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CORRELATIONS BETWEEN STRUCTURE AND SPECTROSCOPIC PROPERTIES IN MEMBRANE MODEL SYSTEMS

FLUORESCENCE OF DANSYLATED PROTEIN AND DANSYLATED LIPID IN PROTEIN–LIPID–WATER PHASES

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SUMMARY

The fluorescence of protein–lipid–water phases dansylated on either the protein or the lipid moiety is studied; the structure of the phases is determined by X-ray diffraction. The fluorescence parameters (emission spectrum, quantum yield, efficiency of energy transfer) are different in the different phases; a correlation is observed between fluorescence and structure which may be interpreted in terms of the two major types of protein–lipid interaction, namely electrostatic and hydrophobic. The location of the dansyl moiety in the various phases is discussed. A comparison is made with the fluorescence of 1-anilino-8-naphthalenesulfonate. This information on the protein–lipid–water phases leads to the interpretation of the fluorescence properties of probes incorporated in biological membranes in terms of membrane structure.

INTRODUCTION

Within the general framework of a search for correlations between spectroscopic properties and structure in biological membranes, we recently reported the X-ray diffraction analysis of a variety of protein–lipid–water systems and the spectroscopic study (ultraviolet absorption, circular dichroism, fluorescence) of a few phases of these systems whose structures had been established[1,2]. The correlations observed were indeed strong and could be interpreted in terms of two types of protein–lipid interaction: electrostatic and hydrophobic. In our first report[1], we introduced two operational criteria to differentiate the two types of interaction, as follows.

(a) The electrostatic interactions are disrupted when the ionic strength is increased. Thus phases of the electrostatic type are dissociated in 1 M NaCl, and the protein separates from the lipid. On the contrary, phases of the hydrophobic type resist this treatment; in order to free the protein, the lipids have to be salted out by divalent cations.

Abbreviations: ANS, 1-anilino-8-naphthalenesulfonate; DNS-, dansyl- or 5-dimethyl amino-1-naphthalenesulphonyl-.

(b) In the lamellar phases the partial thickness of the lipid leaflet is the same in the presence as in the absence of protein if the interactions are of the electrostatic type. If the interactions are of the hydrophobic type the lipid leaflet shrinks in the presence of the protein, suggesting that some of the paraffin chains come in contact with the protein.

A subsequent study of the fluorescence of 1-anilino-8-naphthalenesulfonate (ANS) led us to introduce two additional criteria[2]:

(c) The quantum yield of ANS fluorescence is higher in the phases of the hydrophobic type than in those of the electrostatic type, showing that the microenvironment of the probe is of lower polarity in the former than in the latter.

(d) The efficiency of energy transfer from the protein tryptophans to ANS is higher in the phases of the hydrophobic type than in those of the electrostatic type. Since this parameter is strongly dependent upon the distance separating the two chromophores, this result suggests that ANS comes in closer contact with the protein in the phases of the hydrophobic type than in those of the electrostatic type.

One serious problem in the study of the fluorescence properties associated with ANS is posed by the small size of this probe, an easily diffusible molecule whose precise location in heterogeneous systems is not easy to assess. In the study of protein–lipid–water systems we have tried to circumvent this problem by a systematic analysis of the protein–water and lipid–water phases, and by the determination of the partition coefficients of ANS between different phases. Such a complete set of control experiments would be difficult to carry out on biological membranes. One way of avoiding the difficulty is to use a label covalently bound to one of the membrane components. We report here the results of a series of X-ray and spectroscopic experiments made with 5-dimethylamino-1-naphthalenesulfonyl chloride (DNSCl), a probe covalently bound to either the protein or the lipid and incorporated in the lipid–water and protein–lipid–water phases. We show that the fluorescence parameters of the probe are sensitive to the type of interaction between the protein and the lipid.

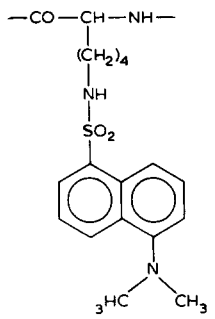
MATERIALS AND METHODS

The source of the lipids and of the protein, as well as the general preparation of the protein–lipid–water phases, are described in a previous article[1]. The protein used throughout this work is lysozyme; the lipids are cardiolipin and phosphatidyl inositol. Among the various phases of these systems, whose structures have already been determined by X-ray diffraction and described in two previous articles[1,2], we chose those that differ in the widest way as to the type of lipid–protein interaction: electrostatic and hydrophobic.

