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Absorption and Fluorescence Spectra of Polyene Antibiotics in the Presence of Cholesterol*

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The alterations in the absorption and fluorescence spectra observed for the polyene antibiotics filipin and nystatin in the presence of cholesterol are due to an exciton interaction (polyene aggregates) and cannot be attributed to a specific sterol-antibiotic complex. Filipin and nystatin molecules partition into the sterol aggregates, these structures being very efficient to induce exciton interaction; the observed splitting profile indicates that the chromophores are in a stacked arrangement (parallel transition dipoles). For filipin incorporated in lipid bilayers, the sterol is able to induce the same type of aggregate, at variance with nystatin.

Polyene antibiotics, *e.g.* filipin, nystatin, and amphotericin B, are antifungal agents, characterized by a low antibacterial activity, that promote the leakage of cellular components if added to the culture medium (1). These facts led to the following hypothesis: (i) the biochemical action site of polyene antibiotics is located at the cell membrane and (ii) the selective toxicity of this class of antibiotics is due to a membrane compound found only in the sensitive cells. Lampen *et al.* (2) proved that these compounds were sterols, being the fungal membranes sterol-rich while the bacterial membranes are almost depleted of these compounds (3). Moreover, Lampen *et al.* (2) showed that the presence of different types of sterols in the membranes potentiated different lytic responses.

The polyene antibiotics are macrolides with amphipatic nature, and for the sterol-antibiotic interaction in membranes, several models were proposed (4). For nystatin, the formation of a channel composed of alternate sterol and nystatin molecules was postulated, based on permeability studies (5). For filipin, there is no consesual model, three main hypothesis being advanced: (i) the antibiotic and the sterol interact in a 1:1 stoicheometry forming large planar aggregates between the two layers of the membrane (4), (ii) filipin associates with the sterol in a 1:1 stoicheometry but at the membrane surface inducing a structural disorder (6), and (iii) the interaction between the antibiotic and the sterol is localized in the upper layer of the membrane promoting the deformation of the bilayer due a surface pressure increase (7).

Both nystatin and amphotericin B are drugs used in the therapy of systemic fungal infections (for review see Ref. 8), whereas filipin is not currently used for medical purposes. This pentaene antibiotic has been instead widely applied as a probe for sterol location in biological membranes ((9), for reviews see (7, 10)).

The only structural information reported for the sterol-polyene interaction in aqueous solutions was obtained from absorption and fluorescence spectroscopy. In this journal the first detailed study was presented (11), and this experimental approach was used to completely describe the sterol-antibiotic complex in aqueous solution or in membranes, namely its stoicheometry (11) and the selective affinity for several types of sterols (11–13). This information is based on changes observed in the antibiotics absorption spectra, marked alterations being reported in the relative intensities of the vibronic progression, in all cases the interaction of the polyene with the sterols increasing the intensities of the transitions at higher energies (5, 11–16); *e.g.* for filipin, the ratio of the 0 ← 0 (357 nm) and 2 ← 0 (321 nm) transitions was used to quantify the interaction (11). This effect was also observed in the excitation spectra of the antibiotics, with concomitant quenching being observed in some cases (16, 17).

For nystatin (a tetraene), the reported solvent response (18) is only the expected red shift of the absorption; upon increase of the polarizability of the medium, no alteration of the spectral profile was observed. The same happens for the parinaric acids (tetraenes) fluorescent probes for which a detailed photophysical study was carried out by Sklar *et al.* (19). In this way, the dramatic spectral alterations induced by a sterol-antibiotic complex are difficult to rationalize.

These facts and the proposed biochemical relevance of the antibiotic-sterol interaction prompted us to carry out an absorption and fluorescence study of filipin and nystatin in the presence of cholesterol to further clarify the reported conclusions in Refs. 11–13 and 15–17.

MATERIALS AND METHODS

Chemicals—Filipin and L-α-1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC)[1] were obtained from Sigma and used as received. Filipin is a mixture of four macrolides (20), with minor differences in their structures, having a pentaene chromophore as a common feature, the fraction known as filipin III (Fig. 1) being the major component. Nystatin was a gift from Squibb (New Brunswick, NJ) and is a mixture of three tetraenes, one of them (Fig. 1) being the major component (80%).

Filipin stock solutions in Tris-HCl (British Drug House (BDH), London) buffer (50 mM, pH 7.4, NaCl 10 mM) with 1% (v/v) in ethanol, were kept in the dark at 4° C, with no alteration being observed in its spectral properties for months. Nystatin stock solutions were prepared in methanol and were always tested for antibiotic degradation before use.

