Binding at Lipid Surfaces: Nystatin

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The binding of the antibiotic, nystatin, to aqueous dispersions of phospholipids was measured by separating a lipid pellet from the system. The pellet was dissolved in methanol, and the amount of nystatin bound was determined spectrometrically. The bindings by dimyristoyl and dipalmitoyl lecithin in the gel phase were strong; these, however, decreased sharply above the temperature for the transition to the liquid crystal form. The binding by egg lecithin was small but increased when codispersed with cholesterol. The presence of cholesterol in whole brain lipids also increased the binding. The greater binding in the gel phase of lecithins containing saturated hydrocarbon chains and the increased binding obtained by the addition of cholesterol to egg lecithin or the presence of cholesterol in brain lipids is related to the increased ordering of the hydrocarbon chains. The variation of the binding with the concentration of nystatin was linear on a log plot with a slope that increased with the disorder or mobility of the lipid molecules.

INTRODUCTION

In order to gain an insight into the nature of the role of phospholipids in membrane behavior, several recent investigations have been directed toward a study of the physical characteristics of aqueous systems of synthetic phospholipids consisting of a single molecular form. Thermal calorimetric, infrared, and X-ray studies (1), as well as turbidity (2), nmr (1,3, 4), electron spin-label (5), and fluorescent probe (6) measurements, have shown the nature of the structural changes that occur as these compounds, in an aqueous medium, undergo a transition from the gel to the liquid crystal (LC) form. Earlier investigations in this laboratory have been concerned with the changes in the ionization, hydration, and reactions with cations of these lipids that accompany the structural changes in this phase transition. In the present paper, these studies are extended to an antibiotic compound, nystatin, which is known to increase the permeability of membranes as shown by studies of its effects on cell membranes (7), bilayer lipid films (8,9,10), and liposomes (11). A recent paper (12) reviews some of these interactions. In all of these, a primary step leading to the observed effects is the binding of nystatin present in the aqueous medium with the membrane surface. We report here on this binding of nystatin to lipids and describe the effect of the gel–LC phase transition as well as cholesterol on this binding.

MATERIALS AND METHODS

Egg lecithin[2] from Sylvana Company, and $diC_{16}PC$ and $diC_{14}PC$ from Mann Research Laboratory or LaMotte Chemical Company were purified by silica gel chromatography. Thin layer chromatograms showed no foreign

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[2] Abbreviations used in this paper are: egg PC, egg lecithin; $diC_{16}PC$, 1-2-dipalmitoyl-L-lecithin; $diC_{14}PC$, 1-2-dimyristoyl-L-lecithin.

bands. Cholesterol was obtained from Eastman Kodak Company. Whole beef brain lipids and the brain lipids freed of cholesterol by extraction with acetone were gifts from Dr. A. Finkelstein. Commercially available nystatin (Squibb) was used. For these experiments the nystatin was dissolved in spectroscopic grade methanol to give a concentrated solution of approximately 2000 μg/ml. In preparing the more concentrated solution, a small residue of solid remained undissolved. This was removed by centrifugation and weighed, and the concentration of the solution was corrected accordingly. The solutions of nystatin were stored at 10°C in the dark. Their concentrations were checked periodically by diluting a portion and measuring its absorption spectrum in the manner used for the other experiments in this investigation.

