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CIRCULAR DICHROISM STUDY OF THE INTERACTION BETWEEN AROMATIC HEPTAENE ANTIBIOTICS AND SMALL UNILAMELLAR VESICLES

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Unlike the non-aromatic heptaene amphotericin B, only two types of complex are revealed by circular dichroism when the aromatic heptaenes interact with lipid vesicles. The first is formed when no permeability is observed. The second one is correlated with the appearence of permeability. The cholesterol concentration and the physical state of the membrane have influence only on the amount of the permeabilizing species. These results indicate important differences in the membrane properties of aromatic and non-aromatic heptaenes.

Aromatic heptaene macrolide antibiotics are particularly interesting since their biological activity on pathogenic yeast like microorganisms is two orders of magnitude higher than that of other groups of large ring polyenes like amphotericin B or nystatin (1,2,3,4).

In this paper the effect of cholesterol concentration as well as of physical state of the membrane on the interaction between aromatic heptaenes and lipid vesicles has been studied by circular dichroism and compared with permeability measurements. Circular dichroism was already used qualitatively to monitor the interaction of candicidin D with erythrocyte membranes (5). On the other hand, in the case of amphotericin B, we have shown earlier (6,7,8) that among spectroscopic methods circular dichroism is the most sensitive and we expected that the same will hold for aromatic heptaenes.

In our previous papers (6,7,8) we have shown that amphotericin B, a non-aromatic heptaene, interacting with lipid vesicles may exist in a number of different forms depending on:membrane physical state,cholesterol/phospholipid ratio,antibiotic/total lipid ratio and time elapsed after the mixing.When amphotericin B is added to the vesicles with phospholipid in

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the gel state,three events are detected as thetime goes.Amphotericin B interacting with cholesterol-free vesicles in the liquid crystalline state forms mixed micelles with phospholipids in 1:1 molar ratio.With cholesterol-containing vesicles in liquid crystalline state Amphotericin B forms three different complexes according to cholesterol/phospholipid and antibiotic/total lipid ratio.

The aim of this study was an attempt to define interactions between lipid vesicles and two representatives of aromatic heptaene macrolide antibiotics : vacidin and candicidin D.

MATERIALS AND METHODS

Sources of lipids and antibiotics are indicated in our previous papers (6,9).The stock suspensions of sonicated unilamellar vesicles were prepared as previously described (6) in 100 mM KCl bufferd with 10 mM Hepes, pH 7.5.Lipids concentrations was 10^{-2}M.For experiments the stock suspension was diluted with the same buffer to desired lipid concentration.In all experiments the same antibiotic concentration was used and it was 10^{-5}M.The concentration of the antibiotics was calculated for pure compounds.Circular dichroism spectra were recorded with a Jobin-Yvon Mark III or Mark V dichrograph.Note that the aggregated state of the aromatic polyene antibiotics in solution and the corresponding Duysens effect,influences the intensity of the absorption bands which is approximately two times smaller than that of the antibiotics embedded in the vesicles.

RESULTS

Vesicles without cholesterol. Fig. 1 shows effect of various concentration of egg-yolk phosphatidylcholine vesicles on circular dichroism spectra

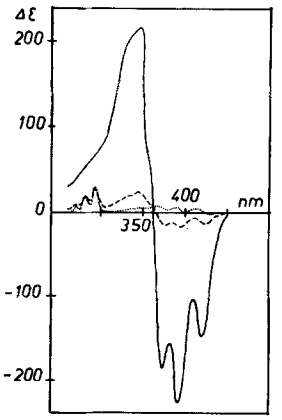

Fig. 1. Circular dichroism spectra of vacidin A in the presence of egg-yolk phosphatidylcholine vesicles at 25°C for various antibiotic/phospholipid ratio, R : ——— without vesicles, — — — R = 5 x 10^{-2}, ... R = 5 x 10^{-3}. Concentration of vacidin A = 1 x 10^{-5}M.