9,10-Diphenylanthracene (scintillation grade) and cyclohexane

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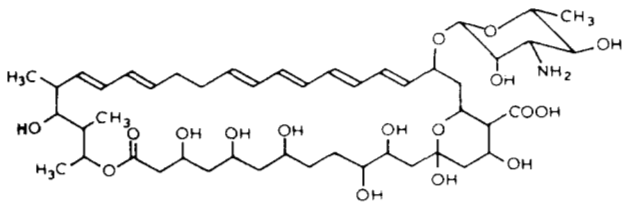
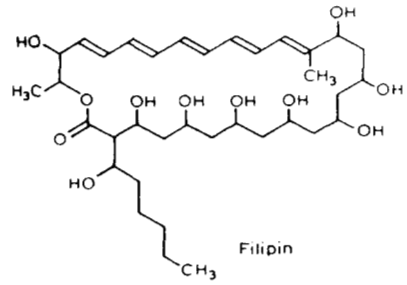
[1] The abbreviations used are: DPPC, L-α-1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; c.m.c., critical micellar concentration.



Nystatin



Filipin

FIG. 1. **Molecular structure of filipin III and nystatin.**

(spectroscopic grade) were supplied by BDH. *n*-Hexane (spectroscopic grade) was from Merck (Darmstadt, Germany).

Cholesterol was from Merck (Darmstadt, Germany) and used without further purification.

Preparation of Phospholipid Vesicles and Cholesterol Suspensions—A film of lipid with or without cholesterol was obtained from evaporation of stock solutions in chloroform (30 mM DPPC and 5 mM cholesterol), kept under vacuum for more than 12 h, and then solubilized in buffer by vortexing and warming the solution above the phase transition temperature of the lipid.

To the lipid solutions, filipin and nystatin were added from a stock solution in Tris-HCl buffer or in methanol, respectively. For the highest concentration of solvent used, 2%, no alteration in the bilayer structure is reported (21). The incubation time for incorporation was 2 h in the dark, a steady intensity of fluorescence being observed in all cases after uptake into the vesicles.

Cholesterol suspensions were prepared in a similar way: a film of cholesterol was obtained from evaporation of the stock solution, kept under vacuum for 12 h and then suspended in buffer by vortexing and warming the solution at 50° C. Addition of the antibiotics was as described before.

Absorption and Fluorescence Measurements—Absorption spectra were recorded on a Perkin-Elmer Lambda 15 spectrophotometer, using 1 × 1-cm cuvettes, at 22 °C.

Steady-state excitation and emission spectra were carried out at 22 °C in a right angle geometry, using 1 × 1-cm or 0.5 × 0.5-cm cuvettes, in a Spex F112 A Fluorolog spectrofluorimeter equipped with a double emission monochromator and a thermostatting unit. Excitation and emission bandwiths were, respectively, 1.80 and 2.25 nm in most measurements. Correction of excitation and emission spectra were performed using a rhodamine-B quantum counter solution and a standard lamp, respectively (22).

Relative quantum yields of fluorescence of filipin and nystatin were determined *versus* 9,10-diphenylanthracene in cyclohexane (ϕ_f = 0.90 ± 0.02 (22)). Fluorescence measurements in lipid solutions were obtained in a magic angle arrangement (23) at 22 °C. Solutions were degassed by nitrogen bubbling.

Fluorescence anisotropies were determined from Equation 1; I_{vv} and I_{vh} are fluorescence intensities where the two subscripts indicate the orientation of the excitation and emission polarizers (h, horizontal; v, vertical), respectively, and $G = I_{hv}/I_{hh}$ is the instrumental factor. Glan-Thompson polarizers were used. The excitation and emission wavelengths were 338 and 480 nm for filipin and 305 and 415 nm for nystatin, respectively, and the background intensities were taken into account.

$$r = (I_{vv} - GI_{vh})/(I_{vv} + 2GI_{vh}) \quad (1)$$

For the purpose of c.m.c. determination, scattered light intensities (λ = 450 nm) were measured in the spectrofluorimeter at a 90° geometry.