Although some experiments required modification, the procedure used for preparing most of the systems studied was the following. In the case of egg PC, for example, a quantity of the lipid dissolved in chloroform–methanol (2:1 by volume) was transferred to a stoppered 30-ml tube. The amount chosen was sufficient to give 6–8 aliquots of 1.2–2.0 mg lipid. The solvent was evaporated by a stream of nitrogen, and the tube placed in vacuum overnight. Five milliliters water (redistilled from Pyrex) was added along with two glass beads, and the tube was placed on a shaker for 30 min. This treatment swelled the lipid and caused it to come free from the glass walls. The coarse dispersion was transferred to a 20-ml sonicating tube, and the system while cooled by an ice–water mixture was exposed to 1 min of low power ultrasonic radiation from an MSE generator. The more uniform dispersion formed this way was then diluted with water to a volume to give the desired number of aliquots of 2.5 ml volume. For lipids mixed with cholesterol, a weighed amount of cholesterol was placed in the tube; the lecithin in solution was added with 1 ml of solvent to aid mixing. After drying, the weight of the lipids was determined. This lipid mixture was then dispersed by the method described. The individual systems were prepared by transferring 2.5 ml of the sonicated dispersion to a 5-ml volumetric flask and adding the required salt solution. The nystatin solution was then added from the concentrated methanolic stock solution using a Hamilton microliter syringe. The concentrations of nystatin did not exceed 6×10^{-4} M because it was found to precipitate from the system at higher concentrations. The final volumes were brought to 5 ml.

The systems were then shaken for 1 hr at the desired temperatures. An initial experiment was performed with each type of lipid system studied. In these, a relatively high concentration of nystatin (5×10^{-5} M) was added to the lipid dispersion, and the spectrum was obtained. The purpose was to determine whether the interaction of nystatin produced spectral changes compared to the spectrum of aqueous systems without lipids. In all other experiments, the systems were centrifuged 20 min at 20,000g at the interaction temperatures in Corex tubes. (The only exception to this procedure was in the case of the beef brain lipids, where the interaction temperature was 23°C and the centrifugation was done at 2°C). The supernatant solution was removed by means of a pipet, and the areas surrounding the pellet and the walls of the tube were wiped thoroughly using cotton swabs. The pellet in each tube was dissolved in 5 ml methanol and mixed thoroughly for spectroscopic analysis.

The method used to determine the amount of nystatin bound was to measure the optical density (OD) of the 319-nm component of the strong multipeaked absorption band in the spectra of the dissolved pellets. This spectrum was the same as reported by others (13). A model 350 Perkin Elmer double beam spectrometer was used with 1-cm path length quartz cuvettes.

The amount of bound nystatin was obtained from the measured OD by the use of a calibration curve obtained in the following way. A dispersion of the lipid was formed and centrifuged as in the binding experiments. The resulting pellet was dissolved in methanol, and measured amounts of nystatin were added. The

OD of the 319-nm band for a series of different nystatin concentrations gave the calibration curve. The weight of the lipid to which the nystatin was bound was determined after the spectrum was recorded. The methanolic solution of the lipid system was transferred from the cuvette and the Corex tube along with methanol rinsings to a weighed dish. After drying, the weight of the lipid was obtained. It was found that each pellet contained 70–80% of the amount of the lipid used, independent of the nystatin concentration or type of lipid.

The uncertainty of the calibration procedure was of the order of 10–15%. However, the greatest uncertainty in the measurements was due to the difficulty in determining the baselines for measurements of the OD. The best procedure was to first obtain the sample spectrum without any reference. The spectrum of the lipid alone dissolved in methanol was then recorded directly under the sample spectrum. Small amounts of undissolved matter and micellar dispersions gave a background spectrum due to scattering. This spectrum served as a guide for sketching the baseline.

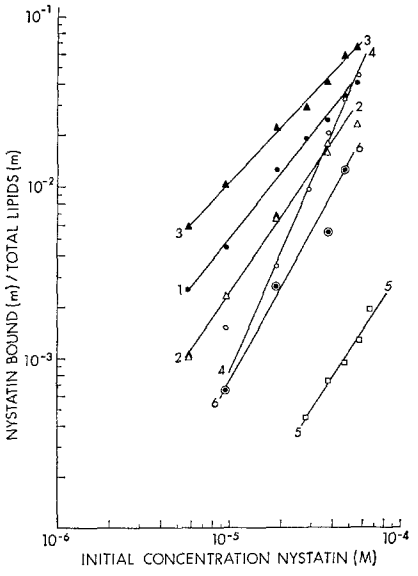

FIG. 1. Binding of nystatin to lipid dispersions. Each system contained 1.2–1.8 mg of lipid sonicated in water. Systems were brought to 0.03 *M* NaCl. Ordinates show nystatin bound to pellet obtained after 1 hr at the indicated temperature. (1) $diC_{16}PC$, 31°C; (2) $diC_{16}PC$, 43°C; (3) $diC_{14}PC$, 15°C; (4) $diC_{14}PC$, 31°C; (5) egg PC, 30°C; (6) egg PC–cholesterol (62%: 38%), 30°C.

