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INTERACTION BETWEEN PHOSPHOLIPID BILAYER MEMBRANES AND THE POLYENE ANTIBIOTIC AMPHOTERICIN B

LIPID STATE AND CHOLESTEROL CONTENT DEPENDENCE

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Summary

The interaction between amphotericin B and egg yolk phosphatidylcholine, dimyristoyl (DMPC) and dipalmitoyl phosphatidylcholine (DPPC) phospholipid bilayer vesicles has been monitored by the circular dichroism (CD) spectra of amphotericin B at a $1 \cdot 10^{-5}$ M concentration. This method has revealed that amphotericin B may be present in a number of different forms depending on the time elapsed after the mixing, the cholesterol content of the vesicles and the vesicles' physical state. Some striking features of these CD detected species are the following: with egg yolk phosphatidylcholine and a molar cholesterol percentage lower than 25, at 25°C several forms are coexistent, their amount is time-dependent; with dipalmitoyl or dimyristoyl phosphatidylcholines without cholesterol or with a cholesterol molar percentage lower than 25, in the gel state, a form different from the former appears very rapidly; with egg yolk phosphatidylcholine, DMPC and DPPC at a molar cholesterol percentage between 25 and 50 a new form is monitored, identical in the three cases and observed in the liquid crystalline state as well as in the gel state. In the case of the three phospholipids without cholesterol a definite interaction with the antibiotic is observed but with different characteristics according to the nature of lipid.

With amphotericin B 'Fungizone' the same species are monitored but their appearance is much slower.

Two explanations are proposed for the origin of the discrepancies between CD and electronic absorption.

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Abbreviations: DMF, dimethylformamide; DPPC, dipalmitoyl phosphatidylcholine; DMPC, dimyristoyl phosphatidylcholine.

Polyene macrolide antibiotics interact with biological membranes containing sterols and promote more-or-less important selective alterations of the membrane permeability leading to cell death. The mechanism of action of these antibiotics is essentially the same on all cells containing sterols, algae, fungi and animal cells, their selectivity being rather poor [1].

However, the different polyene antibiotics exhibit various affinities either for ergosterol (the main sterol in fungi) or for cholesterol (present in animal cells) [2]. This may provide a basis for a systematic search of selective toxicity. A better knowledge of the mode of action at the molecular level is thus needed, which will give information on the interaction of the antibiotics with the sterols and on the importance of the various membrane properties.

The characteristic optical properties of the polyene antibiotics, due to the presence in these molecules of a system of conjugated double bonds, make the study of their interactions with lipids by spectroscopic methods particularly appealing. So far, electronic absorption has been widely used, but the same does not hold true for circular dichroism which, however, is much more sensitive to environmental modifications: only two studies have been made, one by Bittman et al. [3] on the interaction of filipin with phosphatidylcholine vesicles and another by Moulki et al. [31] on the interaction of amphotericin B with erythrocyte membranes, but these authors did not carry out detailed investigation.

Resonance Raman spectroscopy is another selective spectroscopic tool for such studies since, because of resonance, one may obtain the vibrational spectrum only of the polyene moiety which thus acts as a probe of the phospholipid environment. However, only one study [4] has been made by Raman spectroscopy on the interaction of amphotericin B and phospholipids, but at such a high concentration (presumably $3 \cdot 10^{-4}$ M in antibiotic and approx. 0.4 M in phospholipid) that it makes difficult any comparison with the results obtained by other methods such as permeability measurements and that a detergent effect [1] is not to be excluded.

We have therefore decided to use CD for polyene antibiotic/phospholipid bilayer membrane studies and have chosen amphotericin B which has been the most studied of polyene antibiotics. As with other polyene antibiotics, the mode of interaction may be complex, depending on the presence of a sterol and on the sterol/amphotericin ratio. It has been shown that amphotericin B can interact with cholesterol in aqueous solution in a stereochemically and stoichiometrically defined manner [5—8]; in lipid bilayers [9] with cholesterol, amphotericin B at a concentration lower than 10^{-5} M can create aqueous pores of specific size in the membrane, possibly by interacting with cholesterol [10—12]. At higher concentration (10^{-4} M) it may cause vesicle destruction [13] without the necessary presence of cholesterol. A recent study [14] using gel permeation chromatography and NMR has borne out these results and provided new details.

Materials and Methods

L-α-Phosphatidylcholine from egg yolk, type V-E, DL-α- and L-α-phosphatidylcholine dipalmitoyl (DPPC), L-α-phosphatidylcholine dimyristoyl

(DMPC) were purchased from Sigma and used without further purification. Some experiments were performed on a sample of egg yolk phosphatidylcholine given by Dr. Prigent and carefully prepared according to the method of Patel and Sparrow [15]. No discrepancy was observed between the results obtained with these egg yolk phosphatidylcholine samples origination from different sources.

