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MODE OF BINDING OF CYTOCHROME b_5 TO PHOSPHOLIPID BILAYERS IN LAMELLAR STRUCTURE

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Summary

X-ray diffraction studies were made on the multilamellar systems produced by incubation of phospholipid bilayers and the membrane protein, cytochrome b_5, or non-membrane proteins (albumin, ovalbumin and β-lactoglobulin A) at pH 8.1 in aqueous 5 mM $CaCl_2$ solutions.

Detergent-extracted cytochrome b_5 (soluble aggregate) forms two types of lamellar phase with dipalmitoyl phosphatidylcholine bilayers, depending upon the incubation temperature. One type, which has a repeat distance of 114 Å, is formed above 34°C, where the binding of cytochrome b_5 to the bilayers is hydrophobic. The other type, with a repeat distance of 153 Å, is formed below 34°C, where the binding is electrostatic. It is also suggested that cytochrome b_5 is monomeric in the former phase but remains aggregated in the latter phase.

When dimyristoyl phosphatidylcholine is used, the boundary temperature for the two types shifts to 12°C. These boundary temperatures coincide with the thermal pretransition points of hydrated dipalmitoyl phosphatidylcholine and dimyristoyl phosphatidylcholine, respectively.

Trypsin-treated cytochrome b_5 (monomeric) and the three non-membrane proteins exhibit only binding of the electrostatic type to the bilayers, independently of the incubation temperature. The observed repeat distances suggest that in these cases two layers of protein molecules are incorporated between the bilayers.

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Abbreviations: DMPC, dimyristoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine.

Introduction

Lipid-binding properties of membrane proteins have recently become of great interest, and so have been extensively studied using model membrane systems. Cytochrome b_5 is one of the membrane proteins available for such studies. This protein is an amphiphatic membrane protein composed of two segments; a hydrophilic, catalytic segment containing the heme, and a hydrophobic segment responsible for membrane attachment [1,2]. Many reconstitution experiments [3—7] demonstrated that cytochrome b_5 extracted with detergent re-binds to microsomal membranes or sonicated phospholipid vesicles, but that cytochrome b_5 extracted with trypsin cannot bind to such membranes because of the lack of the hydrophobic segment.

Previously, we reported that the addition of 1—10 mM $CaCl_2$ to the dipalmitoyl phosphatidylcholine-water system causes the indefinite swelling of its lamellar phase and, consequently, produces a dispersion of lipid bilayer leaflets [8]. In such a system, the surfaces of the bilayers were suggested as being positively charged by bound Ca^{2+} [9]. By utilizing these facts, we have succeeded in producing lamellar phases consisting of positively charged bilayers and negatively charged proteins. The proteins used were the membrane protein, cytochrome b_5, derived from rabbit liver microsomes and three non-membrane proteins: albumin, ovalbumin and β-lactoglobulin A. All these proteins are negatively charged at the experimental pH of 8.1, since their isoelectric points are lower than 7.0 [10,11]. We have studied the structure of these complexes by means of an X-ray diffraction technique.

In this paper, the binding modes of the above-mentioned two cytochrome b_5 proteins to dipalmitoyl phosphatidylcholine bilayers in the lamellar structure are described, in comparison with those of the three non-membrane proteins. Also this report presents evidence which suggests that the hydrophobic binding of cytochrome b_5 is related to the pretransition [12] of the bilayer phase.

Materials and Methods

Materials. Rabbit liver cytochrome b_5 was prepared following the detergent procedure of Spatz and Strittmatter [2] without any modification. The final product was homogeneous by electrophoresis in 15% SDS-polyacrylamide disc gels. The protein was concentrated to about 30 mg/ml by means of ultrafiltration. This cytochrome b_5 extracted with detergents is abbreviated to 'd-b_5' below. The heme-containing hydrophilic segments of rabbit cytochrome b_5 were obtained from purified d-b_5 by tryptic cleavage in the same manner as that described by Spatz and Strittmatter [2]. d-b_5 and trypsin were incubated in 20 mM Tris-acetate/0.2 mM EDTA buffer, pH 8.1, at a molar ratio of 1 : 400 for 16 h at room temperature. The mixture was then fractionated on a Sephadex G-100 column equilibrated with the same Tris-acetate buffer. The fractions of the hydrophilic segments were collected and concentrated to 10—30 mg/ml. This segment is identical to trypsin-extracted cytochrome b_5 [1] and abbreviated to 't-b_5' below. d-b_5 and t-b_5 were stored below —20°C in 20 mM Tris-acetate/0.2 mM EDTA buffer, pH 8.1. Albumin (bovine, crystallized), ovalbumin (grade II) and β-lactoglobulin A (milk) were purchased from Sigma