The method most frequently used by investigators for the spectroscopic determination of the amount of a bound chromophore has been to obtain the spectrum of the supernatant solution. From the OD of a chromophore band, the concentration of the chromophore in the supernatant is found, and the difference between this and the initial concentration gives the amount removed by binding (13). This method proved unsatisfactory in the present studies because the amount of bound nystatin was usually a small fraction of the total, hence the subtraction of two large concentrations would result in large uncertainties.

RESULTS

A series of experiments conducted to measure the binding of nystatin to different lipid systems is presented in Fig. 1 and Table I. The results show a wide range in the binding, varying with the type of lipid and nystatin concentration. The greatest binding of nystatin was exhibited by $diC_{14}PC$ and $diC_{16}PC$. The effect of cholesterol on some lipid systems which bind nystatin more weakly is shown in Table I. After removal of the cholesterol from mixed brain lipids, the binding is sharply reduced. Adding back cholesterol increases the binding again. A similar change is noted with egg lecithin on the addition of cholesterol.

The logarithmic plots of Fig. 1 show a linear relation between the binding and the initial concentration of the nystatin added to the system. The abscissa in these graphs is given as the total initial nystatin concentration, since no data is available on the micellar characteristics of nystatin in the aqueous medium. Each lipid system shows not only a different amount of binding but also a characteristic slope which changes with the state of the lipid, as shown by the graphs for $diC_{14}PC$ and $diC_{16}PC$ below and above their transition temperatures (23°C and 41°C, respectively). In all these systems, an ionic concentration of 0.03 *M* NaCl was maintained to aid in the separation of the pellet.

TABLE I

THE EFFECT OF CHOLESTEROL ON THE BINDING OF NYSTATIN BY LIPID SYSTEMS (T = 23°C)

Lipid system[a]	Nystatin concentration ($\times 10^{-5}$ M)	NaCl (M)	Nystatin bound (moles) / Total lipid (moles) ($\times 10^{-3}$)
Egg lecithin	1.9	0.03	0.25
	3.8	0.03	0.8
Egg lecithin (62%); Cholesterol (38%)	1.9	0.03	2.2
	3.8	0.03	9
Whole brain lipids (bovine)[b]	2.1	0.15	13
Cholesterol-free brain lipids[c]	2.1	0.15	1.2
	5.5	0.15	3.1
Cholesterol-free brain lipids (47%) + added cholesterol (53%)	2.1	0.15	9.2
	5.5	0.15	25

[a] The composition of mixed lipids is given as approximate mole percent.
[b] An approximate average molecular weight of 640 was used.
[c] An approximate average molecular weight of 790 was used.

An estimation of the magnitude of the errors in these determinations was made from duplicate samples. Two aliquots of the same dispersion were measured with the same concentration of nystatin. Also, systems prepared at different times from different dispersions of the same lipids were compared. The errors ranged from 10 to 15% in the systems with strong binding and from 25 to 40% when the binding was weak.

In Fig. 2 is shown the effects of temperature on the binding of nystatin to four different lipid systems. Two of these, namely, $diC_{14}PC$ and $diC_{16}PC$ are shown at temperatures above and below their gel–LC phase transitions. The egg PC and the mixed egg PC–cholesterol systems are in the LC (lamellar) form throughout the temperature range investigated. The binding to $diC_{16}PC$ increases with temperature to approximately 30°C and then drops sharply at temperatures above 37°C; with $diC_{14}PC$, the binding drops above 18°C. Egg PC and egg PC–cholesterol mixtures show smaller temperature effects, with binding decreasing above 15–25°C. The data in Fig. 2 was obtained with systems prepared with the concentration of 3.8×10^{-5} M nystatin. In another series with 1.8×10^{-5} M nystatin, the binding showed changes with temperature that were parallel to the changes shown in the figure.

