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FLUORESCENT PROBE STUDIES OF MIXED MICELLES OF PHOSPHOLIPIDS AND BILE SALTS

EFFECT OF CHOLESTEROL INCORPORATION

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Summary

The binding of the fluorescent alkylamines, *N*-(2-aminoethyl)-5-dimethylamino-1-naphthalene sulfonamide, *N*-(5-aminopentyl)-5-dimethylamino-1-naphthalene sulfonamide (dansyl cadaverine) and *N*-(10-aminodecyl)-5-dimethylamino-1-napthalene sulfonamide with phospholipid and phospholipid-deoxycholate micelles, has been shown to increase with the length of the alkyl spacer chain. The probes bind more effectively to micelles containing unsaturated phospholipids and do not interact strongly with bile salt solutions at low concentrations. Cholesterol incorporation into mixed micelles results in a quenching of probe fluorescence due to displacement of probe molecules. The enhanced rigidity of the mixed micelles on solubilizing cholesterol is established by a decrease in pyrene excimer fluorescence and by the less effective perturbation of the micellar structure by 1-anilino-8-naphthalene sulfonate. The anionic probe 1-anilino-8-naphthalene sulfonate is also displaced from the mixed micelles when cholesterol is incorporated, suggesting a dominant role for packing and hydrophobic effects in binding both positively and negatively charged probes.

Abbreviations: Dansyl, 5-dimethylamino-naphthalene-1-sulfonyl; ANS, 1-anilino-8-naphthalene sulfonate; AII, *N*-(2-aminoethyl)-5-dimethylamino-1-naphthalene sulfonamide; AX, *N*-(10-aminodecyl)-5-dimethylamino-1-naphthalene sulfonamide; AV, dansyl cadaverine (*N*-(5-aminopentyl)-5-dimethylamino-1-naphthalene sulfonamide).

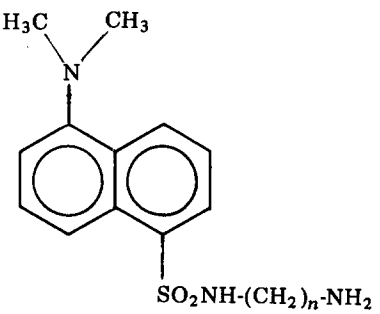
Introduction

Lipids form an important constituent of all biological membranes and an understanding of lipid organisation is a prerequisite to investigations of membrane structure and function. Aqueous dispersions of phospholipids form multilamellar structures called liposomes which have water sequestered between lipid bilayers [1]. Prolonged ultrasonication leads to the breakdown of these structures to unilamellar vesicles which are spheres enclosing a volume of aqueous solution [2,3]. Phospholipid packing in a monolayer or micelle is dependent on the degree of unsaturation of the fatty acid chains. At room temperature, saturated phospholipid monolayers are more condensed than unsaturated ones [4,5].

The solubilisation of cholesterol by mixed micelles of phospholipids and bile salts is an important biological phenomenon. It has been shown that the feeding of unsaturated fats to rats leads to greater unsaturation of fatty acyl chains [6,7]. This, in turn, leads to increased solubilisation and excretion of cholesterol through the bile [8]. Any interference in this process leads to the precipitation of cholesterol and the subsequent formation of gall stones [9].

Relatively few spectroscopic investigations of mixed phospholipid-bile salt micelles have been carried out [7]. A preliminary report from this laboratory [10] described the use of the fluorescent probe, dansyl cadaverine, in the study of mixed phospholipid-bile salt micelles. Probe fluorescence has been shown to be sensitive to fatty acid unsaturation and to the incorporation of cholesterol into mixed micelles.

In this study we describe the use of the positively charged homologous probes *N*-(5-aminopentyl)-5-dimethylamino-1-naphthalene sulfonamide (dansyl cadaverine, AV), *N*-(2-aminoethyl)-5-dimethylamino-1-naphthalene sulfonamide (AII) and *N*-(10-aminodecyl)-5-dimethylamino-1-naphthalene sulfonamide (AX):



n = 2, AII; n = 5, AV; n = 10, AX.

together with the negatively charged probe 1-anilino-8-naphthalene sulfonate (ANS) and the excimer-forming probe pyrene in the investigation of phospholipid, phospholipid-bile salt and phospholipid-bile salt cholesterol aggregates. Sodium deoxycholate is the model bile salt in these studies. Probe fluorescence is sensitive to the degree of phospholipid fatty acid unsaturation and the incorporation of cholesterol into the lipid micelles.

