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# Differences in the Interaction of the Polyene Antibiotic Amphotericin B with Cholesterol- or Ergosterol-Containing Phospholipid Vesicles. A Circular Dichroism and Permeability Study†

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ABSTRACT: The selective toxicity of the polyene antibiotic amphotericin B between pathogenic eukaryotic organisms and animal cells has often been said to originate in the presence of ergosterol in fungal membranes instead of cholesterol, found in membranes of animal cells. We have tested this hypothesis by measuring the proton efflux induced by amphotericin B in egg yolk phosphatidylcholine small unilamellar vesicles. By measuring circular dichroism under the same conditions, we monitored the interaction of the antibiotic and its conformational changes. Sterol-free vesicles are sensitive to amphotericin B, but the sensitivity of sterol-containing vesicles is always greater and increasingly so with increasing sterol concentration. Ergosterol-containing vesicles are more sensitive than cholesterol-containing vesicles. On the other hand, numerous amphotericin B conformers can be detected in sterol-containing vesicles, depending upon both the concentration of sterol and the amphotericin B sterol ratio. It appears that one conformer, or maybe two at high amphotericin B concentration, is responsible for the induced permeability. From their circular dichroism spectra, these two conformers are the same in the presence of ergosterol or cholesterol. The concentration of amphotericin B necessary to obtain the two conformers is higher with cholesterol than with ergosterol, which agrees with the permeability results.

The molecular basis of amphotericin B action on membrane ionic permeability is thought to be due to pore formation by amphotericin B–sterol complexes (Finkelstein & Holtz, 1973; Andreoli, 1974; De Kruijff & Demel 1974). However, alternative hypotheses have been proposed relating the sterol requirement of amphotericin B action to more general physicochemical properties of the membrane (Hsu Chen & Feingold, 1973). Numerous studies carried out on both biological and model membranes using either structural (spectroscopic) or functional (permeability) approaches have revealed that the interactions between amphotericin B and sterol-containing membranes are complex. Recently, Bolard et al. (1980) have shown by circular dichroism spectroscopy that numerous types

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of interactions and of complexes of amphotericin B with membrane lipids can be detected in unilamellar lipidic vesicles depending upon the nature of the lipid, the amphotericin B concentration, and the physicochemical conditions. A correlation between some of these complexes and the amphotericin B induced permeability in vesicles membranes has been demonstrated (Bolard et al., 1981).

It is well-known that amphotericin B is selectively more toxic in pathogenic eukaryotic organisms than in animal eukaryotics. It has been proposed that this selective toxicity is due to the higher affinity of amphotericin B for ergosterol (present in fungal cell membranes) than for cholesterol (present in animal cell membranes) (Gale, 1974; Kotler-Brajtburg et al., 1974; Hammond, 1977). In order to test this hypothesis, comparative studies of permeability have been performed by Teerlink et al. (1980) on multilamellar large vesicles (MLV)[1] containing either ergosterol or cholesterol. Although the results confirmed that the MLV containing ergosterol were more sensitive to amphotericin B, no information on the molecular mechanism of this effect was obtained. In this paper, we report a systematic study of the increase in permeability induced by amphotericin B in small unilamellar vesicles (SUV) made with different proportions of egg yolk phosphatidylcholine and sterol. Under the same conditions the interactions and conformational changes of the amphotericin B have been monitored by CD. Comparative experiments using SUV with either ergosterol or cholesterol have been carried out. The results indicate that only one or two of the several amphotericin B species detected spectroscopically cause the antibiotic-induced permeability. The amphotericin B induced permeability in ergosterol and cholesterol-containing vesicles appears to be caused by the formation of the same active species in both cases. However, the active species are formed at lower concentration of amphotericin B in ergosterol-containing vesicles than in cholesterol-containing vesicles.

## Materials and Methods

L-$\alpha$-Phosphatidylcholine was prepared from egg yolk according to Patel & Sparrow (1978). Cholesterol and ergosterol were purchased from Fluka and Merck, respectively. Both sterols were purified by recrystallization in ethanol before use. FCCP [carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone] was purchased from Boehringer. Amphotericin B was a generous gift from Squibb France. Amphotericin B concentration was determined from the electronic absorption of the mother liquor in dimethyl sulfoxide with $\epsilon_{416} = 121\,400$.

