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The Adsorption of Prothrombin to Phosphatidylserine Multilayers Quantitated by Ellipsometry*

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We investigated by means of an automated ellipsometer the adsorption of prothrombin from a buffer solution by multilayers of 14:0/14:0- and 18:1/18:1-phosphatidylserine (PS) stacked on chromium slides. In this instrument thickness and refractive index of the adsorbed phospholipid and proteins are monitored continuously.

Two equations are derived to relate the mass of stacked phospholipids and the mass of protein adsorbed to the thickness and refractive index. These equations are based upon the Lorentz-Lorenz relation among the molar refractivities, refractive indices, and the densities of binary mixtures.

Experimental validation of these equations is performed by measuring stacked multilayers of known mass of phosphatidylserine and the adsorption of [^{125}I] albumin and [^{3}H]prothrombin on these multilayers.

Using these equations we measured the dissociation constants K_d and the number of binding sites n_b of prothrombin. Values of $K_d = 0.15 \times 10^{-8}$ M and $n_b = 122$ molecules of PS/molecule of prothrombin were observed for di $C_{14:0}$ PS and values of $K_d = 0.45 \times 10^{-8}$ M and $n_b = 54$ molecules of PS/molecule of prothrombin for di $C_{18:1}$ PS. These data compare well to data obtained by other methods available in the literature.

Several crucial steps in the activation sequence of blood coagulation occur at phospholipid-water interfaces (1). In order to allow a quantitative description of these reactions it is essential to know the binding parameters of the enzymes and proenzymes involved at these phospholipid surfaces. To determine these protein-lipid interactions different techniques have been used, such as gel filtration, light scattering, fluorescence quenching, and measurement of surface radioactivity (2-6).

In this paper we present quantitative automatic ellipsometry as a new technique by which the adsorption process of proteins on phospholipid surfaces can be studied. As a model for the phospholipid surfaces we use phospholipid mono- or multilayers which are stacked on a reflecting chromium surface by the dipping technique of Blodgett-Langmuir (7). The optical constants of these layers are measured before, during, and after the interaction with the proteins. From these measurements the amount and density of the protein and lipid in the protein-lipid complex can be calculated directly.

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MATERIALS AND METHODS

The following phospholipids were used: 1,2-dimyristoyl-*sn*-glycero-3-phosphoserine (14:0/14:0 – PS) and 1,2-dioleoyl-*sn*-glycero-3-phosphoserine (18:1/18:1 – PS). They were prepared by enzymatic synthesis from the corresponding glycerophosphocholine (8). Bovine prothrombin was prepared according to the method of Owen *et al.* (9). All other chemicals used were Merck P.A. Chromium-coated glass slides were manufactured by Stabilix, The Hague, Holland ($n = 3.0$; $k = -2.5$). Radioactive ^{3}H-labeled prothrombin was prepared by oxidation with sodium metaperiodate and then by reducing with sodium [^{3}H]borohydride (10). The specific radioactivity of [^{3}H]prothrombin was 1.64×10^{7} cpm/mg. [^{3}H]$NaBH_4$ and ^{125}I-human serum albumin were purchased from Amersham. The composition of buffers is given in the legends to the figures.

Stacking of Monolayers or Multilayers—Stacking was done with a preparative Langmuir trough (Lauda, Type FW-1) according to the method of Blodgett and Langmuir (7). Unless mentioned otherwise the trough was filled with double distilled water and 5 μM $CaCl_2$. On this aqueous subphase a monomolecular film of phospholipids is spread by adding 100 μl of a solution containing ≅2 mg of phospholipid/ml of chloroform and the surface pressure is held constant at 40 dyne/cm. A chromium-coated glass slide is mechanically dipped into this trough and subsequently redrawn at a speed of 2 mm/min. A double layer of phospholipid is deposited on the slide at each repeated dip. The surface area/molecule of phospholipid was determined on this trough at collapse pressure. The exact quantity of phospholipid spread on the trough was determined by phosphorus analysis (11). In this way it was possible to stack phospholipid layers with an exactly known mass.