Amphotericin B was a generous gift of Mrs. Gauthier from Squibb France. Amphotericin B as the Fungizone preparation (containing sodium deoxycholate and sodium phosphate) was obtained from Serva.

Vesicle dispersions were prepared by dissolving weighed amounts of phosphatidylcholine and cholesterol in chloroform and then removing the solvent in vacuo. The appropriate buffer (Tris-HCl, pH 7.5, $5 \cdot 10^{-2}$ M) was added to give the desired concentration and the sample was quickly vortexed, then sonicated to clearness under Ar for approx. 5 min in an MSE 150 W Mk 2 sonicator operating at power 2.5, the sonication bath being maintained in each case above the transition temperature (T_c) of the phospholipid. It has been shown [22] that after a 5 min sonication, vesicles were predominantly unilamellar. Stock solutions of amphotericin B were prepared daily with the buffer. Me_2SO was first added to solubilize the powder but its final concentration was always lower than 0.5%. Fungizone was added either directly from the aqueous stock-solution or after having added Me_2SO or DMF to the stock-solution (the final proportion being lower than 0.5%) in order to compare our results with those of other authors. Between these two preparations minor differences occur only in the intensity of the dichroic doublet at 330 nm.

Electronic absorption spectra were recorded with either a Cary 14 or a Cary 219 spectrophotometer.

CD spectra were recorded with a Jobin-Yvon Mark III dichrograph equipped with a Nicolet 1171 signal averager. Spectral wavelengths are reported to ±0.5 nm. As the major part of our results is based on differences between absorption and CD peak wavelengths it was important to determine them accurately. We thus calibrated our dichrograph carefully with a low-pressure Hg lamp and worked with a constant band pass of 5 Å.

Studies of the vesicles' properties by electronic absorption or circular dichroism may be complicated by the scattering corresponding to the large size of the particles to be studied. In aqueous solution, at a concentration higher than 10^{-5} M, amphotericin B Fungizone exists in an aggregated form, the gyration radius of which is approx. 800 Å [16]; therefore the size parameter for the wavelength used is approx. 1.7 and the observed CD is expected to vary as the photomultiplier geometry is changed [17—19]. Actually tha has not been observed when the acceptance half-angle of the detector has been varied between 9 and 50°. Note that these experiments on amphotericin B Fungizone were done with a cell of 0.001 cm length in order to avoid multiple scattering. As far as the vesicles are concerned, one expects to observe no difference between monomeric amphotericin molecules in solution and monomeric amphotericin molecules embedded in the phospholipid bilayers, since theoretical studies on the optical activity of membrane shells as a function of shell radius have shown that for phospholipid vesicles only very minor differences may occur [17].

Results

Amphotericin B

Electronic absorption spectra (Fig. 1). In Tris-HCl buffer at pH 7, the spectra are concentration dependent (the solutions are obtained by mixing the buffer with a solution of amphotericin B in Me_2SO such that the final Me_2SO concentration does not exceed 0.5%). At low concentration (10^{-8} M) the spectrum is similar to that in Me_2SO: four bands are observed between 420 and 320 nm (409, 385, 365, 347). As the concentration increases, the spectrum is progressively modified and above 10^{-4} M a totally new spectrum is observed: the bands near 400 nm are replaced by 420 (approx.), 385 and 360 nm bands and a