*Permeability Studies.* Vesicle suspensions were prepared by dissolving known amounts of phospholipid and sterol in chloroform in such a way that the total concentration in lipids (phospholipid plus sterol) remained constant. After removal of the solvent, 400 mM sodium phosphate, pH 5.20 (1 mL/30 $\mu$mol of lipid), was added. The sample was sonicated under nitrogen gas at 4 °C. Since ergosterol can be destroyed by sonication, its integrity was always checked by electronic absorption after this treatment. The vesicle suspension was dialyzed to equilibrium against 500 volumes of isotonic sodium sulfate. Proton efflux was measured with a pH-stat (Radiometer, Copenhagen) in the following way: 0.5 mL of vesicle suspension (15 $\mu$mol of lipids) was diluted with 3.5 mL of dialyzing buffer in the pH-stat titrating vessel and equilibrated at 2 °C under nitrogen gas. The pH was brought to 7.40 by addition of 30 mM NaOH in isotonic sulfate. Then, 10 $\mu$L of a $10^{-2}$ M FCCP solution in ethanol was added (final concentration $2.5 \times 10^{-5}$ M). Subsequently, the desired amount of amphotericin B was added as microliter volumes of a freshly made 1 mg/mL solution in dimethyl sulfoxide ($Me_2SO$) with Hamilton syringes. The proton efflux was measured as the volume of 4 mM NaOH solution in isotonic sodium sulfate required to maintain pH 7.40. All experiments were carried out at 22 °C. The total concentration of protons inside the vesicles was measured in the same way but after the addition of Triton X-100. Proton efflux is expressed as a percentage of this total concentration.

*Spectroscopic Studies.* Vesicles were prepared as described above with minor modification concerning lipid concentrations. Amphotericin B was introduced either as its $Me_2SO$ solution or as suspension in buffer containing less than 0.5% in $Me_2SO$, no significant differences being observed between the two procedures. All experiments were carried out at 22 °C.

CD spectra were recorded with a Jobin-Yvon Mark III dichrograph equipped with a Nicolet 1171 signal averager. Spectral wavelengths are given ±0.5 nm. The dichrograph was carefully calibrated with a low-pressure Hg lamp, and a constant bandwidth of 5 Å was used. The path length of the cells was 1 cm for the lower concentrations of amphotericin B and 0.1 cm for the higher concentrations. We have already considered the problems arising from the light-scattering properties of the vesicles (Bolard et al., 1980) and stated that in our particular case they do not lead to distorsions of the amphotericin B signals; in particular, the cell path length was without influence on the quantitative results. In a general manner, in our amphotericin B studies we have not detected any artifact originating from light scattering, except at concentrations higher than $10^{-4}$ M. This is not the case with other polyene macrolides such as aromatic heptaenes.

Electronic absorption spectra were recorded with a Cary 219 (Varian) spectrometer. They were analyzed into Gaussian curves with a Du Pont 310 curve analyzer. $^{31}P$ NMR spectra (with proton noise decoupling) were recorded at 40.5 MHz on a Varian XL 100-12 WG spectrometer at 29–30 °C. The chemical shifts were measured from 85% $H_3PO_4$ as an external reference.

## Results

*Permeability Studies.* The measurement of cation permeability on vesicles by the proton–cation exchange method has been fully described previously (Bolard et al., 1981). It has been demonstrated that, under the experimental conditions chosen, the permeability to cations induced by polyene antibiotics in vesicles submitted to a pH difference between intravesicular and external medium can be conveniently measured by monitoring the proton flux occurring in exchange through the proton carrier FCCP. Upon amphotericin B addition in the suspension medium, the proton flux develops rapidly and reaches a plateau within 10–15 min except at very low amphotericin concentrations. The percentage of proton released at this plateau varies with the amphotericin B concentration. $^{31}P$ NMR experiments carried out as previously described (Prigent et al., 1980) show that when this plateau is reached, two populations of vesicles are present in the sample: one, the internal medium of which is at the same pH as the external medium, and the other remaining at the initial pH value.