Validation of the Lorentz-Lorenz Equations—This validation was performed by using stacked phospholipid layers of known mass and by using radioactive proteins. The phospholipid layers were measured in air and in buffer. The validation using the proteins was performed in the following way. Chromium-coated glass slides were stacked with phospholipid multilayers and placed in a cuvette filled with buffer. The protein was added to the cuvette. After adsorption the cuvette was repeatedly rinsed with a volume of buffer 10 times that of the cuvette in order to avoid errors due to radioactive proteins in the adhering water. The cuvette was removed and the protein was desorbed from the slide with a 1 M HCl solution and the amount of radioactivity was counted. Adsorptions of [^{3}H]prothrombin and [^{125}I] albumin to di $C_{14:0}$ PS[1] were performed at pH 5 to avoid desorption during the change of the content of the cuvette. With di $C_{18:1}$ PS, protein desorption was sufficiently slow to allow measurement at pH 7.5.

Determination of the Equilibrium Constant—To study the adsorption of prothrombin on di $C_{14:0}$ PS, we stacked a double layer of di $C_{14:0}$ PS on the chromium slide. This slide was put in the cuvette filled with 0.05 M Tris-HCl buffer, pH 7.5, and 10 mM Ca^{2+}, 0.1 M NaCl. In order to obtain maximal adsorption of prothrombin, the slides had to be conditioned at 40-50 °C for a few minutes. Prothrombin was adsorbed at 37 °C. The protein concentrations used varied between 0.1 and 40 μg/ml. The adsorption of prothrombin to di $C_{18:1}$ PS was done under the same conditions as for di $C_{14:0}$ PS except for the conditioning, which in this case had no influence on the amount of prothrombin adsorbed.

[1] The abbreviation used is: PS, phosphatidylserine.

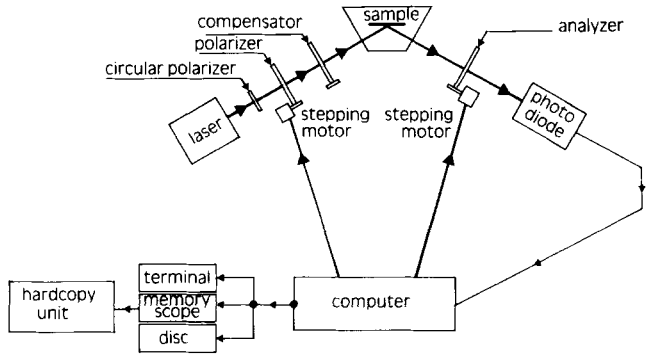

FIG. 1. **Schematic representation of the automated ellipsometer.**

Ellipsometry—The ellipsometer is an optical instrument that measures the changes in the polarization of light due to reflection (*cf.* Fig. 1). These changes are influenced by the presence of a thin film of substance on the reflecting surface. The refractive index n and thickness d of, for instance, an adsorbing layer of protein can be measured at short intervals (1–10 s) because the positions of the polarizer and analyzer are monitored. The instrument used is a modified Rudolph & Sons ellipsometer Type 43303-200 E. The instrument is automated by computer-controlled stepping motors on the two polarizers indicated in Fig. 1 as the polarizer (P) and the analyzer (A). The measurement consists of finding the positions of P and A corresponding to minimal transmission of light to the photodiode. A complete description of the instrument is given in Refs. 12 and 13. The method of computation is based on Refs. 14 and 15. It can be summarized as follows. The ratio R_p/R_s, where R_p is the reflection coefficient for light polarized parallel to the plane of incidence and R_s is the reflection coefficient for light polarized perpendicular to the plane of incidence, is given by

$$R_p/R_s = \tan \Psi \cdot \exp(i\Delta) \tag{1}$$

where Ψ and Δ can be directly determined from the readings of, respectively, A and P and $i = \sqrt{-1}$. n and d of phospholipid layers stacked on chromium slides were analyzed according to the system presented in Fig. 2. The reflection coefficients R_p and R_s are dependent upon the angle of incidence ϕ_1, the wavelength of light λ, the refractive indices n_1, n_2, and n_3, and the thickness d_2. In fact Equation 1 can be written (16) as

$$C_1(\exp D)^2 + C_2(\exp D) + C_3 = 0 \tag{2}$$

where C_1, C_2, and C_3 are complex functions of the refractive indices, Ψ and Δ and

$$D = -4\pi i n_2 \sqrt{1 - (n_b \cos \phi_1/n_2)^2 d_2/\lambda} \tag{3}$$

The value of n_1 is determined by refractometry and the (complex) value of n_3 is determined ellipsometrically for the chromium slide in buffer, before it is coated with phospholipid. Substituting these values, and an arbitrary (real) value for n_2, in Equation 2 will generally yield a complex value for d_2. The correct value for d_2 must however be real, so Equation 3 is solved by an iterative procedure in which n_2 is adjusted such that the complex part of d_2 is minimized.