Associated with these temperature effects on binding are changes in the slopes as shown in Fig. 1. The slope of the logarithmic plot of the binding for $diC_{14}PC$ at 15°C is 1.0 but changes to 2.5 at 31°C. For $diC_{16}PC$ the change in slope is smaller; from 1.2 at 31°C to 1.5 at 43°C. Egg PC and mixed egg PC–cholesterol systems had slopes of 1.7–1.8 which showed no significant change from 20 to 30°C.

To find the pH dependence of the reaction of nystatin with lipids, a series of experiments were made with aliquots of dispersions of ap-

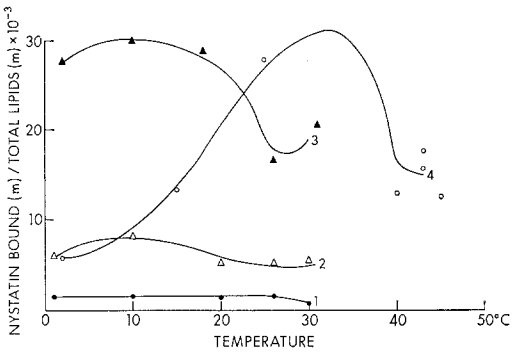

FIG. 2. Effect of temperature on the binding of nystatin to lipids. Each system contained 1.2–1.8 mg of lipid sonicated in water at 2°C ,then brought to the indicated temperature and adjusted to 0.03 M NaCl and 3.8×10^{-5} M nystatin. (1) Egg PC; (2) egg PC–cholesterol (62%: 38%); (3) $diC_{14}PC$; (4) $diC_{16}PC$.

proximately equimolar egg PC–cholesterol in 0.05 *M* Tris buffer +0.1 *M* NaCl. The pH was adjusted to several levels by the addition of HCl or NaOH. When the dispersion was brought to the desired pH, nystatin was added to give a concentration of 4.5×10^{-5} *M*. After standing 1 hr and centrifugation, the pH of the supernatant solution was redetermined. This showed either no difference from the original value or a maximum change of 0.05 pH units. The binding of nystatin was found to drop sharply in the systems where the pH was higher than 7–8.

In an attempt to ascertain the nature of the effect of elevated pH, one system with nystatin added to equimolar egg PC–cholesterol in Tris buffer was brought to pH 9 and allowed to stand for 45 min. The pH was then lowered to 7 and it remained for 45 min at this level. The amount of nystatin found on the lipid particles was low and equal to the small value for the system that remained at pH 9 for the entire period. In still another experiment, two systems were used. In one, the nystatin without lipid was maintained in a similar buffer solution at pH 9 and then brought to pH 7 at which point the lecithin–cholesterol dispersion was added. This was paralleled by another experiment in which the lipid mixture alone was kept at pH 9, and after standing was brought to pH 7, and then addition of the nystatin was made. In both experiments, the binding of nystatin was reduced sharply from the amount observed for systems at pH 7. The reduction was greatest in the system in which the nystatin was maintained at an elevated pH level before adding the lipid dispersion.

A few experiments were able to show that the binding of nystatin to the lipids increased with increased dispersion and surface area of the particles. A dispersion in water of equimolar egg PC–cholesterol was prepared by mechanical shaking with the aid of a few glass beads. In a solution of 0.15 *M* NaCl and 5.1×10^{-5} *M* nystatin, it gave a binding ratio of 4.2×10^{-3} mole nystatin/mole lipid. For comparison, a like system that had been exposed to ultrasonic radiation for 1 min gave a binding ratio of 6.4×10^{-3} mole nystatin/mole lipid. A dispersion of cholesterol produced by vigorous sonication in water combined with a ratio of 1.3×10^{-2} mole nystatin/mole cholesterol in 0.15 *M* NaCl and 4.9×10^{-5} *M* nystatin. Another dispersion was formed by injecting a methanolic solution of cholesterol into an aqueous solution of NaCl and nystatin. It was now finely dispersed and combined in the ratio of 5.2×10^{-2} mole nystatin/mole cholesterol, an increase by a factor of four.