Under these conditions, the amount of protons released was used as an index of the vesicle permeabilization by amphotericin B. This parameter was measured at different con-

[1] Abbreviations: CD, circular dichroism; NMR, nuclear magnetic resonance; EPR, electron paramagnetic resonance; EPC, egg phosphatidylcholine; $Me_2SO$, dimethyl sulfoxide; SUV, small unilamellar vesicle; MLV, multilamellar vesicle; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone.

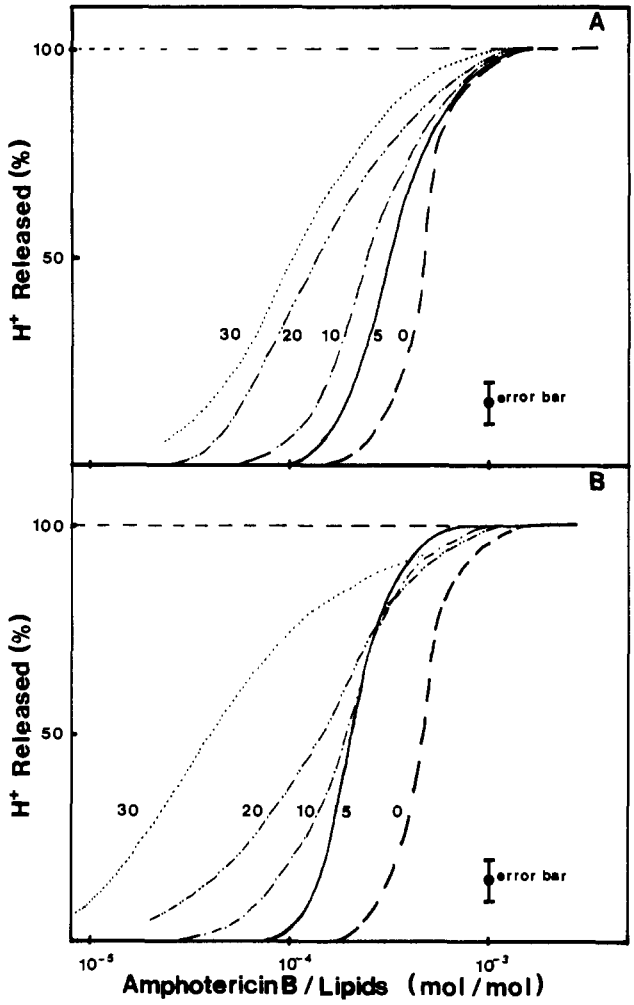


FIGURE 1: Proton release from cholesterol- (A) and ergosterol- (B) containing egg yolk phosphatidylcholine vesicles as a function of the amphotericin B/total lipids ratio in moles/mole. The total lipid concentration was 3.75 mM at the beginning of the $H^+$ efflux. This figure gives the mean curves obtained from several experiments.

centrations of amphotericin B. In Figure 1, the results of such experiments in vesicles containing various concentrations of either cholesterol (Figure 1A) or ergosterol (Figure 1B) are plotted vs. amphotericin B concentration expressed in moles per mole of lipids. This molar ratio is called hereafter the ratio *R*.

From these plots, the following information can be obtained: (1) Sterol-free vesicles are sensitive to amphotericin B; this corroborates the observation previously made by Cybulska et al. (1981). (2) The sensitivity of sterol-containing vesicles is always greater than sterol-free vesicles and increases with sterol concentration. (3) Ergosterol-containing vesicles are more sensitive than cholesterol-containing vesicles. This is more clearly demonstrated in Figure 2 where the percentage of protons released is compared in ergosterol and cholesterol vesicles as a function of sterol concentration for two different *R* values.

Also in Figure 1, it can be seen that the dose–response sigmoidal curves obtained at different sterol concentrations reach the maximum value at about the same *R* values ($10^{-3}$) and that the curves are steeper at lower concentrations of the sterol. In other words, in the low *R* range, the sensitivity of the results to amphotericin B (expressed as the percentage of protons released) increased markedly with increasing sterol concentration. This effect is more marked with ergosterol than with cholesterol.