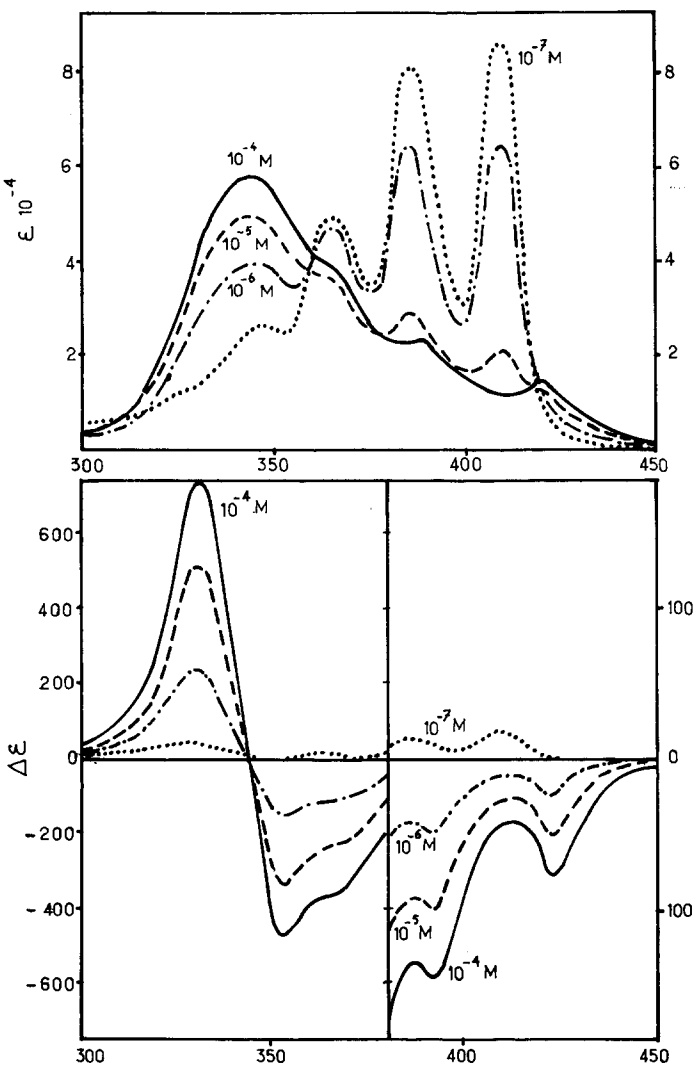

Fig. 1. Electronic absorption and circular dichroism spectra of amphotericin B at various concentrations.

new intense but flattened band is observed at approx. 340 nm. The spectra of Fungizone follows the same concentration dependance and are similar [16] to those of pure amphotericin B except that at high concentration the main band is shifted to 329 nm.

Circular dichroism spectra (Fig. 1). In Tris-HCl buffer at pH 7 the spectra are concentration dependent. At a 10^{-4} M concentration a very intense bisignated curve is observed [26,21] ($\Delta\epsilon = -106$ at 356 nm and $\Delta\epsilon = +140$ at 325). Its center corresponds to the absorption peak at 340 nm. (The same bisignated curve is observed at 329 nm with amphotericin B 'Fungizone'.) A shoulder is located at 368 nm and two weaker bands ($\Delta\epsilon = -77$ and -44) at 393 and 423 nm. At a very low concentration (approx. 10^{-7} M) four positive bands (as in Me_2SO) are observed at 409, 385, 365 and 347 nm, with a weak $\Delta\epsilon$ (approx. 10). It is important to note (see below) that at an intermediate concentration, e.g. 10^{-5} M, the absorption spectrum in the 400 nm region is that of the low-concentration species, while the CD spectrum is that of the aggregated species, this discrepancy being due to the very intense excitonic CD of this latter form relative to the weak CD of the former.

Interaction of $1.25 \cdot 10^{-5}$ M amphotericin B and phospholipid vesicles (1.75 mM egg yolk phosphatidylcholine, 1.5 mM DMPC, 1.5 mM DPPC)

Electronic absorption. In all the cases studied with the phospholipid vesicles, the free amphotericin B bands at 420 and 385 nm are shifted in approx. 30 s to 413 and 388 nm, as observed by Chen and Bittman with DMPC [22]. In the 300—350 nm region the results vary with the lipid content of the vesicles and with the temperature, and the broad band observed at approx. 340 nm with free amphotericin may be shifted or may vanish: above the phase-transition temperature with egg yolk phosphatidylcholine or DMPC vesicles, without cholesterol, it is shifted to 328 nm in less than 5 min and then remains stationary, while in the presence of cholesterol it is shifted to 332 nm. In this latter case the intensity of the band is very weak for 7% cholesterol but increases with the cholesterol content. Below the phase transition temperature with DPPC vesicles, without cholesterol, no band is observed near 330 nm, while with cholesterol a band is observed at 335 nm, the intensity of which increases when the cholesterol content increases; with DMPC vesicles without cholesterol a band is observed at 330 nm.

Circular dichroism. (a) Egg yolk phosphatidylcholine vesicles at 25°C, i.e. in the liquid crystalline state (Fig. 2). The time dependence of the amphotericin B CD at 419 nm in the presence of egg yolk phosphatidylcholine vesicles is given in Fig. 3 for various cholesterol contents.

Without cholesterol the strong negative bands of amphotericin B at 423 and 393 nm slowly decrease and are replaced in 1 h by weak negative bands at 419 and 390 nm and a positive band at 430 nm. On the other hand the cross-over point of the excitonic doublet of free amphotericin B at 340 nm is very rapidly shifted to 328 nm (exhibiting the same behaviour as the corresponding absorption maximum) and the amplitude of the doublet is divided by five.

With cholesterol: (i) 10 and 20% cholesterol. In 20 min the negative bands of free amphotericin B at 423, 393 and 370 nm are replaced by smaller negative bands at 420, 392 and 372 nm. The two former are strongly dissymmetric.