Chemical Co. These powdery proteins were stored at about 0°C and dissolved in 10 mM Tris-acetate buffer (pH 8.1) before experiments.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were obtained from Sigma. These lipids were, respectively, dissolved in chloroform and stored below −20°C.

All the above chemicals were used without further purification.

Preparation of protein-lipid complexes. Six kinds of complex, i.e., (1) *d*-b_5-DPPC, (2) *d*-b_5-DMPC, (3) *t*-b_5-DPPC, (4) albumin-DPPC, (5) ovalbumin-DPPC and (6) β-lactoglobulin A-DPPC, were prepared as follows. Aliquots of a stock chloroform solution containing lipid at 2 mM were pipetted off. The solvent was removed on a rotary evaporator under vacuum at 40°C and finally in an evacuated desiccator overnight at room temperature. The dried lipids were then suspended at 3.7 mg/ml in 10 mM Tris-acetate buffer, pH 8.1, containing 5 mM $CaCl_2$. Each protein solution (2.5—28 mg/ml) was mixed with this bilayer suspension (3.7 mg/ml) in approx. 0.3 ml total volume. The mixtures were incubated at various temperatures within the range 0—45°C for 30 min. In some cases, the incubation at 0°C was allowed to continue overnight to avoid, if any, the time-dependence of protein-lipid binding. These incubations yielded heavy precipitates. In the cases of *d*-b_5 and *t*-b_5, the resulting precipitates were colored red. The accuracy of the temperature control during incubation was ±0.2°C. All samples were always handled below 5°C after incubations.

Determination of protein and lipid contents. *d*-b_5 and *t*-b_5 were determined from the absorbance at 413 nm (extinction coefficient $\epsilon = 117\ mM^{-1} \cdot cm^{-1}$ [13]) and other proteins by using the method of Lowry et al. [14]. Lipid was determined gravimetrically or from phosphorus content [15]. The weight ratio of protein to lipid in the mixture was varied from 1 : 5 to 1 : 1. Under these conditions, the weight ratio of protein to lipid in the precipitate was maximally approx. 1 : 1.3 for *d*-b_5 and approx. 1 : 5 for albumin. But the ratios for the others were not determined.

X-ray diffraction experiments. The mixtures containing precipitates were sealed in thin-walled X-ray glass capillaries (1.0 mm internal diameter). To obtain concentrated specimens, the mixtures were centrifuged at 15 000 × *g* for 30 min at 4°C by holding the capillaries in the narrow holes of modified centrifuge tubes. Diffraction patterns were recorded photographically (Fuji Medical KX film) on an Elliott toroidal focusing camera using Ni-filtered CuKα radiation (λ = 1.542 Å) from a Rigaku Denki rotating anode microfocus generator (RU-3HM). Exposure time was 6—20 h. Samples were maintained at 5°C during exposure. The absorbance of the films was measured on a Nalumi Type C microdensitometer. The spacings were calibrated with $Pb(NO_3)_2$ powder.

Results

Formation of protein-lipid lamellar phases

Protein-lipid complexes precipitated at various temperatures in the mixtures of DPPC bilayers and *d*-b_5, *t*-b_5, albumin, ovalbumin and β-lactoglobulin A, were subjected to X-ray diffraction. For all precipitates, several low-angle reflections and the so-called 4.2 Å reflection were recorded on X-ray films. Fig. 1 shows the low-angle patterns of X-ray photographs obtained for *d*-b_5 and