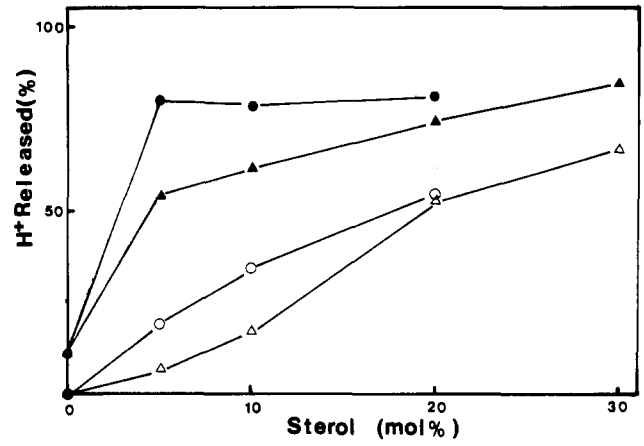


FIGURE 2: Maximal proton release (in % of total titratable proton) as a function of cholesterol (triangles) and ergosterol (circles) concentration in vesicles under the influence of amphotericin B at two concentrations: $R = 3 \times 10^{-4}$ (●, ▲) and $R = 1.5 \times 10^{-4}$ (○, △) in moles of amphotericin/mole of lipid.

At higher amphotericin B concentration (higher *R* values) in conditions in which more than 60% proton are released, the curves obtained at the various sterol concentrations are overlapping. Moreover, it seems that the curves for high sterol content (especially ergosterol) cross the curves for low sterol content in such a way that, in this range, the sensitivity of vesicles to amphotericin B is probably not a monotonic function of sterol concentration but exhibits a maximum at around 10% ergosterol.

*Circular Dichroism Studies.* The experiments were carried out in two different ways. First, microliter amounts of amphotericin B solution in $Me_2SO$ (2–5 $10^{-7}$ M increments) were added successively in the vesicles, and spectra were recorded immediately after each addition; second, a single addition of the desired amount of amphotericin B as an aqueous suspension was made. In this case, a slow change in the spectrum takes place from that of aggregated Amphotericin B in aqueous solution to that of the antibiotic in the vesicle membrane. The time required for this change to occur depended upon the amount of amphotericin B added, being larger for larger addition (e.g., for the addition of amphotericin B to give an *R* value of $10^{-2}$, the spectrum was recorded 90 min after). In both types of experiments identical spectra were eventually obtained for a given *R* value. The addition of up to 1% $Me_2SO$ or ethanolic FCCP solution does not interfere with the spectra.

CD spectra are given in Figure 3. Their spectroscopic significance has already been considered (Bolard et al., 1980). In each block of this figure are presented representative spectra recorded at different *R* values in sterol-free vesicles (A) and in vesicles containing 4, 10, and 20 mol % ergosterol. The corresponding spectra recorded with cholesterol vesicles are not presented: spectra similar to those recorded in presence of ergosterol were obtained, although in different ranges of *R* values.

*Sterol-Free Vesicles.* The spectra recorded confirm and expand the results of previous studies (Bolard et al., 1980). In the range $0.33 \times 10^{-4} < R < 6 \times 10^{-4}$, that is, from $10^{-7}$ to $2 \times 10^{-6}$ M (mol of amphotericin B/L of suspension), the spectrum ($A_1$) exhibits three positive bands at 413 ($\Delta\epsilon = 12$), 389 ($\Delta\epsilon = 12$), and 370 nm ($\Delta\epsilon = 12$). This characteristic spectrum of three equal bands regularly spaced will be referred to as "type I" spectrum hereafter. Beyond $2 \times 10^{-6}$ M ($R > 6 \times 10^{-4}$), a different spectrum ($A_2$) is obtained, which has been interpreted as indicating amphotericin B–phospholipid mixed micelle formation (Bolard et al., 1980).