Proteins adsorbed on phospholipid were analyzed according to the system presented in Fig. 3. Equations 2 and 3 remain valid but the complex functions C_1, C_2, and C_3 now also depend upon n_3 and d_3. Values of n_3 and d_3 are determined by ellipsometric measurement before the protein is added to the cuvette.

Calculation of the Adsorbed Mass from the Refractive Index and Thickness of an Adsorbed Layer—The Lorentz-Lorenz relation for the refractive index n of a mixture of substances can be written as (17):

$$\frac{n^2 - 1}{n^2 + 2} = A_1N_1 + A_2N_2 + A_3N_3 + \ldots.$$

where A_i and N_i are, respectively, the molar refractivity of substance i and the number of moles of substance i per unit volume. For a pure substance we may write

$$\rho^\circ = M \cdot N = \frac{M}{A}\frac{n^2 - 1}{n^2 + 2}$$

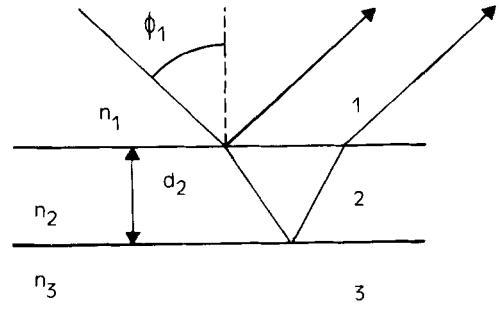

FIG. 2. **Analysis of phospholipid layers.** *1*, buffer; *2*, phospholipid; *3*, chromium.

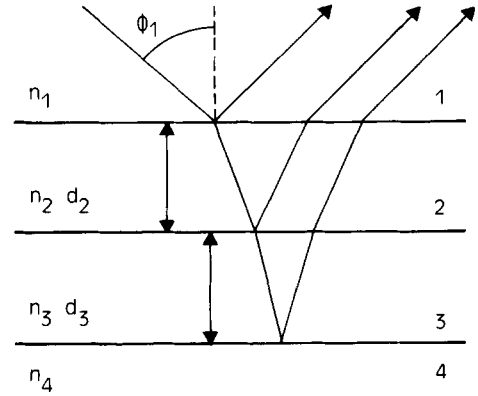

FIG. 3. **Analysis of protein adsorption to phospholipids.** *1*, buffer; *2*, protein; *3*, phospholipid; *4*, chromium.

where ρ° is the density in mass per unit volume. If we consider an adsorbed layer of thickness d we find for the adsorbed mass of a pure substance

$$m = d \cdot \rho^\circ = \frac{0.1\ M \cdot d}{A}\left(\frac{n^2 - 1}{n^2 + 2}\right) \tag{4}$$

where the thickness d is expressed in nanometers and the adsorbed mass m is expressed in micrograms per square centimeter. For a mixture of buffer (b) and protein (p) we have

$$\frac{n^2 - 1}{n^2 + 2} = A_bN_b + A_pN_p = \frac{A_b}{M_b}\rho_b + \frac{A_p}{M_p}\rho_p \tag{5}$$

Assuming that we have an ideal mixture, the volume fraction of protein is $V_{20}\rho_p$, where V_{20} is the partial specific volume of protein at 20 °C, and $V_{20}\rho_p^\circ = 1$ with ρ_p° the density of the pure protein. The remaining volume fraction $(1 - V_{20}\rho_p)$ has the density of pure buffer, *i.e.* ρ_b°. Thus the density of buffer in the mixture is $\rho_b = \rho_b^\circ(1 - V_{20}\rho_p)$ and we obtain

$$\frac{n^2 - 1}{n^2 + 2} = \frac{A_b}{M_b}\rho_b^\circ(1 - V_{20}\rho_p) + \frac{A_p}{M_p}\rho_p$$

$$= \frac{n_b^2 - 1}{n_b^2 + 2}(1 - V_{20}\rho_p) + \frac{A_p}{M_p}\rho_p$$

where n_b is the refractive index of pure buffer.