Materials and Methods

AII, AV and AX were prepared by dansylation of the appropriate alkylamine. An illustrative procedure has been described earlier [11]. ANS, sodium deoxycholate and dipalmitoyl phosphatidylcholine were obtained from Sigma Chemical Co., U.S.A. Pyrene and cholesterol were of the best analytical grade. Soya phosphatidylcholine containing about 70% linoleic acid was a gift from Natterman and Co., Cologne, Germany and was purified by chromatography over alumina. Egg lecithin was prepared essentially by the methods described in the literature [12,13]. Mixed micelles of phospholipids, bile salts and cholesterol were prepared by the procedure of Saunders and Wells [14]. Phosphatidylcholine and cholesterol were dissolved in chloroform and the solvent was evaporated under reduced pressure. The last traces of solvent were removed by passing a stream of nitrogen. The residue was dissolved in 0.025 M Tris-HCl buffer (pH 8.0) and sodium deoxycholate dissolved in the same buffer was added. The solution was cooled and sonicated for 45 s using a Branson sonifier (20 kHz, 75 W) and incubated for 2 h at 37°C. Mixed micelles without cholesterol and also dispersions of phosphatidylcholine alone were prepared in a similar fashion.

Alkylamine solutions were prepared in 0.025 M Tris-HCl buffer (pH 8.0) and ANS solutions in 0.025 M sodium phosphate buffer (pH 8.2). Pyrene was incorporated into the lipids by the addition of concentrated ethanol solutions of pyrene, such that the final alcohol concentration did not exceed 0.4% (v/v). Fluorescence measurements were made on a Perkin-Elmer Model 203 spectrofluorimeter. All spectra are uncorrected. The excitation wavelength for all alkylamine probes was 340 nm. For the binding experiments, alkylamine excitation and emission were fixed at 340 nm and 500 nm, respectively. ANS experiments were carried out using an excitation wavelength of 390 nm. The emission wavelength for the binding studies was 480 nm. For pyrene an excitation wavelength of 335 nm was used. The emission maxima for the monomer and dimer bands, were 390 and 470 nm, respectively. All measurements were carried out at ambient room temperature (25—27°C).

Results

Fig. 1 shows the effects of the addition of phospholipids and mixed micelles on the fluorescence spectra of AV and AX, respectively. While AV shows no change in the wavelength of maximum emission (λ_{max}) and fluorescence intensity in the presence of dipalmitoyl phosphatidylcholine and the mixed phospholipid-bile salt micelle, the addition of soya phosphatidylcholine (70% linoleic acid) and the corresponding mixed micelle results in a blue shift of λ_{max} and an enhancement of fluorescence intensity. The shorter chain probe, AII, showed small changes in the presence of soya phospholipid micelles and no change on the addition of dipalmitoyl phosphatidylcholine and the mixed micelle with bile salt (figure not shown). Fig. 1B shows that the longer homolog, AX, interacts with dipalmitoyl phosphatidylcholine and to a larger extent with soya phospholipid. The incorporation of bile salts into the lipid dispersions causes a further enhancement of AX fluorescence. The

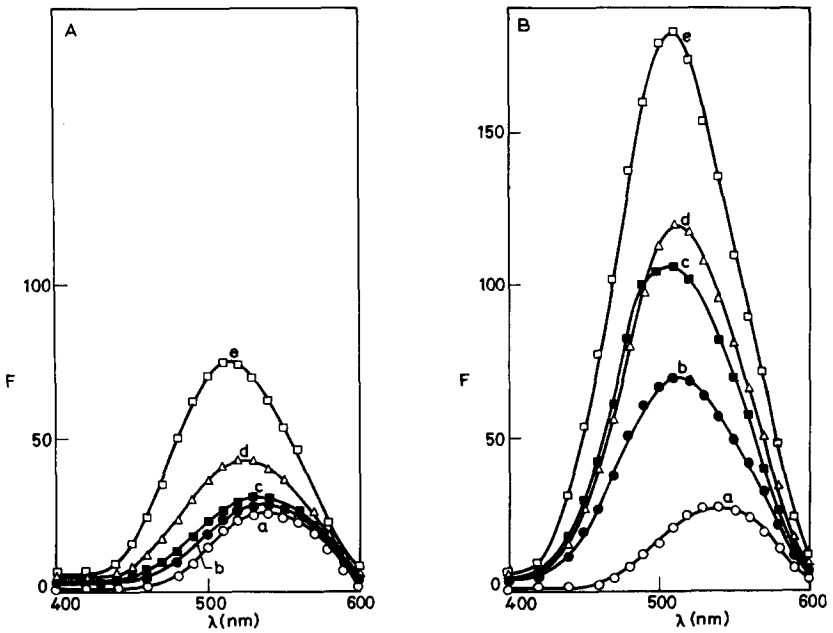


Fig. 1.A. Fluorescence spectra of AV in phospholipid and mixed phospholipid-bile salt micelles. AV concentration, 25 μM. Lipid and bile salt (sodium deoxycholate) concentration, 100 μg/ml. a, buffer; b, dipalmitoyl phosphatidylcholine; c, dipalmitoyl phosphatidylcholine + bile salt; d, egg phospholipid; e, egg phospholipid + bile salt. B. Fluorescence spectra of AX in phospholipid and mixed phospholipid-bile salt micelles. AX concentration, 25 μM. Lipid and bile salt, 100 μg/ml. a, Buffer; b, dipalmitoyl phosphatidylcholine; c, dipalmitoyl phosphatidylcholine + bile salt; d, egg phospholipid; e, egg phospholipid + bile salt.

