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Effect of bovine prothrombin fragment 1 on the translational diffusion of phospholipids in Langmuir-Blodgett monolayers

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Previous work has shown that bovine prothrombin fragment 1 binds to supported planar membranes composed of phosphatidylcholine and phosphatidylserine in a Ca^{2+}-specific manner (Tendian et al. (1991) Biochemistry 30, 10991; Pearce et al. (1992) Biochemistry 31, 5983–5995). In the present work, fluorescence pattern photobleaching recovery has been used to examine the effect of membrane-bound fragment 1 on the translational diffusion coefficients of two fluorescent phospholipids in fluid-like phosphatidylserine/phosphatidylcholine Langmuir-Blodgett monolayers. The results show that saturating concentrations of fragment 1, in the presence of Ca^{2+}, reduce the diffusion coefficient of nitrobenzoxadiazolyl-conjugated phosphatidylserine (NBD-PS) and nitrobenzoxadiazolyl-conjugated phosphatidylcholine (NBD-PC) by factors of approximately four and two, respectively. Ca^{2+} or fragment 1 alone do not have a statistically significant effect on NBD-PS or NBD-PC diffusion. In addition, a nonspecific protein (ovalbumin) does not change the diffusion coefficients of the fluorescent phospholipids either in the absence or presence of Ca^{2+}. The fractions of the fluorescent phospholipids that are laterally mobile are approximately 0.9 for all samples. These results are interpreted with several models for possible mechanisms by which extrinsically bound proteins might retard phospholipid diffusion in membranes.

Introduction

The membrane-mediated conversion of prothrombin to thrombin is a key process in thrombosis and hemostasis. This process also serves as a paradigm for several enzymatic processes in blood coagulation that require the association of proteins containing γ-carboxyglutamic acid residues with membranes that contain negatively charged phospholipids (such as platelet surfaces). Of particular interest is how transport and reaction are coupled at the membrane surface during the assembly and activation of the prothrombinase enzyme complex and during subsequent prothrombin cleavage [1–3].

One method for obtaining quantitative information about the physical dynamics of proteins at membrane surfaces is to use substrate-supported planar membranes and techniques in fluorescence microscopy [4–6]. Previous work has shown that bovine prothrombin and its fragment 1 bind to supported membranes composed of mixtures of palmitoyloleoylphosphatidylcholine (POPC) and bovine brain phosphatidylserine (PS) in a Ca^{2+}-specific manner and that fluorescence microscopy can be used to measure both equilibrium and kinetic rate constants for membrane binding [7,8].

In this work, fluorescence pattern photobleaching recovery (FPPR) is used to examine the effect of bovine prothrombin fragment 1 on the translational diffusion coefficients of two fluorescent phospholipids, nitrobenzoxadiazolyl-conjugated phosphatidylserine (NBD-PS) and nitrobenzoxadiazolyl-conjugated phosphatidylcholine (NBD-PC), in PS/POPC Langmuir-Blodgett monolayers. The effects are interpreted with several models for possible mechanisms by which extrinsically bound blood coagulation proteins might alter phospholipid diffusion in membranes.

Materials and Methods

Reagents. Bovine prothrombin fragment 1 was produced as previously described [8]. Ovalbumin (Sigma, St. Louis, MO); tetradecyltrichlorosilane (Lancaster

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Abbreviations: FPPR, fluorescence pattern photobleaching recovery; NBD-PC, 1-acyl-2-[12-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino]-dodecanoyl]phosphatidylcholine; NBD-PS, 1-acyl-2-[12-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino]dodecanoyl]phosphatidylserine; POPC, palmitoyloleoylphosphatidylcholine; PS, bovine brain phosphatidylserine; TBS, Tris-buffered saline.

Synthesis, Windham, NJ); and bovine brain sodium salt phosphatidylserine (PS), 1-palmitoyl-2-oleoyl-3-*sn*-phosphatidylcholine (POPC), 1-acyl-2-[12-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoyl]phosphatidylcholine (NBD-PS), and 1-acyl-2-[12-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoyl]phosphatidylserine (NBD-PS) (Avanti Polar Lipids, Birmingham, AL) were obtained commercially.