From this relation it is easily verified that the adsorbed mass of protein in an adsorbed mixed layer of thickness d in nanometers is given by

$$m = d \cdot \rho_p = \frac{0.3\ d \cdot f(n)}{\frac{A_p}{M_p} - V_{20}\frac{n_b^2 - 1}{n_b^2 + 2}}(n - n_b)$$

$$f(n) = \frac{n + n_b}{(n^2 + 2)(n_b^2 + 2)} \tag{6}$$

From Formula 6 it follows that the molecular weight, the molar refractivity, and the partial specific volume of the adsorbed or stacked molecular species have to be known in order to obtain m from d and n.

Molar Refractivity—The molar refractivity of a molecular species can in principle be obtained from the known data of its constituent material. Using the different values of the molar refractivities of the atoms or atom groups (Table I), we calculated the molar refractivities of the different compounds that are used from their molecular structures (17). Knowing this molar refractivity it is easy to calculate the M/A or A/M values for the mass formulas. In order to calculate the M/A for the proteins, we first calculated the M/A values of the different amino acids and then calculated the M/A values of the proteins by taking the weighted average of their amino acids. If part of the protein consisted of carbohydrate we included their calculated M/A values in our calculations. Calculated values of the M/A values of proteins were checked with data on albumin solutions of different densities and refractive indices known from the literature (19). The M/A for albumin thus calculated was 4.12, whereas the value calculated from data in the literature was 4.14. No data are available on the refractive index as a function of prothrombin concentration because of the large quantities that are needed for such experiments; so for prothrombin we calculated M/A = 4.23 from the amino acid composition (20).

Partial Specific Volumes—The values of the partial specific volumes of the proteins were taken from the literature (Table I). For phospholipids only a few data are available (21) and we determined the partial specific volumes in the following way. From the change in area of the monomolecular film on the Langmuir trough the quantity of adsorbed phospholipid per cm^2 was calculated. The thickness of this layer was measured in air by ellipsometry and, based on the high refractive index of the layers, we assumed that the water content of these stacked phospholipid layers on chromium in air was too low to influence the thickness significantly. This assumption is supported by the validity of the one-component formula (*cf.* below) and also by direct observations that stacked lipid layers in air do not contain any water (22). The partial specific volume was calculated according to the following relation

$$V_{20} = \frac{\text{thickness}}{\text{mass/cm}^2}$$

The thicknesses of these stacked layers are given in Table III. The calculated partial specific volumes are shown in Table II.

Accuracy of Ellipsometric Measurements and Mass Calculation—In Fig. 4 the registration of ellipsometer readings during a prothrombin adsorption on 4 layers of di $C_{18:1}$ PS on chromium is shown. The total change in analyzer and polarizer values during protein adsorption is about 0.9° for the polarizer and 0.6° for the analyzer. These changes correspond to an adsorbed mass of about 0.30 $\mu g/cm^2$.

As illustrated in Fig. 5, for protein adsorptions, experimental scatter

TABLE I

Molar refractivities of the atoms or atom groups according to Vogel (18)

Atom or atom group	Molar refractivity A	Atom or atom group	Molar refractivity A
C	2.591	N(sec)	2.582
H	1.028	C_6H_5	25.463
=O	2.122	S	7.729
>O	1.643	Cl	5.844
—OH	2.553	Ca	7.27
N(prim)	2.376	P	7.15

TABLE II

Different constants used in the calculations

Compound	M	A	M/A	V_{20}
14:0/14:0 PS	680[a]	183.6	3.70	
	700	187.2	3.73	0.906
18:1/18:1 PS	791[a]	216.6	3.65	
	811	220.2	3.68	0.889
Albumin	66,500		4.12	0.729
		16.070	4.14	
Prothrombin	73,000		4.23	0.700
Sialic acid	302	66.13	4.57	
Mannose	180	37.15	4.85	
Galactose	180	37.15	4.85	
(*N*-Acetylglucosamine)	198	46.95	4.22	

[a] Including ½ Ca^{2+} because of Ca-PS complex.