fluorescence intensity values of AX in the presence of the various lipid dispersions follow the sequence dipalmitoyl phosphatidylcholine < dipalmitoyl phosphatidylcholine + bile salt < soya phospholipid < soya phospholipid + bile salt. Similar behavior is noted for AV. In contrast to their behavior in the mixed micelles, the fluorescence parameters of AII and AV are not affected by the addition of bile salts even at concentrations as high as 100 μg/ml, when micellar aggregates are likely to be present [15]. AX, however, shows small changes in the presence of bile salts. The results obtained for the three alkylamine probes are summarised in Table I.

Fig. 2A presents the effect of cholesterol incorporation into a dipalmitoyl phosphatidylcholine dispersion on AX fluorescence. Intensity reductions of 17% and 66% are observed on the addition of 5 and 30 μg/ml cholesterol, respectively. Similar results were obtained in mixed micelles containing deoxycholate. Fig. 2B shows 6% and 27% quenching of AX fluorescence in soya phospholipid at the same concentrations of cholesterol. Similar results were obtained for AV and AX on the incorporation of cholesterol into mixed micelles (figure not shown).

The fluorescence changes of AV and AX in the presence of various lipid dispersions have been quantitated in terms of binding stoichiometries (n) and dissociation constants (K_D) [16]. The differences in fluorescence parameters observed in the presence of various lipids and the quenching of probe fluores-

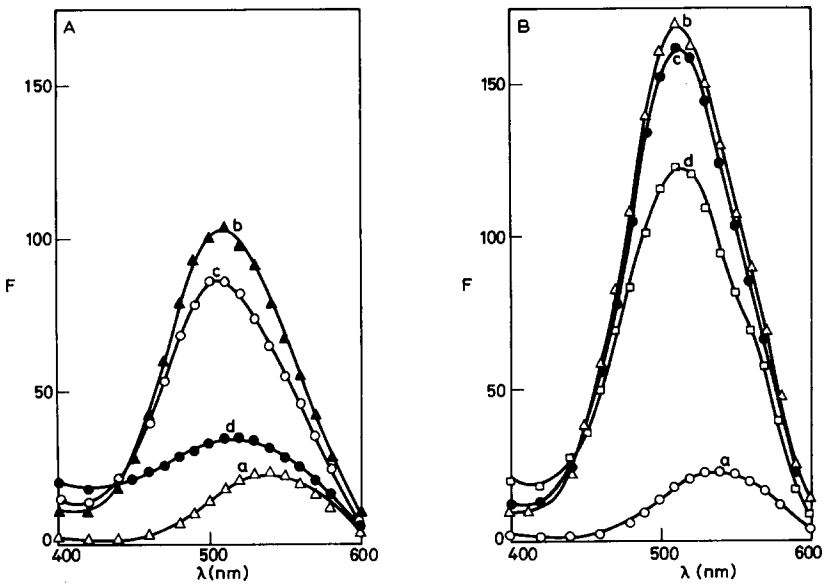


Fig. 2.A. Cholesterol effect on AX fluorescence in dipalmitoyl phosphatidylcholine micelles. AX, 10 μM; lipid, 100 μg/ml. a, Buffer; b, lipid; c, lipid + 5 μg/ml cholesterol; d, lipid + 30 μg/ml cholesterol. B. Cholesterol effect on AX fluorescence in soya phospholipid micelles. AX, 10 μM; lipid, 100 μg/ml. a, Buffer; b, lipid; c, lipid + 5 μg/ml cholesterol; d, lipid + 30 μg/ml cholesterol.

cence on cholesterol incorporation may arise due to one or more of the following factors: (a) an alteration in the number of probe binding sites which is reflected in the value of n; (b) changes in the extent of probe binding resulting in variations in the K_D values; (c) alterations in the bound quantum yield leading to different values of F_m, the limiting probe fluorescence.

Fluorescence titrations, varying probe and lipid concentrations, have been carried out. Fig. 3 shows the double reciprocal plots of the AX fluorescence intensity as a function of the lipid concentration. The limiting fluorescence

TABLE I

THE EFFECT OF LIPIDS ON ALKYLAMINE AND ANS FLUORESCENCE

Probe concentration, 25 μM; lipid and bile salt concentration, 100 μg/ml for alkylamine probes and 130 μg/ml for ANS.