of vacidin A in aqueous solutions.In aqueous media at a concentration 10^{-5}M the aromatic heptaenes form strong dissymetric aggregates (9),the spectra of which exhibit characteristic excitonic doublet centered at about 360 nm (solid line in Fig. 1).This doublet vanishes when phospholipid vesicles are added,its decrease being related to the antibiotic/phospholipid ratio,R.At low R the CD doublet totally disappears and weak positive bands $\Delta\varepsilon \sim 5$ between 415 and 330 nm are observed. In addition, in the "aromatic" region of spectra between 300 and 270 nm, two positive bands are observed. At high R, that is 5×10^{-2} for instance, some amount of vacidin remains free as evidenced by the CD spectrum of the pellet which can be sedimented by low speed centrifugation. This spectrum is exactly that from free vacidin. Electronic absorption measurements shows that the major part remains in suspension, which indicates that the vesicles have not been destroyed.

The same characteristics of changes are observed for phospholipids in liquid crystalline state (egg-yolk phophatidylcholine) as well as in gel state (dipalmitoylphosphatidylcholine vesicles at 25°C).

Spectra obtained for candicidin D, an other aromatic heptaene, are similar and only quantitative differences in the extent of the doublet decrease and the intensity of positive bands are evidenced.

Vesicles containing cholesterol. The circular dichroism spectra of aromatic heptaenes in the presence of egg-yolk phosphatidylcholine vesicles containing cholesterol are dependent on the antibiotic/total lipid and cholesterol/phospholipid ratio (Fig.2).

For vesicles containing below 10 moles % of cholesterol circular dichroism spectra are similar to that obtained for vesicles without cholesterol. Above 20 moles % cholesterol significant differences occur. Two strong positive bands near 415nm ($\Delta\varepsilon_1 = 64$) and 392 nm ($\Delta\varepsilon_2 = 68$) as well as two negative bands near 383 nm ($\Delta\varepsilon_3 = -25$) and 365 nm ($\Delta\varepsilon_4 = -44$) are observed. In the "aromatic" region the positive bands are flattened. This

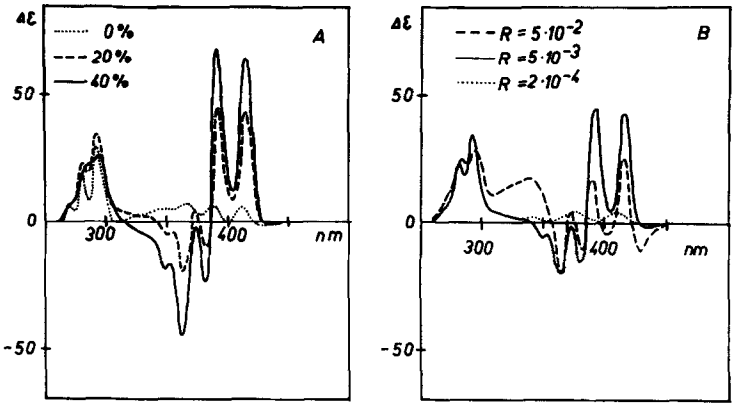

Fig. 2. Circular dichroism spectra of vacidin A in the presence of egg-yolk phosphatidylcholine vesicles with cholesterol. Concentration of vacidin A = 1 x 10^{-5}M.
A. Various amount of cholesterol for antibiotic/total lipid ratio R = 5 x 10^{-3}.
B. 20 moles % of cholesterol for various R.

type of spectrum may be interpreted as an excitonic doublet centered at 387 nm and exhibiting vibronic structure.

In order to confirm suggestion that this type of spectrum is representative for the species inducing permeability we compared permeability dose-response curve (10) with circular dichroism in the same conditions. As a measure of the amount P of that species per mole of lipids we use :

$$P = \Delta\varepsilon_1 \cdot R$$

The comparison of spectroscopic and permeability results is shown in Fig.3.