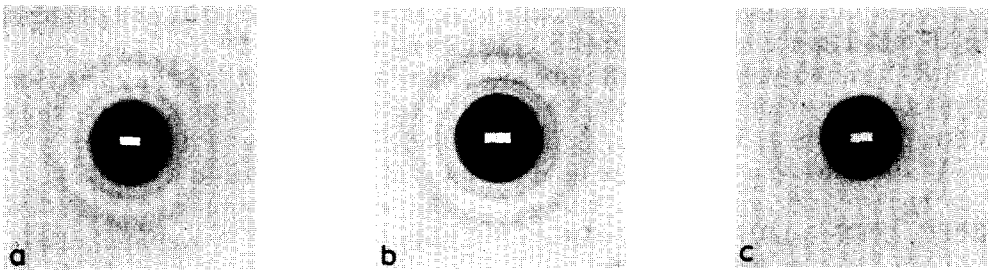

Fig. 1. Low-angle X-ray diffraction patterns of the precipitates of protein-lipid mixtures. (a) d-b_5-DPPC mixture incubated at 38°C and (b) at 0°C; (c) t-b_5-DPPC mixture incubated at 0°C. Patterns are for samples chilled at 5°C and show several orders of a repeat distance, d, of (a) 114 Å, (b) 153 Å and (c) 100 Å, respectively.

t-b_5; (the high-angle patterns around 1/4.2 $Å^{-1}$ are shown later.) a set of low-angle reflections from each precipitate could be indexed as integral orders of one-dimensional lattice. This indicates that these protein-lipid complexes have structure of the lamellar type. This lamellar structure would probably consist of alternate layers of protein and lipid, as was described by other authors on similar systems [16—20].

Effect of incubation temperature on repeat distance

Table I gives the repeat distances, d, of these protein-lipid lamellar phases together with the incubation temperatures of the mixtures. In addition, the molecular weights of the component proteins are listed in Table I. In the case of d-b_5, two repeat distances were observed depending upon the incubation temperature: the lamellar phase with $d = 153$ Å was formed when d-b_5 was incubated with the suspension of DPPC bilayers below 34°C, whereas the lamellar phase with $d = 114$ Å was formed for the incubation above 34°C. Moreover, the first phase formed was once transformed into the latter phase

TABLE I

REPEAT DISTANCES OF LIPID-PROTEIN LAMELLAR PHASES FORMED AT VARIOUS TEMPERATURES

Repeat distance, d, was measured for the samples chilled at about 5°C. Each value of d represents the mean ± S.D. for five to ten preparations.

System	Incubation temperature (°C)	Repeat distance d(Å)	Molecular weight of protein
d-b_5-DPPC	0—34	153 ± 2	16 700 *
	34—45	114 ± 2	
t-b_5-DPPC	0—38	100 ± 2	11 700 *
β-Lactoglobulin A-DPPC	0—38	106 ± 1	36 500 **
Ovalbumin-DPPC	0—38	127 ± 1	45 000 **
Albumin-DPPC	0—38	140 ± 3	67 000 **

* Taken from the data of Spatz and Strittmatter [2], including the molecular weight of the heme.
** From the data of Andrews [21].

when heated above 34°C, but not the reverse. The existence of this irreversibility assures that the samples retain the respective phases, originated in incubation, as long as they are maintained below 34°C after incubation (actually, maintained below 5°C as described in Materials and Methods). Here, it should be noted that this boundary temperature coincides with the pretransition temperature (34—35°C [12,22,23]) of hydrated DPPC.

In the cases of t-b_5 and the three non-membrane proteins, only one lamellar phase was respectively formed: their repeat distances remain constant over the range of incubation temperature, 0—38°C (see Table I).

In order to confirm the incubation-temperature dependence of the repeat distance observed with d-b_5-DMPC complexes, similar experiments were performed with DMPC instead of DPPC. Table II shows the results for d-b_5-DPPC complexes in comparison with those for d-b_5-DMPC complexes. The lamellar phase with d = 149 Å was formed when the suspension of DMPC bilayers and d-b_5 was incubated below 11°C, whereas the lamellar phase with d = 110 Å was formed for the incubation above 12°C. Thus, d-b_5-DMPC complexes also exhibited the same dependence, though the boundary temperature for the two types of lamellar phase shifted to 11—12°C. This boundary temperature is very close to the pretransition temperature (13—14°C [12,22,23]) of hydrated DMPC. It is clear that there is a correlation between the boundary of the two lamellar phases and the pretransition temperatures of these lipids. In both systems, however, the incubation-temperature dependence of the repeat distance was not observed above and below the main transition temperatures of these lipids (41—42°C for DPPC and 23—24°C for DMPC [12,22,23]). As shown in Table II, the repeat distance of the lamellar phase with DMPC in each type is smaller than that of the phase with DPPC by 4 Å. This arises from the difference in the length of the hydrocarbon chain.