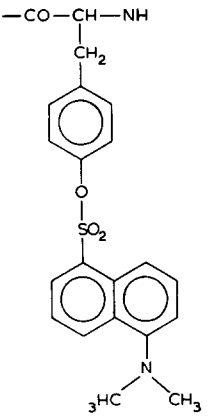
Preparation of DNS-lysozyme

The moderate dansylation of lysozyme is performed in the following way. 0.3 ml of a 0.2 M DNSCl solution in acetonitrile is added to 1 g of lysozyme dissolved in a mixture of 250 ml borate buffer (0.2 M, pH 9.5) and 12 ml of dimethylformamide. The dansylation is allowed to proceed for 10 min at room temperature; another 0.3 ml of the DNSCl solution is then added. After another 10 min the reaction is stopped by the addition of 1.5 ml cysteine solution (0.1 M). The mixture is then dialyzed for 48 h against several changes of 0.05 M NH_4HCO_3, centrifuged, and the supernatant

lyophilized. Chromatographic analysis of the hydrolyzed protein indicates that approximately 1 residue of lysine and 0.5 residue of tyrosine are dansylated per molecule of protein, in the form respectively of ε-DNS-lysine and *O*-DNS-tyrosine.



ε - DNS - lysine



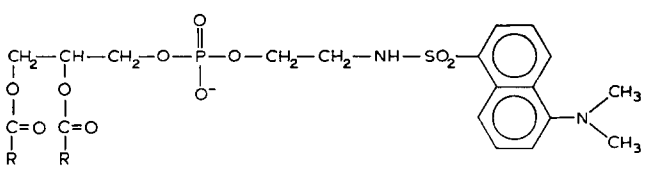
O - DNS - tyrosine

The ε-amino group of the six lysine residues of lysozyme are distributed over the surface of the protein and readily accessible[3]; the dansylation of these residues probably occurs at random. The same should be true for at least two of the three tyrosine residues which are easily iodinated[4].

The circular dichroism of DNS-lysozyme in the regions of absorption of the peptidic and aromatic chromophores is identical with that of the untreated protein. The moderate dansylation therefore seems to perturb neither the secondary nor the tertiary structure.

Preparation of DNS-phosphaditylethanolamine

L-Dipalmitoylethanolamine (630 mg, 1 mmole), DNSCl (313 mg, 1.16 mmole) and triethylamine (0.5 ml) are shaken at 35° in benzene (50 ml) until the ninhydrin-positive spot ($R_F = 0.52$) on thin-layer chromatography (silica gel F_{254}; chloroform–methanol–water (65:25:4, by vol.)) disappears. After being washed with dilute bicarbonate and HCl, the solution is dried and evaporated. The pale yellow residue is dissolved in diethyl ether (10 ml). The addition of acetone (20 ml) at −15° precipitates the DNS-derivate which is collected by centrifugation and washed twice with cold acetone. After being dried, 700 mg of a nearly white powder are obtained. Thin-layer chromatographic analysis indicates, besides the presence of the expected DNS-derivate ($R_F = 0.65$), the occurrence of about 5 % of another fluorescent compound ($R_F = 0.54$), probably a lyso derivate of the main product. Pure DNS-phosphatidylethanolamine is obtained by preparative thin-layer chromatography with chloroform–methanol (1:1, by vol.)



N-DNS-phosphatidylethanolamine

Preparation of the phases

In one set of experiments DNS-lysozyme was first diluted with untreated lysozyme in a 1 to 3 ratio. This solution was then mixed with either cardiolipin or phosphatidylinositol to prepare the DNS-lysozyme–lipid–water phases.