Langmuir-Blodgett films. Monolayers composed of either PS/NBD-PS/POPC (28:2:70, mol/mol/mol) or PS/NBD-PC/POPC (30:2:68, mol/mol/mol) were spread from hexane/ethanol (9:1) at 100 Å^2/molecule and room temperature on water (18 MΩ · cm) in a Langmuir trough (Vickers Model 4). The monolayers were compressed to 35 dyne/cm at 1–2 Å^2/molecule per min. Monolayers were deposited at 5 mm/min and at constant pressure on tetradecyltrichlorosilane-treated fused quartz slides [9,10]. Monolayers were washed with 1 ml of Tris-buffered saline (TBS; 0.05 M Tris, 0.1 M NaCl, pH 7.4) containing either 10 mM $CaCl_2$ or 1 mM Na_2 EDTA. For measurements in the presence of proteins, monolayers were then treated with 250 μl TBS containing either 10 mM $CaCl_2$ or 1 mM Na_2 EDTA, and either 5 μM fragment 1 or 5 μM ovalbumin.

Fluorescence microscopy. The fluorescence microscope was composed of an argon ion laser (Innova 90-3, Coherent, Palo Alto, CA), an inverted optical microscope (Zeiss IM-35, Eastern Microscope, Raleigh NC), and a single-photon-counting photomultiplier (31034A, RCA, Lancaster, PA) interfaced to an IBM PC AT computer [9] The translational mobilities of NBD-PC and NBD-PS were measured with FPPR [11]. Parameters were as follows: laser wavelength, 488.0 nm; objective, 40X 0.75 N.A; observation laser power, 5–10 μW; bleaching laser power, 0.5 W; bleaching pulse duration, 200–500 ms; depth of bleaching, 60–80%; ruling periodicity in the sample plane, 16 μm; radii of illuminated and observed areas, ≥ 90 μm and 75 μm.

Data analysis. Recovery curves were fit to the functional forms for FPPR as previously described (Eqn. 1 in Ref. 9) *. This analysis gave best-fit values for the diffusion coefficients and fractional recoveries for each recovery curve.

In general, the primary source of experimental uncertainty in the measured diffusion coefficients and fractional mobilities could be spot-to-spot variability within a given Langmuir-Blodgett film or variability between different independently prepared films. Thus, the best-fit values of the free parameters were averaged with two methods: in one method, the means and standard deviations were obtained by averaging over all recovery curves; in the other method, means and standard deviations were obtained by averaging over the means from each independently prepared monolayer. This analysis showed only very small differences (between the two methods) for the calculated means and standard deviations. The results shown in Tables I and II are those obtained by averaging over the means from each monolayer.

The statistical significance of changes in the NBD-PS and NBD-PC diffusion coefficients that accompanied the presence of Ca^{2+}, fragment 1, and/or ovalbumin was addressed using *t*-tests [12]. The calculated values of *P* were strongly dependent on whether the number of trials was taken to equal the number of monolayers or the number of individual recovery curves. Because the measured diffusion coefficients were more equivalent for measurements taken on a single monolayer than on different monolayers, the larger values of *P* (those calculated by using only the means from each monolayer) were taken to be more accurate and are shown in Table III.

The possibility that some or all of the recovery curves would best be described by the functional form for two distinct probe populations with different diffusion coefficients was examined by analysis with the *F*-statistic

$$F = \frac{(N-5)(\chi_1^2 - \chi_2^2)}{2\chi_2^2} \quad (1)$$

where χ_i^2 was the chi-squared goodness-of-fit statistic for the FPPR functional form for *i* diffusing components and *N* was the number of data points (usually 600). In this analysis, *F* values well above the critical value of 3.0 indicate that the increased accuracy of the two-component fit is statistically significant [9].

Results

The surface pressures of PS/POPC monolayers at the air/water interface increased monotonically during compression to smaller average molecular areas, ranging from approx. 5 dyne/cm at 100 Å^2/molecule to approx. 35 dyne/cm at 65 Å^2/molecule. These data were consistent with previous measurements of the pressure-area curves for monolayers of a similar phospholipid composition [13]. The pressure-area curves were not significantly altered by the presence of 2 mol% NBD-PC or NBD-PS, also consistent with results for similar systems [14,15]. The ratios of the differences in the surface areas of air/water interface PS/POPC monolayers before and after deposition, to

* A typographical error in Eqn. 1 of Ref. 9 reads "$-\exp(-36\pi^2 Dt/a^2)$" rather than "$+\exp(-36\pi^2 Dt/a^2)$". All FPPR data in the work described herein and in Refs. 7–10 and 15 were analyzed with an equation containing the correct sign (+).

the areas of the tetradecyltrichlorosilane-treated fused silica substrates, were approx. 1.2. This transfer ratio is consistent with previous measurements for phospholipid Langmuir-Blodgett monolayers and bilayers [4,5].