Studies were made to determine whether the binding of nystatin to the lipid is reversible. These showed that the nystatin could be desorbed when the supernatant solution was removed and replaced by a solution that did not contain nystatin. One of the several experiments to establish this point proceeded as follows. Aliquots of a dispersion of whole brain lipids were brought to a concentration of 0.3 *M* NaCl and 2×10^{-5} *M* nystatin and allowed to stand 1 hr. These were then centrifuged, and the supernatant solutions were removed. The pellets were resuspended in another equal volume of 0.3 *M* NaCl alone. The total amount of nystatin in this resuspended system was determined spectrometrically and was found to be 2.6×10^{-2} μmole. This nystatin had been bound to the lipid surfaces before the particles were resuspended. This suspension was centrifuged, and the supernatant solution was removed. The resuspended pellet was then found to have retained 0.76×10^{-2} μmoles nystatin, or 71% of the nystatin had been removed by resuspending the pellet in NaCl solution. Other similar experiments showed in a like manner that a major fraction (~70–80%) of the nystatin bound to the lipid was released when the particles were resuspended in an aqueous medium.

A somewhat different experiment was able to show that nystatin does not readily diffuse through the lipid layers. This experiment was performed by entrapping nystatin within the multilayered lipid structures in the following manner. A solution of equimolar quantities of egg PC and cholesterol in chloroform–methanol was evaporated in a glass tube leaving a solid

deposit of the mixed lipids to which was added a solution containing 0.15 *M* NaCl and 2.6×10^{-5} *M* nystatin. The lipids were dispersed on a shaker with the aid of a few glass beads. The volume of the dispersed system was sufficient to divide into several equal aliquots. One of these was centrifuged, and the pellet was dissolved for analysis. This showed that a total of 2.71×10^{-3} mole nystatin/mole lipids had been either entrapped within the lipid or was bound to the surfaces. Two other aliquots were centrifuged, but the pellets, after removal of the supernatant, were resuspended in 0.15 *M* NaCl and allowed to stand for 20 min and 1 hr, respectively. After centrifugation, the pellets were dissolved in methanol, and the amount of nystatin that had not escaped from the lipid was found to be 1.75×10^{-3} mole/mole lipid for both the 20-min and 1-hr samples. This showed that 65% of the total nystatin present initially in the pellet was entrapped and did not pass through the lipid layers during 1 hr.

DISCUSSION

In discussing the results of this investigation, we will direct our attention to several issues concerning the interaction of nystatin with lipids. These are principally: (*1*) the effect of lipid structure and the changes in structure resulting from temperature changes and (*2*) the role and importance of cholesterol. In addition, we will briefly discuss the effects of pH on the binding.

The effect of lipid structure on the binding of nystatin is seen most dramatically in Fig. 2, with the sharp decrease in binding for both $diC_{14}PC$ and $diC_{16}PC$ at the temperatures at which they undergo the transition from the gel to the LC (lamellar) phase. In contrast, one sees that for both egg PC and the egg PC–cholesterol mixtures there is little temperature effect. These lipids are solely in the LC phase in the temperature range shown. In Fig. 1 one can distinguish the two different lipid phases by the slopes of the logarithmic binding curves. The slopes for $diC_{14}PC$ and $diC_{16}PC$ which are in the gel phase have values of 1.0 and 1.2, respectively (curves 3 and 1), while for the lipids in the LC phase the slopes are larger than 1.7. (The slope of $diC_{16}PC$ in the gel phase at 43°C is only 1.5, probably because the temperature is too close to the transition. In fact, several points taken at 45°C but not shown in Fig. 1 gave a slope of 1.7.)