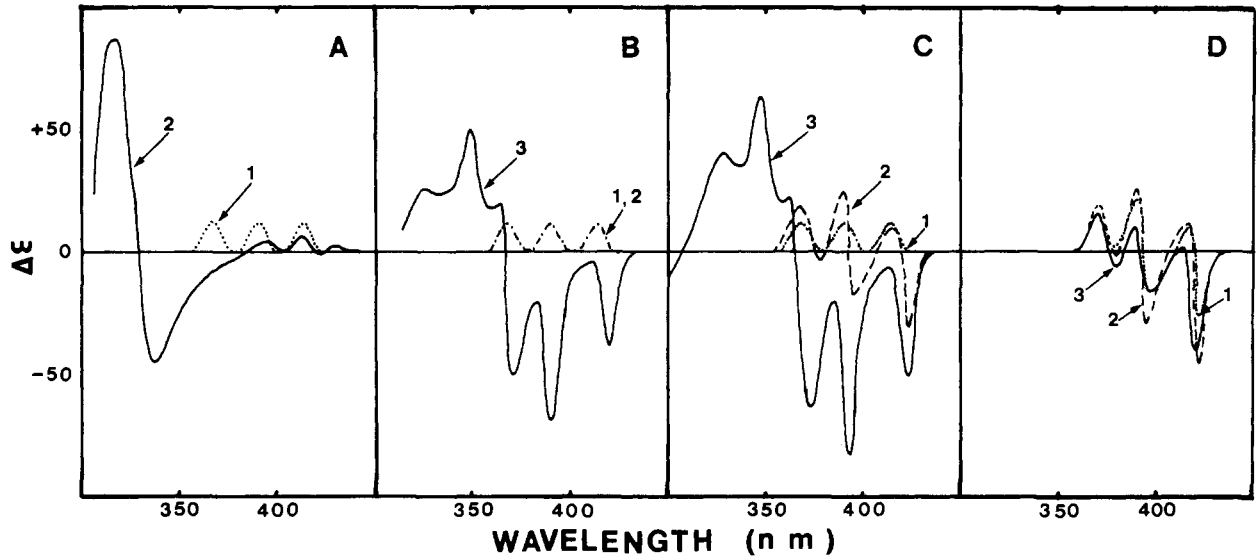


FIGURE 3: Circular dichroism spectra of amphotericin B in the presence of ergosterol-containing vesicles at 0% (A), 4% (B), 10% (C), and 20% (D). For sterol-free vesicles (A), (1) $R = 10^{-4}$ and (2) $R = 10^{-3}$. For sterol-containing vesicles (B, C, and D), (1) $R = 10^{-4}$, (2) $R = 4 \times 10^{-4}$, and (3) $R = 5 \times 10^{-2}$. $R$ is the molar ratio between amphotericin B and total lipids. $\Delta\epsilon$ is the differential molar dichroic absorption coefficient ($10^3$ cm$^2$ mol$^{-1}$). This value is determined from the intensity of the signal recorded on the dichrograph, referred to the total amphotericin B concentration. Theoretically speaking, this is not correct because what we actually observe is a mixture of amphotericin B species, each having their own $\Delta\epsilon$: free amphotericin B (monomeric and aggregated), bound amphotericin (species corresponding to spectra of type I, II, and III), and mixed micelles. Most of the time in the experimental conditions here described, free amphotericin B may be considered as negligible, and the CD observed is the resultant of two species only. In this figure are shown the extreme cases where a single species is dominant.

*Sterol-Containing Vesicles.* In the range of $R < 1.33 \times 10^{-4}$ ($\sim 4 \times 10^{-7}$ M), the same type I spectrum is recorded, with vesicles containing either up to 13 mol % ergosterol or up to 30 mol % cholesterol ($B_1$ and $C_1$). For $R > 1.33 \times 10^{-4}$ and with vesicles containing more than 10 mol % of either sterol, a new spectrum progressively appears ($C_2$ and $D_2$), consisting of six bands alternatively positive and negative between 450 and 360 nm. This spectrum will be referred to as "type II" spectrum.

In the range of $R > 5 \times 10^{-3}$ ($>1.5 \times 10^{-5}$ M), a different spectrum is obtained ($B_3$ and $C_3$), which may be described as an intense excitonic doublet centered at 370 nm, exhibiting a fine structure (320, 393, 373, 360, 350, and 320 nm). This spectrum will be referred to as "type III" spectrum. This spectrum, which is observed in vesicles containing up to 30 mol % cholesterol, disappears in ergosterol-containing vesicles beyond approximately 20 mol % of this sterol ($D_3$).