In all cases, there was no effect of the relative concentration of protein to lipid on the repeat distance within the mixing ratios (1 : 1—1 : 5 by weight) of this study.

Repeat distance and size of protein molecule

Fig. 2 is a plot of the repeat distance d in Table I vs. the apparent diameter, D, of the component protein. D values were calculated from the molecular weights of the proteins, assuming that these proteins take a spherical form with

TABLE II

EFFECT OF INCUBATION TEMPERATURE ON d-b_5-PHOSPHOLIPID LAMELLAR PHASE

See legend of Table I.

Phospholipid	Incubation temperature (°C)	Repeat distance d(Å)
DPPC	0, 19, 25, 30, 32, 33, 34	153 ± 2
	34, 35, 37, 38, 41, 45	114 ± 2
DMPC	0, 5, 8, 10, 11	149 ± 2
	12, 14, 15, 24, 38	110 ± 1

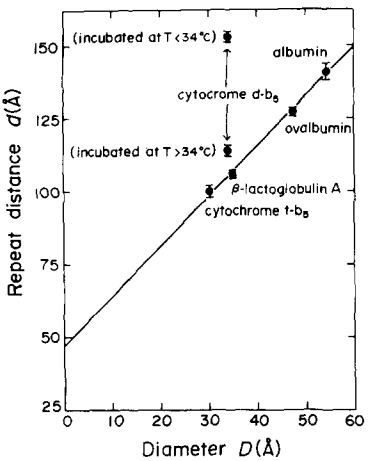

Fig. 2. Repeat distances (d) of the lamellar phases plotted against the apparent diameters (D) of the component proteins. D was calculated according to the relationship $(M/N_0)\bar{\nu} \cdot 10^{24} = (4/3)\pi(D/2)^3$, where M and $\bar{\nu}$ are the molecular weight (listed in Table I) and the specific volume (taken as 0.75 cm^3/g on the average [24]) of the protein, respectively, and N_0 is Avogadro's number. Note that in calculating D of β-lactoglobulin A, a half-molecular weight was used because this protein molecule consists of two identical subunits [25]. The straight line was obtained by the least-squares method.

a uniform density (for calculation, see legend of Fig. 2). As can be seen from Fig. 2, the data points for the four proteins except d-b_5 obviously show that the repeat distance d is a linear function of the diameter D of the protein. This relationship is fitted by:

$$d = 47 + 1.7\,D\ (\text{Å}) \qquad (1)$$

The first term, 47 Å, on the right-hand side, which is given at $D = 0$, denotes the thickness of the DPPC bilayer in these protein-lipid lamellar phases. This value (47 Å) is in good agreement with that (46 Å) for a DPPC bilayer at 5°C in our previous report [26]. The second term therefore corresponds to the thickness of the interbilayer space involving protein and water. The coefficient, 1.7, in this term approximates to 2. This suggests that two layers of protein molecules are incorporated between the bilayers.

On the other hand, two data points for d-b_5 deviate from the straight line. In the case of the complex prepared above 34°C, however, the deviation is very small. Therefore, it appears that the lamellar phase of this example, as well as those of other proteins, involves two protein layers between the bilayers. In the case of the complex prepared below 34°C, the deviation is large. The interbilayer distance of this lamellar phase (153 — 47 = 106 Å) corresponds numerically to about 3 times the apparent diameter (34 Å) of the d-b_5 molecule calculated according to the above assumption.

Protein-lipid interactions

The interactions between proteins and lipid bilayers in the constructed lamellar phases were characterized by two experimental results: the high-angle diffraction patterns, and the dissociating effect [17] of salts or chelating agents on the protein-lipid complexes.