In a second set of experiments DNS-phosphatidylethanolamine was mixed with either cardiolipin or phosphatidylinositol, and this mixture was added to an untreated lysozyme solution leading to lysozyme–lipid + DNS-phosphatidylethanolamine–water phases. The molar ratio lipid/DNS-phosphatidylethanolamine is so chosen as to give a final ratio of one DNS-phosphatidylethanolamine molecule to two lysozyme molecules. This corresponds to a lipid/DNS phosphatidylethanol/amine ratio of 30–50 according to the type of phase.

Routine X ray diffraction experiments were performed on all protein–lipid–water phases to make sure that no change in structure results from the introduction of either DNS phosphatidylethanolamine or DNS lysozyme.

Spectroscopic analysis

The conditions of the fluorescence experiments are those described previously[2]. The quantum yield of DNSCl fluorescence is determined by exciting at different wavelengths between 320 and 400 nm. Absolute values are obtained by comparing the integrated energies of the fluorescence spectrum to that observed with ANS in ethanol (0.37) (ref. 5). The efficiency of the energy transfer from tryptophan to DNSCl is determined in the lysozyme–lipid + DNS phosphatidylethanolamine–water phases. This involves a comparison of the fluorescence of DNSCl when excited between 300 and 320 nm (where both the protein and DNSCl absorb) and above 320 nm (where only DNSCl absorbs).

RESULTS AND DISCUSSION

The fluorescence of DNSCl, like that of ANS, is sensitive to the polarity of the microenvironment: the lower the polarity, the higher the quantum yield and the larger the blue shift of the emission spectrum[6]. Moreover, the fluorescence of DNSCl is known to be sensitive to the nature of the molecule to which it is attached[7]. In Table I we present the fluorescence parameters observed in a variety of solvents with the three dansylated compounds used in this work: DNS-phosphatidylethanolamine, and the two dansylated residues of DNS lysozyme, ε-DNS-lysine and *O*-DNS-tyrosine. In a given solvent the fluorescence of ε-DNS-lysine and DNS-phosphatidylethanolamine are identical; the fluorescence of *O*-DNS-tyrosine is much weaker. It must be noted that DNSCl and ANS are differently sensitive to polarity: for environments of low or medium polarity the wavelength of the maximum of the emission spectrum (λ^{E}_{max}) is more sensitive than the quantum yield in the case of DNSCl; the opposite occurs with ANS (see comparison Table I).

DNS-phosphatidylethanolamine fluorescence

In a series of control experiments we studied the fluorescence of DNS-phosphatidylethanolamine incorporated in the lamellar lipid–water phases containing cardiolipin and phosphatidylinositol (molar ratio DNS-phosphatidylethanolamine/lipid = 1/30). The results are reported in Table II. Over the whole range of existence

TABLE I

FLUORESCENCE PARAMETERS OF DANSYLATED MOLECULES IN VARIOUS SOLVENTS; COMPARISON WITH ANS

Abbreviations: QY, quantum yield; λ^{E}_{max}, wavelength of the maximum of the emission spectrum.

Solvent	*Dansylated molecule*	*DNS-*		*ANS*[5]	
		QY	λ^{E}_{max} (*nm*)	QY	λ^{E}_{max} (*nm*)
Hexane*	Phosphatidyl-ethanolamine	0.50	475	0.98	454
n-Butanol		0.40	500	0.56	464
Ethanol (100%)		0.35	510	0.37	466
Ethanol (100%)	Lysine	0.35	510		
Ethanol (60%)		0.20	523	0.062	489
Ethanol (10%)		0.045	538	0.004	510
Ethanol (100%)	Tyrosine	0.020	540		
Ethanol (10%)		0.002			

* ANS is insoluble in hexane; the reported parameters are those of ANS in a highly apolar environment[5].

of the two lamellar phases the fluorescence parameters are independent of the water content, in agreement with the observation that in the lamellar phases of these systems the thickness of the lipid leaflet (d_1) and thus the average area per polar group (S), are independent of concentration. In a search for correlations between S and fluorescence we incorporated DNS-phosphatidylethanolamine in a lamellar