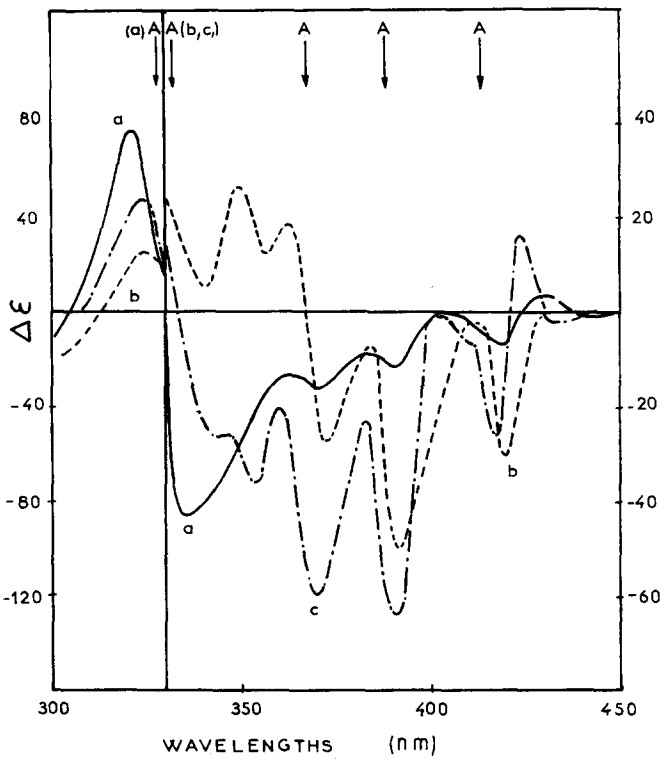

Fig. 2. CD spectra at 25°C of $1.25 \cdot 10^{-5}$ M amphotericin B in the presence of 1.7 mM egg yolk phosphatidylcholine vesicles with various cholesterol contents. Spectra have been recorded approx. 1 h after the mixture. The background due to the dichroic scattering of the vesicles has been substracted. mol% cholesterol: a, ———, 0; b, - - - - - -, 10; c, · — · — ·, 50. Vertical arrows indicate the absorption maximum wavelengths.

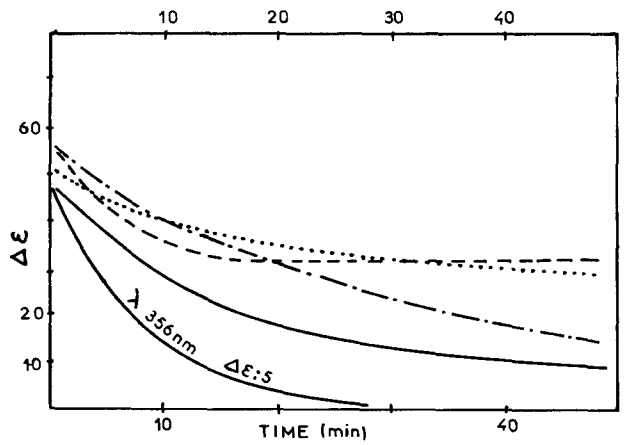

Fig. 3. Circular dichroism decrease at 419 nm and 356 nm as a function of time after the mixing of amphotericin B (final concentration, $1.25 \cdot 10^{-5}$ M) with lecithin vesicles (final concentration, 1.7 mM) with various cholesterol contents: at 356 nm, ———, 10%; at 419 nm, ———; 0%, - - - - - -, 10%; · — · — ·, 33%; · · · · · ·, 50%. The recording of spectra was triggered 1 min after the mixture of the components.