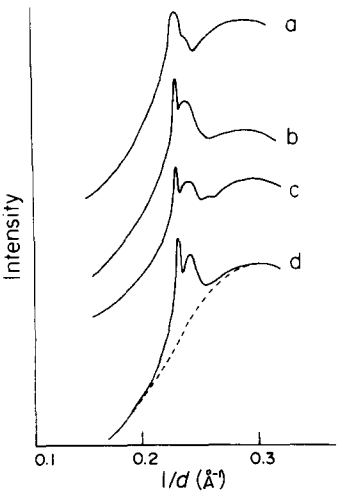

Fig. 3. Microdensitometer tracings of the 4.2 Å region of some lamellar phases at 5°C. (a) *d*-b_5-DPPC lamellar phase produced at 38°C and (b) at 0°C; (c) *t*-b_5-DPPC lamellar phase produced at 0°C; (d) lamellar phase of DPPC-water system without proteins. All the profiles include the background scattering from water. (— — —) indicates the scattering intensity level of water.

Fig. 3 shows the intensity profiles of the high-angle reflections from some systems at 5°C. Curves a and b are for *d*-b_5-DPPC complexes prepared above and below 34°C, respectively. Curve c is for the *t*-b_5-DPPC complex. Curve d is for the DPPC-water system without proteins. This profile shows a high-angle diffraction pattern from the gel phase of DPPC (the Lβ' phase) and is characterized by a sharp reflection at 1/4.2 $Å^{-1}$, followed by a more diffuse reflection [27]. Curve a shows a fusing of two peaks indicated in curve d. This evidently suggests that the ordered array of the hydrocarbon chains of the bilayer is more or less disturbed by the penetration of other molecules, presumably the hydrophobic 'tail' of *d*-b_5. On the other hand, curves b and c show no detectable change in their profiles as compared with curve d. Similarly, no change was observed for the complexes with albumin, ovalbumin and β-lactoglobulin A (not shown in Fig. 3). This indicates that in these cases the internal structure of the DPPC bilayer is not disturbed by proteins.

Table III shows the results on the dissociating effect of 0.5 M KCl and 10 mM EDTA on the protein-lipid complexes. The addition of KCl increases the ionic strength in the complexes and EDTA removes the bound Ca^{2+} from the bilayer surfaces. Therefore, if the interaction of protein and lipid bilayer is electrostatic, either of these treatments will prevent them from associating. In order to examine whether the proteins used here still bind to DPPC bilayers in the presence of KCl or EDTA at high concentrations, the proteins were assayed for the pellets which were obtained by centrifugation of the mixtures treated with 0.5 M KCl or 10 mM EDTA. In the case of the *d*-b_5-DPPC complex prepared above 34°C, analysis of the pellet showed that the dissociation of protein and bilayer did not occur (line 1 of Table III); this was visually distinct owing to the color characteristics of cytochrome b_5. This was also confirmed by the fact that the high-angle profile of this complex (curve a in Fig. 3) was

TABLE III

DISSOCIATING EFFECT OF KCl (0.5 M) AND EDTA (10 mM) ON PROTEIN-LIPID COMPLEXES

The dissociation between proteins and lipid bilayers was ascertained by measuring the amount of protein in the pellets which were obtained by ultracentrifugation (100 000 × *g* for 1 h at 4°C) of the mixtures after the addition of 0.5 M KCl or 10 mM EDTA solution. (+) Dissociated; the amount of remaining protein in the pellet is negligibly small. (—) Undissociated; the protein content is nearly constant before and after the treatments. A portion of each treated sample was examined by X-ray diffraction (see text).

System	Dissociating effect	
	KCl	EDTA
d-b_5-DPPC		
(1) incubated at $T > 34$°C	—	—
(2) incubated at $T < 34$°C	+	+
(3) *t*-b_5-DPPC	+	+
(4) β-Lactoglobulin A-DPPC	+	+
(5) Ovalbumin-DPPC	+	+
(6) Albumin-DPPC	+	+

not affected by the addition of these agents. These results, together with curve a of Fig. 3, suggest that the interaction between *d*-b_5 and the bilayer is predominantly hydrophobic. On the contrary, the *d*-b_5-DPPC complex prepared below 34°C and all the other complexes were readily dissociated into proteins and bilayers in 0.5 KCl or 10 mM EDTA solution (lines 2—6 of Table III), and the proteins were recovered in the supernatant after centrifugation of the mixtures. From these treated samples, the lamellar patterns giving a repeat distance of 65 Å were observed, indicating the formation of the lamellar structure of DPPC alone. These observations and the results on the high-angle reflections (e.g., curves b and c) are strong evidence for the electrostatic interaction between proteins and bilayers.