Physical-chemical studies show that the gel phase of lecithin consists of aggregates of bilayers (1). The hydrocarbon chains are quite rigid and approximately perpendicular to the aqueous lipid interface. In the LC phase these are transformed to lamellar arrays of bilayers, which may assume the form of multi-layered vesicles (liposomes). In this phase the hydrocarbon chains and the polar head groups are no longer rigid. There is also increased lateral spacing between the lipid molecules with the resulting increased hydration and ionization of the polar groups.

The most plausible relationships between these structures and our binding data can be stated as follows: (a) the binding of nystatin is enchanced by increased rigidity of the hydrocarbon chains, while (b) the slope of the logarithmic binding curve is increased by increased fluidity, mainly of the polar groups of the molecules. Let us now examine the reasonableness of these correlations. It is known from monolayer studies that nystatin interacts with lipid monolayers by penetrating between the hydrocarbon chains (11). (The molecular structure of nystatin contains a long ring with a tetraene and diene unsaturations, giving a rod-like configuration.) Hence, this penetration is aided in the case of the gel phase by the greater order of the hydrocarbon chains and inhibited by the disorder in the LC phase. With respect to correlate (b), the slope of the binding curves is a measure of the cooperativity of the interaction between the nystatin molecules and the lipid molecules. One can visualize that the penetration of one polyene molecule reduces somewhat both the mobility of the neighboring hydrocarbon chains and polar head groups in a manner that facilitates the interaction with successive polyenes. Another example of this type of effect can be seen in the work of Pache and Chapman (14). They showed by calori-

metric and spin-label measurements that, when the antibiotic chlorothricin penetrates into the hydrocarbon region of egg lecithin, it reduces the chain mobility of the hydrocarbons. Haynes (15) has also found that the effects of the gel–LC transition on the binding characteristics of $diC_{16}PC$ lecithin for other antibiotics which penetrate lipid layers, e.g., valinomycin and monactins, are similar to what we report here, the binding decreasing above the transition temperature of the lecithin.

Until now we have not considered the amount of available surface as a factor in the binding. However, it may be responsible for the large increase in binding shown by $diC_{16}PC$ between 2°C and 30°C. Several types of measurements indicate that changes occur in lecithin–water at temperatures below the mesomorphic transition. Differential thermal analysis measurements (16) show a broad endothermal range between the ice–melting temperature and the transition to the LC phase. This is also accompanied by a large decrease in the turbidity of the system (2) together with increased hydration and ionization (17). As the temperature approaches the transition temperature, one can view the changes that take place as an increased penetration of water between the layered polar faces. This penetration of water, with an associated increase in the availability of surface sites, could lead to increased binding. We also have to consider a decrease in binding sites that may arise in the gel-LC transition due to the formation of closed vesicles with the inner layers not exposed to the antibiotic in the solution. This probably accounts for some of the decreased binding at the transition temperature. We showed this is an experiment in which a dispersion of $diC_{16}PC$ was divided into two aliquots. One was brought to 30°C, and four portions were reacted with four different concentrations of nystatin at 30°C and then maintained at this temperature. The other aliquot was first maintained for an equal time at 43°C, then brought to 30°C, and reacted with similar concentrations of nystatin at 30°C. The bindings for the latter were about 70–80% of the former. The slopes for the binding in both series were about 1.2, characteristic of the gel structure. Since others have shown that on cooling from above the transition temperature to the gel phase the vesicle structures are maintained (3), we ascribe this loss in binding to a loss in binding sites. We will also present some arguments to indicate that it is improbable that the binding curves shown in Fig. 1 result from an increase in surface area. The binding was found to vary with nystatin concentration in the manner of: $B \alpha C_n^x$. If this resulted mainly from a change in area, the latter would also depend on a power of C_n. It is unlikely that each of the four lipid systems shown would behave in this unique manner.

In the present study, we find that the addition of cholesterol to egg PC and sterol-free brain lipids greatly enhances the binding of nystatin. The explanation for this appears to lie in the effect of cholesterol on the structure of these lipids. Table I summarizes the changes in binding that takes place with the addition of cholesterol.