Probe:	AII		AV		AX		ANS	
Lipid	I *	λ_{max} (nm)	I	λ_{max} (nm)	I	λ_{max} (nm)	I	λ_{max} (nm)
Dipalmitoyl phosphatidylcholine	1.3	540	1.1	540	2.6	510	—	—
Soya phosphatidylcholine	1.8	525	1.7	520	4.4	510	—	—
Bile salt	1.0	540	1.0	540	1.3	530	1.1	510
Dipalmitoyl phosphatidylcholine + bile salt	1.2	540	1.2	540	4.1	510	5.3	475
Soya phosphatidylcholine + bile salt	2.5	520	2.8	510	7.2	510	6.5	475
Egg lecithin + bile salt	—	—	—	—	—	—	6.9	475

* I, relative fluorescence intensity using a buffer value of 1.0.

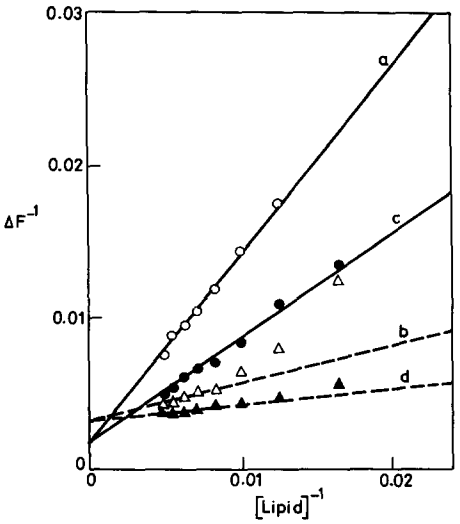


Fig. 3. Double reciprocal plots of changes in AX fluorescence (ΔF arbitrary units) as a function of lipid concentration. AX, 10 μM; lipids, 0—200 μg/ml. a, Dipalmitoyl phosphatidylcholine; b, dipalmitoyl phosphatidylcholine + bile salt; c, soya phospholipid; d, soya phospholipid + bile salt.

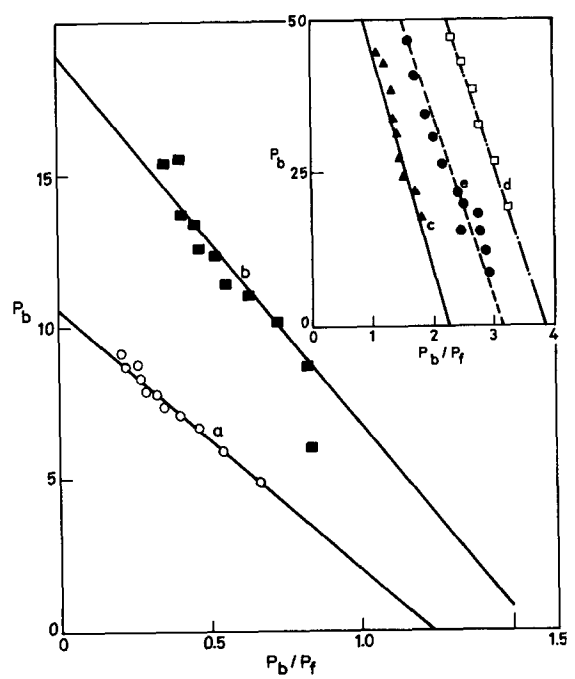


Fig. 4. Scatchard plots for the binding of AX to phospholipid and mixed micelles. AX varied from 0 to 50 μM. Phospholipids and bile salts, 100 μg/ml; cholesterol, 30 μg/ml. a, Dipalmitoyl phosphatidylcholine; b, soya phospholipid. Inset: c, dipalmitoyl phosphatidylcholine + bile salt; d, soya phospholipid + bile salt; e, soya phospholipid + bile salt + cholesterol.

TABLE II

BINDING PARAMETERS FOR THE INTERACTION OF AV, AX AND ANS WITH PHOSPHOLIPID AND MIXED PHOSPHOLIPID BILE SALT MICELLES

Data are obtained from Figs. 3, 4 and 6. Values for AV not shown.

Lipid	Probe	n (μmol/g)	K_D (M) (X10^5)
Dipalmitoyl phosphatidylcholine	AX	10.6	0.86
Dipalmitoyl phosphatidylcholine + bile salt	AX	82.0	3.64
Soya phosphatidylcholine	AX	19.0	1.22
Soya phosphatidylcholine + bile salt	AX	122.5	3.18
Soya phosphatidylcholine + bile salt + cholesterol	AX	96.5	3.06
Soya phosphatidylcholine	AV	13.8	2.12
Soya phosphatidylcholine + bile salt	AV	43.0	2.65
Dipalmitoyl phosphatidylcholine + bile salt	ANS	108.0	1.20
Soya phosphatidylcholine + bile salt	ANS	122.0	0.87
Soya phosphatidylcholine + bile salt + cholesterol	ANS	100.0	1.00
Egg lecithin + bile salt	ANS	140.0	2.60
Egg lecithin + bile salt + cholesterol	ANS	80.0	3.20

value (F_m, fluorescence of 1 μmol of bound probe) is obtained from the reciprocal of the Y-intercept divided by the probe concentration used. A value of 52.6 is obtained for F_m in phospholipid dispersions while F_m = 31.3 for the mixed micelles. Incorporation of cholesterol into soya phospholipid-bile salt micelles leaves F_m unchanged at 31.3 (figure not shown). Binding data,

