Influence of Counterions on the Interaction of Pyridinium Salts with Model Membranes

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The interaction of pyridinium salts (PS) with red blood cells and planar lipid membranes was studied. The aim of the work was to find whether certain cationic surfactant counterion influence its possible biological activity. The counterions studied were Cl^-, Br^-, I^-, ClO_4^-, BF_4^- and NO_3^-. The model membranes used were erythrocyte and planar lipid membranes (BLM). At high concentration the salts caused 100% erythrocyte hemolysis (C_{100}) or broke BLMs (CC). Both parameters describe mechanical properties of model membranes.

It was found that the efficiency of the surfactant to destabilize model membranes depended to some degree on its counterion. In both, erythrocyte and BLM experiments, the highest efficiency was observed for Br^-, the lowest for NO_3^-. The influence of all other anions on surfactant efficiency changed between these two extremities; that of chloride and perchlorate ions was similar. Some differences were found in the case of BF_4^- ion. Its influence on hemolytic possibilities of PS was significant while BLM destruction required relatively high concentration of this anion. Apparently, the influence of various anions on the destructive action of PS on the model membrane used may be attributed to different mobilities and radii of hydrated ions and hence, to different possibilities of particular anions to modify the surface potential of model membranes. This can lead to a differentiated interaction of PS with modified bilayers. Moreover, the effect of anions on the water structure must be taken into account. It is important whether the anions can be classified as water ordering kosmotropes that hold the first hydration shell tightly or water disordering chaotropes that hold water molecules in that shell loosely.

Introduction

Pyridinium salts (PS) studied belong to amphiphilic biologically active compounds whose efficiencies to destroy various microorganisms by damaging their membranes depends on a number of various features of the amphiphile. The most important are: polarity, which depends on steric properties of the polar head of the compound and its net charge or charge distribution, and lipophilicity depending in turn on number and length of alkyl chains of this compound (Sarapuk *et al.*, 1986; Kleszczyńska *et al.*, 1990). As already shown by our previous experiments bromides and chlorides of the same amphiphile can influence the interaction of the compound with biological and/or model membranes (Sarapuk *et al.*, 1998; Kleszczyńska *et al.*, 1998; Kleszczyńska and Sarapuk 1998) to a different degree thus determining its biological activity. The objective of this study was to find a possible influence of other anions on the efficiency of the surfactant studied. The problem has its practical implications. The anions studied were Cl^-, Br^-, I^-, ClO_4^-, NO_3^- and BF_4^-. They present two groups of ions that are referred to as chaotropic (first five of mentioned ions) and kosmotropic (BF_4^-) compounds affecting solute solubility. Chaotropes increase this solubility disordering water structure while kosmotropes cause opposite effects (Collins and Washabough, 1985; Washabough and Collins, !986; Collins, 1995; 1997; de-Paula and Schreier, 1996).

PS compounds are potential pesticides (preliminary tests showed their activity against some bacteria, unpubl results) with an oxymethylene group incorporated between polar head and alkyl chain of PS. This group should serve two purposes. The elongated hydrophobic part of PS increases its bi-

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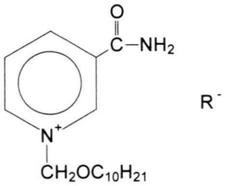
ological efficiency (Kleszczyńska and Sarapuk, 1998; Sarapuk and Kubica, 1998) and at the same time it facilitates the degradation of the molecule. Model membranes (erythrocytes and planar lipid membranes) were chosen because they were shown to verify each other qualitatively, i. e., the results of studies on the interaction of various biologically active compounds with erythrocytes and BLMs were in good qualitative agreement (Sarapuk *et al.*, 1986; 1998; Kleszczyńska *et al.*, 1990; 1998; Kleszczyńska and Sarapuk, 1998).

Materials and Methods

Fresh heparinized pig erythrocytes were used in hemolytic experiments. The blood was centrifuged for 3 min at 1000×*g*, the plasma removed and the cells washed four times with isotonic phosphate (131 mM NaCl, 1.79 mM KCl, 0.86 mM $MgCl_2$, 11.79 mM $Na_2HPO_4 \cdot 2H_2O$, 1.80 mM $NaH_2PO_4 \cdot H_2O$, pH 7.4). The erythrocytes were then treated for half an hour at 37 °C with the same solutions containing various concentrations of the compounds studied. Four different hematocrites H (the ratio of the erythrocyte volume to that of the whole sample) were studied (2%, 4%, 6% and 8%). After modification samples were taken, centrifugated and the supernatant assayed spectrophotometrically for hemoglobin content (Spekol 11, Carl Zeiss, Jena) at 540 nm. The extent of hemolysis was the hemoglobin concentration in the supernatant of completely hemolyzed cells.

