentrations of the compounds studied ranged from 0.5 mM to 5 mM. It follows from Fig. 3 that the highest AS Vn activity towards the acceleration of SO_4^{2-} ions transport has AS V14 compound. This support the suggestion (Rucka *et al.*, 1983) about optimum (maximum) activity of AS V14 compound in action on the bacteria *Sphaerotilus natans*.

The compound AS VB (devoided of the alkyl chain) does not show any effect on the rate of SO_4^{2-} ions transport through the liposome membranes even at 50 mM concentration. However, the AS VA compound activity in this process is larger than AS Vn with the same alkyl chain length (n = 12). The relatively larger the AS VA than AS V12 activity can be related with larger dimension of its polar head group which can cause higher deformation in the liposome membranes structure. On the other hand, the lack of the AS VB compound activity may suggest that it does not enter into the membranes structure because its molecules have no the hydrophobic parts.

Investigation by the ESR spin probe method showed that the AS Vn cause an increase of the EYL liposome membranes fluidity (Podolak *et al.*, 1994; 1996). Measure of fluidity was, among others, the spectroscopic parameter τ (quantity inversely proportional to the rotational velocity of the spin probe as well as to the membranes fluidity).

In the case of the mixed liposomes EYL-AS Vn (LM) formed in buffer solution, the highest fluidity of membranes (the smallest parameter τ value)

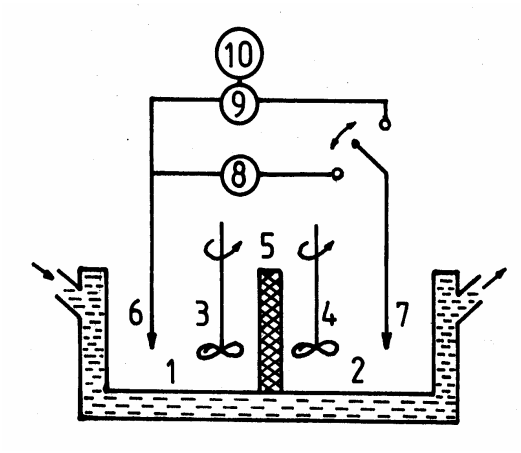

Fig.5 Scheme of the measuring set-up. (1) measuring chamber, (2) reference chamber, (3 and 4) stirres, (5) membrane, (6) measuring and (7) reference electrodes, (8) nanoamperemetr, (9) milivoltmetr, (10) recorder.

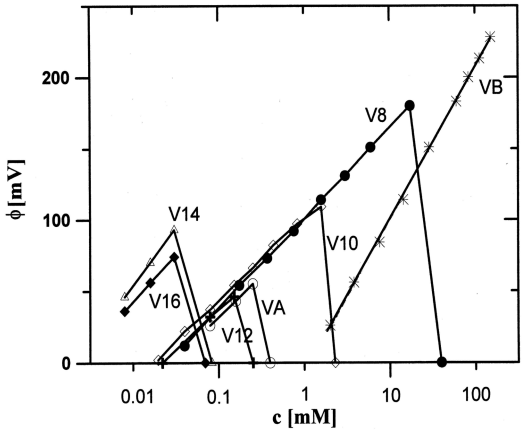

Fig.6 IF membrane potential (φ) vs. logarithm of the AS V concentration c (μM) in the measuring : (Δ) V14, (◆) V16, (◇) V10, (●) V8, (+) V12, (○) VA, (✳) VB (Podolak *et al.*, 1992).

is caused by AS V10 compound. In the case of the EYL liposomes (LL), after adding the AS V*n* to their buffer suspension the highest fluidity is caused by AS V12 (Fig. 4).

The results of the liposome membranes fluidity investigation support, as one can suppose, the suggestion (Rucka *et al.*, 1983; Kuczera *et al.*, 1985) about the existence of a definite alkyl chain length most favorable for the biological activity of the AS V*n* molecule. However, this suggestion finds no confirmation in the results of the speed of the erythrocyte hemolysis and BLM stability (Kleszczyńska *et al.*, 1990).