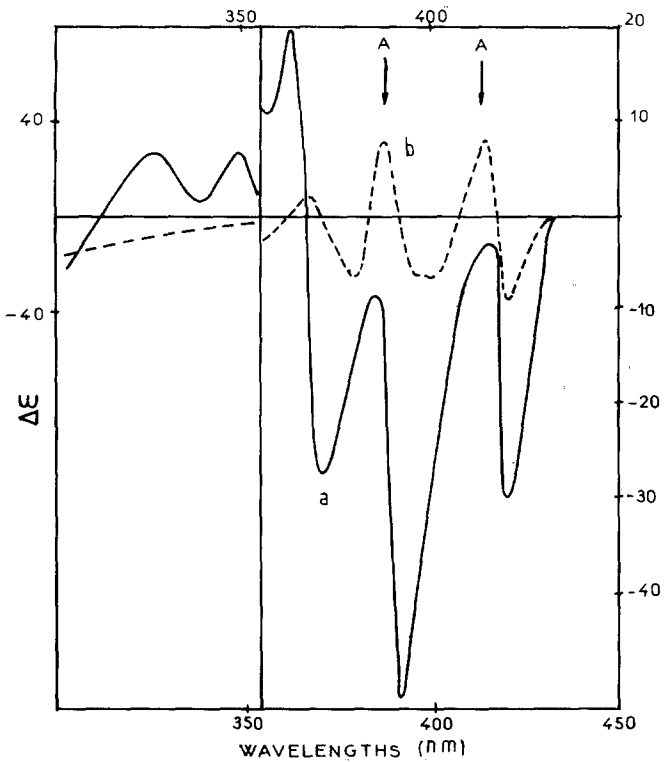

Fig. 4. Circular dichroism spectra of $1.25 \cdot 10^{-5}$ M amphotericin B in the presence of (a) 1.7 mM egg yolk phosphatidylcholine vesicles and (b) 42 mM egg yolk phosphatidylcholine vesicles with the same cholesterol content (14 mol%) in both cases. Spectra taken 1 h after the mixing.

Very rapidly (Fig. 3) a bisignated curve is observed with a trough at 353 nm and a positive peak at 327 nm; it is 15 times smaller than that from free amphotericin B. This doublet progressively decreases and in 1 or 2 h is replaced by three positive bands, at 364, 350 and approx. 325 nm. The intensity of these three bands is poorly reproducible from one experiment to another and may vary by 50%.

The dissymmetry of the 420 and 392 nm bands may be explained by varying the proportions of the constituents: when increasing the amphotericin B/phospholipid vesicle ratio (assuming an average number of 2300 phospholipids per vesicle [23]) from 30/1 in the above experiments to 1/1 (20 mM egg yolk phosphatidylcholine, 22 mM cholesterol and $1.25 \cdot 10^{-5}$ M amphotericin B) and keeping the phospholipid sterol ratio constant, two positive bands are observed in addition at 415 and 388 nm (Fig. 4).

(ii) 33 and 50% cholesterol. In approx. 20 min the negative bands of free amphotericin B at 423, 393 and 370 nm are replaced by a positive band at 423 nm, a negative band at 417 nm, a shoulder near 410 nm and two negative bands at 391 and 370 nm. The CD intensity of these bands increases with the cholesterol content of the vesicles, being 4 times greater at 50% cholesterol than at 33%. A doublet with a crossover point at 333 nm is observed, approx. 10 times smaller than that from free amphotericin B.

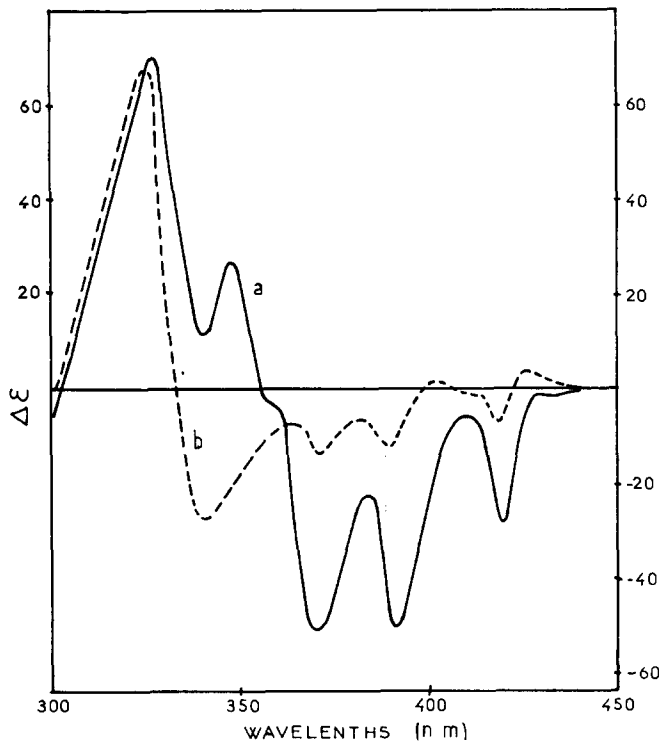

Fig. 5. Time dependence of the circular dichroism of $1.25 \cdot 10^{-5}$ M amphotericin B after its mixing with 1.5 mM DMPC vesicles with 10 mol% cholesterol at 30°C. a, ———, Δt = 30 min; b, - - - - - -, Δt = 3 h.

(b) DMPC vesicles at 30°C, i.e. in the liquid crystalline state.

Without cholesterol the spectra are similar to those observed with pure egg yolk phosphatidylcholine but two weak positive bands near 400 nm are superimposed and may be residual traces of the gel state bands.

With 10% cholesterol one does observe the same initial step as with egg yolk phosphatidylcholine plus 10% cholesterol: negative bands near 400 nm and either a doublet or three positive bands near 350 nm, but the negative bands are not dissymmetric and the spectra move after 2 or 3 h to spectra similar to those obtained with 33% cholesterol (Fig. 5).