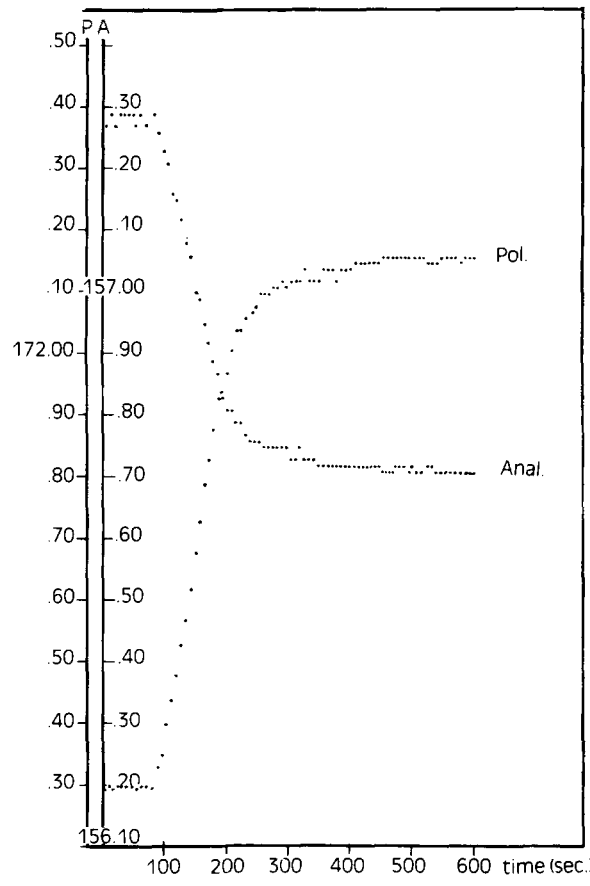

FIG. 4. **Prothrombin adsorption on 4 layers di $C_{18:1}$ PS stacked on chromium.** Prothrombin concentration, 10 $\mu g/ml$; buffer, 0.05 M Tris-HCl, pH 7.5, 10 mM $CaCl_2$, 0.1 M NaCl. Analyzer and polarizer values are indicated on the figure. Analyzer and polarizer axes are different. Time is indicated in seconds.

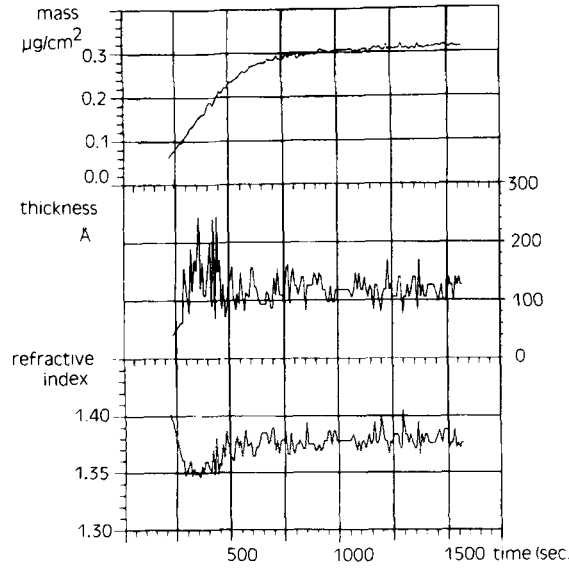

FIG. 5. **The thickness, the refractive index, and the mass of the adsorption of Fig. 4 as a function of time.**

of 0.02–0.04° in polarizer and analyzer readings (*cf.* Fig. 4) results in considerable variation in calculated thickness and refractive index. However, this is not a random scatter. A high incidental value of calculated thickness corresponds with a low value of the refractive index and vice versa. This explains why the adsorbed mass of protein can be calculated with much more accuracy then either refractive index or thickness (*cf.* Fig. 5). This sensitivity of calculated values of

TABLE III

Experimental validation of the mass relations

All values given are mean ± standard deviation. Phospholipid layers are stacked as described under "Materials and Methods." Buffer, 0.05 M Tris-HCl, pH 7.5, 10 mM $CaCl_2$, 0.1 M NaCl. Prothrombin adsorption on 8 layers of di $C_{14:0}$ PS: prothrombin concentration, 20 μg/ml, 0.05 M Hepes, pH 5. Prothrombin adsorption on 8 layers of di$C_{18:1}$ PS: prothrombin concentration 20 μg/ml, 0.05 M Tris-HCl, pH 7.5, 10 mM $CaCl_2$, 0.1 M NaCl. Albumin adsorption on 8 layers of di $C_{14:0}$: albumin concentration, 20 μg/ml, 0.05 M Hepes, pH 5.

