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THE MECHANISM OF THE ACTION OF PRYMNESIUM TOXIN ON MEMBRANES

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SUMMARY

The mechanism of the lytic action of prymnesin, a toxin produced by the alga, *Prymnesium parvum*, was studied using liposomes as a model membrane system. Prymnesin showed severe damage to liposomes containing cholesterol but did not affect those without cholesterol. The requirement of cholesterol for the susceptibility to prymnesin was much more strict than the reported requirement for the susceptibility to polyene antibiotics. The net charge on the membranes was shown not to be important for the reaction.

Prymnesin, the toxin produced by *Prymnesium parvum* is known to cause lysis of various erythrocytes [1] and nucleated cells such as Ehrlich ascites cells, Hela cells and normal liver cells [2, 3]. The toxin produces damage to membranes causing leakage of the intracellular constituents into the medium. The mode of action of prymnesin is not known, although the toxin was reported to behave as a surface-active agent [4].

In the present study, we established a model system for studying the action of prymnesin using liposomes. The compositional requirements of the liposomal membranes determining prymnesin susceptibility will be described in this paper.

Egg yolk lecithin and *Escherichia coli* phospholipids were prepared in our laboratory. Other lipids used for preparing liposomes were obtained from commercial sources. Prymnesin was purchased from Makor Chemicals, Jerusalem, Israel.

Liposomes were prepared by the method described previously [5, 6]. The release of glucose marker was assayed enzymatically according to the method developed by Kinsky et al. [6]. The reaction of liposomes with the toxin was always started by adding proper volumes of an ethanolic solution of the toxin (100 000 hemolytic units/ml). Liposomes did not show any leakage of glucose by adding ethanol up to a final concentration of 0.4 %; all experiments were, therefore, performed in solutions containing 0.4 % ethanol. The extent of the toxin-induced release of glucose marker is expressed as the percentage of trapped glucose which was released during a certain period of time (min).

Aliquots of liposome preparation (2.5 μl) derived from egg lecithin, dicetyl phosphate and cholesterol in the molar ratio of 1 : 0.1 : 1 were incubated with various

concentrations of prymnesin for 30 min at room temperature. Prymnesin gave the maximum effect on the liposomes at the concentration of 200 hemolytic units/ml (Fig. 1). At the concentration below 50 hemolytic units/ml, prymnesin showed no appreciable effect on the liposomes. The reaction proceeded very rapidly, reaching the plateau within 10 min when a sufficient amount of the toxin was added (Fig. 2).

An experiment to determine the influence of cholesterol incorporation into liposomes on prymnesin sensitivity is shown in Fig. 3. The response of the liposomes of egg lecithin containing 50 mole% of cholesterol with prymnesin was extremely rapid, while the response of liposomes with 17 mole% of cholesterol was much slower. In liposomes with less than 10 mole% of cholesterol, at most 10 % of trapped glucose was released after 30-min incubation even at the highest concentration of prymnesin

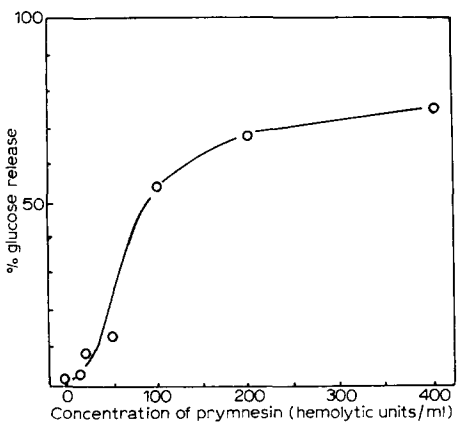

Fig. 1. Effect of the concentration of prymnesin on glucose release from liposomes. Liposomes were prepared from egg lecithin, dicetyl phosphate and cholesterol in the molar ratio of 1 : 0.1 : 1. Liposomes were reacted for 30 min with prymnesin in the buffered solution containing 0.4 % of ethanol.