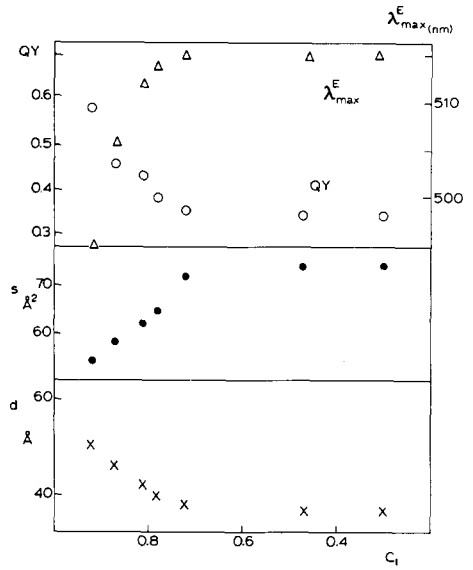


Fig. 1. Phase Lα of the lipid–water system containing 90% lecithin and 10% phosphatidic acid with added DNS-phosphatidylethanolamine (molar ratio lipid/DNS-phosphatidylethanolamine = 30/1). Thickness of the lipid leaflet (d_1), area per polar group at the lipid–water interface (S), quantum yield (QY) and wavelength of the maximum of the emission spectrum (λ^{E}_{max}) as a function of the lipid concentration (c_1). Note that as c_1 decreases, d_1 and QY decrease, S and λ^{E}_{max} increase, in the range $c_1 > 0.7$; at $c_1 < 0.7$ all the parameters become constant.

lipid–water phase formed of a mixture of egg lecithin and phosphatidic acid (90:10) in which S varies with concentration. The results reported in Fig. 1 show a striking correlation: as S decreases, the quantum yield increases and the emission spectrum is shifted to the blue. In the concentration range where the lipid leaflet remains constant the observed values of the fluorescence parameters clearly show that the microenvironment of the DNS- moiety is a region of intermediate polarity between those of water and of paraffin (see Fig. 4); further, the variations with S ($c_1 > 0.7$) suggest that the fluorescent probe is located at the lipid–water interface and that as S decreases the chromophore is forced deeper into the paraffin chains. Such behavior has already been observed with ANS[2].

The fluorescence parameters of DNS-phosphatidylethanolamine incorporated in the lamellar lysozyme–lipid + DNS-phosphatidylethanolamine–water phases of the electrostatic type are reported in Table II. If one compares these results with those obtained with the lipid–water phases one notices that in the presence of protein the emission spectrum is shifted to the blue by some 5–10 nm and that the quantum yield increases; thus it may be inferred that the presence of protein leads to a decrease of the polarity around the DNS- chromophore.

TABLE II

FLUORESCENCE PARAMETERS OF VARIOUS PHASES WITH DNS-PHOSPHATIDYLETHANOLAMINE

Abbreviations: CL, cardiolipin; PI, phosphatidylinositol; DNS-PE, DNS-phosphatidylethanolamine; c_l, weight concentration of lipid; c_p, weight concentration of protein; ET, efficiency of energy transfer (tryptophan to DNSCl). Nomenclature of the phases: lipid–water systems, L_α lamellar; protein–lipid–water systems, L_e lamellar electrostatic type, L_h lamellar hydrophobic type, $_2H$ two-dimensional hexagonal. For other abbreviations see Table I.

System	*Phase*	c_l	c_p	QY	λ^E_{max} (*nm*)	ET (%)
CL–DNS-PE–water	L_α	0.2–0.8		0.20	515	
PI–DNS-PE–water	L_α	0.2–0.8		0.35	510	
Lysozyme–CL + DNS-PE–water	L_e	0.34	0.38	0.40	502	20
Lysozyme–PI + DNS-PE–water	L_e	0.35	0.33	0.40	504	25
Lysozyme–CL + DNS-PE–water	L_h	0.31	0.52	0.55	485	45
Lysozyme–PI + DNS-PE–water	$_2H$	0.39	0.42	0.55	490	45