Fluorescence decays were measured using the time-correlated single photon counting technique. The excitation source was a nitrogen-filled flash lamp (Edinburgh Instruments, 119 F), operated at 80 KHz. Excitation (λ = 337 nm for filipin and λ = 316 nm for nystatin) and emission wavelengths (λ = 480 nm for filipin and λ = 410 nm for nystatin) were selected using monochromators (Jobin Yvon, H20) with emission band passes of 8.0 and 10.0 nm, respectivly. Collection of pulse and sample profiles, detected with a cooled (−30 °C) Phillips XP2254 B photomultiplier, was alternated with maximum cycle times of 15 min. Decays were observed for 3 decades of intensity, either with 0.268 or 0.122 ns/channel resolution, and 10^4 counts were accumulated in the peak channel. Data analysis was performed with the δ-function convolution method (24), (using *p*-bis-[2-(5-phenyloxazolyl)]benzene as a reference), in order to avoid "color" shift of the photomultiplier. The best fit parameters (lifetimes, τ_i, and pre-exponentials, a_i) were obtained from nonlinear least-squares iterative program, minimizing χ^2 (23). The adequacy of the fit to the decay data was judged from the χ^2 value and inspection of the weighted residuals plots.

Fluorescence decays were complex and described by a sum of two or three exponentials, the mean lifetime $\bar{\tau}$ being obtained from Equation 2.

$$\bar{\tau} = \Sigma a_i \tau_i^2 / \Sigma a_i \tau_i \quad (2)$$

Radiative and nonradiative decay rate constants, k_f and k_{nr}, respectively, were calculated according to Equations 3 and 4.

$$k_f = \phi_f / \bar{\tau}_f \quad (3)$$

$$k_{nr} = 1/\bar{\tau}_f - k_f \quad (4)$$

The mean wavenumber of the absorption, $\langle \bar{\nu} \rangle$, was calculated from the absorption spectrum (in wavelength), using Equation 5.

$$\langle \bar{\nu} \rangle = \frac{\int \epsilon(\lambda) \lambda^{-3} d\lambda}{\int \epsilon(\lambda) \lambda^{-2} d\lambda} \quad (5)$$

RESULTS

In Fig. 2 the scattered light intensities *versus* concentration of filipin and nystatin buffer solutions are presented. In both cases the scatter intensity is roughly constant up to a concentration of 1 μM for filipin and 3 μM for nystatin, where a sudden increase is observed due to the formation of aggregates.

Absorption spectra of filipin in buffer solution were carried out in the presence and absence of cholesterol. In Fig. 3*a* is depicted the absorption spectrum at a concentration where the antibiotic is aggregated in water, and the expected pattern with a clear vibrational progression of a polyene is observed; the monomer absorption spectrum is identical to the one of the aggregate (result not shown). Interestingly, near the con-

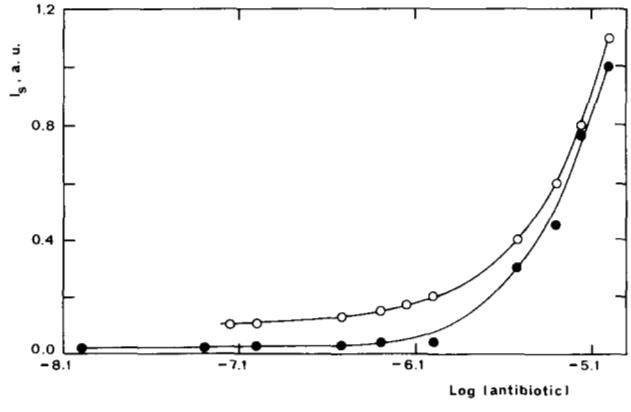


FIG. 2. **Scattered light intensities (λ = 450 nm) as a function of antibiotic (●, filipin; ○, nystatin) concentration in buffer.** The ordinate scale for nystatin is shifted by 0.1 unit.

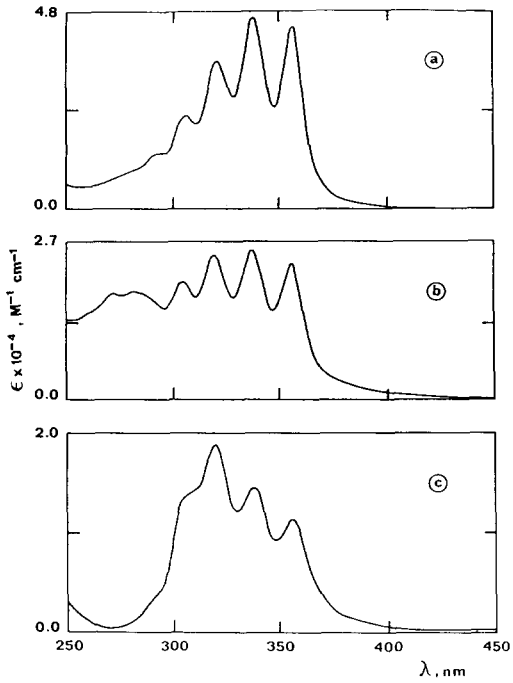


FIG. 3. **Absorption spectra of filipin in buffer.** *a*, 8.0 μM (aggregate); *b*, 1.5 μM (≅c.m.c.); *c*, 1.5 μM in a 50 μM cholesterol suspension.