Up to these $R$ values only a small amount of amphotericin B remains in solution: (1) No CD signal corresponding to free aggregated amphotericin B is observed. (2) By low-speed centrifugation, which would sediment the aggregated free amphotericin B, if any, no modification is brought to the absorption or CD spectra. (3) By dialysis, which retains the vesicles but not the monomeric amphotericin B, we found that approximately 1% amphotericin B was free. Therefore, in those experimental conditions, it is possible to neglect the CD signal of free amphotericin B.

In the range of $R > 2 \times 10^{-2}$ ($>6 \times 10^{-5}$ M), an intense spectrum is superimposed to the spectra already described. This spectrum indicates, first, that free amphotericin B aggregates are present in the aqueous medium (Bolard et al., 1980) and, second, that mixed micelles of amphotericin B–phospholipid (at low sterol concentration) and of amphotericin B–sterol–phospholipid (at high sterol concentration) are present (Bolard & Chêron, 1982).

The sterol dependency of the intensity of the type II spectrum, monitored at 423 nm, and of the type III spectrum, monitored at 393 nm, are given in Figure 4. The intensity of the type II spectrum increases continuously with sterol concentration. Its amplitude is 6 times larger in ergosterol-containing vesicles than in cholesterol-containing vesicles at all concentrations of sterol. The intensity of the type III spectrum exhibits a strong maximum around 8 mol % ergosterol. In cholesterol, this maximum is less prominent and shifted to between 15 and 20 mol %. At their respective maxima, the amplitude of the type III spectrum is about 3

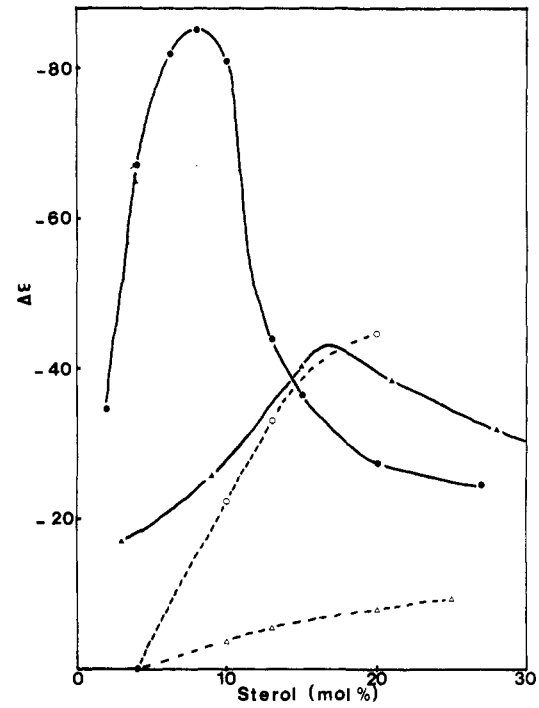


FIGURE 4: Differential molar dichroic absorption coefficient ($10^3$ cm$^2$ mol$^{-1}$) as a function of concentration of cholesterol (triangles) and ergosterol (circles), in percent of total lipid moles, at two amphotericin B concentrations corresponding to the type II spectrum (△, ○) ($R = 4 \times 10^{-4}$ monitored at $\lambda = 423$ nm) and to the type III spectrum (▲, ●) ($R = 5 \times 10^{-2}$ monitored at $\lambda = 393$ nm). We have selected the wavelengths where the CD variation of each species is the most specific.

times larger in ergosterol-containing vesicles than in cholesterol-containing vesicles. However, at sterol concentrations above approximately 14%, the intensity of the spectrum is slightly higher in cholesterol-containing vesicles.

## Discussion

CD recording and permeability measurements were carried out in the same experimental conditions in order to allow the direct comparison of the two sets of data. A typical example of such a comparison may be done for vesicles containing 10 mol % ergosterol. Three concentration ranges can be distinguished: Below $3 \times 10^{-7}$ M, a small but significant permeability is observed (up to 20% $H^+$ released in the example chosen). In this region, only type I spectrum is observed. Between about $3 \times 10^{-7}$ and $1.6 \times 10^{-6}$ M, the permeability induced increases from 20% up to 90% $H^+$ released. The corresponding spectrum is of type II. Beyond $1.6 \times 10^{-6}$ and up to $1.5 \times 10^{-5}$ M, a maximum permeability is nearly always obtained. In this concentration range, type III spectrum progressively appears and takes the place of type II spectrum.