The spatial distribution of both NBD-PC and NBD-PS in the PS/POPC monolayers appeared uniform within optical resolution for all sample types. This observation rules out the possibility that fragment 1 and Ca^{2+} induce large (≥ 1 μm) domains in which NBD-PC or NBD-PS significantly partition.

The PS/POPC monolayers were expected to be fluid-like because of the presence of an unsaturated acyl chain on POPC and the heterogeneous nature of the bovine PS acyl chains. FPPR measurements showed

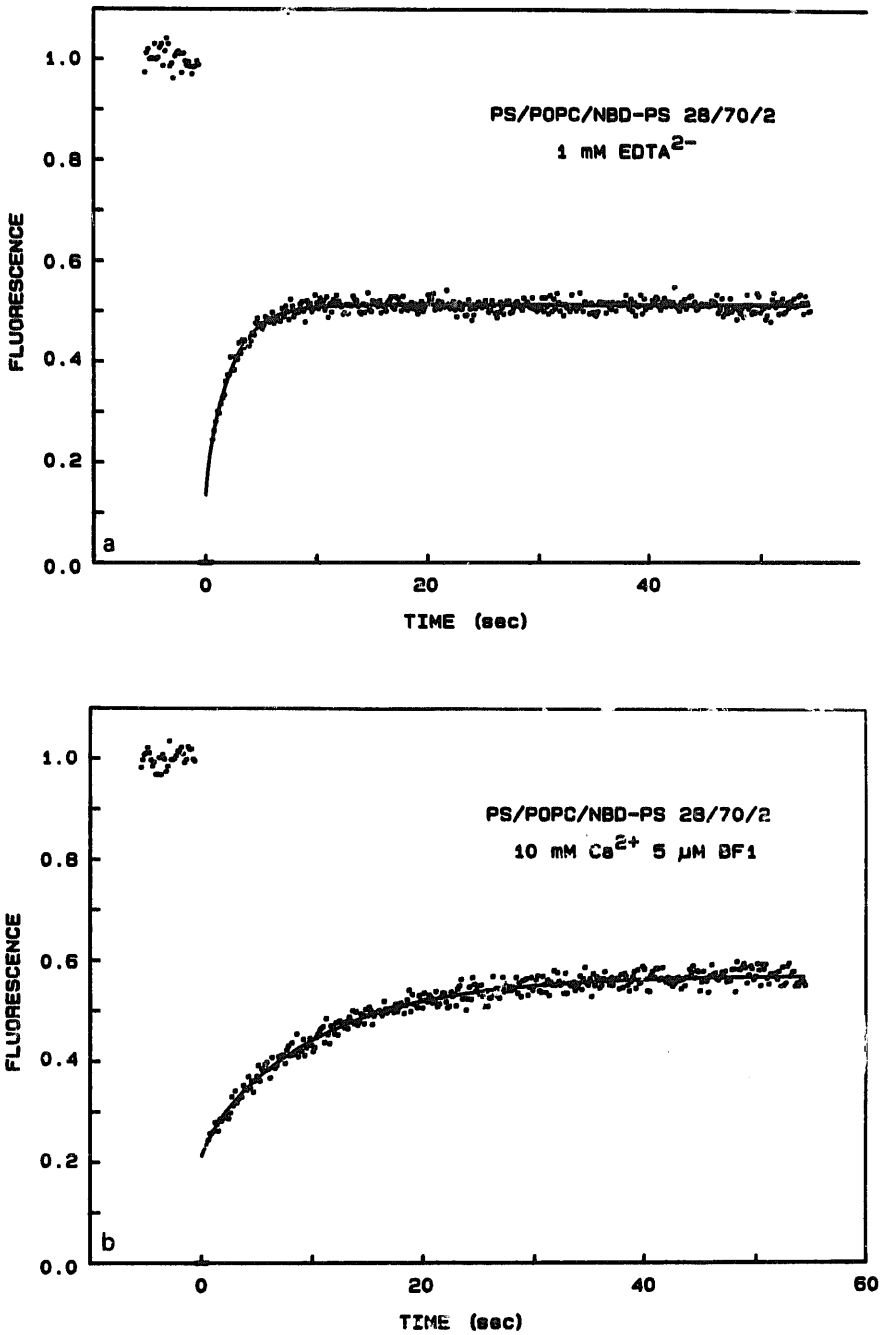

Fig. 1. Fluorescence pattern photobleaching recovery curves. Shown are typical curves for PS/POPC/NBD-PS (28:70:2, mol/mol/mol) monolayers in TBS with (a) 1 mM EDTA or (b) 10 mM Ca^{2+} and 5 μM fragment 1. The lines show the best fits to Eqn. 1 of Ref. 9 (see footnote on p. 260). The best-fit parameters gave values for the diffusion coefficients and fractional mobilities equal to (a) $2.82 \cdot 10^{-8}$ cm^2/s, 0.93 and (b) $0.61 \cdot 10^{-8}$ cm^2/s, 0.97.

TABLE I

Diffusion of NBD-PS in PS / POPC monolayers

The composition of the buffered solution adjacent to the monolayers was Tris buffer with EDTA, Ca^{2+}, fragment 1 (F1) or ovalbumin (Ova). Diffusion coefficients (D) and mobile fractions (f) were obtained from FPPR curves by nonlinear curve-fitting and are the means and standard deviations obtained by averaging over all recovery curves. The parameters m and n denote the total number of monolayers and recovery curves, respectively.

EDTA 1 mM	Ca^{2+} 10 mM	F1 5 μM	Ova 5 μM	D (10^{-8} cm^2/s)	f	m	n