Discussion

Hydrophobic and electrostatic bindings

It is a generally accepted idea that *d*-b_5 binds tightly to membranes by penetrating its hydrophobic segment into membranes [3—7]. The present data also indicate that *d*-b_5 binds to the planar bilayers of DPPC and DMPC with the direct contact of the hydrophobic segment of *d*-b_5 and the hydrocarbon core of the bilayer. In the present systems, however, such hydrophobic behavior of *d*-b_5 is observed only when *d*-b_5 is incubated with the bilayer suspension above the pretransition temperature of the bilayer, and when the incubation is performed below the pretransition temperature the electrostatic binding occurs instead of the hydrophobic one. This result implies that the hydrophobic tail of *d*-b_5 cannot penetrate into the bilayers in the low-temperature phase below the pretransition temperature and behaves differently. It is known that in aqueous solution devoid of detergents, *d*-b_5 aggregates into a water-soluble oligomer with a molecular weight of about 120 000 [1,2]. This oligomer is believed to be an octomer from its molecular weight [1,2]. Also Robinson and Tanford [6] reported the Stokes' radius of approx. 60 Å for the oligomer (porcine *d*-b_5).

These facts, combined with the present data, lead us to expect that in the lamellar phase formed below the pretransition temperature the octomers of d-b_5 are sandwiched between the bilayers without disaggregating into monomers.

Since such an unexpected effect of incubation temperature on the binding of d-b_5 to the bilayers is equally obtained with both systems of DPPC and DMPC having different pretransition temperatures (Table II), the alteration of the binding type described above is probably attributable to the structural changes of the bilayer accompanying the pretransition. This seemingly contradicts the observations of Faucon et al. [28] that d-b_5 binds to DPPC vesicles below the pretransition temperature (at 20°C). However, there appears to be no discrepancy between both sets of data. Although the nature of the pretransition of the DPPC (or DMPC) bilayer has not been established yet, it has been reported that the packing of the hydrocarbon chains of the planar bilayer becomes somewhat disordered at this transition [29—31]. On the other hand,the high-angle reflection pattern of sonicated vesicles has shown that the chain packing is rather disordered even below the pretransition temperature (Inoko, Y., unpublished data). Also Sheetz and Chan [32] reported the loosened structure of vesicles with large curvatures. These facts appear to suggest that a disordering of the packing of the hydrocarbon chains is essential for the tail of d-b_5 to penetrate into the bilayers. A similar conclusion has been proposed by Mateu et al. [33]. Thus, the above disagreement can be considered to be due to the differences of the systems employed.

The two sets of experimental data (Fig. 3 and Table III) also suggest that t-b_5 is merely adsorbed electrostatically on the surfaces of the bilayers. This means that, when cytochrome b_5 loses its hydrophobic segment, this protein, i.e., t-b_5, becomes equivalent to water soluble proteins such as albumin or β-lactoglobulin A examined here with respect to lipid-binding behavior.

Molecular arrangement between proteins and bilayers

The plot of d vs. D (Fig. 2) provides interesting information about the structure of the protein-lipid lamellar phases. In the cases of t-b_5, albumin, ovalbumin and β-lactoglobulin A, it is suggested that two layers of protein molecules are sandwiched between the bilayers. This picture is based on the relationship (Eqn. 1) that the interbilayer distance approximately corresponds to twice the apparent diameter of each protein molecule calculated with a spherical approximation. This interpretation for Eqn. 1 is probably correct, since these proteins are regarded as globular on the whole [24].

In the case of d-b_5, however, it is complicated. When the lamellar phase with hydrophobic interaction is formed, its repeat distance (114 Å) is comparable to that (100 Å) of the t-b_5-DPPC lamellar phase. From this, if d-b_5 embeds its hydrophobic segment almost completely into the bilayer and leaves only its hydrophilic segment out of the bilayer, a period of 114 Å can be simply accounted for by regular stacking of the bilayers bearing proteins on both sides. That is, this phase, as well as that of t-b_5, involves two layers of the hydrophilic segments between the bilayers. But there is a difference of 14 Å between the repeat distances of both phases. This difference may be due to a change of tilt of the hydrocarbon chains as a result of the penetration of the tail of d-b_5. In fact, the electron-density profile of the d-b_5-DPPC membrane derived from the