centration of aggregate formation (≅1 μM), a new band centered at 275 nm is apparent (Fig. 3*b*). Upon the addition of cholesterol (Fig. 3*c*), a strong alteration is observed for both the pre-micellar and aggregated forms of filipin (these changes are also observed in the excitation spectra (Fig. 4*a*). The vibrational progression seems to be affected with an increase of intensity at 323 nm and, in addition, the band at 275 nm is not apparent. With nystatin, no alterations were observed in the absorption spectra upon aggregation, but similar changes occurred in the presence of cholesterol.

The effect of cholesterol is also revealed on the excitation spectra of both antibiotics, as shown in Fig. 4. For filipin (Fig. 4*a*), the alterations observed upon increase of cholesterol concentration are qualitatively similar to the ones observed in the absorption spectra, the magnitude of changes being dependent on the sterol concentration. No alterations on the emission spectrum of this antibiotic are observed upon the presence of cholesterol (Fig. 4*b*).

Similar alterations are observed on the excitation spectra of nystatin (Fig. 4*c*). An increase in intensity at higher energies is observed, and the 0 ← 0 transition red-shifts 3 nm. The emission spectrum of this molecule is clearly distinct for the different types of aggregates as depicted in Fig. 4*d*. For monomeric nystatin, an unstructured emission spectrum centered at 410 nm is observed; at concentrations higher than 3 μM, when the aggregate is formed, a blue-shifted and clearly structured emission is obtained. In the presence of cholesterol, the fluorescence spectrum of nystatin is also affected, the emission becoming less broad.

The photophysical parameters for the aqueous solutions of the two antibiotics are presented in Table I. For nystatin the emission in the absence of cholesterol is very weak ($\phi_f <$ 0.003), and it does not allow a precise determination of the singlet state deactivation rate constants. This tetraene chromophore undergoes a large increase in anisotropy upon changing from the monomer ($r = 0.05$) to the aggregated species ($r = 0.28$) and in the presence of cholesterol the lifetime largely increases.

The quantum yield of filipin also increases upon its aggregation ($\phi_f = 0.26$), but in contrast, both its mean lifetime and anisotropy are almost invariant, even in the presence of cholesterol.

For both antibiotics a study of the excitation profile was carried out in a model system of membranes (liposomes of DPPC). As shown in Fig. 5, the presence of the sterol is essential to induce spectral alterations in filipin. For nystatin, no significant spectral alteration is observed in the absence or presence of sterol (up to 50 μM).

DISCUSSION

The light scattering experiment (Fig. 2) indicates that these amphipatic molecules self-associate in water. The expected variation of intensity *versus* concentration is obtained and the critical micellar concentration is clearly determined (c.m.c. = 1 μM for filipin and c.m.c. = 3 μM for nystatin). The formation of micellar aggregates of polyene macrolide antibiotics is pointed out in Refs. 2 and 25, and the value for filipin is in agreement with one reported (15).

For both antibiotics the quantum yield, ϕ_f, increases upon aggregation, as shown in Table I. The filipin pentaene chromophore lifetime is quite insensitive to the aggregation in buffer, but its anisotropy does not increase upon aggregate formation. Considering that these aggregates are quite large as revealed by light scattering experiments (radius ≅ 50 nm),[2] this implies that the chromophore must have an efficient depolarization dynamics, the anisotropy not being controlled by the aggregate size. On the contrary, for the tetraene chromophore nystatin, a large increase of anisotropy is observed, but as the mean lifetime remains very short, it is not possible to draw any conclusions about the flexibility of the polyene within the aggregate.

The absorption spectra of both the monomer and aggregate of filipin are identical (Fig. 3*a*) and show the vibrational resolution of polyene chromophores (19). Near the critical micellar concentration, the new band observed (Fig. 3*b*) is assigned to a ground state interaction of the chromophores (exciton interaction). When there are dipolar interactions between the transition dipoles of two monomers, two new excited states are defined, and there is an efficient transfer of excitation between them (26). The formation of pre-micellar aggregates with peculiar properties is reported in Ref. 27, and a similar result was verified for amphotericin B (25).