+	−	−	−	2.82±0.51	0.91±0.06	4	20
−	+	−	−	2.16±0.41	0.92±0.04	4	19
+	−	+	−	2.39±0.43	0.93±0.06	4	19
−	+	+	−	0.62±0.24	0.90±0.07	4	21
+	−	−	+	2.18±0.07	0.93±0.04	3	15
−	+	−	+	1.92±0.25	0.92±0.04	3	15

TABLE II

Diffusion of NBD-PC in PS / POPC monolayers

The composition of the buffered solution adjacent to the monolayers was Tris buffer with EDTA, Ca^{2+}, fragment 1 (F1) or ovalbumin (Ova). Diffusion coefficients (D) and mobile fractions (f) were obtained from FPPR curves by nonlinear curve-fitting and are the means and standard deviations obtained by averaging over all recovery curves. The parameters m and n denote the total number of monolayers and recovery curves, respectively.

EDTA 1 mM	Ca^{2+} 10 mM	F1 5 μM	Ova 5 μM	D (10^{-8} cm^2/s)	f	m	n
+	−	−	−	3.03±0.25	0.88±0.03	3	18
−	+	−	−	2.54±0.61	0.88±0.05	4	20
+	−	+	−	2.46±0.21	0.87±0.08	3	17
−	+	+	−	1.15±0.23	0.86±0.05	3	17
+	−	−	+	2.61±0.31	0.92±0.03	3	15
−	+	−	+	2.50±0.24	0.90±0.01	3	15

TABLE III

Effect of solution properties on NBD-PC and NBD-PS diffusion coefficients in PS / POPC monolayers

The effects of (a) Ca^{2+} in the presence of fragment 1 (F1), (b) F1 in the presence of Ca^{2+}, (c) Ca^{2+}, (d) F1, (e) ovalbumin (Ova), (f) ovalbumin in the presence of Ca^{2+}, and (g) probe head group are shown. Ratios and P values were obtained using the values for D in Tables I and II. Uncertainties in the ratios were calculated by conventional propagation methods. Values of P were obtained using two-tailed t-tests [12].