AS V COMPOUNDS MICELLIZATION

All of the AS V compounds but one (AS VB) show amphiphilic properties. The compounds well dissolve in water up to some characteristic concentration called the critical micelle concentration (CMC). After exceeding the CMC value, the AS V molecules create micelles — liotropic liquid-cristalline structures. CMC values of the compounds discussed in the paper have been determined by calorimetric method (Różycka-Roszak, Przestalski & Witek, 1988) and in the case of AS VA also by the ESR spin probe method (Podolak, Pawluk, Różycka-Roszak, Witek & Przestalski, 1988). The CMC values decrease with increasing AS V alkyl chain length (Table 2). The AS VB compound, which molecules have no alkyl chain, well dissolves in water in wide range of concentrations and does not form micelles. Podolak *et al.* (1992) studied the effect of AS V compounds on the electric potential of membranes in the form of filters impregnated with fatty acid ester (IF). The IF membranes were mounted between measuring and reference chambers filled with buffer solution (Fig. 5). The introduction of the AS V compounds into the measuring chamber (1) caused the generation of a potential drop between the surfaces of membrane. This potential drop increased with increasing AS V concentration (Fig. 6). At lytic concentration (LC), characteristic to each of the compounds studied, the IF membrane suffered destruction and the potential drop falls to zero. LC values of the AS V studied are collected in Table 2.

The LC values like CMC decrease with increasing the AS V molecule alkyl chain length, but are at least one order of magnitude lower. The IF membrane lysis results probably from creation in the membrane surrounding solution the mixed micelles containing AS V and IF impregnated fatty acid ester molecules. This process is supposed to remove the fatty acid ester molecules from IF membrane, conducting to its destruction.

The AS VB compound does not cause the membrane lysis even at very high concentration (164 mM) because it can not create micelles.

CONCLUSIONS

The results of various studies concerning the AS compounds discussed in the paper suggest that their influence on the natural and model membranes is closely connected with ability to the micelles formation. The critical micelle concentration (CMC) and the lytic concentration (LC), conditioning the mixed micelle formation, increase

monotonically with increasing the AS molecule alkyl chain length. The AS activity shows the tendency to stabilization for the compounds with 14 and 16 carbon atoms in the alkyl chain. Such conclusions result from the studies concerning the erythrocyte, bilayer lipid and IF membranes (the first group of the studies). In the case of the liposome membranes and *Sphaerotilus natans* bacteria (the second group of the studies), the maximum activity for the AS compound with the definite alkyl chain length (10, 12 or 14 carbon atoms) was observed.

The differences in the dependencies of AS activity on the alkyl chain length are caused, as it can supposed, by different AS concentration ranges used in these two groups of the studies. In the first of them, the AS concentrations were much lower than CMC as well as LC of these compounds (Figs 1, 2, 6 and Table 2). In the second group, the AS concentrations were comparable or even higher than CMC and much higher than LC values (Tables 1, 2 and Figs 3, 4). Therefore, the effect of the favorable (for AS activity) alkyl chain length observed in this group of studies is probably caused by formation of purely or mixed micelles of these compounds, in water solution surrounding the membranes. This process is finally supposed to conduct to partition of AS molecules between membranes and micelles. The partition coefficient value can depend on the compounds concentration, the type of membranes and the manner of sample preparation. An increase of the AS molecule alkyl chain length may cause an increase of the micelle formation process competitivity. Therefore, the effective activity of AS compounds (at a constant concentration of the compounds) can achieve maximum for the definite alkyl chain length (the second group of the studies).

SYMBOLS LIST

AS	Quaternary Ammonium Salt,
AS V	Quaternary Ammonium Salts belonging to the V group (AS V*n*, AS VA and AS VB),
AS Vn	AS V belonging to V*n* group of the general chemical formula, $(CH_3)_3\,N^+ - CH_2COOC_nH_{2n+1}\,Cl^-$, where *n*=6, 8, 10, 12, 14 or 16 ,
AS VA	AS V of the chemical formula, $(CH_3)_2N^+(C_{12}H_{25})-CH_2COOCH_2Cl\;\;Cl^-$
AS VB	AS V of the chemical formula, $(CH_3)_3\,N^+ - CH_2COOCH_2CH_2Cl\;Cl^-$,
EYL	Egg Yolk Lecithine,
LL	EYL liposomes,
LM	mixed liposomes of EYL and AS V*n*,
ESR	Electron Spin Resonance,
τ	ESR spectroscopic parameter inversely proportional to the rotational velocity of the spin probe as well as to the membrane fluidity,
τ_k	parameter τ of the sample devoided of AS,
BLM	Bilayer Lipid Membranes,
FI	Filter Impregnated with lauric fatty acid ester,
a(100)	AS V*n* concentration causing 100% hemolysis of the pig blood erythrocytes (mM),
CC	Critical Concentration of the AS Vn compound at which the lifetime of BLM does not exceed 5 minutes (mM),
CMC	Critical Micellar Concentration (mM),
LC	Lytic Concentration - AS concentration caused of FI membrane destruction (mM),
α	rate constant of SO_4^{2-} ions permeation through EYL liposome membranes for AS modified,
α_0	rate constant of SO_4^{2-} ions permeation through unmodified EYL liposome membranes.

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