The very discrimination of the protein–lipid–water phases into electrostatic and hydrophobic types was based on the analysis of the properties of the lipid–water interface[1]; these properties were believed to be the same in the presence and in the absence of protein for the phases of the electrostatic type. It could thus be feared that the attachment of a fairly bulky group like DNSCl to the polar moiety of the lipid might upset the sharp distinction between the two types of phase. In order to investigate this possibility we studied a series of lysozyme–lipid + DNS-phosphatidylethanolamine–water phases, presumably of the electrostatic type, as a function of the DNS-phosphatidylethanolamine/lipid ratio. The result is that for ratios as high as 0.2 the dissociating effect of NaCl, as well as the thickness of the lipid leaflet (determined by X-ray diffraction) are independent of the presence of DNS-phosphatidylethanolamine, and consequently two properties which are characteristic of the phases of the electrostatic type are clearly preserved in the presence of DNS-

phatidylethanolamine. The differences of the fluorescence parameters between the lysozyme–lipid DNS-phosphatidylethanolamine–water phases of the electrostatic type and the lamellar lipid–water phases can probably be explained by the small perturbations due to the electrostatic interactions between the protein and the lipid, leading to a local decrease of S without involving hydrophobic interactions between the DNS- chromophore and the protein (see Fig. 4).

The fluorescence parameters of DNS-phosphatidylethanolamine incorporated in the lamellar and two-dimentional lysozyme–lipid + DNS-phosphatidylethanolamine–water phases of the hydrophobic type are also reported in Table II. As compared with the phases of the electrostatic type, the quantum yield increases considerably and the emission spectrum is shifted to the blue by some 15 nm. This result suggests that the environment of the probe is less polar in phases of the hydrophobic type than in those of the electrostatic type, in agreement with the notion that as a result of the hydrophobic interactions the paraffin chains of the lipid come in contact with the hydrophobic core of the protein[1] (see Fig. 4).

We measured the efficiency of energy transfer from the lysozyme tryptophans to the DNS- moiety of DNS-phosphatidylethanolamine (Table II). This parameter is twice as high in the phases of the hydrophobic type as in those of the electrostatic type. Since the energy transfer is highly dependent on the relative distance between the donor and acceptor chromophores[8], this result suggests (assuming that other parameters remain constant) that the DNS- moiety is closer to the protein tryptophans in the phases of the hydrophobic type than in those of the electrostatic type.

DNS-lysozyme fluorescence

Since lysozyme may be dansylated on the lysine and tyrosine residues we first studied the fluorescence of ε-DNS-lysine in the lamellar lipid–water phases (Table III, Fig. 2). The fluorescence parameters of ε-DNS-lysine suggest that, as for DNS-phosphatidylethanolamine, the DNS- moiety is located at the lipid–water interface, partly in contact with the paraffin chains, partly in contact with the polar head of the lipid. As expected, the fluorescence of *O*-DNS-tyrosine (not reported) is negligible in the lipid–water phases: it may be assumed that it is also negligible in the DNS-lysozyme-containing phases. As a consequence, in the phases containing DNS-lysozyme, both ε-DNS-lysine and *O*-DNS-tyrosine residues absorb strongly at the

TABLE III

FLUORESCENCE PARAMETERS OF VARIOUS PHASES WITH DNS-LYSOZYME AND ε-DNS-LYSINE

For abbreviations see Tables I and II. In the protein–lipid–water phases the quantum yields are calculated by taking into account only the absorption of the DNS-lysine residues (see text for explanation).

System	*Phase*	c_l	c_p	QY	λ^{E}_{max} (*nm*)
CL–ε-DNS-lysine–water	L_α	0.2–0.8		0.35	510
PI–ε-DNS-lysine–water	L_α	0.2–0.8		0.35	510
DNS-lysozyme–CL–water	L_e	0.34	0.38	0.40	510
DNS-lysozyme–PI–water	L_e	0.35	0.33	0.40	510
DNS-lysozyme–CL–water	L_h	0.31	0.52	0.60	495
DNS-lysozyme–PI–water	L_h	0.39	0.42	0.60	495

exciting wavelengths, whereas the fluorescence results solely from the ε-DNS-lysine residues. Since the ratio of dansylated lysines to tyrosines cannot be determined with sufficient accuracy, the determination of the quantum yield associated with ε-DNS-lysine residues is rather inaccurate. Thus, in the interpretation of the fluorescence spectra, emphasis will be put on the wavelength of the maximum of the emission spectrum rather than on the quantum yield.

