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Biophysical Investigations of Model Membranes Modified by Some Bis-Ammonium Salts with Potential Biological Activity

INTRODUCTION

The surface and biological activities (e.g. antimicrobial) of many ammonium salts have been known from tests performed on fungi, algae and bacteria *in vivo*. Several reports [1–5] have indicated a qualitative relationship between the results obtained with some ammonium salts in biological tests and those obtained for different model membranes. It is reasonable to assume that the biological activity of a newly synthesized compound can be determined by studying its interaction with model membranes. In the present study erythrocyte membranes and bimolecular planar phospholipid membranes formed from lipid extract of erythrocytes were used as model membranes. The choice of the models was determined by the results of previous works [3,4,6–8] on the interaction of different mono-ammonium salts with the above models showing encouraging correlation between the results obtained for RBC and those for BLM. Moreover, it was found that the effectiveness of the compounds depends on the length of the alkyl chains both in the case of mono- and bis-ammonium salts.

The aim of the present work was to find out whether the polar groups of the compounds investigated play a role in the interaction with the lipid phase and alter the mechanical properties of membranes.

MATERIALS AND METHODS

Fresh heparinized pig blood cells were used in the hemolytic experiments. To obtain the salt concentration dependence of hemolysis, the respective salts were added in proper amounts to the RBC suspension in 131.91 mmol/l NaCl; 4.79 mmol/l KCl; 0.86 mmol/l $MgCl_2$; 11.79 mmol/l Na_2PO_4 $2H_2O$; 1.80 mmol/l NaH_2PO_4 H_2O and 5.84 mmol/l glucose, pH 7.4. Spectrophotometrical measurements at 540 nm were

performed at 37°C during 1 hour. The experimental procedure was described elsewhere [3]. Total lipid extract from erythrocytes was prepared according to Dodge *et al.* [9] and used in BLM experiments. The membranes were formed of 1.5% (w/v) solution of the lipid extract in 1:1 (v/v) mixture of n-butanol:n-decane on a hole (1.75 nm in diameter) in a Teflon partition of a two-compartment measuring chamber. The voltage of 20 mV was applied to the membranes from an external d.c. source by means of two identical calomel electrodes. Agar-KCl bridges were used to avoid electrode contamination. The bath solution was the same as in hemolytic experiments. The salts were added to the bath solution by steps until their concentration was high enough to bring about destruction of the BLMs in 5 minutes; the time needed for BLMs to achieve the bimolecular of "black" arrangement was about 10 min at ca 22°C (room temperature). This salt concentration is further on called the critical concentration (cc). Once the salt reached its cc in the bath solution no new membranes could achieve the bimolecular structure, the transition to which was monitored electrically and also optically. All the studied bis-ammonium salts, of general structure shown in Fig. 1, were synthesized in the Institute of Organic and Polymer Technology, Technical University, Wrocław.

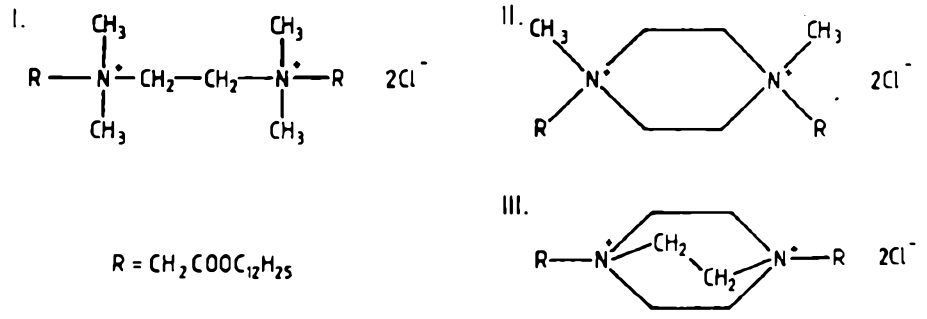

Fig. 1. Chemical formulae of the bis-ammonium salts studied. I — N,N,N',N'-tetramethyl-ethylene-bis-glycinedodecylester dichloride; II — N,N'-di-methyl-piperazine-bis-glycinedodecylester-dichloride; III — triethylene-bis-glycinedodecylester dichloride

RESULTS

The results of hemolytic experiments are shown in Fig. 2. The concentration of BAS inducing 100% hemolysis of erythrocytes (C_{100}) was arbitrarily taken as a measure of hemolytic activity of BAS. The values of C_{100} are calculated with the biggest experimental error and such an approach had the aim to achieve the closest relation between C_{100} and cc values. Once the concentration of BAS in the bath solution reached its C_{100} or cc value (BLM experiments), the model membranes broke down. Values of C_{100} and cc are specified in Table 1.