In the central region, which represents the quasitotality of the dose–response curve, type II spectrum is observed practically in a pure state. The possibility that this spectrum could represent a combination of types I and III spectra can be disregarded for the following reasons: First, a combination of these two spectra in various proportions (using the least-squares method) never results in a type II spectrum. Second, the signal amplitude at 393 nm of this spectrum increases first rapidly and then remains constant in the whole concentration range. Third, the signal amplitude only increases beyond $1.6 \times 10^{-6}$ M with the progressive contamination by type III spectrum. Finally, the variation of the optical density measured at 413 nm is linear in the whole range.

The main conclusion of these comparisons is that the amphotericin B concentration ranges in which the type II spectrum is recorded depend upon both the nature and the concentration of sterols in the vesicles, and this dependency is identical with that observed for the amphotericin B induced permeability. For instance, with vesicles containing 10 mol % cholesterol instead of ergosterol, both the permeability and the appearance of type II spectrum are shifted by a factor of 3 in amphotericin B concentration. At higher concentration of ergosterol, both permeability and type II spectrum appear at amphotericin B concentrations as low as $10^{-7}$ M (i.e., for $R$ values of about $10^{-5}$). It should be stressed that this amphotericin B concentration corresponds to about 0.1 amphotericin B molecule per vesicle (Prigent et al., 1980). Such a high efficiency is made possible by the rapid exchange of amphotericin B between vesicles (Van Hoogevest & De Kruijff, 1978; Bolard et al., 1981) but implies a very high degree of cooperativity in the amphotericin B–sterol interaction.

Therefore, it appears that in a concentration range of amphotericin B just sufficient to induce cation permeability on vesicles, a unique permeant species of amphotericin B–sterol complex, characterized by its CD spectra (type II), is present in the vesicle membrane. This permeant species is formed optimally when the amphotericin B concentration is low, the sterol concentration is high, and in the presence of ergosterol rather than cholesterol. This is consistent with the hypothesis that it is the preferential interaction of amphotericin B molecules with ergosterol that is responsible for the greater sensitivity of ergosterol-containing membranes.

At higher concentrations of amphotericin B, type III spectrum takes the place of type II spectrum. It is more difficult to establish the relationship between this new species and its permeabilizing effect, since this amphotericin B concentration range is mostly supramaximal for permeability. Moreover, the intense type III spectrum may mask completely the presence of a type II spectrum.

The type III spectrum, in contrast to the type II spectrum, presents a maximum at about 20 mol % cholesterol in vesicles. The fact that in the same range of amphotericin B concentration, a permeability maximum is observed in MLV containing 20% cholesterol (Teerlink et al., 1980) may indicate that type III spectrum could correspond also to a permeant entity.

It remains now to consider the very low range of $R$ in which only type I spectrum is recorded. This spectrum cannot be distinguished from the spectrum observed in the pure phospholipid vesicle case at amphotericin B concentration as low as $5 \times 10^{-8}$ M. This spectrum appears to be identical with the spectrum recorded for the monomeric form of amphotericin B in aqueous solution in the same concentration range but red shifted by about 4 nm. This can be interpreted as type I spectrum corresponding to amphotericin B in monomeric form either adsorbed or dissolved in the vesicle bilayer, the red shift being due to the change in the molecule environment.

The type I spectrum is observed still with vesicles containing 30 mol % cholesterol, concentration at which there is no pure phosholipidic domain in the bilayer (Mabrey et al., 1978; Estep et al., 1978; Gershfeld, 1978). This fact is in favor of the hypothesis that, at very low $R$, type I spectrum is due to amphotericin B monomers adsorbed at the bilayer surface.

Amphotericin B apparently induces cation permeability on sterol-free egg yolk phosphatidylcholine vesicles. Considering the probable significance of type I spectrum recorded with these vesicles, it may be considered that it is due to a detergent-like effect of amphotericin on these relatively fragile membranes. The type I spectrum evolves rapidly with increasing amphotericin B concentration in a spectrum that was interpreted as mixed-micelle formation (Figure 4, $A_2$) (Bolard et al., 1980). If this hypothesis holds true, the observed permeabilization of sterol-free SUV is not in contradiction with the observation that no permeability induction to $Na^+$ ions (Singer, 1975) or a very weak permeability to $K^+$ ions (Teerlink et al., 1980) is obtained in sterol-free MLV: in this case, the detergent effect would affect only the very first layers and, therefore, would induce the liberation of a very small portion of the liposome internal volume.