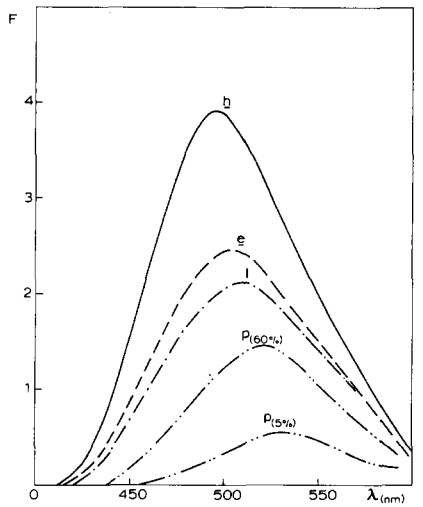


Fig. 2. Emission spectra of various phases with DNS-lysozyme and ε-DNS-lysine. Excitation wavelength, 335 nm. —··— (p), dansyl lysozyme solutions (60 and 0.5 %); —·— (l), ε-DNS-lysine in the cardiolipin–water or phosphatidylinositol–water lamellar phases; — — — (e), lamellar DNS-lysozyme–lipid–water phases of the electrostatic type; ——— (h), lamellar DNS-lysozyme–lipid–water phases of the hydrostatic type. Ordinates: fluorescence intensity divided by the absorbance of the DNS-lysine chromophore at the excitation wavelength.

A problem specific to the DNS-lysozyme–lipid–water phases is raised by the fact that the DNS- moiety of ε-DNS-lysine residues can be located in regions of lipid–protein contact as well as in regions of protein–protein contact. The interactions between the lipid and the protein will of course affect differently the fluorescence of ε-DNS-lysine residues located in the two regions.

Some information on the fluorescence of the ε-DNS-lysine residues involved in the protein–protein contacts can be gained by the study of DNS-lysozyme solutions as a function of protein concentration (Table IV, Fig. 2). Between 60 % (the highest protein concentration used here) and 10 % the fluorescence remains constant; below 10 % the quantum yield decreases and the emission spectrum is shifted to the red; a plateau is reached at about 1 %. Since lysozyme is known to polymerize at high concentration[9,10], the variations between 10 and 1 % can be ascribed to the polymer–monomer transition, suggesting that some ε-DNS-lysine residues are involved in protein–protein contacts. (In lysozyme crystals at least one protein–protein contact involves a lysine side chain[3].)

The fluorescence of the DNS-lysozyme–lipid–water phases of the electrostatic type is identical with that of the lamellar lipid–water phases containing ε-DNS-lysine; each emission spectrum has its maximum at 510 nm (Table III, Fig. 2). By

TABLE IV

FLUORESCENCE PARAMETERS OF DNS-LYSOZYME–WATER SOLUTIONS

For abbreviations see Tables I and II.

c_p	QY	λ^E_{max} (nm)
0.6	0.25	520
0.3	0.25	520
0.1	0.25	520
0.05	0.20	525
0 02	0 15	528
0.01	0.12	530
0.005	0.10	530

contrast, in the case of the lamellar phases containing DNS-phosphatidylethanolamine the addition of protein (phases of the electrostatic type) shifts the emission spectrum to the blue (see Table II). This difference might perhaps be related to the site of attachment of the fluorescent probe, althought it may be remarked that the fluorescence spectrum of ε-DNS-lysine residues involved in the protein–protein contacts is in fact shifted to the red with respect to that of ε-DNS-lysine incorporated in the lipid–water phases (λ^E_{max} at 520 and 510 nm, respectively). The discrepancy may thus be justified by assuming that the observed fluorescence spectrum of the DNS-lysozyme–lipid–water phases is the sum of two spectra, one (centered around $\lambda^E_{max} = 520$ nm) associated with the ε-DNS-lysine residues involved in the protein–protein contacts, the other (centered below $\lambda^E_{max} = 510$ nm) associated with the ε-DNS lysine residues involved in the lipid–protein contacts. Thus the data of Table III do not conflict with the notion that, as for DNS-phosphatidylethanolamine, the polarity of the environment of the probe is lower for ε-DNS-lysine residues located at the lipid–protein interface than for the ε-DNS-lysine molecules added to the lipid–water phase.