	Thickness	Refractive index	One-component formula	Two-component formula	Direct determination
	nm		*μg/cm²*	*μg/cm²*	*μg/cm²*
Twelve layers di $C_{14:0}$ PS in air ($N = 10$)	2.43 ± 0.06	1.53 ± 0.02	0.28 ± 0.01	0.31 ± 0.02	0.27 ± 0.01
Twelve layers di $C_{18:1}$ PS in air ($N = 10$)	1.93 ± 0.06	1.54 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.22 ± 0.01
Twelve layers di $C_{14:0}$ PS in buffer ($N = 10$)	2.98 ± 0.15	1.49 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.27 ± 0.01
Twelve layers di $C_{18:1}$ PS in buffer ($N = 10$)	2.52 ± 0.10	1.48 ± 0.01	0.26 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
[^{3}H]Prothrombin on di $C_{14:0}$ PS ($N = 3$)	2.79 ± 0.77	1.90 ± 0.20	0.53 ± 0.06	0.73 ± 0.03	0.51 ± 0.03
[^{3}H]Prothrombin on di $C_{18:1}$ PS ($N = 3$)	5.17 ± 1.12	1.46 ± 0.02	0.60 ± 0.10	0.37 ± 0.01	0.35 ± 0.02
[^{125}I]Albumin on di $C_{14:0}$ PS ($N = 3$)	1.42 ± 0.14	1.73 ± 0.06	0.23 ± 0.01	0.29 ± 0.01	0.25 ± 0.01

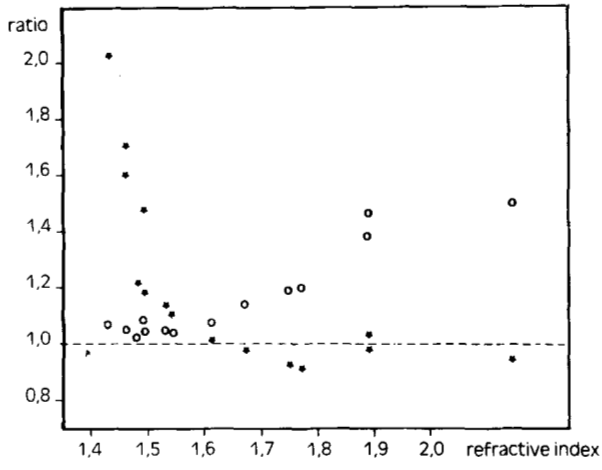

FIG. 6. **Mass calculated, with the one-component formula and the two-component formula, divided by the direct determined mass measured on the Langmuir trough or by radioactivity presented as a function of the refractive index.** ★, one-component formula; ○, two-component formula.

thickness and refractive index to experimental scatter in P and A readings is dependent on the specific optical properties of the system under study. It is much less, for instance, in experiments with stacked multilayers of PS alone.

RESULTS

Calculation of Adsorbed Mass from the Amount of di $C_{14:0}$ PS and di $C_{18:1}$ PS Deposited—Total adsorbed mass was calculated for 12 stacked monolayers of di $C_{14:0}$ PS and di $C_{18:1}$ PS and compared with the quantities of phospholipid disappearing from the Langmuir trough. The results are shown in Table III. Values of the refractive index, the thickness per layer, the mass calculated by the two-component formula, and the mass calculated by the one-component formula are given as the mean values ± standard deviation. If we compare the values of the phospholipid layers measured in air with the values measured in buffer we observe an increase in thickness and a decrease in refractive index for both phospholipids when they are in buffer, indicating swelling by penetration of water.