By means of nmr (3,4) and spin label resonance (5) spectroscopy, it has been shown that cholesterol added to egg lecithin restricts the motion of the $N(CH_3)_3$ groups to a small degree but reduces the mobility of the hydrocarbon chains to a large degree. The authors of the nmr study point to the similarity in the effects of cholesterol on egg lecithin to that obtained by cooling $diC_{16}PC$ below the transition temperature. Similar results have been obtained for the addition of cholesterol to the LC phase of $diC_{16}PC$ (4,18). Furthermore, consistent with these results are the finding of Butler *et al.* (19), who showed by electron spin label resonance that the presence of cholesterol in beef brain lipids caused orientation of the hydrocarbon chains in the direction perpendicular to the lamellar faces. We can thus qualitatively account for the increased binding produced when cholesterol is added to egg lecithin and sterol-free brain lipids as due to the effects of the increased order of the hydrocarbon chains.

Two other explanations can be suggested for

the increased binding that results when cholesterol is present in the lipids: (a) a nystatin-cholesterol complex may form, and (b) surface areas of the liposomes may increase. The first is questionable, since the spectra of nystatin added to dispersions of cholesterol mixed with egg PC and cholesterol mixed with sterol-free brain lipids was the same as the spectra in dispersions of cholesterol-free lipids. This contrasts with the work of Norman *et al.* (20), who found the spectrum of filipin to change in the presence of cholesterol. This is ascribed to the formation of a filipin-cholesterol complex. For the second, we refer to some recent studies by Finer *et al.* (21,22) who show that, for egg PC, low power sonication similar to our procedure forms small spherical vesicles (260 Å) composed of a single bilayer as well as large multilamellar particles with diameters ranging from 0.2 to 4 μm. The distribution between the two types depends on the sonication time. From their data, we find that our procedure gave equal numbers of the two types. An approximate calculation shows that the small spheres contribute more than 90% of the total surface area of the egg PC dispersions. These investigators also show that the spherical vesicles are responsible for the narrow line nmr spectrum of egg PC. Adding cholesterol to egg PC causes a broadening of the lines associated with the hydrocarbon chains protons but almost no change in the line due to the protons of $-N(CH_3)_3$ group. Since this group is oriented at the surface of the particle in the presence of water, we infer from these results that the number and size of the small vesicles is roughly the same in dispersions of egg PC and egg PC–cholesterol. To this can be added the observation that the nmr spectrum of sonicated dispersions of $diC_{16}PC$ in the LC phase gives a line for the $-N(CH_3)_3$ protons similar to that obtained for egg PC and egg PC–cholesterol (4), suggesting that the surface areas of these systems are similar.

In discussing the effect of pH on the binding of nystatin we must recognize that changes in pH may also produce changes in the structure of the dispersed lipid which may lead to changes in binding. The major effect is seen to be a sharp decrease in binding at elevated pH levels. Bringing the nystatin alone to a pH >9 produced an irreversible decrease in binding. This could be explained as due to the base-catalyzed hydrolysis of the lactone ring which alters the structure and characteristics of nystatin. Bringing the lecithin to pH 9 and then to neutral resulted in a smaller decrease in binding of nystatin than in the preceding experiment in which the pH of nystatin was changed. The effect of elevated pH on lecithin is to increase the ionization of the phosphoryl choline moiety. This increases the hydration of the lipid. When the pH is brought to neutral, the lecithin remains in the highly hydrated state for a long period. It was shown by Levine and Wilkins (23) that increased hydration of lipid bilayers tend to disorient the hydrocarbon chains, in fact leading to the conditions we find for reduced binding.

SUMMARY

It is our belief that studies such as those described here may aid in formulating some general concepts concerning the effect of molecular order on the reactions of biological membranes. Using well-defined single compounds, as the synthetic phospholipids which undergo precise physical changes, we showed how these changes may alter the binding characteristics of the lipid. Furthermore, the incorporation of cholesterol into more complex membrane matter, such as the brain lipids, produces changes in the reaction with nystatin similar to that shown by adding cholesterol to egg lecithin. Our work leads to the view that the degree of order of the hydrocarbon chains of the membrane lipids may alter the binding characteristics.

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