	Higher D condition	Lower D condition	Ratio	P
a	NBD-PS, EDTA, F1	NBD-PS, Ca^{2+}, F1	0.26±0.11	0.0004
	NBD-PC, EDTA, F1	NBD-PC, Ca^{2+}, F1	0.47±0.10	0.002
b	NBD-PS, Ca^{2+}	NBD-PS, Ca^{2+}, F1	0.29±0.12	0.0007
	NBD-PC, Ca^{2+}	NBD-PC, Ca^{2+}, F1	0.45±0.14	0.02
c	NBD-PS, EDTA	NBD-PS, Ca^{2+}	0.77±0.20	0.09
	NBD-PC, EDTA	NBD-PC, Ca^{2+}	0.84±0.21	0.25
d	NBD-PS, EDTA	NBD-PS, EDTA, F1	0.85±0.22	0.24
	NBD-PC, EDTA	NBD-PC, EDTA, F1	0.81±0.10	0.04
e	NBD-PS, EDTA	NBD-PS, EDTA, Ova	0.77±0.14	0.17
	NBD-PC, EDTA	NBD-PC, EDTA, Ova	0.86±0.12	0.14
f	NBD-PS, Ca^{2+}	NBD-PS, Ca^{2+}, Ova	0.89±0.20	0.42
	NBD-PC, Ca^{2+}	NBD-PC, Ca^{2+}, Ova	0.98±0.25	0.91
g	NBD-PC, EDTA	NBD-PS, EDTA	0.93±0.19	0.55
	NBD-PC, Ca^{2+}	NBD-PS, Ca^{2+}	0.85±0.26	0.35
	NBD-PC, EDTA, F1	NBD-PS, EDTA, F1	0.97±0.19	0.79
	NBD-PC, Ca^{2+}, F1	NBD-PS, Ca^{2+}, F1	0.54±0.23	0.03
	NBD-PC, EDTA, Ova	NBD-PS, EDTA, Ova	0.84±0.10	0.16
	NBD-PC, Ca^{2+}, Ova	NBD-PS, Ca^{2+}, Ova	0.77±0.12	0.09

that the two fluorescent lipids, NBD-PS and NBD-PC, underwent long-range translational mobility in the monolayers with diffusion coefficients ($\approx 10^{-8}$ cm^2/s) and fractional mobilities (≈ 0.9) indicative of a fluid-like phospholipid environment (Fig. 1). The effects of different combinations of 10 mM Ca^{2+}, 5 μM fragment 1, and/or 5 μM ovalbumin on the measured diffusion coefficients and fractional mobilities of NBD-PS and NBD-PC are shown in Tables I and II.

The statistical significance of changes in the NBD-PS and NBD-PC diffusion coefficients that accompanied the presence of Ca^{2+}, fragment 1, and/or ovalbumin was addressed using *t*-tests [12] as shown in Table III. The diffusion coefficient of NBD-PS in the presence of both fragment 1 and Ca^{2+} was reduced by factors of 3.8 and 3.4 relative to the diffusion coefficients in the presence of fragment 1 (without Ca^{2+}) or Ca^{2+} (without fragment 1), respectively. These reductions in the NBD-PS diffusion coefficient were significant at the $P = 0.0004$ and $P = 0.0007$ levels, respectively. Ca^{2+} and fragment 1 also reduced the diffusion coefficient of NBD-PC. These effects were smaller, corresponding to factors of 2.1 ($P = 0.002$) and 2.2 ($P = 0.02$), relative to the diffusion coefficients in the presence of fragment 1 (without Ca^{2+}) or Ca^{2+} (without fragment 1), respectively. In the presence of 10 mM Ca^{2+}, the equilibrium dissociation constant of fragment 1 at PS/POPC planar membranes (30:70, mol/mol) is approx. 1 μM [7,8]. Thus, when monolayers were treated with 5 μM fragment 1 and 10 mM Ca^{2+}, the phospholipid surfaces were $\approx 83\%$ saturated with fragment 1.

Several control measurements were carried out to confirm that the decreases in the diffusion coefficients of NBD-PS and NBD-PC due to the presence of both Ca^{2+} and fragment 1 were the result of biologically specific binding of fragment 1 to the monolayer surfaces. Ca^{2+} (alone) or fragment 1 (with 1 mM EDTA) did not significantly affect the diffusion coefficient of NBD-PS or NBD-PC. Similarly, a nonspecific protein (ovalbumin) did not change the diffusion coefficient of either fluorescent lipid, either in the presence of Ca^{2+}or EDTA. Finally, the mean diffusion coefficients for NBD-PS were in general slightly lower than those for NBD-PC, but the differences were not statistically significant except in the presence of both fragment 1 and Ca^{2+} ($P = 0.03$).