The results of the mass calculation with the different mass formulas show that for the layers measured in air the results obtained with the one-component formula correspond best with the directly determined mass measured on the trough,

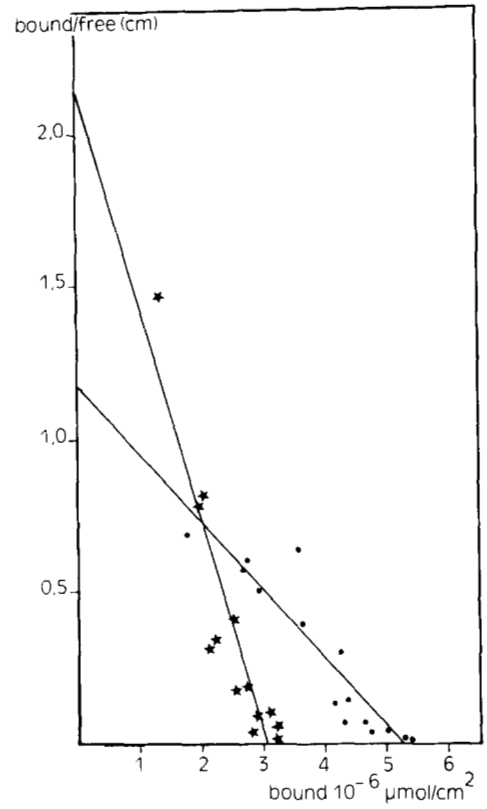

FIG. 7. **Scatchard plot.** Ratio for the equilibrium values of prothrombin surface concentration micromoles/cm² to its free concentration micromoles/cm³ against the surface concentration micromoles/cm². Lines through the data points are least squares lines. ○, di $C_{18:1}$ PS; ★, di $C_{14:0}$ PS.

whereas the layers measured in buffer are better calculated by the two-component formula.

Quantitation of [^{125}I]Albumin and [^{3}H]Prothrombin—Calculations of adsorbed mass of protein based on the one-component and the two-component formulas were compared with direct estimates of adsorbed radioactivity. Adsorptions of [^{3}H]prothrombin on 8 layers of di $C_{14:0}$ PS and 8 layers of di $C_{18:1}$ PS and adsorption of [^{125}I]albumin on 8 layers of di $C_{14:0}$ PS are shown in Table III. This table shows a considerable variation in refractive index and thickness among differ-

ent experiments. If we look at the results of the two formulas we see that the mass in some of the experiments should be calculated by the one-component formula and in other experiments by the two-component formula. To find criteria for using one of these formulas, the calculated mass divided by the directly determined mass is presented in Fig. 6 as a function of the refractive index. This figure shows that the mass should be calculated by the two-component formula if the refractive index value is between buffer values $n = 1.3335$ and $n = 1.5$–1.6, depending on the substance adsorbed. For refractive indices higher than $n = 1.5$–1.6 we have to use the one-component formula.

Adsorption of Prothrombin to di $C_{14:0}$ PS and di $C_{18:1}$ PS—To determine the dissociation constant and the number of binding sites of prothrombin to di $C_{14:0}$ PS and di $C_{18:1}$ PS we adsorbed prothrombin to these layers (0.1–40 μg/ml) at different concentrations. Fig. 7 presents the Scatchard plot of prothrombin adsorptions. We obtain two different sets of data depending upon the phospholipid used. This results in a K_d of prothrombin for di $C_{14:0}$ PS of $K_d = 0.15 \times 10^{-8}$ mol/liter and number of binding sites $n = 3.08 \times 10^{-6}$ μmol/cm^2, which corresponds to 122 mol of PS/mol of prothrombin. The values for the di $C_{18:1}$ PS-prothrombin interaction are $K_d = 0.45 \times 10^{-8}$ mol/liter and number of binding sites $n = 5.2 \times 10^{-6}$ μmol/cm^2, which corresponds to 54 mol of PS/mol of prothrombin.

DISCUSSION AND CONCLUSIONS

Calculation of Adsorbed Mass—Calculations of adsorbed mass of protein should be done by two different formulas depending on the value of the refractive index. As long as the refractive index of the adsorbed layer is between the value of pure buffer and pure protein or pure phospholipid we have to use the formula derived for the mixtures. The one-component formula should be used however if we are dealing with pure substances. This formula also is applicable for values of the refractive index which are higher than calculated for the pure substance. The existence of these high refractive indices can only partly be explained by the experimental scatter in the measurements (see "Materials and Methods"). These inaccuracies cannot explain discrepancies like a refractive index of $n = 1.46 \pm 0.02$ for adsorption of prothrombin on di $C_{18:1}$ PS and a refractive index of $n = 1.90 \pm 0.20$ for adsorption of prothrombin on di $C_{14:0}$ PS.