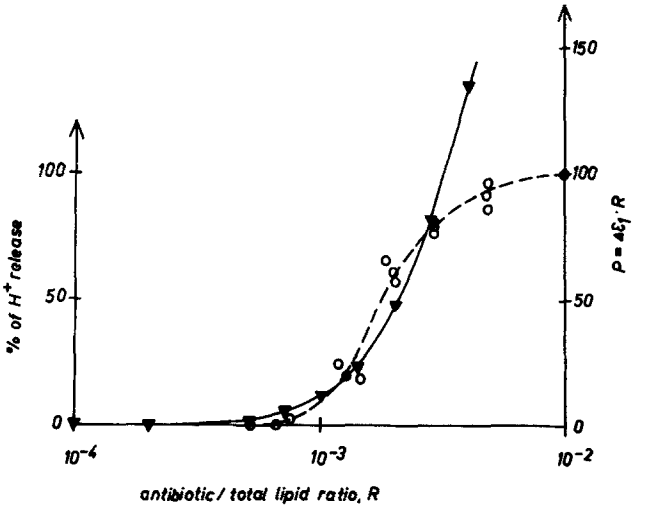

Fig. 3. Comparison between the extent of permeability (10) o — — o and the amount of antibiotic-cholesterol comples ▼——▼ for vacidin A.

At low antibiotic/lipid ratio, namely for R at which no permeability is induced, spectra similar to those obtained with sterol-free vesicles are observed, that is small positive bands (Fig. 2B). When R is progressively increased and permeability begins to be induced, spectrum typical for cholesterol containing vesicles appears. The amount of that species increases strongly with increasing R. For higher R, namely above 10^{-2}, the bilayer begins to be saturated in vacidin and some amounts of the antibiotic remain free in solution and decrease the intensity of the positive signals (Fig. 2B).

For candicidin D changes in the spectra have the same characteristics and only quantitative differences in the intensity of bands are evidenced and for this reason are not shown.

For dipalmitoylphosphatidylcholine-cholesterol vesicles at 25°C (in gel state) we obtained very similar results. Only amount of permeabilizing species at the same cholesterol concentration and antibiotic/total lipid ratio is significantly lower.

DISCUSSION

In this paper we present the effect of cholesterol concentration and of the membrane physical state on the interaction of aromatic polyene macrolides with small unilamellar vesicles. These two parameters appeared to be important for interaction of amphotericin B (6,7,8). Circular dichroism spectroscopy has been used for monitoring the antibiotic-lipid interaction. For identification of permeabilizing species the result of permeability measurements obtained at the same condition by C.M. Gary-Bobo et al. (10,11) have been compared.

Our circular dichroism studies reveal the existence of two types of interaction of the aromatic polyene antibiotics with the small unilamellar vesicles. The first one is observed in the presence of sterol-free vesicles or at low antibiotic/lipid ratio R in the presence of cholesterol containing vesicles. In both cases no induced permeability is observed. The spectra are characterized by three weak positive bands in the heptaene

region and are similar to those observed with free aromatic heptaene antibiotics in monomeric form in water (9), with a small red shift. We suggest that it indicates that the antibiotics are adsorbed in monomeric form on the surface of the lipid bilayer.

In the presence of cholesterol containing vesicles, for R such as permeability is induced (according to the dose-response curve) and with enough cholesterol concentration a second type of interaction may be detected. We suggest the formation of polyene-cholesterol complex responsible for the permeability inducement (Fig.3). The amount of that species increases with cholesterol concentration until saturation above 30 moles % (Fig.2). With the same cholesterol level it is lower for dipalmitoylphosphatidylcholine than for egg-yolk phosphatidylcholine vesicles.

The results presented in this paper, similarly to those already published (10,11) indicate therefore important differences in the properties of aromatic heptaenes and amphotericin B (non-aromatic heptaene). Upon the interaction of lipid vesicles aromatic heptaenes exist only in two forms : antibiotic-phospholipid and antibiotic-cholesterol. However only the latter one is responsible for permeability induction. The physical state of the membrane and sterol concentration have influence only on quantity of that complexes.

On the other hand it is very interesting to note that our spectra show a great similarity with those obtained (5) with candicidin in aqueous solution in the presence of cholesterol or in the presence of erythrocyte membranes.

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