It is not surprising that this interaction is not evident at concentrations above the critical micellar concentration. The requirements for exciton interaction are very strict, depending on the relative orientations and distances of the transition moments of the monomers, thus revealing the structural differences between the two agreggates. Upon the presence of cholesterol a strong alteration is observed as shown in Fig. 3*c*. The excitonic transition moves to lower energies, and the profile of relative intensities of the components is similar to the ones observed with polyene alcohols related to retinol at 77 K (28, 29) or with a parinaric acid derivative in aqueous solution (30). The greater intensity of the higher energy component of the exciton doublet implies a parallel arrangement of the transition moments, and a card-stacked arrangement for the two chromophores was proposed (29). Cholesterol aggregates are very efficient both on compartimentalizing the antibiotic and on promoting this geometry, and it should be emphasized that the complex is distinct from the one formed in the pre-micellar region.

While the geometry of the pre-micellar- and sterol-induced

[2] M. A. R. B. Castanho, A. Coutinho, and M. J. E. Prieto, manuscript in preparation.

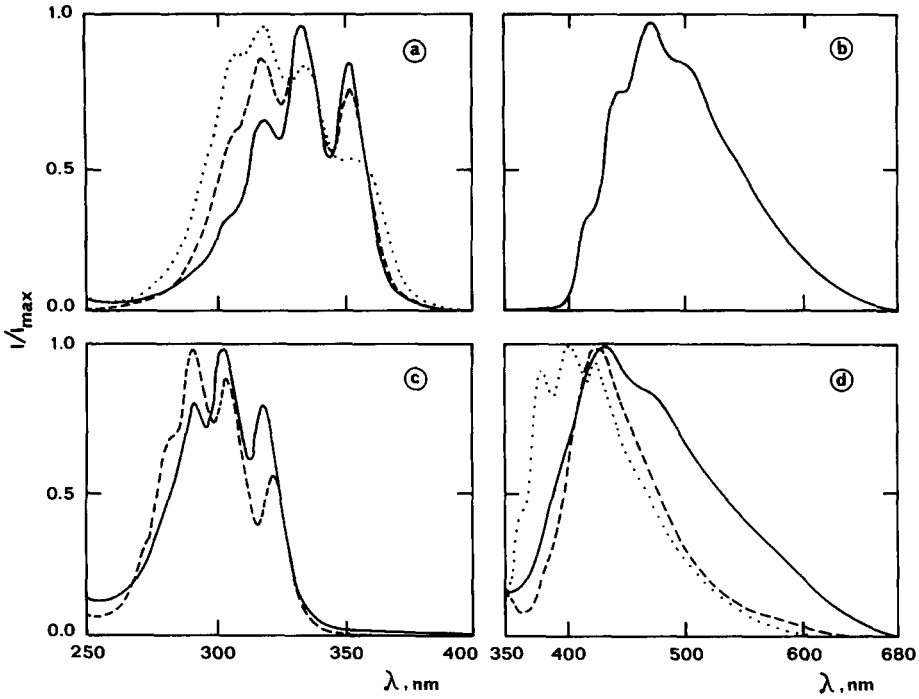


FIG. 4. **Corrected and normalized excitation (*a* and *c*) and emission (*b* and *d*) fluorescence spectra of filipin (*a* and *b*) and nystatin (*c* and *d*).** *a*, 1.5 μM filipin in the absence (——) and in the presence of 50 μM cholesterol suspension (- - - -), and 15 μM filipin in the presence of 500 μM cholesterol suspension (· · · ·); *b*, monomeric or aggregated filipin, with or without cholesterol; *c*, 1.4 μM nystatin in the absence (——) and in the presence of a 50 μM cholesterol suspension (- - - -); *d*, nystatin in the absence (——, 1.4 μM; · · · ·, 26.2 μM) and in the presence (1.4 μM) of a 50 μM cholesterol suspension (- - - -). (Excitation and emission wavelengths were 338 and 480 nm for filipin and 320 and 410 nm for nystatin, respectively.)

TABLE I

Quantum yields (ϕ_f), anisotropies (r), mean lifetimes ($\bar{\tau}$), and radiative (k_f) and nonradiative (k_{nr}) rate constants for nystatin (N) and filipin (F) in aqueous solution in the presence and in the absence of cholesterol (C)

Antibiotic	[F] or [N]	[C]	ϕ_f	$\bar{\tau}$	r	k_f	k_{nr}
	μM			ns		10^7 s^{-1}	
F	0.5[a]	0.0	0.08	14.0	0.02	0.53	6.50
	15.0[b]	0.0	0.26	12.4[c]	0.02	2.10	6.00
	1.5	50.0	0.31	13.6[d]	0.02	2.28	5.07
N	1.0[a]	0.0	<0.0001		0.05		
	10.0[b]	0.0	<0.003	<1	0.28		
	1.0	50.0	0.02[e]	8.2[d]	0.31		