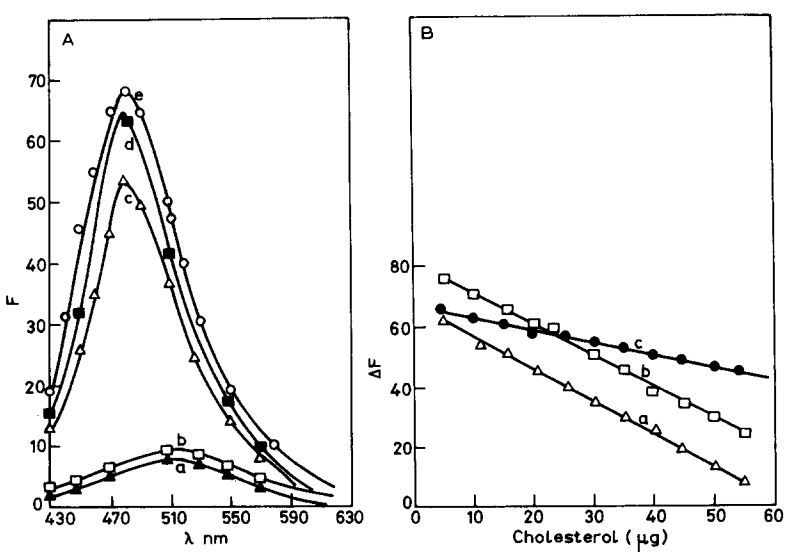


Fig. 5.A. Fluorescence spectra of ANS in mixed micelles. ANS, 25 μM; phospholipid and bile salt, 130 μg/ml. a, Buffer; b, bile salt; c, dipalmitoyl phosphatidylcholine + bile salt; d, soya phospholipid + bile salt; e, egg lecithin + bile salt. B. Effect of cholesterol incorporation on ANS fluorescence intensity in mixed micelles. ANS, 25 μM; phospholipid and bile salt, 130 μg/ml. a, Dipalmitoyl phosphatidylcholine; b, egg phosphatidylcholine; c, soya phosphatidylcholine.

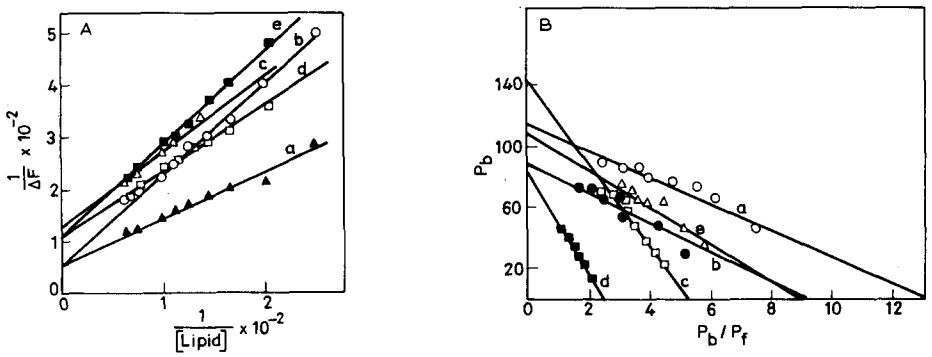


Fig. 6.A. Double reciprocal plots of changes in ANS fluorescence intensity (ΔF, arbitrary units) against varying lipid concentration. ANS, 25 μM. Lipids, 30—150 μg/ml. Cholesterol incorporated to the extent of 30% by weight. a, Egg lecithin + bile salt; b, a + cholesterol; c, dipalmitoyl phosphatidylcholine; d, soya phosphatidylcholine; e, d + cholesterol. B. Scatchard plots for the binding of ANS to micelles. Phospholipid and bile salt, 130 μg/ml; cholesterol, 30 μg/ml. ANS varied from 9 to 47 μM. a, Soya phosphatidylcholine; b, a + cholesterol; c, egg lecithin + bile salt; d, c + cholesterol; e, dipalmitoyl phosphatidylcholine + bile salt.

obtained by measurement of emission intensities as a function of probe concentration, is represented by the Scatchard plot [16] in Fig. 4. The binding parameters obtained from these plots, together with those for AV, are listed in Table II.