Planar lipid membranes (BLM) were formed from a solution of 1.5% (w/v) azolectin (Sigma Chem., St Louis) in n-butanol:n-decane (1:1) (v/v) on a 1.7 mm hole in a partition of two-compartmental chamber filled with a 0.9% NaCl bath solution. 0.01 M solutions of the compounds studied were prepared and appropriate volumes of these solutions were pipetted directly into the measurement chamber until the compound concentrations reached a value that caused BLM breakdown in no more than 3 min. These concentrations are referred as critical ones (CC).

3-Carbamoyl-1-(decyloxymethyl)pyridinium salts of general formulae shown in Fig. 1 were obtained like follows. Pyridinium chloride was prepared by dissolving nicotinamide (2.44 g, 0.02 mol) in 15 ml N,N-dimethylformamide and adding an equimolar amount of chloromethyldecyl ether. The mixture was stirred at room temperature for 2 h and 10 ml of ethyl acetate was added. The solid substrate was filtered and then recrystallized from EtOH with a yield of 95%. Particular salts were prepared by mixing a concentrated aqueous NaX solution (X = Cl, Br, I, ClO_4, BF_4 and NO_3) with an aqueous pyridinium chloride solution at room temperature. A precipitate was obtained which was redissolved in warm water and precipitated again after cooling. The solution was filtered and recrystallized from EtOH. The purity of salts were checked by ^1H-NMR and ^{13}C-NMR spectra. They were recorded on a Varian Model XL 300 spectrometer at 300 MHz for ^1H and 75 MHz for ^{13}C in CDCl at 20 °C with tetramethylsilane as internal reference. Satisfactory elemental analyses were obtained.



R = Cl, Br, J, ClO_4, BF_4, NO_3

Fig. 1. General formula of bifunctional surfactants.

Results

Stability of azolectin BLMs, the measure of which was their lifetime, gradually decreased with the increase of surfactant concentration in the bath solution. The obtained values of so-called critical concentrations (CC) of compounds, i.e., concentrations causing destruction of BLMs in time shorter than 3 min are documented in Table I.

The results of studies on erythrocyte hemolysis present typical hemolytic curves shown in Fig. 2. The most and the least active compounds, like PS bromide (A) and nitrate (B) were chosen as examples. The hemolytic curves allowed for an estimation of the concentrations of compounds causing 100% hemolysis (C_{100}). Such approach was chosen because the C_{100} is qualitatively similar to the CC parameter featuring BLM experiments. Namely, both parameters describe total membrane disruption. Estimated values of C_{100} are shown in Table II.

Table I. Critical concentration (CC) of pyridinium salts (PS) causing BLM brakdown in no more than 3 min.

Type of salt	PSCl	PSBr	PSJ	$PSClO_4$	$PSBF_4$	$PSNO_3$
CC[mM]	0.240±0.02	0.083±0.02	0.110±0.02	0.280±0.02	0.480±0.02	1.100±0.02

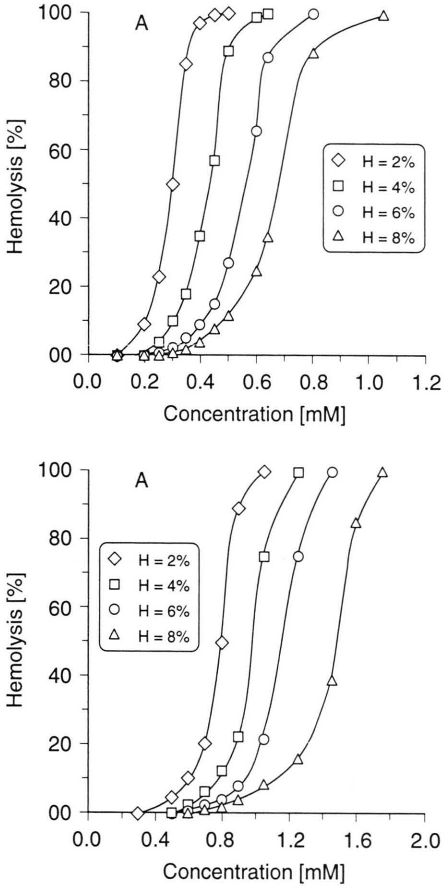


Fig. 2. Dependence of the degree of red blood cell hemolysis on concentration of pyridinum bromide (A) and pyridinium nitrite (B) for four hematocrites – H (H - volume percentage of erythrocytes in a suspension).