In the DNS-lysozyme–lipid–water phases of the hydrophobic type, the quantum yield is higher than in the phases of the electrostatic type, and the emission spectrum has its maximum at 495 instead of 510 nm (Table III, Fig. 2). This again is consistent with the presence of hydrophobic interactions between the protein and the lipid, leading to a highly apolar environment of the ε-DNS-lysine residues located in regions of lipid–protein contact. However, as noted above for the phases of the electrostatic type, some of the ε-DNS-lysine residues are probably located in regions of protein–protein contact as the emission spectrum is shifted some 5–10 nm to the red as compared with the phases of the hydrophobic type containing DNS-phosphatidylethanolamine (see Tables II and III).

CONCLUSIONS

We may conclude that in the protein–lipid–water systems the fluorescent properties associated with the DNSCl chromophores display a clear correlation with the structure of the phases, and more specifically with the nature of the protein–lipid interactions, namely electrostatic and hydrophobic. We reached a similar conclusion earlier in the study of ANS incorporated in the protein–lipid–water phases[2]; it is

clear, nevertheless, that the interpretation is more direct for DNSCl, firmly bound to the protein or lipid moieties, than for ANS, an easily diffusible molecule.

In the phases containing DNS-phosphatidylethanolamine, in which the location of the DNS- moiety is restricted within the regions of protein–lipid and lipid–water contacts, a more precise discussion of the environment of the probe can be attempted. Molecular models show that for DNS-phosphatidylethanolamine the position and orientation of the DNS- moiety with respect to the polar head is rather loosely restricted. Among the various positions shown in Fig. 3 the two extremes, with the DNS- moiety either sticking out of, or deeply embedded in, the paraffin chains,

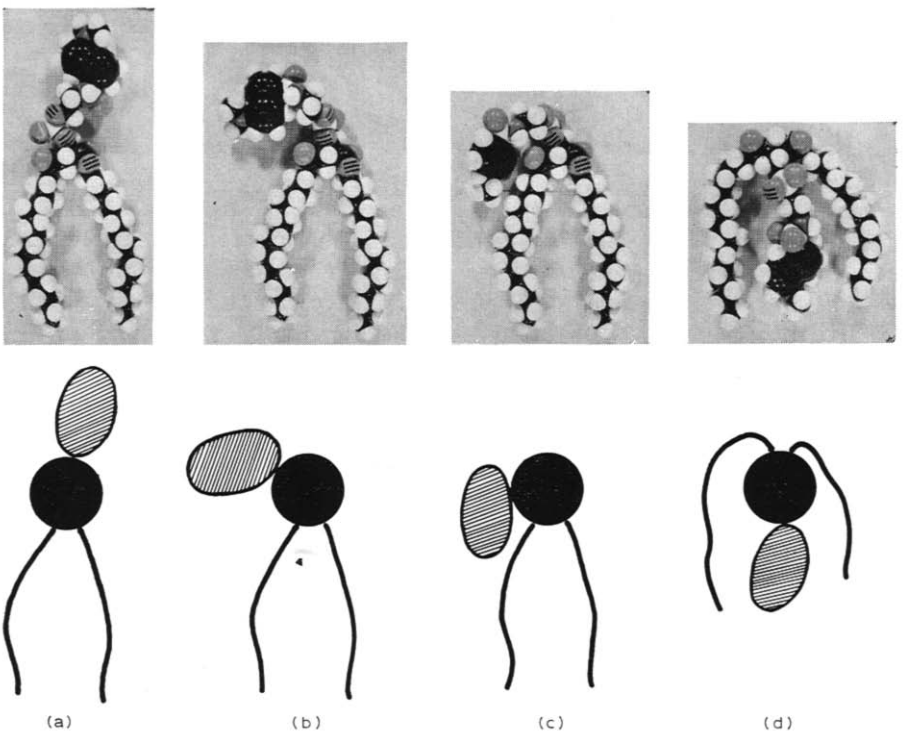


Fig. 3. CPK molecular models of DNS-phosphatidylethanolamine and schematic representation used in Fig. 4. The black area represents the polar head of the lipid; the hatched area the DNS-moiety.

appear to be inconsistent with the fluorescence observed with the lipid–water phases and protein–lipid + DNS-phosphatidylethanolamine–water phases of the electrostatic type. Of the two intermediate models, the one with the DNS- moiety in contact with the paraffin chains (c in Fig. 3) appears to be more satisfactory, as it provides a suitable microenvironment of intermediate polarity and involves only minor perturbations of the lipid–water interface. In the phases of the hydrostatic type the DNS- chromophore appears to be embedded in a medium of still lower polarity at a shorter distance of lysozyme as compared to the electrostatic type phases. A schematic and tentative representation of the location of the DNS-phosphatidylethanolamine probe in the various phases is given in Fig. 4.