Fig. 5A presents the fluorescence spectra of ANS in the presence of bile salts and mixed micelles. Intensity enhancements and a blue shift in the λ_{max} values can be observed in the mixed micelles. As in the case of the alkylamine probes, deoxycholate alone, at a concentration of 130 μg/ml, has no effect on ANS fluorescence. The fluorescence parameters of ANS are listed in Table I. The effects of cholesterol incorporation into mixed phospholipid-bile salt micelles, on ANS fluorescence, are shown in Fig. 5B. Cholesterol quenches probe fluorescence to different extents in the three kinds of micelles, with the maximum quenching being observed for micelles containing dipalmitoyl phosphatidylcholine.

Fig. 6A shows the double reciprocal plot of ANS fluorescence intensity with varying lipid concentrations. The incorporation of cholesterol into mixed micelles of bile salts with egg lecithin and soya phospholipid does not alter the F_m value. The Scatchard plots for the interaction of ANS with the mixed micelles in the presence and absence of cholesterol are shown in Fig. 6B and the binding parameters are summarised in Table II.

Fig. 7 shows the variation of the pyrene dimer/monomer fluorescence intensity ratio as a function of the amount of cholesterol incorporated into soya phospholipid-bile salt micelles. Pyrene excimer fluorescence is a sensitive indicator of lipid phase mobility [17]. With increasing amounts of cholesterol, pyrene excimer fluorescence decreases with a consequent reduction in the dimer/monomer intensity ratio. This presumably follows from the decrease in lipid fluidity on cholesterol incorporation.

Amphipathic molecules like ANS have been shown to exert a disrupting influence on the structure of lipid micelles and lipoproteins [18—20]. The

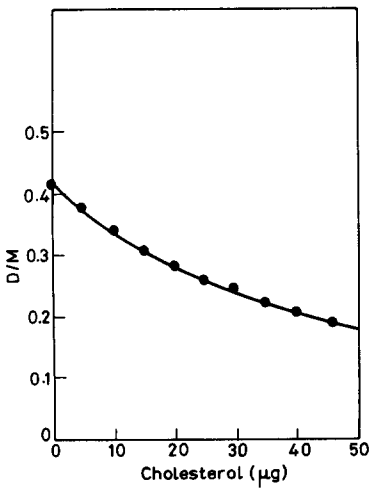


Fig. 7. Pyrene dimer/monomer (D/M) fluorescence ratio as a function of cholesterol content in soya phospholipid-bile salt micelles. Pyrene, 1.8 μM; phospholipid and bile salt, 130 μg/ml.

extent of perturbation by ANS is likely to vary with the type of lipid packing. It has been shown earlier that pyrene excimer fluorescence may be used to monitor ANS-induced perturbation at very low concentrations [19]. Fig. 8A shows the emission spectra of pyrene incorporated into soya phospholipid-bile salt micelles in the presence of ANS. The intensity of the excimer peak at 470 nm increases on addition of ANS, while a concomitant decrease is observed in the monomer fluorescence at 390 nm. The D/M ratios of pyrene

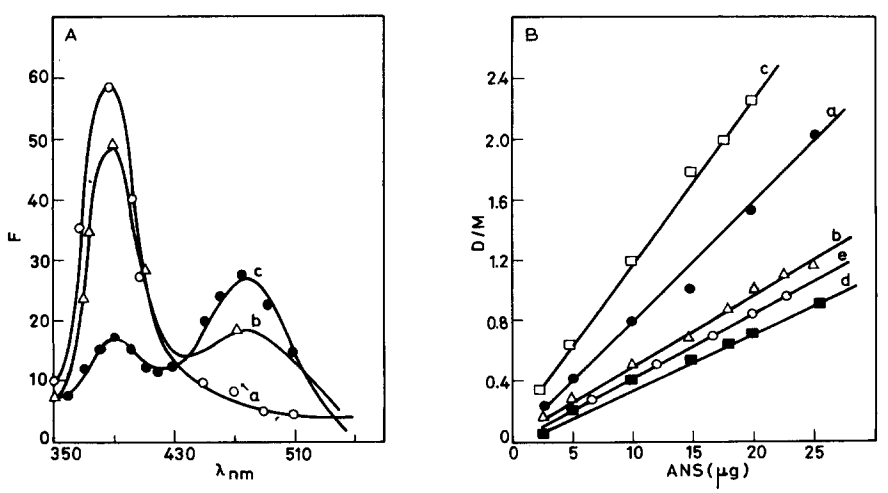


Fig. 8.A. Emission spectra of pyrene in soya phospholipid-bile salt in the presence of ANS. Phospholipid and bile salt, 130 μg/ml, pyrene, 1.8 μM. a, Pyrene free; b, pyrene + mixed micelle, c, pyrene + mixed micelle + 5 μg/ml ANS. B. Pyrene dimer/monomer (D/M) fluorescence ratio as a function of ANS concentration in various lipid micelles. Phospholipid and bile salt 130 μg/ml, cholesterol, 40 μg/ml. a, Soya phospholipid + bile salt; b, a + cholesterol; c, egg lecithin + bile salt; d, c + cholesterol; e, dipalmitoyl phosphatidylcholine + bile salt.