(c) DPPC or DMPC in the gel state (25 and 10°C, respectively) (Fig. 6).

Without cholesterol: in 3 min with DPPC or in 15 min with DMPC the negative bands of free amphotericin vanish and are replaced by three positive bands at 422, 395 and 379 nm. Instead of the large doublet of free amphotericin B near 340 nm, with DPPC a weak positive band is observed near 330 nm and with DMPC a stronger band than the preceding one is observed ($\Delta\epsilon \approx 64$) but decreases with time, and its amplitude is only 30 after 1 h. In both cases, a negative band at 430 nm ($\Delta\epsilon = 6$) appears after 1 h.

With cholesterol: and DPPC for 7,15 and 26% cholesterol the same spectra as without cholesterol are observed except that the 330 nm band increases slowly with percentage cholesterol ($\Delta\epsilon = 40$ for 26% cholesterol).

Beyond 30% cholesterol a new spectrum appears, more and more intense

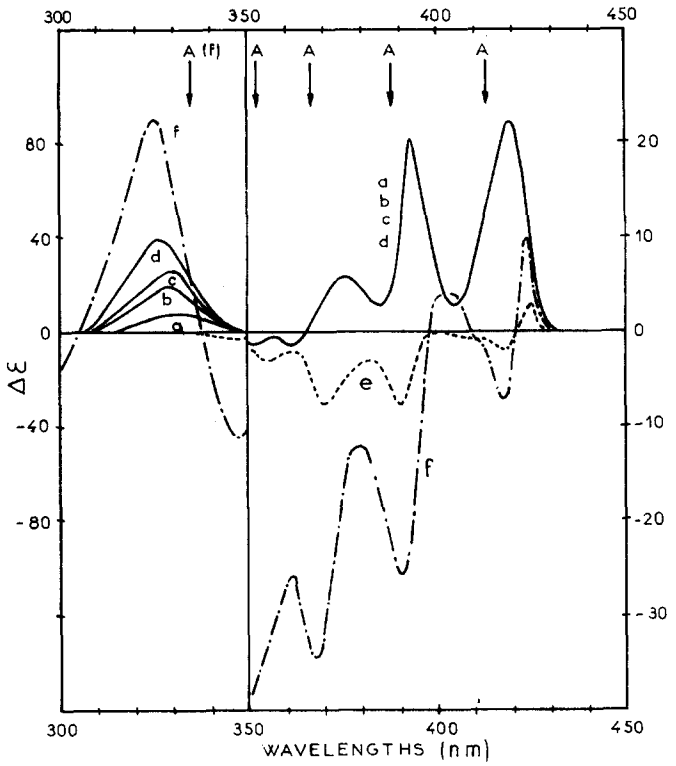

Fig. 6. CD spectra at 25°C of $1.25 \cdot 10^{-5}$ M amphotericin B in the presence of 1.5 mM DPPC vesicles with various cholesterol contents. Spectra have been recorded about 1 h after mixing. The background due to the dichroic scattering of the vesicles has been substracted. mol% cholesterol: a, b, c, d, ———, 0, 7, 15, 26, respectively; e, · · · · · ·, 35; f, · — · — ·, 50. Vertical arrows indicate the absorption maximum wavelengths.

with increasing cholesterol content of the vesicles and which is the same as that obtained in the liquid crystalline state. With 50% cholesterol the dichroic doublet is observed with a crossover point at 335 nm (at the same wavelength as the absorption peak) and a high intensity.

Interaction of amphotericin B 'Fungizone' with phospholipid vesicles

A similar cholesterol content dependence is observed but the reactions are much slower in such a way that the final steps are most of the time not observed in a 1-day-long experiment.

Interpretation

Relationship between the electronic absorption and the CD peak wavelengths

A striking result of these experiments on the interaction of amphotericin B with phospholipid vesicles is the discrepancy between the electronic absorption and the CD of the polyenic transitions near 400 nm: firstly, the wavelengths of the peaks are not the same and secondly, electronic absorption monitors a single species, while CD monitors several when the temperature or the amount

of cholesterol is varied. Two explanations of these facts may be proposed.

In the first explanation, the consideration of the free amphotericin B spectra may be illuminating. In Tris-HCl buffer at very low concentration (10^{-7} or 10^{-8} M) or at a relatively high concentration (10^{-4} M), absorption and CD peak wavelength are in close relationship (or CD crossover point and absorption peak in the case of 10^{-4} M amphotericin at 340 nm). At high concentration, as reported by other workers [16], amphotericin B is present as micelles or aggregates, giving rise to a very important excitonic CD, while at low concentration (i.e. below the critical micellar concentration) it is in monomeric form with very low $\Delta\epsilon$.