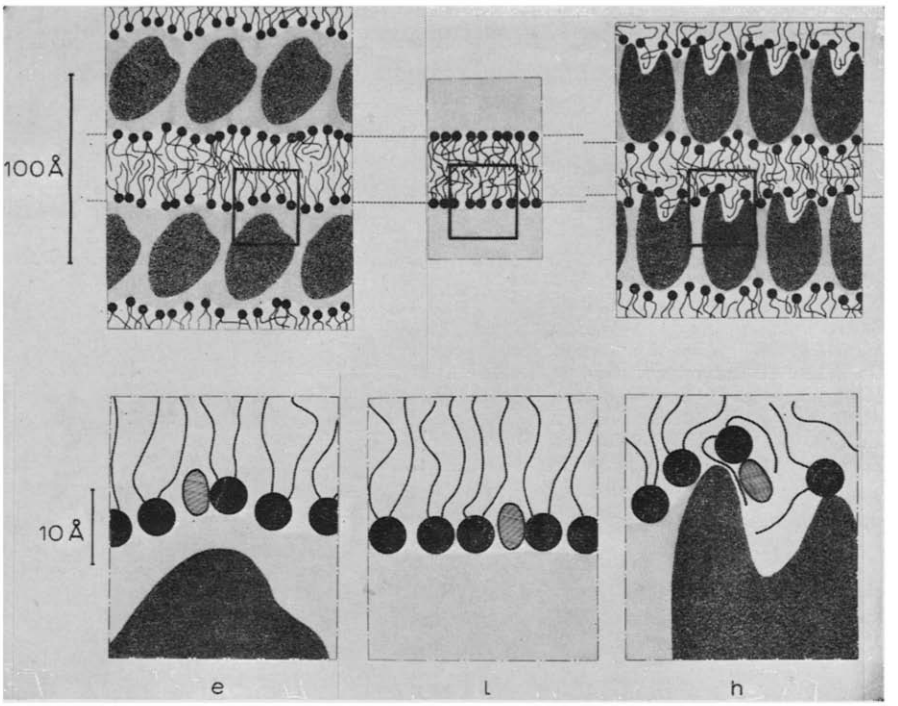


Fig. 4. Lysozyme–lipid–water system; structure of the lamellar phases (see ref. 1). The sections are perpendicular to the plane of the lamellae. The densely and lightly hatched areas represent the protein molecules and the water regions (both of arbitrary shape). The lower frames are enlarged representations of the areas of the upper frames enclosed in the rectangles, and show the position of the DNS-phosphatidylethanolamine molecule. Middle frames, lamellar lipid–water phase; left side frames, lamellar phases of the electrostatic type; right side frames, lamellar phases of the hydrophobic type. Note that in the protein–lipid–water phase of the electrostatic type, as in the lipid–water phase, the DNS- moiety is located at the lipid–water interface, more deeply embedded in the hydrophobic regions in the former than in the later, whilst in the protein–lipid–water phases of the hydrophobic type it is embedded in a non-polar medium in closer contact with the protein than in the phase of the electrostatic type.

DNS-phosphatidylethanolamine thus appears to be a promising probe for the study of correlations between physical structure and physiological functions of biological membranes. We have recently succeeded in incorporating DNS-phosphatidylethanolamine into intact membranes and in studying its fluorescence; the results will be reported elsewhere.

ADDENDUM

While the manuscript was in preparation a report appeared in the literature on the method of preparation of DNS-phosphatidylethanolamine and on the fluorescence properties of this probe in organic solvents and lipid suspensions[11].

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