fluorescence as a function of ANS concentration with different types of phospholipid-bile salt and phospholipid-bile salt-cholesterol micelles are given in Fig. 8B. The incorporation of cholesterol into the phospholipid-bile salt micelles reduces the degree of perturbation by ANS as reflected in the D/M ratios. It can be seen from these plots that the perturbation produced in the soya phospholipid-bile salt-cholesterol and egg lecithin-bile salt-cholesterol micelles is significantly greater than in dipalmitoyl phosphatidylcholine-bile salt micelles. No attempt was made to investigate the perturbation of the cholesterol containing dipalmitoyl phosphatidylcholine mixed micelles, because ANS interacts very weakly with such micelles.

Discussion

The absence of change in the fluorescence parameters of AII and AV in the presence of dipalmitoyl phosphatidylcholine implies that these two probes do not penetrate the tightly packed arrangement of the phospholipid molecules. Electrostatic interactions between the phosphate group and the positively charged probes are not likely to contribute substantially to the binding. Soya phospholipid contains nearly 70% linoleic acid and the introduction of two *cis* double bonds into its fatty acid chains leads to the binding of AII and AV as shown by the intensity enhancements and blue shifts of probe fluorescence (Table I). The introduction of *cis* double bonds in the phospholipid fatty acid chains leads to a lowering of the phospholipid transition temperature (dipalmitoyl phosphatidylcholine, 41°C, and egg yolk lecithin, 25°C, [21]); fluidization of the hydrocarbon phase and increased permeability of the liposome to various molecules [22]. These observations can be explained by the local disorders caused by the introduction of the double bonds, with the consequent loosening of the bilayer packing. Fluorescence polarisation values of perylene and retinol also argue for the greater rigidity of dipalmitoyl phosphatidylcholine in comparison with egg phospholipid [23,24]. The enhanced binding of AX to the dipalmitoyl lipid may be rationalised by the fact that the longer alkyl chain enhances lipid solubility and allows efficient packing of the probe into the bilayers. The sequence of probe binding, AX > AV > AII, observed in the presence of soya phospholipid dispersions also argues for more facile probe incorporation with increasing chain length.

Bile salts are known to form primary micelles which are aggregates of 9—10 molecules, stabilised by hydrophobic interactions between the nonpolar steroid surfaces, with the hydrophilic groups pointing outwards. At higher bile salt concentrations, secondary micelles which are aggregates of primary micelles may be formed [15]. The spectra of AII and AV are not affected by the presence of bile salts at a concentration of 100 μg/ml. The AX spectrum, however, shows small changes under the same conditions. At these concentrations, bile salts are likely to exist as micelles with low aggregation numbers. The probes may then interact with the micellar surface. However, the results suggest only a weak binding by AX and negligible interaction with AII and AV. Interestingly, at very high bile salt concentrations (greater than 2 g/l), AV-bile salt interaction can be observed, presumably due to the formation of secondary bile salt micelles [25]. These results establish that the alkylamines function as probes of fatty acid unsaturation and lipid packing in phospholipid micelles but

interact only weakly with bile salt micelles. However, in mixed soya phospholipid-bile salt micelles, enhanced intensity and wavelength changes are observed for AII, AV and AX (Table I) as compared to the phospholipid micelles. The sequence of binding, AX > AV > AII, is maintained for the mixed micelles.

The incorporation of bile salt into dipalmitoyl phosphatidylcholine dispersions leads to a 30 nm blue shift and an intensity enhancement of AX fluorescence. However, AII and AV do not interact strongly even with these mixed micelles. NMR studies of mixed phospholipid-bile salt micelles have suggested that the fatty acid side chains are in a more fluid state than in phospholipid dispersions [26]. This is evident from the appearance of high resolution proton NMR signals, even in unsonicated egg lecithin dispersions, on the addition of bile salts. This fluidisation is also accompanied by a dramatic decrease in turbidity. The increase in lipid fluidity is responsible for the enhancement of probe fluorescence intensity. The magnitude of this effect is presumably less in the case of saturated phospholipid-bile salt micelles and thus no incorporation of AII and AV can be observed.

Fig. 2 shows that incorporation of cholesterol into phospholipid dispersions results in a decrease of alkylamine fluorescence. It has already been shown that this class of probe is sensitive to the extent of micellar fluidity. Cholesterol incorporation is known to have a profound effect on phospholipid packing [27, 28]. Fluidisation of the bilayer below the phase transition temperature and restriction of fatty acid chain motion above it have been suggested [29]. Fluorescence polarisation values of retinol, retinyl acetate and perylene have shown an increase in the rigidity of phospholipid micelles on incorporation of cholesterol [23,24]. Such changes could lead to alterations in probe binding parameters, leading to a reduction in probe fluorescence.

