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Effect of organic mercury on the electrical resistance of phosphatidylserine bilayers

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Acidic phospholipids have been shown to form complexes with methyl mercury chloride, at physiological pH, in vitro. To check if this interaction had any effect on the physical properties of membranes made with these lipids, the specific resistance of phosphatidylserine bilayers was monitored, as a function of time, in the absence and in the presence of methyl mercury chloride in the bathing solution. While the resistance of the bilayer remained constant in the absence of the toxic, it dropped an average of 17% in four hours in the presence of 100 μM methyl mercury chloride. Such observations suggest that the physical integrity of these membranes is modified by the interaction with organic mercury. This result may be relevant to the observed degeneration of nerve membranes in Minamata disease.

It has recently been shown that organic mercury interacts with acidic phospholipids, at physiological pH, in vitro [1]. The formation of complexes between organic mercury and either phosphatidylserine (PS) or phosphatidylinositol was inferred from the pH-titration of these lipids (in the form of liposomes or microvesicles) in the absence and in the presence of methyl mercury chloride. Such interactions, should they also occur in vivo, could constitute an important factor in the destabilization of the intracytoplasmic membranes of the central nervous system noted in the Minamata disease [2]. By specifically affecting the endoplasmic reticulum membrane, they could be indirectly responsible for the disturbance in the synthesis of integral proteins noted in this disease [1]. The present communication reports an attempt at evaluating the effect of methyl mercury chloride on the electrical resistance of PS bilayers. The objective of this work was to check if the interaction noted between organic mercury and acidic phospholipids could affect the physical integrity of membranes made with these lipids. Electrical resistance was chosen as an index of the integrity of the bilayer. PS was chosen because it is the most common acidic phospholipid found in the brain and the other parts of the nervous system [3].

PS, from bovine brain, was obtained from Sigma, *n*-decane from Eastman, methyl mercury chloride from Matheson coleman and Bell and potassium chloride and potassium hydroxide, ACS, from Anachemia. The water used was distilled and deionized.

The solutions bathing the membranes were not buffered but initially adjusted to pH 7.4 with a dilute potassium hydroxide solution. (Exploratory work had failed to reveal a pH buffer which was not interacting with methyl mercury chloride at pH 7.4. For example, carbonate, phosphate and Tris buffers were found to interact with this toxic compound.) The pH was recorded at the end of each experiment. The membrane forming solution contained 2% PS in decane [4]. The aqueous methyl mercury chloride solution was 2 mM with respect

to methyl mercury chloride and 0.16 M with respect to potassium chloride.

The membranes were prepared by the hairbrush technique across a hole about one millimeter in diameter separating two compartments containing a 0.16 M potassium chloride solution. One compartment where the methyl mercury chloride solution was eventually added was arbitrarily defined as the inside; gentle agitation was obtained in this compartment. Measurement of the electrical resistance was made with a pair of reference calomel electrodes, one on each side of the membrane. The instrumental arrangement, electrical circuits and technical procedures have been described in detail elsewhere [5].

Every time the membrane resistance was measured (roughly, every 30 min), two successive readings were made, one with the inside electrode positive and one with the inside electrode negative. This precaution was judged necessary because some additives can be brought to or away from the membrane depending on the polarity of the electrode [6]. In the present study, identical results were obtained in the two cases.

Methyl mercury chloride being volatile and toxic, the recommendations of Klein and Herman [7] were carefully adhered to in the handling of this chemical.

Fig. 1 shows the specific resistance of the PS bilayer plotted as a function of time in the presence and in the absence of methyl mercury chloride. In the case of the control experiment (without methyl mercury chloride) it can be seen that the resistance of the membrane remained fairly constant during the four hours that these experiments lasted. The very slight increase in resistance that can be noted in Fig. 1 was thought to reflect the fact that the pH dropped slightly (to approx. 7.0) during this time period. The resistance of PS bilayers has indeed been shown to be a function of the pH of the aqueous solutions in which they are bathed, the resistance increasing with a decrease in pH [4]. The experimental value obtained in this study for the specific resistance of the unmodified PS bilayer agreed with that reported in the literature for PS bilayers studied in roughly identical conditions [4]. In the presence of a final concentration of 100 μM methyl mercury chloride in the inside compartment, the specific resistance of the

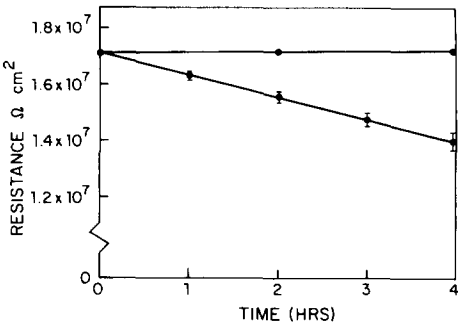

Fig. 1. Specific resistance of the PS bilayer. Upper curve: without methyl mercury chloride; lower curve: in the presence of 100 μM methyl mercury chloride on one side of the membrane.

PS bilayer decreased slowly but constantly during the course of the experiment, as can be seen in Fig. 1. After four hours, it had dropped an average of 17% of the original value. During the same time period, the pH had dropped to approx. 6.8.

It can be noted that a relatively high concentration (100 μM) of methyl mercury chloride had to be used in order that a significant modification in the resistance of PS bilayers be observed. (For comparison, Kasuya reported that only a 10 μM concentration of this compound was necessary to block the outgrowth of nerve fibers in tissue culture [8]). It can be assumed, however, that the effect observed here would have probably also been observed at lower concentrations, although at a slower rate. The effect on the resistance of PS bilayers did not seem to be associated with any ion selectivity since sodium chloride could be substituted for potassium chloride in the bathing solution, without significantly modifying the results.

PS bilayers made from solutions in *n*-alkanes of small and medium chain length retain some solvent molecules in the center of the bilayer [9,10]. The choice of *n*-decane, here, was a compromise. On the one hand, the amount of solvent retained in this case is much less than that of bilayers made from solutions in shorter *n*-alkanes [9,10] and, on the other hand, the stability of bilayers made from solutions in *n*-decane was much better than that obtained with bilayers made from solutions in longer *n*-alkanes (which might explain the fact that *n*-decane has extensively been used in such cases). For example, exploratory research had shown that

unmodified PS bilayers made from solutions in *n*-decane lasted more than six hours while those made from *n*-hexadecane (which is completely extruded from bilayers [9,10]) lasted five to ten minutes. In any event, the presence of a small amount of solvent molecules in the center of the bilayer might not represent an important problem since it has been noted that, in the presence of an applied potential, solvent molecules are squeezed into microscopic 'lenses' [11] (thus, freeing most of the bilayer from solvent molecules). In the procedure followed to obtain the resistance of the bilayer, a constant current pulse was applied during a few seconds, which caused a potential to develop across the membrane: the reading was made when the potential had reached an equilibrium value [5]. Thus, it is suggested that the resistance values obtained here for the unmodified PS bilayer and those for the bilayer modified by the interaction with methyl mercury chloride were obtained in such a way that they are representative of the bilayer itself.

This study did not permit to define a molecular mechanism to account for the decrease in resistance of the PS bilayer in the presence of methyl mercury chloride. A chemical reaction, consecutive to the formation of the complex PS-methyl mercury chloride, and involving a slow modification of the PS molecule is not impossible [12]. However, a simple modification of the cohesion of the lipid molecules in the bilayer, modification brought about by the formation of the complex, would be enough to account for the observation. The important point is that the results suggest that the interaction between organic mercury and acidic phospholipids can destabilize the membranes containing these lipids.

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