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EFFECT OF SELECTED PESTICIDES AND TEMPERATURE ON THE ORDER OF LIPOSOME STRUCTURE

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A b s t r a c t. Studies were carried out on the effect of chloride of N-dodecyl-betaine 2-chloroethyl-ester (BE V-B), and chloride of betaine 2-chloroethyl-ester (BE V-B), added to liposomes formed of egg yolk lecithin, on the order parameter of a spin probe incorporated in the temperature range 2-50 °C. The BE's used here modified ESR spectra to various degrees and lowered the order parameter of the spin probe. At temperatures lower than 20 °C compound V-B caused smaller changes in the order parameter than V-A, whereas above that temperature it caused greater changes. The ESR spectra of liposomes containing BE's exhibited splitting into two components that corresponded to different order parameter values. The molecular mechanism of interaction between the compounds studied and liposomes is disscused.

INTRODUCTION

The substances in this work belong to quaternary ammonium salts, derivatives of betaine esters (BE's). These are biologically active compounds possessing, among others, algicidal, bactericidal, and fungicidal activity [7]. They are very effective as growth inhibitors [7]. The lowest biologically effective concentration is about 6×10^{-6} M in the case of algae and about 3×10^{-4} M in the case of bacteria. Depending on their structure the compounds modify to various degrees the properties of artificial phospholipid membranes. In particular, they cause a marked increase in the rate of sulphate ion transport across liposome membranes [4] and decrease in electric resistance of black lipid membranes [9]. Using the ESR method, we studied the effect of BE's of various alkyl chain lengths on the order parameter and the rotational correlation time of a spin probe incorporated into the liposome structure [6]. Changes in both of the quantities mentioned indicate that BE's induce an increase in liposome fluidity. Since the molecular mechanism of that phenomenon has not yet been fully described, it seems advisable to study the effect of other BE's and temperature on the degree of order of the liposome structure.

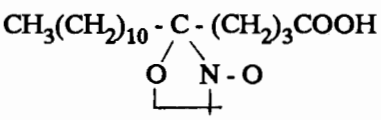
MATERIALS AND METHODS

Two compounds were chosen for the study, both derivatives of betaine esters, synthesized in the Institute of Organic and Polymer Technology of the Technical University of Wrocław. The general chemical formula of BE's has the form

$$(CH_3)_2 - \underset{R}{N^+} - CH_2 - COOC_2H_4Cl \qquad Cl^-$$

where $R = C_{12}H_{25}$ (for BE V-A) and $R = CH_3$ (for BE V-B). The lecithin (EYL) was prepared from the yolks of fresh hen eggs by a method described in [11], and stored in

chloroform solution at -20 °C under nitrogen. The spin probe used was 4-(2n-undecyl-3 oxyl-4, 4-dimethyloxazolidin-2-yl) butyric acid (produced by REANAL, Hungary) of chemical formula



The BE was dissolved in methanol and mixed (in the correct ratio) with a chloroform solution of EYL and the spin probe. The mixture thus obtained was dried for 1 h with a vacuum pump. To the dry sample a veronal-acetate buffer of pH=7.5 was added, and the sample was shaken for twelve or so minutes to obtain multilamellar liposomes. EYL concentration in the buffer solution was 4×10^{-2} M; the spin probe concentration was 4×10^{-4} M. The molar ratio of BE's to EYL varied from 0.1 to 0.5, or it was constant and equal to 0.4. The ESR spectra were taken with a Varian E-3 X-band spectrometer. Based on the spectra, the order parameter (S) was calculated in the same way as in [3].

RESULTS

Both the compounds studied, when added to EYL liposomes, lowered the order parameter value (S) of the spin probe, their effectivity increasing with increased concentrations of BE's (Fig. 1). Compound V-B (when compared with V-A) caused greater changes of parameter S.

Figs 2 and 3 represent exemplary ESR spectra, obtained with samples containing V-A (Fig. 2) and V-B (Fig. 3) for two different temperatures. The spectra of admixed liposomes have two components, one with a higher degree of order (h) and one with a lower degee of order (l). The l component appeared in the ESR spectrum at higher temperatures: above 20 °C with V-B and 30 °C with V-A. With increasing temperature (l) the order parameter (S) decreased with all the samples studied (Fig. 4). V-A admixed with liposomes caused a parallel (aproximately) displacement of the relation $S = f(T)$

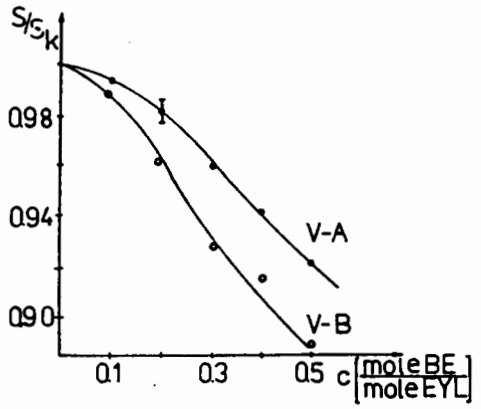


Fig. 1. Dependence of the relative order parameter (S/S_k) of the spin probe incorporated into EYL liposomes modified by BE V-A and BE V-B at contrentration (c) of BE's. The order parameter of control sample was $S_k = 0.6$. The measurements were conducted at 23 °C.