[a] Monomeric antibiotic.
[b] Aggregated antibiotic.
[c] Complex decay with three components.
[d] Complex decay with two components.
[e] Lower limit (see text).

exciton should be similar, the interaction energies, V, are quite different. For filipin the mean wavenumber of monomer absorption, obtained from Equation 5 is $<\bar{\nu}> = 30{,}500$ cm^{-1}. In this way, values of $V = 5864$ cm^{-1} (pre-micellar exciton) and $V = 653$ cm^{-1} (sterol-induced exciton) are obtained, when considering nearest-neighbor interactions only. From the integration of the absorption spectrum of the monomer (31) a transition moment of 1.6 Å is calculated. Not surprisingly, if these values were worked out in a point dipole approximation (31), too large interchromophore distances would be obtained. However, this approximation is not valid for describing the interactions of close packed large dipoles (32), but it can be infered that, for the same geometry, the chromophores in the exciton induced by the sterol would be at a larger distance than the ones forming the pre-micellar aggregate, a factor of two being an upper limit.

Exciton interaction can also be observed in the excitation spectra of these antibiotics (Fig. 4), and in this case artifacts due to light scattering are not so critical as in absorption techniques. In Fig. 4*a*, it is shown that the excitonic interaction depends on the sterol concentration. This is understood considering that at higher sterol concentrations, incorporation of the antibiotic into the sterol aggregates increases. In most cases (*e.g.* dyes), the aggregates are nonfluorescent (33); at variance, we observed strong emissions of these aggregated antibiotics.

For nystatin, similar sterol-induced spectral alterations were obtained as depicted in Fig. 4*c*. For this antibiotic, distinct emissions were observed for the several species (Fig. 4*d*); namely, upon aggregation, the spectrum of the monomer undergoes a blue-shift, and the emission is more structured pointing out to a less polar environment of the tetraene cromophore. The excitonic emission is again red-shifted relative to the one of the aggregate in water and a concomitant shift of 3 nm is observed in the excitation spectrum.

It should be emphasized that, as described in the Introduc-

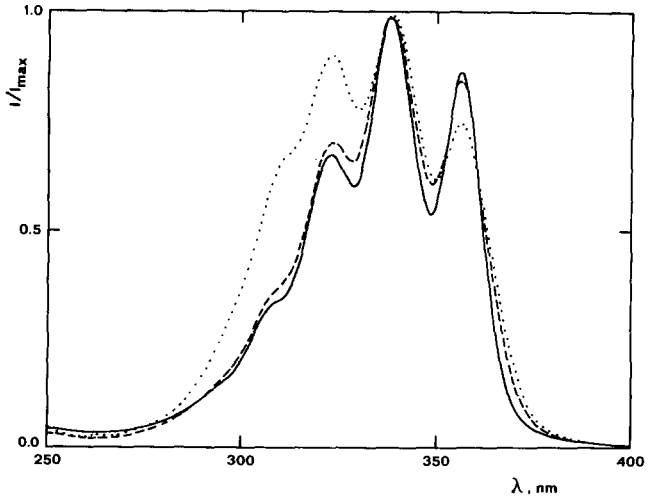


FIG. 5. **Corrected and normalized excitation spectra for filipin (3.9 μM) in buffer (——) and in the presence of liposomes of DPPC (100 μM), with (· · · ·) and without (- - - -) cholesterol (50 μM).**

tion section, the sterol-induced spectral alterations of nystatin and filipin were reported previously, but in all cases the effect was attributed erroneously to the formation of an antibiotic-sterol complex (11–13, 15–17). For amphotericin B in aqueous solutions, the formation of aggregates was demonstrated (34), and relevant circular dichroism and absorption studies elucidating the aggregate structure were reported (32, 35), but these works seemed to be largely overlooked regarding their biological importance. The present study clearly shows that sterol-induced spectral alterations are due to an antibiotic-antibiotic complex, thus invalidating any conclusions regarding stoichiometry and specificity of the sterol-antibiotic complex (11–13). In addition, the parameter that has been used to evaluate the antibiotic-sterol interaction (the ratio of the $0 \leftarrow 0$ and $2 \leftarrow 0$ transition intensities (*e.g.* Ref. 11) is inadequate, because it is significant only for a very specific antibiotic-antibiotic interaction. A consequence of this fact is that filipin is thought not to interact with lipidic bilayers without sterols, because the membranes do not affect this ratio. In fact, fluorescence anisotropy studies demonstrate that filipin does interact strongly with pure DPPC vesicles; namely, for filipin the anisotropy increases from $r = 0.02$ to $r = 0.25$ due to antibiotic immobilization when incorporated in the vesicle. In this way, looking for filipin presence in membranes either via freeze-fracture techniques (7) or fluorescence intensity measurements only (9), instead of spectral alterations, cannot be used as a criteria for sterol presence. Taking into account these considerations, the antibiotic-sterol interaction is an open question, deserving further study.