intensity data shows an increase of 3—4 Å in the bilayer thickness compared to that of pure DPPC bilayer (Inoko, Y., unpublished data). However, an increase of 14 Å cannot be perfectly complemented by this change (3—4 Å). Even if the chains became perpendicular to the bilayer plane and were fully extended, the predicted increase in the bilayer thickness is less than 10 Å (for a change in tilt angle of 30°). This therefore requires another interpretation in addition to that above. For example, part of the hydrophobic segment may be out of the bilayer. As suggested from the amino acid analysis for the hydrophobic segment [2,34], at least the point of proteolytic cleavage which separates the hydrophobic and hydrophilic segments should be located outside the bilayer. In any event, the above-mentioned scheme is basically valid.

On the other hand, when the lamellar phase with electrostatic interaction is formed, the observed repeat distance increases to 153 Å. As discussed above, d-b_5 is inferred to exist as an octomer in this phase, and the Stokes' radius (60 Å [6]) of this octomer suggests that only one layer of octomer is sandwiched between the bilayers. If one regards the octomer of d-b_5 as a spherical particle with uniform density, its molecular weight (133 600 = 8 × 16 700) yields a radius of 34 Å. This value corresponds to about half the above Stokes' radius and permits the presence of two layers of octomers. However, a spherical approximation gives the smallest possible radius for a given particle, and therefore it is not reasonable to use the above assumption for the aggregate of d-b_5 molecules with an asymmetric shape [35]. Presumably, the actual size of the d-b_5 octomer is larger than the value calculated as a sphere. The former case (one layer) seems to be more probable.

On the basis of the above discussions, the possible models for the structure of d-b_5-DPPC and t-b_5-DPPC lamellar phases are presented in Fig. 4. Fig. 4a and b displays the 'hydrophobic' and 'electrostatic' types of binding between d-b_5 and the bilayer, respectively. Fig. 4c displays the electrostatic binding

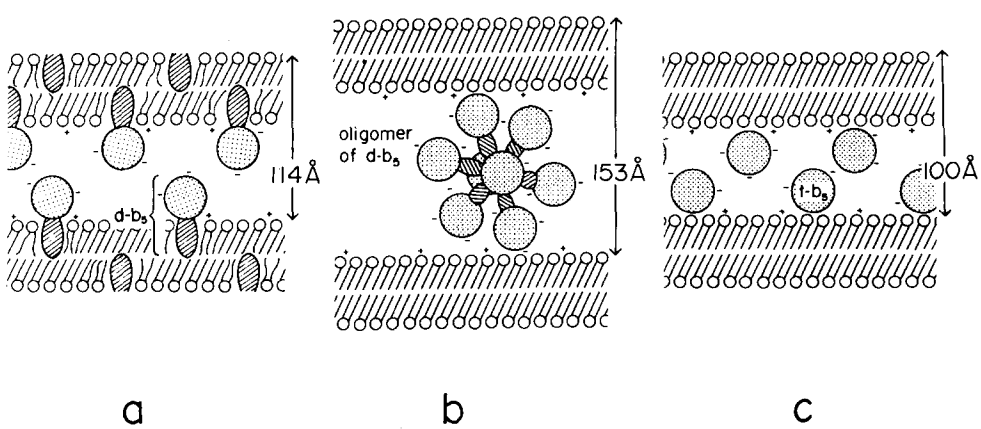

Fig. 4. Schematic representation of the structures of two types of d-b_5-DPPC lamellar phase and the t-b_5-DPPC lamellar phase. d-b_5-DPPC lamellar phase produced when the mixture was incubated above (a) and below (b) the pretransition temperature of the DPPC bilayer. (c) t-b_5-DPPC lamellar phase; this structure is independent of the incubation temperature. The exact shape of the protein molecule is arbitrary. (+) indicates a positive charge due to Ca^{2+} bound on the bilayer surface, and (—) indicates a net negative charge of protein.

between t-b_5 and the bilayer. The situation depicted in Fig. 4c, with the exception of the repeat distance, can be applied to the lamellar phases prepared with albumin, ovalbumin and β-lactoglobulin A.

Finally, there remains one question. That is, why is the disaggregation of the d-b_5 octomer related to the pretransition of the bilayer? The present data superficially give this correlation but provide no further information about this question.

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