(when compared with unadmixed liposomes) towards lower values of S (Fig. 4, b^h and a). However, in the case of the V-B admixture the changes od the parameter S (compared with the unadmixed sample) increased with rising temperature (Fig. 4, c^h and a). It follows from Fig. 4 that below 20 °C compound V-A caused greater changes in the order parameter compared with V-B, whereas above that temperature greater changes were caused by compound V-B.

DISSCUSION

The presence of compounds V-A and V-B results in a disturbance in the EYL liposome structure, which is expressed in the lowering of the order parameter. Compound V-A has a single 12 carbon alkyl chain and positively charged head group, and thus has amphiphilic properties. Introduction of a single-chain V-A to liposomes may weaken the hydrophobic binding between double-chain lecithin molecules. On the other hand, the positively charged head groups of V-A surrounded by electrically neutral heads of EYL may bring about repulsive forces and thus lead to loosening of the region's structure [2]. As a consequence one can observe throughout the whole temperature change a decrease in the degree of order of V-A admixed liposomes compared

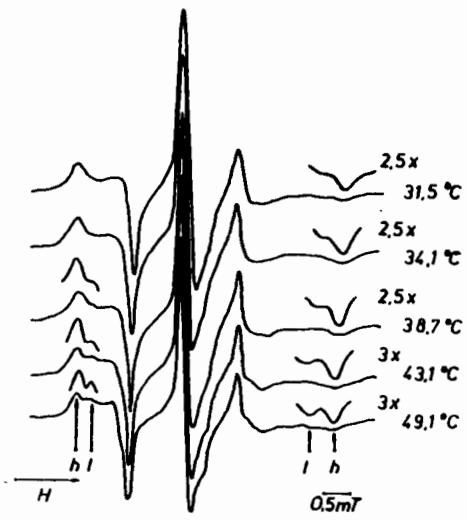


Fig. 2. ESR spectra of the spin probe incorporated into EYL liposome structure containing admixture of BE V-A of concentration 0.4 mole BE/mole EYL, obtained for several chosen temperatures. Symbols h and l mean the higher and lower order ESR spectra components, respectively.

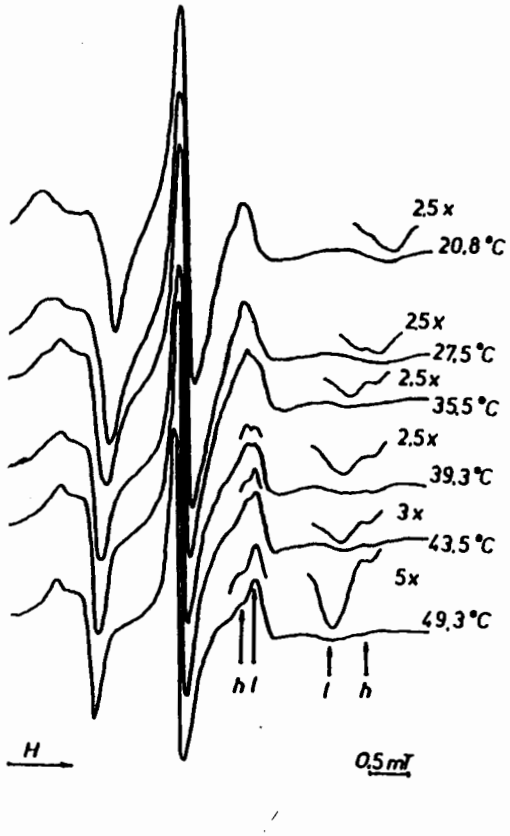


Fig. 3. ESR spectra of the spin probe incorporated into EYL liposome structure, containing admixture of BE V-B of concentration 0.4 mole BE/mole EYL, obtained for several chosen temperatures (symbols h and l as in Fig. 2).

with liposomes without admixture (Fig.4). Compound V-B has no alkyl chain, but only a positively charged head group (the same as V-A) and this determines its hydrophilic properties. One can suppose that at low temperatures, as was observed in studies using the Tempo spin probe [5], compound V-B is located mainly in the aqueous phase and only a small portion of it is present in the lipid bilayer. With rising temperature, fluidity of EYL liposomes increases and, presumably, the content of V-B in liposomes also. At about 20 °C a decrease in the activation energy of the process of fluidizing the EYL liposomes was observed [6]. Above that temperature the V-B content of the liposomes seems to increase significantly. The presence of V-B in liposomes results in a greater disturbance of their structure than the presence of V-A. This is due, presumably, to the lack of a hydrophobic chain and greater motional freedom of the molecules. As a result, one observes larger changes in the ordering of the liposomes caused by V-B than by V-A at temperatures above 20 °C (Fig. 4). This conclusion is supported by the results obtained in ref. [10], where it is shown that the largest disturbances are caused by small molecules introduced to erythrocyte membranes.