It should be noted that in these experiments the stability of BLMs decreased gradually with increasing salt concentration in the bath solution. The shortening of

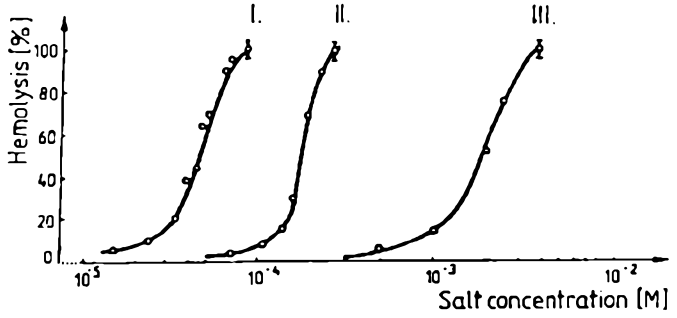

Fig. 2. Dependence of the degree of red blood cell hemolysis on the concentration of the chlorides studied. Each point is the mean value of percent hemolysis for at least five experiments. Standard deviation changes from about ±2% (10% hemolysis) to ±5% (100% hemolysis). Hematocrit was 2%

salt	C_{100} [mol/l]	cc [mol/l]
I	$8.5 \cdot 10^{-5}$	$4.1 \cdot 10^{-5}$
II	$2.6 \cdot 10^{-4}$	$1.9 \cdot 10^{-5}$
III	$3.7 \cdot 10^{-3}$	$3.2 \cdot 10^{-4}$

BLM life-time with increasing salt concentration was accompanied by a change in BLM specific resistance (SR). A typical SR change was over one order of magnitude. For instance, the SR of BLM in bath solution containing salt III in a concentration of $1.6 \cdot 10^{-5}$ mol/l, i.e., well below the cc value for this salt, decreased from $1.1 \cdot 10^{7}$ ohm cm^2 to $6.1 \cdot 10^{5}$ ohm cm^2 during about 20 minutes. Similar effects were observed for all the salts studied and the higher BAS concentration was the shorter was the BLM life-time, i.e., the worse was the BLM stability.

DISCUSSION

The results obtained of both hemolytic and BLM experiments show that the efficiency of the bis-ammonium salts studied in changing the properties of membrane models used depends on the polar head structure of BAS, as their alkyl chains were of the same length. The interaction mechanism leading to inducing changes in stability of model membranes is based in the possibility of incorporation of the hydrophobic alkyl chains of BAS molecules into the hydrophobic interior of these membranes. The depth of the incorporation and thus the strength of the interaction must be, among others, governed by the polar head structure of bis-ammonium salt. Even more, the comparison of the results obtained for both types of experiments given in Table 1 suggests that the lipid phase of

the RBC membrane is the place where BAS molecules incorporate. Once built into the bilayer structure, BAS molecules disturb its organisation and change also its mechanical properties, thus resulting in the appearance of hemolysis of RBC [3,4,6,7,10] and diminished stability of BLM. According to some authors the change of mechanical properties of a membrane and/or their rupture occurs via the formation and development of pores, the expansion of which can result in irreversible membrane breakdown. The pore expansion can be followed by a change in the specific resistance, as in the case of BLM measurements. Another approach to the problem is presented by Sersen *et al.* [13]. It is suggested that the insertion of surfactant molecules into the lipid part of the membrane perturbs the packing density of lipid molecules at the end of the alkyl chain of the surfactant. The resultant free volume depends on the difference between the lengths of the lipid and surfactant alkyl chains and provides a greater possibility for *trans-gauche* isomerization of lipid chains. Such approach correlates to a certain degree with the hypothesis of De Kruijff *et al.* [14] based on the so-called shape concept idea. According to this idea molecules of a surfactant of inverted cone shapes when introduced into a noncomplementary neighborhood can induce packing defects [5].

Generalizing the picture one can say that incorporating "foreign" material into membranes changes their mechanical equilibrium and thus the state of balance between the compressive and repulsive forces. The changes in the mechanical equilibrium may also be the result of the hydrophobic mismatch between the surfactant and lipid materials as proposed by Mouritsen [16]. As it was mentioned above, the ability of inducing mechanical changes of the models used depends on the possibility of incorporation of BAS alkyl chains into the lipid phase of model membranes. The best possibility in that sense has salt I of non-ring polar head structure. On the other hand that possibility is worst in the case of salt III, whose more bulky and less flexible in comparison with salt II, polar head is the reason for the observed weaker membranolytic efficiency. The results obtained seem to confirm such conclusion as in both RBC and BLM experiments the sequence of the BAS efficiency toward model membranes is: I>II>III. Moreover, these results seem to confirm the hydrophobic mismatch and the free volume ideas and show the importance of the polar head structure of surfactants in their interaction with membranes, especially when the surfactants' alkyl chain lengths are the same. In many other cases, when dealing with surfactants differing also in the alkyl chain length and/or how numerous they are, the interaction with the membranes will also depend on these factors as was shown in [8].

The influence of the compounds studied on the lipid phase and the mechanical changes they cause are probably the reasons for their biological activity.

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