The possibility that some or all of the recovery curves might not be well described by the simplest functional form for FPPR data was examined with an *F*-statistic which compares the values of the chi-squared goodness-of-fit statistic for the best fits to the equations for samples with one population of fluorescent molecules or with two populations of fluorescent molecules that have different diffusion coefficients [9]. The values of *F* for all sample types were either below the critical value of 3.0 or only slightly larger. Thus, within experimental uncertainty, the FPPR data were best described by the functional form for a single diffusing species.

Discussion

The result that the diffusion coefficients of NBD-PS and NBD-PC were reduced when the PS/POPC monolayer surfaces were coated with both Ca^{2+} and fragment 1 is in qualitative agreement with results from other model systems. Previous studies using fluorescence anisotropy [16], ESR [17] and FPPR [15] have demonstrated that both phospholipid acyl chain flexibility and phospholipid diffusion can be restricted when proteins extrinsically or intrinsically [19] bind to phospholipid surfaces. In addition, fragment 1, in the presence of Ca^{2+}, increases the phase transition temperatures of vesicles containing negatively charged phospholipids [20]. However, the reduced lipid diffusion coefficients are not consistent with a previous set of measurements describing excimer formation by pyrene-labelled phosphatidylglycerol and phosphatidylcholine in phospholipid vesicles. These measurements showed that the addition of saturating amounts of fragment 1 and Ca^{2+} did not significantly change the lateral diffusion coefficients of the labelled phospholipids [21]. A possible explanation for this discrepancy is that, as compared to FPPR, excimer techniques measure short range diffusion, which may not be sensitive to the presence of obstacles (see below).

The results in Tables II and III demonstrate that the NBD-PC diffusion coefficient is reduced by a factor of approximately two when the monolayer surfaces are saturated with fragment 1 and Ca^{2+}. Plausible explanations for this effect include the following: (1) The bound fragment 1 might increase the monolayer viscosity either by reducing the flexibility of the phospholipid acyl chains or by partially penetrating into the hydrocarbon region of the monolayer. (2) The local solution viscosity might be increased by the effectively high concentration of fragment 1 near the monolayer surface; estimating the thickness of the fragment 1 layer on the monolayers as 30 Å and using the known fragment 1 molecular weight (23500) and the previously measured two-dimensional density of bound fragment 1 (≥ 22000 molecules/μm^2 at 83% saturation) [8], one finds that the effective concentration of fragment 1 near the monolayer surfaces is ≥ 12 mM (or 290 mg/ml). (3) If complexes of PS and fragment 1 were present (see below), the complexes could act as obstacles around which the NBD-PC molecules must diffuse; theoretical calculations have indicated that the diffusion coefficients of tracer molecules can be reduced by at least a factor of two when a significant fraction of the monolayer area is occupied by obstacles [22,23]. (4) If complexes of PS and fragment 1 were

present, it is plausible that the NBD-PC molecules became transiently 'trapped' in the complexes, thereby lowering the apparent lateral diffusion coefficient.

When the PS/POPC monolayers were coated with fragment 1 and Ca^{2+}, the lateral diffusion coefficient of NBD-PS was reduced 4-fold. Whereas a combination of general effects (see above) might cause a slight reduction in the diffusion coefficient of both NBD-PC and NBD-PS, more chemically specific effects may contribute additional restrictions to the random motions of NBD-PS. A likely explanation for the increased reduction of NBD-PS diffusion is that the bound fragment 1 molecules recognize a small number of negative phospholipids as their binding sites [20,21,25–28]. If the bound fragment 1 molecules diffuse more slowly than the free NBD-PS, then the association of the NBD-PS with the putative fragment 1-phospholipid complexes would restrict the diffusion of the NBD-PS.

The FPPR data for NBD-PS would not be well described by the functional form for one diffusing population if the putative complexes of fragment 1 and NBD-PS were long-lived relative to the fluorescence recovery times of 2–10 s, if a significant fraction of the NBD-PS existed in both free and bound states at equilibrium, and if the diffusion coefficients of bound and free NBD-PS molecules were significantly different. Because the FPPR data do not support the existence of different NBD-PS populations which have different diffusion coefficients, we first assume that the putative complexes of fragment 1 and phospholipid are short-lived.