In ref. [4] it is shown that compound V-A accelerates permeation of sulphate ions across liposome membranes, whereas V-B has no effect on the transport. Hence it follows that the rate of sulphate ion permeation across EYL liposome membranes is determined by factors other than the ordering of the membrane structure, or that in the study, V-B did not incorporate into the liposomes or did it in small measure. The splitting of the ESR spectra observed for samples admixed with BE (Figs 2 and 3) is probably connected with two different probe binding sites in the liposome structure. For EYL liposomes

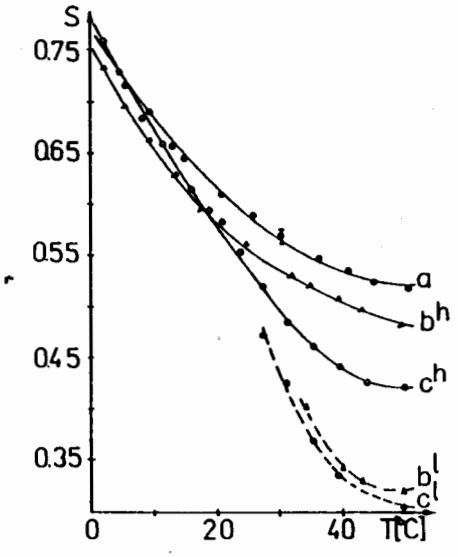


Fig. 4. Dependence of the order parameter (S) on temperature (T) for samples containing: a-EYL liposomes without admixtures; b-EYL liposomes admixed with BE V-A; c-EYL liposomes admixed with BE V-B. Indices h and l mean the curves obtained for the high and low order ESR spectra components, respectively.

without admixtures it was found [1,8] that, depending on the pH of the buffer solution, the ESR spectra (in the case of spin-labelled fatty acids) split into two components (at a pH of from 3 to 7) or remained unsplit (above pH 7 and below pH 3). The presence of two components in an ESR spectrum follows, according to [8], from two possible probe binding sites in the polar interface of the lecithin layer. One of them is the choline group, the other the phosphate group. The spin probe binds to these sites with its carboxyl group. The ionized carboxyl group binds with the choline group (this corresponds to the higher order of the probe), and the unionized with the phosphate group (lower ordering). In the present work, the pH of the solution was constant and equal to 7.5, so the carboxyl group can be assumed to be ionized. As a result, the ESR spectra of lecithin liposomes without admixtures contained only the component that corresponds to higher order (Fig. 4a). The BE admixtures introduced to the polar interface affected the positively charged group $N^+(CH_3)_3$, when incorporating into the liposomes. This group is assumed to bind to the negative phosphate part of the polar interface and could thus constitue a second place where the negative carboxyl group of the spin probe could bind. That second binding site was displaced relative to the first one towards the centre of the lecithin bilayer and corresponded to lower ordering of the spin probe. Therefore, it seems, in the admixed liposome ESR spectra (Figs 2 and 3) the components of higher order (*h*) and lower order (*l*) appeared. In the case of compound V-A the *l* component had lower intensity than the *h* within the whole temperature range studied, although its intensity decreased with rising temperature (Fig. 2). However for V-B the intensity of the *l* component, at higher temperatures, was dominant over that of the *h* component (Fig. 3). This may signify that the spin probe could easily change from state *h* to state *l* as the ordering of the liposome structure decreased, due to both temperature rise and the presence of V-B.

CONCLUSIONS

From the studies conducted it follows that compounds V-A and V-B cause disturbances in the EYL liposome structure, in particular by lowering its ordering; V-B locates both in the hydrophilic layer whereas V-A locates in the hydrophilic and hydrophobic layers. The content of both compounds in liposomes increases with rising temperature. Since the changes in the order parameter are considerably greater for V-B than V-A, this may indicate that compound V-B penetrates predominantly the hydrophilic surface of the lipid bilayer, especially above 20 °C.

Assuming that the fungicidal activity of the compounds studied is related to the ordering of the cell membrane structure, one can expect that increased temperature should markedly increase the biological activity of the compounds, of compound V-B in particular.

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WPŁYW NIEKTÓRYCH PESTYCYDÓW I TEMPERATURY NA BUDOWĘ STRUKTURY LIPOSOMÓW

Przeprowadzono badania nad wpływem chlorku (N-dodecylo-betaina 2-chloroetyl-ester) (BE V-A) i chlorku (betaina 2-chloroetyl-ester) (BE V-B) dodanych do liposomów otrzymanych z lecytyny żółtka jaja kurzego, na parametr uporządkowania spinów sondy (BE) wprowadzonej w zakresie temperatur 2-20 oC. BE użyte tutaj jako sonda modyfikuje spektrum ESR w różnym stopniu i obniża parametr uporządkowania spinów sondy. W temperaturach niższych od 20 oC związek V-B powodował mniejsze zmiany w parametrze uporządkowania aniżeli związek V-A, chociaż dla temperatur wyższych od 20 oC powodował on zmiany większe. Spektrum ESR liposomów zawierających (BE) wykazały rozdzielenie na dwa składniki odpowiednio związane z różnymi parametrami uporządkowania spinów. W pracy przedstawiono molekularny mechanizm współzależności pomiędzy badanymi związkami a liposomami.