Table II. Concentration of pyridinium salts (PS) causing 100% hemolysis of red blood cell (C_{100}).

Type of salt	C_{100} [mM]			
	Hematocrit [%]			
	2	4	6	8
$PSCl^-$	0.95±0.05	1.10±0.05	1.30±0.05	1.45±0.05
$PSBr^-$	0.46±0.05	0.64±0.05	0.80±0.05	1.05±0.05
PSJ^-	0.60±0.05	0.85±0.05	1.00±0.05	1.20±0.05
$PSClO_4^-$	0.86±0.05	1.10±0.05	1.25±0.05	1.40±0.05
$PSBF_4^-$	0.56±0.05	0.72±0.05	0.88±0.05	1.10±0.05
$PSNO_3^-$	1.05±0.05	1.25±0.05	1.45±0.05	1.75±0.05

Hematocrit – volume percentage of erythrocytes in a suspension.

Discussion

BLM and erythrocyte studies showed that pyridinium salts studied act as destabilizers of model membranes when used in high enough concentrations. Based on our results they can be classified as potentially good biologically active substances. These results show that their efficiency to destroy model membranes are clearly differentiated and depends on the kind of the counterion. Similar influence of various anions on efficiencies of some surfactants was observed earlier (Gallova *et al.*, 1990; Sarapuk *et al.*, 1998; Kleszczyńska *et al.*, 1998; Kleszczyńska and Sarapuk, 1998). It was also shown that altering the anion in the original salt solution apparently influenced the rate of transport of a given cation through liquid membranes (Christensen *et al.*, 1979) and that cationic surfactant adsorption to cationic-anionic monolayers depends on the kind of the halide anions (Góralczyk, 1993; 1994). All these effects must be related to the physicochemical properties of particular anions and their differentiated interaction with components of membranes which in turn influences the interaction of a surfactant cation with anion-modified membranes. Physicochemical properties of ions are determined, among others, by their dimensions and mobilities. Electrophoretic mobilities or dimensions of hydrated or effective ion radii are taken into account (Dołowy, 1997). The last factor determines the charge density of ions, and the sequence of the coulombic interaction strength between the ions studied and the choline group of lipid molecules of the model membranes should be like follows:

$$Br^- > I^- > Cl^- > NO_3^- > ClO_4^- > BF_4^- . \qquad (1)$$

Such sequence can not be assembled on the basis of the presented experiments. Especially, the results obtained for BF_4^- (erythrocytes experiments) and NO_3^- (both types of experiments) ions do not fit into that sequence. Apparently also other properties of ions play a role in modification of model membranes. These other properties may be the capacity of particular ions to order (kosmotropes) or disorder (chaotropes) water molecules as was mentioned earlier. Such subdivision is especially important as chaotropes, like bromide ion, were found to bind to lipid molecules (de-Paula and Schreier, 1996). This can explain the small modification of BLM by BF_4^- classified as a kosmotrope. It is evident that chaotropes-induced membrane modification, especially the alteration of the electronic surface properties of a membrane, is of major influence for cationic surfactant incorporation into such modified membrane. The chaotrope facilitate this incorporation which is evidenced by a smaller concentration of surfactant needed for membrane destruction. Additionally, the chaotrope increase the surfactant solubility in aqueous media thus increasing its partition coefficient. According to Collins (1995; 1997) chaotropic properties for the anions studied should follow the following sequence:

$$I^- > ClO_4^- > NO_3^- > Br^- > Cl^- , \quad (2)$$

while BF_4^- is kosmotrope.

However, it must be noted that somewhat divergent opinions concern chloride ion which can be described as "marginally chaotropic" (Collins, 1995; 1997) or as kosmotropic (de-Paula and Schreier, 1996).

None of the presented sequences fits exactly into the results obtained. Such fitting can be achieved when BF_4^- and NO_3^- ions are disregarded. Then the interaction of anions with both model membranes follow sequence (1) pointing to a hydrated radius as indicator to modify these anions. The smaller the radius of a particular ion the stronger it modifies membranes. The kosmotropic behaviour of BF_4^- can partially explain the results obtained with BLM while results obtained for NO_3^- demand further studies.

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