From the results in Table II it is clear that the F_m value for AX is slightly lower and the K_D value slightly higher in the mixed micelles as compared to pure phospholipid. Neither of these changes can account for the enhancement of probe fluorescence in the mixed micelles. The binding stoichiometry (n), however, increases from 10.6 μmol/g in dipalmitoyl phosphatidylcholine to 82 μmol/g in the mixed micelle and from 19 μmol/g in soya phospholipid to 122 μmol/g in the mixed micelle. For AV, the F_m and K_D values in soya phospholipid and the corresponding mixed micelle are nearly the same. The n value however, increases from 13.8 μmol/g in soya phospholipid to 43 μmol/g in the mixed micelle. The results indicate that for both AV and AX the number of probe molecules incorporated into the mixed micelles is greater than that associated with pure phospholipid dispersions, resulting in an enhancement of emission intensity in the former. For AX in soya phospholipid-bile salt micelles it is seen that, while F_m and K_D remain largely unaltered, the binding stoichiometry of AX falls from 122 μmol/g in the mixed micelle to 96 μmol/g on incorporation of cholesterol. This clearly establishes that the reduction in probe fluorescence by cholesterol is due to the displacement of AX from its binding sites in the mixed micelle. A similar result was obtained earlier for the binding of AV with mixed soya phospholipid-bile salt micelles [10]. It is significant that cholesterol incorporation does not alter the λ_{max} of bound AV and AX (Fig. 2), suggesting a similar probe environment in the presence and absence of cholesterol.

The emission spectra of the negatively charged probe, ANS, in the presence of mixed phospholipid-bile salt micelles (Fig. 5A and Table I) show an intensity enhancement and a blue shift of 35 nm. As in the case of the positively charged alkylamines, the presence of unsaturated linoleic acid in egg lecithin and soya phosphatidylcholine leads to enhanced ANS fluorescence compared to the value obtained in fully saturated dipalmitoyl phosphatidylcholine. Furthermore, as in the case of AII and AV, practically no binding could be observed between ANS and bile salt micelles at concentrations of 130 μg/ml.

In the case of mixed phospholipid-bile salt micelles, electrostatic interactions are possible between the positively charged alkylamines and the net negative charge of the bile salts. Such interactions can clearly by ruled out in the case of the negatively charged ANS molecule. ANS is known to bind near the choline head groups of dipalmitoyl phosphatidylcholine. X-ray diffraction studies of lecithin dispersions in the presence of ANS show a reduction in bilayer thickness from 62 Å to 38 Å. The naphthalene ring penetrates only a short distance between the fatty acid side chains, and the polar $N^+(CH_3)_3$ groups are pushed apart in the bilayer by their interaction with the SO_3^- of ANS [30]. In lipid dispersions, the fully saturated fatty acid side chains are likely to be rigidly stacked, making penetration of probe molecules difficult. The phenyl ring of ANS is likely to be more readily incorporated into the hydrocarbon phase of the bilayer in the case of disordered packing arrangements.

The incorporation of cholesterol into the mixed micelles causes a diminution of ANS fluorescence. Fig. 6A shows that the incorporation of cholesterol into soya and egg phospholipids does not alter the value of F_m. Furthermore, the K_D values (Table II) in the presence of the mixed micelles are within one order of magnitude. As in the case of AV and AX, the diminution in fluorescence intensity on the addition of cholesterol seems to be due mainly to the fall in the values of binding stoichiometry. The value falls from 122 to 100 μmol/g in soya phospholipid-bile salt micelles and from 140 to 80 μmol/g in egg lecithin-bile salt micelles. The effect of cholesterol in displacing ANS from the mixed micelles parallels that observed for the alkylamines. This suggests that both probes are more sensitive to lipid packing and hydrophobic interactions than electrostatic effects. The effect of cholesterol in reducing lipid fluidity in soya phospholipid-bile salt micelles is clearly seen from the reduction in pyrene excimer fluorescence (Fig. 7). The results in Fig. 8 also establish that cholesterol incorporation into mixed phospholipid-bile salt micelles reduces the effectiveness of ANS as a perturbant of lipid structure.

Conclusion

The use of three different fluorescent probes in studies of mixed phospholipid-bile salt micelles has demonstrated that bile salt incorporation fluidises the phospholipid matrix. Furthermore, the fluidity of the hydrocarbon phase in mixed micelles is enhanced by fatty acid unsaturation. The solubilisation of cholesterol into the mixed micelles leads to a reduction in fluidity and is accompanied by a reduction in the number of bound alkylamine and ANS molecules. The similar behavior of the positively and negatively charged probes suggests that the binding of these molecules is more sensitive to changes in lipid packing and comparitively insensitive to electrostatic effects.

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