The same type I spectrum is recorded at very low $R$ with sterol-containing vesicles on which a small but significant permeability is induced. However, it is probable that the permeant entity corresponding to type II spectrum is already present although at concentrations too weak to be detectable.

Several studies have been performed that have attempted to prove the existence of a definite amphotericin B–sterol complex in membranes. In these experiments, the sterol content of the bilayer and the amphotericin B concentration were varied, and the stoichiometry (and ipso facto the existence of the complex) was estimated from the dependence of some property of the lipid bilayer on the amphotericin B/sterol ratio. For instance, molar ratios of 1/3.9 and 1/0.7 have been proposed from absorption measurements or from the amphotericin B induced $K^+$ efflux from lecithin MLV (De Kruijff et al., 1974), while monitoring the order parameter changes of nitroxide spin-labeled membrane components gave a stoichiometry of approximatively 1/1 with lecithin in SUV (Ohki et al., 1979) and with lecithin planar multilayers (Oehlschlager & Laks, 1980). It is important to note that these results may not be relevant to interactions at the level of the cell membrane

because the location of the amphotericin B–sterol complex inside the phospholipid bilayer of the SUV has been questioned (Aracava et al., 1981; Bolard & Chêron, 1982).

Direct evidence for an amphotericin B–sterol complex in phospholipid–sterol membranes was proposed from fluorescence energy transfer with dehydroergosterol (Archer, 1975a) but was later retracted (Archer, 1975b). Recently, another kind of direct evidence was derived from Scatchard analysis of the binding of AmB to lecithin–sterol SUV monitored by electronic absorption, and a single complex of 1/1 stoichiometry was proposed (Readio & Bittman, 1982). However, electronic absorption spectroscopy can detect only three conformers, free amphotericin B in monomeric or aggregated form and amphotericin B bound to the lipids, and cannot provide specific information. CD spectroscopic data do not allow a quantitative analysis of this stoichiometry: the complexes are too numerous, and their respective spectra overlap too much to find a concentration range large enough to study the spectral characteristics of a single species as a function of the amphotericin B/sterol molar ratio. Therefore, the stoichiometry of amphotericin B–sterol complexes remains unresolved.

Some structural informations may be obtained from the CD spectra by taking advantage of the interpretations of carotenoid CD spectra already put forward by Noack & Thomson (1979) and Sturzenegger et al. (1980). According to the classification proposed by Sturzenegger et al., type I spectra, as well as that of monomeric amphotericin B in water, are of the nonconservative type while type II spectra, with higher $\Delta\epsilon$, are typically conservative. For carotenoids, it now seems well established that conservative spectra arise when the terminal rings, bearing strongly polarizing substituents at asymmetric centers, have a restricted conformational mobility with respect to the polyene chain. It is therefore tempting to extend this notion to our particular case and to say that type II species are in a more rigid environment than type I species. Type III spectrum is not so characteristic, and it is more difficult to classify it. One may, however, point out its striking similarity with that of free self-associated aromatic polyene antibiotics such as vacidin or candicidin, in water (Mazerski et al., 1982).

In conclusion of this study, numerous amphotericin B conformers can be detected in sterol-containing vesicles by their CD spectroscopic properties, depending upon both the concentration of sterol and the amphotericin B/sterol molar ratio. One of these conformers, and perhaps a second at high amphotericin B concentration, is responsible for the permeability induced in the vesicles. From their CD spectra, these two conformers are the same either with ergosterol or with cholesterol, but the concentration of amphotericin B necessary to obtain these permeant entities is 10 times higher for cholesterol than for ergosterol. This fact accounts for the greater sensitivity to amphotericin B of the ergosterol-containing membranes as compared to cholesterol-containing membranes.

**Registry No.** Amphotericin B, 1397-89-3; ergosterol, 57-87-4; cholesterol, 57-88-5.

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