At an intermediate concentration the peaks wavelength near 400 nm are no longer in coincidence and this because the monomeric and aggregated forms ϵ are approximately the same, while the $\Delta\epsilon$ of the aggregated form is much greater than that of the monomeric form. Thus electronic absorption monitors well the exact amounts of the different amphotericin B forms, while CD allows us to detect associated species in minor amounts. For instance in free amphotericin B at a 10^{-6} M concentration the absorption spectrum (409, 384 nm) represents mainly the monomeric species (approx. 90% of the total amphotericin) while the CD spectrum (420, 388) represents only the aggregated form (Fig. 1) with its characteristic doublet near 340 nm.

The second explanation, which does not keep the former from being valid, may be found in the fact that similar discrepancies between absorption and CD wavelengths have already been observed in compounds with spectra exhibiting fine vibronic structure: aromatic compounds of the type $C_6H_5CR_1R_2R_3$ [27] and visual pigments [28]. The problem may be solved as follows. By taking account of the electron-vibration interaction, the electronic wavefunction of the chromophore can be expressed by the Herzberg-Teller expansion. Those terms which contain nuclear displacements make a contribution to the electric transition dipole moment different from that to the magnetic transition dipole moment. This results in differences in shape between the absorption and the CD spectra [29].

The different species monitored by CD (Table I)

Chen and Bittman [22], by following the rates of increase of the absorbance at 385 nm, have shown that an initial step was performed in the first minute following the beginning of the reaction and that another reaction followed. By CD we see that in the first minutes the excitonic CD doublet near 400 nm characteristic of the aggregation either vanishes or decreases strongly and shifts. The slow decrease in the following 30 min of the negative CD bands of free amphotericin at 423, 393 and 368 nm corresponds to the second reaction detected by absorption.

We can say that in the first moments of the reaction the monomeric free amphotericin is adsorbed onto the vesicles, that a more-or-less important part of the aggregated amphotericin (total in the case of DPPC without cholesterol in the gel state) is disaggregated either to be adsorbed onto the vesicles or to remain as monomers in the solution and that the remaining part of aggregated amphotericin is rearranged in some way, since the CD crossover point is shifted.

Phospholipid vesicles without cholesterol; detergent effect. We must notice

TABLE I

SPECIES MONITORED BY CIRCULAR DICHROISM BETWEEN 430 AND 360 nm AND OCCURRING AFTER THE INITIAL STEP OF ADSORPTION ONTO THE VESICLES

LEC, egg yolk phosphatidylcholine.

mol% cholesterol	Gel state	Liquid crystalline state
0	A	D_1 or D_2 (LEC) D_1' or D_2' (DMPC)
10	A	(LEC) $C + C' \rightarrow C''$ (DMPC) $C'' \rightarrow B$
50	B	B

that even without cholesterol the CD spectra are modified. This indicates some interaction between amphotericin B and the phospholipid vesicles, in agreement with Chen and Bittman's results.

(i) Below the phase transition temperature, namely in the gel state: a species 'A' with a high rate of formation is detected: in 3 min it is totally formed.

(ii) Above the phase transition temperature, namely in the liquid crystalline state: a striking feature of the CD spectrum of amphotericin B in the presence of pure egg yolk phosphatidylcholine vesicles is that the excitonic doublet, which is expected to represent amphotericin B remaining in the aqueous phase, does not exhibit the same peaks and crossover point wavelengths as the antibiotic alone in buffer. A possible explanation of these discrepancies may be found in the fact that amphotericin B is a soluble amphiphile, i.e. a detergent. According to Helenius and Simmons [30] the action of a detergent on a phospholipid bilayer divides into three stages depending on the detergent concentration: (i) at low concentration, non-disruptive penetration of detergent molecules into the bilayer; (ii) when saturation of the bilayer has occurred, appearance of a second phase, i.e. detergent-phospholipid mixed micelles, in addition to detergent saturated bilayers; (iii) whole disruption of the bilayer into mixed micelles at high detergent concentration. The last stage is well known for amphotericin B at 10^{-4} M [13] and it is tempting to assume that the concentration range of our experiments with pure egg yolk phosphatidylcholine (and of most permeability measurements) corresponds to stage number two. In this case, amphotericin B would be present in solution as mixed micelles with membrane lipids and slight differences in molecular packing in comparison with pure micelles would explain the wavelength changes of the doublet. We must note that Fungizone, which is assumed to consist of deoxycholate-amphotericin B mixed micelles, also has an excitonic doublet slightly blue-shifted compared to that of pure amphotericin B. Furthermore, if the suspension obtained after a 2 h interaction of amphotericin B with egg yolk phosphatidylcholine vesicles be centrifuged at $1500 \times g$ for 1 h (i.e., without vesicle sedimentation), an 80% decrease of the CD intensity occurs in the supernatant. Spectrophotometric determination of the antibiotic and lipid phosphorus analysis [32] indicated that the resulting pellet contained a major part of the added amphotericin as well as phospholipid, to approximately the same molar content. Note that the vesicles suspension used in this particular experiment were centrifuged

at 100 000 × *g* for 1 h prior to amphotericin B addition so that the phosphorus detected in the pellet cannot originate from undispersed phospholipids.