Considering the relevance of this conclusion, some more arguments should be carried out regarding the excitonic nature of the absorption band. In fact, a genuine spectral alteration due to the interaction with sterol would be very unlikely on spectroscopic grounds; the change of the absorption (or excitation) spectra could be rationalized on the basis of (i) alteration of the vibronic coupling and modification of the Franck-Condon factors, (ii) induced structural changes on the antibiotic geometry, and (iii) formation of a ground state complex absorbing at lower wavelengths and superimposed to the intrinsic chromophore absorption. The first two arguments are closely related and can be ruled out considering the rigidity of the macrolide structure. The band under study is the allowed (strong) $^1B_u^+ \leftarrow {}^1A_g$ transition (36, 37), and it would not undergo dramatic alterations upon orbital mixing induced by slight molecular distortion (vibronic effect). Any speculative deviation from the all-trans geometry of the antibiotic, and the concomitant appearence of a cis-band (29) is also prevented by the macrolide structure. The formation of a ground state complex of charge transfer nature is not feasible on thermodynamic grounds, *i.e.* cholesterol with only a double bond chromophore (or even a saturated sterol as reported (11–13)) cannot participate as an acceptor regarding the pentaene or tetraene chromophores. In addition it should be stressed that the experiments in Fig. 4*a* were carried out at a constant filipin/sterol molar ratio. In case that the spectral alterations were due to a filipin-sterol complex, the same spectrum should be obtained at variance with the observed in Fig. 4*a*.

The photophysical parameters for both molecules associated with cholesterol are presented in Table I. For filipin, both the nonradiative and the radiative rate constants are invariant upon exciton formation. The tetraene chromophore is very sensitive to the rigidity of the medium, and a large increase in lifetime and quantum yield is observed. The quantum yield in the presence of cholesterol, $\phi_f = 0.02$, is not higher due to the fraction of nonfluorescent antibiotic in the water. The lifetimes behavior is similar to the one observed for parinaric acids (38).

The result depicted in Fig. 5 constitutes evidence that for filipin incorporated in a model system of membranes, no exciton type of absorption is observed in the absence of sterol, *i.e.* even in case that the antibiotic is aggregated in the membrane the previously described stereochemical requirements for exciton interaction are not fulfilled. Interestingly, for nystatin, the presence of sterol in the membrane, in the range of concentrations studied, is not able to induce a proper geometry and/or vicinity of the two chromophores. We are presently involved in a study of fluorescence quenching to elucidate the location of these chromophores in the bilayer.

CONCLUSIONS

A fluorescence study of the polyene antibiotics nystatin and filipin in aqueous solution and in a model system of membranes was carried out in the presence and absence of cholesterol. It was concluded that: (i) these antibiotics aggregate in aqueous solution, its c.m.c. being 1–3 μM as determined from ligth scattering intensities; anisotropy measurements reveal that the pentaene chromophore depolarization dynamics is not controlled by the aggregate size; (ii) the antibiotics partitionate strongly to the sterol aggregates but the spectral alterations are due to an exciton interaction between the polyenes and cannot be ascribed to any antibiotic-sterol complex; (iii) sterols are very efficient on inducing a proper geometry of the aggregates (close vicinity and parallel arrangement of the transition dipoles); (iv) the photophysical parameters of filipin micelles are quite similar to the filipin interacting with the sterol; in contrast, nystatin undergoes a large increase in lifetime and quantum yield, indicating a greater rigidity of the molecule upon its interaction with cholesterol; (v) while sterol can induce exciton formation in filipin incorporated in lipid bilayers, this effect is not observed for nystatin.