For the case in which the NBD-PS molecules rapidly exchange between free and bound states, the measured diffusion coefficient D would be given by [29]

$$D = \alpha D_b + (1-\alpha) D_f \tag{2}$$

where D_b and D_f are the NBD-PS diffusion coefficients in the bound and free states and α is the fraction of the NBD-PS molecules that is bound to fragment 1 at equilibrium. If D_f is equal to the diffusion coefficient of NBD-PS in the presence of Ca^{2+} alone (without fragment 1), then $D/D_f \approx 0.3$; if D_f is equal to the diffusion coefficient of NBD-PC in the presence of both fragment 1 and Ca^{2+}, then $D/D_f \approx 0.5$ (see Table III). In addition, there are two physical restrictions given by the conditions that $\alpha \leq 1$ and $D_b \geq 0$. These restrictions, the assumed ratios of D/D_f, and Eqn. 2 imply that $0.7 \leq \alpha \leq 1$ or $0.5 \leq \alpha \leq 1$ and that $0 \leq D_b/D_f \leq 0.3$ or $0 \leq D_b/D_f \leq 0.5$.

The result that the diffusion coefficient of the bound fragment 1 is at least 2- or 3-fold lower than the free phospholipid diffusion coefficient (i.e., $D_b/D_f \leq 0.3$ or $D_b/D_f \leq 0.5$) is consistent with previous measurements of the diffusion coefficients of other proteins tightly bound to planar model membrane surfaces. These measurements have shown that the diffusion coefficients of extrinsically bound proteins are usually at least 2-fold lower than the phospholipid diffusion coefficients and are often much lower [9,30,31].

The previously estimated fragment 1 surface density, $2.2 \cdot 10^4$ molecules/μm^2 at 83% saturation [8], and the known phospholipid density, $1.5 \cdot 10^6$ phospholipids/μm^2, imply that the model system contains ≈ 68 phospholipids (20 PS and 48 POPC) per fragment 1 molecule. Assuming that the NBD-PS probe molecules accurately report the behavior of the unlabelled PS molecules, then the data imply that between 10 ($\alpha = 0.5$) and 20 ($\alpha = 1$) PS molecules are associated with a putative phospholipid-fragment 1 complex at any given time. Previous estimates for the number of PS molecules that group to form a fragment 1 binding site have ranged from 3 to 20 [20,21,25–28].

Approximate information may be obtained about the average time for which an NBD-PS molecule is transiently bound to a fragment 1 molecule by viewing the association of NBD-PS with fragment 1 as a reversible isomerization (between free and bound states). The ratio of the average times for which the NBD-PS molecules are freely diffusing (τ_f) rather than transiently bound to fragment 1 (τ_b) is

$$\frac{\tau_b}{\tau_f} = \frac{\alpha}{1-\alpha} \tag{3}$$

The characteristic diffusional length d may be estimated as the difference between the center-to-center distance for fragment 1 molecules $(\mu m^2/22000)^{1/2} \approx 67$ Å (see above) and the fragment 1 diameter (36 Å) [32]. The resultant $d = 31$ Å, the measured $D_f \approx 3 \cdot 10^{-8}$ cm^2/s, and the expression

$$\tau_f = \frac{d^2}{4D_f} \tag{4}$$

imply that $\tau_f \approx 1$ μs. Using this value of τ_f together with the minimum and maximum values of α for NBD-PS in Eqn. 3, one finds that $\tau_b \geq 1$ μs. In addition, τ_b must be less than the surface residency time for fragment 1 on planar membranes, which is on the order of seconds [8].

Even though the photobleaching data do not have a clear two-component feature, it is possible that the putative complexes of phospholipids and fragment 1 are long-lived but the data are too noisy to distinguish the two components. In this case, the diffusion coefficients of the bound (D_b) and free (D_f) labelled phospholipids would have to be approximately equivalent (within a factor of two or three) and the fractional fluorescence recoveries associated with each component would also have to be approximately equal. These

results would imply a conclusion not unlike the rapid-exchange case (see above), that a significant fraction of the phospholipids are associated with fragment 1 molecules at any given time.

Insight into the dynamics of molecular events at membrane surfaces is important for understanding surface-requiring enzymatic processes, such as those in blood coagulation. Results from the present study indicate that prothrombin fragment 1 decreases the lateral mobility of both phosphatidylserine and phosphatidylcholine in Langmuir-Blodgett monolayers. In the future, combining fluorescence photobleaching with evanescent interference patterns [33] should provide additional information about the diffusional properties of the loosely bound fragment 1.

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