These data indicate that very few amphotericin B is bound to pure egg yolk phosphatidylcholine vesicles at the equilibrium, in agreement with the results of Gent and Prestegard [14] and of van Hoogevest and de Kruyff [12]. However, the presence of amphotericin B/phospholipid mixed micelles in the aqueous phase indicates that transient association of the antibiotic with the membranes must occur, leading to a light loss of lipids of the vesicles.

Phospholipid vesicles with cholesterol. The species observed by CD are essentially similar (except for some minor differences (Table I)) for egg yolk phosphatidylcholine, DPPC and DMPC when the lipidic state (i.e., gel or liquid cristalline state) and the percentage of cholesterol are the same.

(i) Below the phase transition temperature, namely in the gel state, up to 26 mol% cholesterol the same species, 'A', as without cholesterol is observed to approximately the same extent. As the quantity of cholesterol contained in the vesicles is increased beyond 30%, a new species, 'B', appears. The amount of this species 'B' is maximum when the molar percentage of cholesterol reaches 50. It is totally formed in 1 h.

(ii) Above the phase transition temperature, namely in the liquid crystalline state. With 50 mol% cholesterol the same species 'B' is obtained as with 50% mol cholesterol below T_c. As the amount of cholesterol is lowered, the intensity of the dichroic peaks typical of 'B' decreases. It is not surprising to find the same species as below T_c since it is well known that cholesterol has a 'dual effect on fluidity' of phospholipid bilayers: in the gel state it increases the fluidity while in the lipid crystalline state, it has a condensing effect. At sufficiently high concentration cholesterol abolishes the phase transition.

Below 20 mol% cholesterol a mixture of two species, C and C', appears in 10 min. With egg yolk phosphatidylcholine the form C' is transformed in 1 h in a form C'' and then remains stationary, while with DMPC the form C'' is transformed into form 'B', the forms C and C' being not observed; the faster the transformation, higher is the mol% cholesterol. Without cholesterol other species are observed (D with egg yolk phosphatidylcholine, D' with DMPC) which are formed in 20 min and are weakly dichroic.

Relationship between the species monitored by CD and the species responsible for the permeability to ions

Permability alteration measurements in the presence of amphotericin B have been done on liposomes or phospholipid monolamellar bilayer vesicles by several authors [2,11,12,14,24,25]. With egg yolk phosphatidylcholine liposomes at room temperature (i.e. in the liquid crystalline state) it has been shown that amphotericin B at a concentration of approx. 10^{-5} M induces a leakage of glucose or of Na^+ which is very weak or absent in the absence of cholesterol, increasing with the cholesterol percentage and either reaching a plateau for a 20 mol% cholesterol [24] or decreasing afterwards [2,25]. On the other hand, with DPPC vesicles at room temperature (i.e. in the gel state) the permeability to glucose, maximum in the absence of cholesterol, decreases when the mol% cholesterol increases [24].

The C or C' forms, observed with egg yolk phosphatidylcholine in the liquid

crystalline state, non-existent without cholesterol and at a maximum between 10 and 20% cholesterol may thus be related to the appearance of permeability. The C′ form may be a better candidate to the permeable form than the C form, since the latter has been assumed to correspond to the species monitored by electronic absorption (above mentioned) and is not specific to this cholesterol percentage. The C″ form seems to be discarded, since its rate of formation is slow while the Na^+ efflux is total in 5 min [10] and would (better) correspond to the faster appearance of the C′ form. The C″ form may be in relation to that which has been observed by electron microscopy [1] after a 1 day incubation.

In the same way, the A form may be related to the appearance of the permeability to glucose in the gel state, although the dependence of the intensity of the dichroic signal characteristic of this species on the cholesterol content does not follow exactly the cholesterol content dependence of the glucose efflux: the former is stationary when the cholesterol content increases, while the latter decreases [23]. One must insist on the characteristics of this permeability-inducing species relative to the form C or C′. The A form, which does not need cholesterol, appears almost instantaneously, is totally different from the C forms (the signs of the dichroism are opposite), and exhibits almost no trace of the aggregated species responsible for the dichroic doublet near 340 nm.

In the present state of our knowledge of the electronic transitions of polyenes in a lipidic environment, and on the CD of chiral polyenes, it would be premature to propose structural models for these different species. However, CD seems to be the most sensitive spectroscopic tool for following the interactions of the polyene antibiotics with membranes.

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