REFERENCES

1. Kinsky, S., Luse, S., and Van Deenen, L. L. M. (1966) *Fed. Proc.* **25,** 1503–1510
2. Lampen, J. E., Arrow, P. M., and Safferman, R. S. (1960) *J. Bacteriol.* **80,** 200–206
3. Weete, J. D. (1973) *Phytochemistry* **12,** 1843–1864
4. De Kruijff, B., and Demel, R. A. (1974) *Biochim. Biophys. Acta* **339,** 57–70

5. De Kruijff, B., Gerritsen, W. J., Oerlemans, A., Van Dijck, P. W. M., Demel, R. A., and Van Deenen, L. L. M. (1974) *Biochim. Biophys. Acta* **339,** 44–56
6. Elias, P. M., Friend, D. S., and Goerke, J. (1979) *J. Histochem. Cytochem.* **27,** 1247–1260
7. Severs, N. J., and Robenek, H. (1983) *Biochim. Biophys. Acta* **737,** 373–408
8. Medoff, G., Brajtburg, J., and Kobayashi, G. S. (1983) *Annu. Rev. Pharmacol. Toxicol.* **23,** 303–330
9. Drabikowski, W., Łagwińska, E., and Sarzała, M. G. (1973) *Biochim. Biophys. Acta* **291,** 61–70
10. Miller, R. G. (1984) *Cell Biol. Int. Rep.* **8,** 519–535
11. Norman, A. W., Demel, R. A., De Kruyff, B., and Van Deenen, L. L. M. (1972) *J. Biol. Chem.* **247,** 1918–1929
12. Norman, A. W., Demel, R. A., De Kruijff, B., Geurts van Kessel, W. S. M., and Van Deenen, L. L. M. (1972) *Biochim. Biophys. Acta* **290,** 1–14
13. Schroeder, F., Holland, J. F., and Bieber, L. L. (1972) *Biochemistry* **11,** 3105–3111
14. Milhaud, J., Bolard, J., Benveniste, P., and Hartmann, M. A. (1988) *Biochim. Biophys. Acta* **943,** 315–325
15. Schroeder, F., Holland, J. F., and Bieber, L. L. (1973) *Biochemistry* **12,** 4785–4789
16. Bittman, R., Chen, W. C., and Anderson, O. R. (1974) *Biochemisty* **13,** 1364–1373
17. Bittman, R., and Fischkoff, S. A. (1972) *Proc. Natl. Acad. Sci. U. S. A.* **69,** 3795–3799
18. Petersen, N. O. (1985) *Can. J. Chem.* **63,** 77–85
19. Sklar, A., Hudson, B. S., Peterson, M., and Diamond, J. (1977) *Biochemistry* **16,** 813–819
20. Bergy, M., and Eble, T. (1968) *Biochemistry* **7,** 653–659
21. Chalpin, D. B., and Kleinfeld, A. M. (1983) *Biochim. Biophys. Acta* **731,** 465–474
22. Eaton, D. F. (1988) *J. Photochem. Photobiol.* **82,** 523–530
23. Phillips, D., and O'Connor, D. V. (1984) *Time Correlated Single Photon Counting*, pp. 171–190, 254–261, Academic Press, London
24. Zucker, M., Szabo, A. G., Bramall, L., Krajcarski, D. T., and Selinger, B. (1985) *Rev. Sci. Instrum.* **56,** 14–22
25. Tancrède, P., Barwicz, J., Jutras, S., and Gruda, I. (1990) *Biochim. Biophys. Acta* **1030,** 289–295
26. Cantor, C. R., and Schimmel, P. R. (1980) *Biophysical Chemistry* Vol. II, pp. 390–398, W. H. Freeman & Co, New York
27. Israelachvili, J. (1985) *Intermolecular Surface Forces*, pp. 229–245, Academic Press, London
28. Chihara, K., Takemura, T., Yamaoka, T., Yamamoto, N., Schaffer, A., and Becker, R. S. (1979) *Photochem. Photobiol.* **29,** 1001–1008
29. Das, P. K., and Becker, R. S. (1980) *Photochem. Photobiol.* **32,** 739–742
30. Sklar, L. A., Miljanich, G. P., and Dratz, E. A. (1979) *Biochemistry* **18,** 1707–1716
31. Suzuki, H. (1967) *Electronic Absorption Spectra and Geometry of Organic Molecules*, Chapters 4 and 20, Academic Press, London
32. Ernst, C., Grange, J., Rinnert, H., Dupont, G., and Lematre, J.(1981) *Biopolymers* **20,** 1575–1588
33. Neckers, D. C. (1989) *J. Photochem. Photobiol. A Chem.* **47,** 1–29
34. Mazerski, J., Bolard, J., and Borowski, E. (1982) *Biochim. Biophys. Acta* **719,** 11–17
35. Hemenger, R. P., Kaplan, T., and Gray, L. J. (1983) *Biopolymers* **22,** 911–918
36. Becker, R. S. (1988) *Photochem. Photobiol.* **48,** 369–399
37. Houdson, B., and Kohler, B. (1974) *Ann. Rev. Phys. Chem.* **25,** 437–460
38. Sklar, L. A., Hudson, B. S., and Simoni, R. D. (1977) *Biochemistry* **16,** 819–828