It is presently assumed that refractive indices that are higher than the refractive index of the pure components indicate interactions between the adsorbant and the adsorbing molecules that are more complicated than simple apposition. One might think of penetration of the protein into the lipid, shrinking or swelling of the lipid layers, etc. The validation of the formulas also shows that the assumption of ideal behavior of the protein solution, even for very high concentrations, is justified. This result was previously found for solutions with protein concentration as high as 40% protein (23, 24) in refractive index and density studies. This ideal behavior also means that the refractive index increment of these proteins is a constant at all concentrations. The good correlation between calculated mass and the radioactive labeling protein mass for prothrombin and albumin justifies the calculation of the value of M/A from the amino acid composition. As shown in Table III, the refractive index of the stacked phospholipids is lower in buffer than in air. This indicates that water molecules penetrate the phospholipid layer. In stacked phospholipid layers a water gradient was found with fluorescent probes (22). The good results of the mass calculation mean that it is also possible to determine the partial specific volume of the adsorbed or stacked substances by ellipsometry.

Binding Parameters of Prothrombin—Direct comparison of our data with those found in the literature is impossible because our results are obtained with synthetic pure compounds whereas binding parameters found in the literature are obtained with mixtures of synthetic compounds or inhomogeneous biological preparations, and in systems in which protein adsorption onto micelles is measured in the presence of Ca^{2+}, where PS cannot be used because of aggregation of the micelles. The adsorption of prothrombin to di $C_{18:1}$ PS gives results comparable with the results of Lecompte and Miller (5) who used ox brain PS which is mainly composed of di $C_{18:1}$ PS and found values of $n = 6.2 \times 10^{-12}$ mol/cm^2 and $K_d = 0.12 \times 10^{-7}$ to 0.8×10^{-8} mol/liter. In the concentration range of 0.1–40 μg/ml, no indication for a biphasic adsorption as suggested by Lecompte and Miller was found however. To obtain maximal adsorption of prothrombin on di $C_{14:0}$ PS layers, the stacked layers had to be heated in buffer for several minutes at 50 °C. This heating was not necessary for the adsorption on di $C_{18:1}$ PS. A possible explanation for this discrepancy can be the fact that di $C_{18:1}$ PS layers are stacked above the phase transition temperature whereas the di $C_{14:0}$ PS layers are stacked below this temperature. On heating di $C_{14:0}$ PS layers in the buffer, a change in the layers is observed at about 38 °C depending on pH and Ca^{2+} concentration. Even after conditioning the slides, adsorption of prothrombin to di $C_{14:0}$ PS is only 60% of the amount adsorbed on the di $C_{18:1}$ PS surface. If we compare the number of phospholipid molecules/prothrombin molecule we find $n = 122$ di $C_{14:0}$ PS/prothrombin and $n = 54$ di $C_{18:1}$ PS/prothrombin. These values give an area/prothrombin molecule of 5300 Å^2 and 3200 Å^2, respectively. Comparing the areas occupied with the cross-sectional area of the prothrombin molecule (25), the two areas are the same magnitude, suggesting a monolayer. As di $C_{18:1}$ PS has a lower surface charge than di $C_{14:0}$ PS these differences found indicate that the negative charge is probably not the only factor that is responsible for the prothrombin phosphatidylserine interaction.

In the present study binding experiments with prothrombin were performed on pure PS phospholipid layers and in the concentration range between 0.1–40 μg/ml of prothrombin. Our method is advantageous in that the binding of prothrombin to phospholipid using light scattering (3, 4) cannot be performed in the lower range of these protein concentrations and cannot be studied for pure PS because of aggregation of vesicles in the presence of Ca^{2+}. In contrast, it is not possible to measure prothrombin adsorption at concentrations exceeding ≅5 μg/ml with the Langmuir trough technique with radiolabeled proteins (5) because the phospholipid layer is solubilized at higher prothrombin concentrations.

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