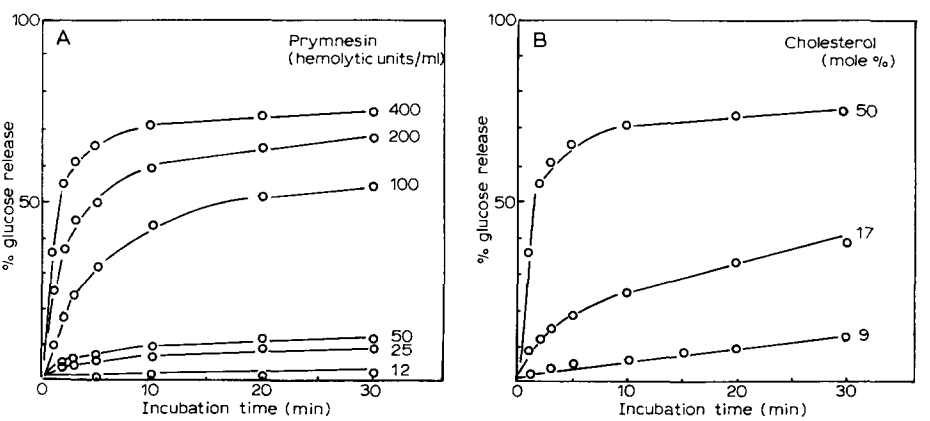

Fig. 2. Time course of glucose release induced by prymnesin. (A) The same liposomes as described in the legend for Fig. 1 were incubated with various concentrations of prymnesin for different times at room temperature. (B) Liposomes of egg lecithin with different amounts of cholesterol were incubated with the toxin at the concentration of 400 hemolytic units/ml at room temperature.

(400 hemolytic units/ml). The influence of the fatty acid components of lecithin and the structure of phospholipids on the measured effect of prymnesin is given in Fig. 4. In the case of liposomes with egg lecithin, the incorporation of more than 17 mole% of cholesterol was essential for the detectable susceptibility toward the toxin while dipalmitoyllecithin liposomes required a much smaller amount of cholesterol for the toxin sensitivity. Liposomes of *E. coli* phospholipids (mainly composed of phosphatidylethanolamine, cardiolipin and phosphatidylglycerol) showed almost the same dependence of cholesterol as those with dipalmitoyllecithin, suggesting that the head groups of phospholipids may not be important for the reaction with the toxin. The susceptibility of dipalmitoyllecithin liposomes against the toxin was highly dependent on the temperature (Fig. 4A). At lower incubation temperatures, a decreasing sensitivity of liposomes to prymnesin was observed. When incubated at 1 °C liposomes containing less than 10 mole% of cholesterol did not show any reactivity, while egg lecithin liposome showed almost the same reactivity at 1 °C as observed at 20 °C (Fig. 4B). Glucose release could not be observed from dipalmitoyllecithin liposomes containing 50 mole% of cholesterol when incubated with prymnesin at 1 °C.

Positively charged liposomes which contained egg lecithin, stearylamine and cholesterol (molar ratio, 1 : 0.1 : 1) were shown to be damaged to almost the same degree as negatively charged liposomes with dicetyl phosphate instead of stearylamine (Fig. 3). This fact indicates that the net charge of the liposomal membranes is not important for the interaction with prymnesin.

The polyene antibiotics have been used for studying membrane structure and function because they have a remarkable property of interacting with membrane sterols [7]. One source of the evidence for the central role of sterols in the reaction between these antibiotics and membranes is that the incorporation of cholesterol into egg lecithin liposomes increased the sensitivity of liposomes to filipin [8]. It is of special interest that prymnesin produced by alga showed a similar requirement of

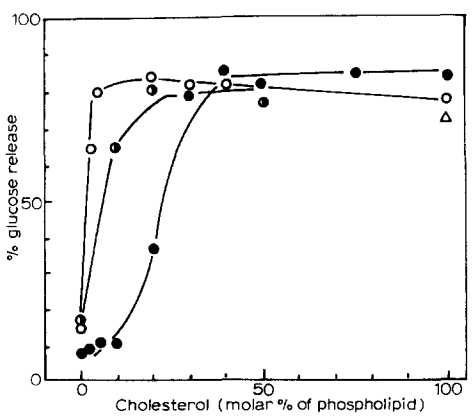

Fig. 3. Effect of cholesterol incorporation into liposomes of various phospholipids on the extent of glucose released by prymnesin at 400 hemolytic units /ml. Liposomes with prymnesin were incubated at room temperature for 30 min. Liposomes were prepared from egg lecithin and dicetyl phosphate (●–●), dipalmitoyl lecithin and dicetyl phosphate (○–○), *E. coli* phospholipids (◑–◑), egg lecithin and stearylamine (△).

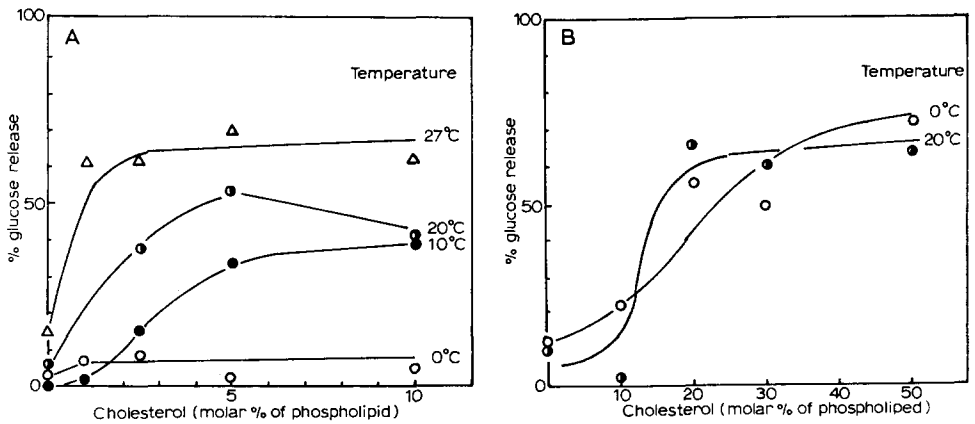

Fig. 4. Effect of temperature on the glucose release from liposomes after incubation for 30 min with prymnesin (400 hemolytic units/ml). (A) Liposomes of dipalmitoyllecithin with varying amounts of cholesterol were incubated with prymnesin at different temperatures as described in the figure. (B) Liposomes of egg lecithin with varying amounts of cholesterol were incubated under the same condition as those of dipalmitoyllecithin.

cholesterol for the reaction with lipid membranes, because this suggests that prymnesin also interacts primarily with membrane sterols. However, in contrast to the polyene antibiotics, the sensitivity of liposomes to prymnesin requires cholesterol quite strictly. Polyene antibiotics were reported to affect liposomes without cholesterol in some cases [9]. Furthermore, the influence of the fatty acid components of lecithin on the susceptibility seemed to be completely different between the polyene antibiotics and prymnesin. The presence of cholesterol in liposomes derived from dipalmitoyllecithin was reported to suppress their susceptibility toward amphotericin B [9]. The same authors described that digitonin which is also known to interact specifically with membrane sterol increasingly reacted with liposomes of dipalmitoyllecithin with increasing amounts of cholesterol incorporated [9]. Therefore, the dependence of prymnesin sensitivity on cholesterol is rather close to that of digitonin sensitivity. The reason why dipalmitoyllecithin liposomes need a smaller amount of cholesterol to be susceptible toward the toxin than those of egg lecithin is not known, but it is reasonable to assume that the fluidity of the membranes influences the sensitivity to prymnesin. The temperature dependence of the susceptibility of dipalmitoyllecithin liposomes to the toxin also indicates the importance of the fluid state of the membranes in their interaction with the toxin. The result, however, seems incompatible with the observation that dipalmitoyllecithin liposomes were more sensitive than those of egg lecithin, because the bilayer composed of egg lecithin is apparently more fluid than that of dipalmitoyllecithin at room temperature. The first interaction between the toxin and the membranes may require some fluid states of the membranes. Once the toxin interacted with the membranes, the less flexible membrane of dipalmitoyllecithin [5] may be more suitable for further inducing a permeability change than that of egg lecithin. Below 10 °C, the dipalmitoyllecithin may be too compact to interact with the toxin and allow it to penetrate into the hydrophobic region. In a study of the polymyxin B action on liposomes, we observed that cholesterol incorporation and the saturation of fatty acid components of lecithin reduced the susceptibility to poly-

myxin B (unpublished). It is worthwhile noting that the composition of liposomal membranes had an opposite effect on prymnesin sensitivity.

The chemical nature of prymnesin is still unknown, though it was reported to be a proteolipid-like material [11]. The exact mode of action of prymnesin must await determination of the chemical nature of the toxin. Ulitzur and Shilo [10] showed that prymnesin lysed spheroplasts or protoplasts of some bacteria whose membranes had no cholesterol. This is incompatible with the present suggestion that the effect of prymnesin depends on the binding to membrane sterols. It should be kept in mind that the preparations of the toxin are not pure but contain several components. It may be possible that various components in the toxin preparation may have different modes of action. In fact, it was impossible in our assay to obtain lysis of *E. coli* spheroplasts (10^9/ml) by adding prymnesin at the concentration of 1000 hemolytic units/ml which is enough to lyse sheep erythrocyte (10^{10}/ml). Much higher concentrations of the toxin seem to be required for lysis of membranes lacking cholesterol.

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