Interaction of Prothrombin and Blood-Clotting Factor X with Membranes of Varying Composition†

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ABSTRACT: The interaction of prothrombin and factor X with membranes of widely varying composition is reported. These protein–membrane interactions require the presence of acidic phospholipid; phosphatidylserine is the most effective from the standpoint of protein–membrane affinity. The maximum protein binding capacity is proportional to the phosphatidylserine content of the membrane up to about 15% and the stoichiometry is 9.2 ± 1.3 phosphatidylserine residues per prothrombin molecule and 5.2 ± 1.5 per factor X molecule. The protein apparently causes clustering of the phosphatidylserine residues in these membranes of low phosphatidylserine content. Above about 20% phosphatidylserine, the factor limiting protein–membrane interaction appears to be protein packing density on the membrane surface. The maximum protein binding capacity at 50% phosphatidylserine is 1.2 g of protein per g of phospholipid. Phosphatidic acid is similar to phosphatidylserine in its ability to bind these proteins except for somewhat larger dissociation constants. Phosphatidylethanolamine and phosphatidylglycerol are less effective in all respects for promoting these protein–membrane interactions. Small amounts of phosphatidylserine mixed with these latter phospholipids, however, have a major effect on protein–membrane binding so that the dissociation constants are more characteristic of membranes of high phosphatidylserine content.

Description of the known properties of prothrombin- and factor X–membrane binding can be accomplished with the following equilibria (Nelsestuen and Lim, 1977):

$$\begin{array}{l} P + i\mathrm{Ca} \overset{1}{\rightleftarrows} P_{i\mathrm{Ca}} \overset{2}{\rightleftarrows} P'_{i\mathrm{Ca}} \\ \qquad\qquad + \qquad + m\mathrm{Ca} \overset{4}{\rightleftarrows} P'\cdot PL_{i+j+m\mathrm{Ca}} \\ PL + j\mathrm{Ca} \overset{3}{\rightleftarrows} PL_{j\mathrm{Ca}} \end{array} \quad (1)$$

In eq 1, P is protein (prothrombin or factor X), P′ is the protein after undergoing a calcium-dependent protein transition, P·L is the phospholipid expressed as the concentration of protein binding sites, $P'_{i\mathrm{Ca}}$ and $PL_{j\mathrm{Ca}}$ are complexes of protein and phospholipid with calcium, and $P'\cdot PL_{i+j+m\mathrm{Ca}}$ is the protein–phospholipid complex. The number of functional calcium ions in the complex (the sum of i, j, and m) is 6 or greater and, under conditions of 0.5 mM calcium, does not exceed 8.9 ± 1.2 (Nelsestuen and Lim, 1977). The values of i, j, and m are variable and are proposed to be interdependent. For example, the value of m decreases from several calcium ions to zero as the free calcium concentration is increased (Nelsestuen and Lim, 1977).

For the evaluation of protein binding to membranes, the most useful parameter is the value $K_4^{\mathrm{Ca}*}$ which is defined as:

$$K_4^{\mathrm{Ca}*} = \frac{[P'_{i\mathrm{Ca}} + P_{i\mathrm{Ca}}][PL_{j\mathrm{Ca}}]}{[P'\cdot PL_{i+j+m\mathrm{Ca}}]} \quad (2)$$

This equation applies at calcium concentrations above about 0.3 mM calcium (Nelsestuen and Lim, 1977). From the value of $K_4^{\mathrm{Ca}*}$ and a known total protein and total PL concentration, the quantity of protein bound to the membrane can be determined.

This communication examines the values of $K_4^{\mathrm{Ca}*}$ for prothrombin and factor X binding to membranes of varying composition. The relationship between the concentration of protein binding sites (PL) in eq 1 and the molar phospholipid concentration and composition is also determined. A preliminary presentation of these results has been given (Nelsestuen, 1977).

Materials and Methods

The proteins and phospholipids were prepared and quantitated as described elsewhere (Nelsestuen and Lim, 1977). The phospholipid compositions reported are the compositions which were mixed (i.e., before sonication and chromatography). Acidic phospholipids were mixed with phosphatidylcholine, a phospholipid which is inert in the interaction between the proteins under investigation and phospholipid. Whenever a membrane composition is expressed as a percentage or mole fraction of acidic phospholipid, the remaining phospholipid is phosphatidylcholine. Protein–membrane binding was measured by relative 90° light scattering in a Perkin-Elmer Hitachi Model MPF-2A fluorescence spectrophotometer and the free and bound protein concentrations were determined as described by Nelsestuen and Lim (1977). Protein–membrane binding capacities and dissociation constants were estimated from double-reciprocal plots (Nelsestuen and Lim, 1977). For prothrombin, these plots showed no abnormalities or significant deviation from linearity at all calcium concentrations and phospholipid compositions used in these studies. Factor X, however, appeared to stimulate aggregation of the phospholipids at high calcium concentrations (>5 mM). As for aggregation of the vesicles by calcium ions alone (Nelsestuen and Lim, 1977), this problem was more acute for membranes of high phosphatidylserine content. For this reason, most membrane compositions were tested for factor X binding at calcium concentrations below 2.5 mM only.

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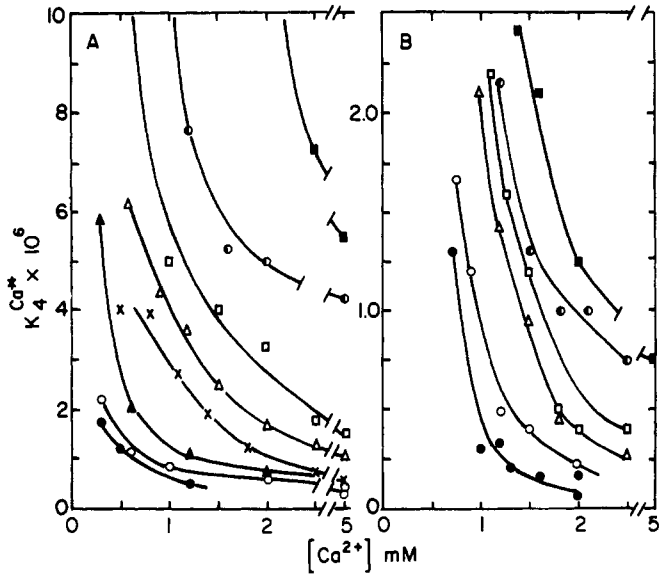

FIGURE 1: The dissociation constants for prothrombin and factor X binding to membranes of various phosphatidylserine contents at varying calcium concentrations. The values of K_4^{Ca*} (eq 2) were determined from double-reciprocal plots (Nelsestuen and Lim, 1977). A gives the values for prothrombin and B gives the values for factor X (note the different scale of the ordinate). The mole fractions of phosphatidylserine in these membranes are: 0.06 (■), 0.08 (◐), 0.10 (□), 0.12 (△), 0.16 (x), 0.19 (▲), 0.26 (○), and about 0.80 (100% Folch fraction III) (●).

Results

The values of K_4^{Ca*} for prothrombin and factor X binding to single bilayer vesicles containing various amounts of phosphatidylserine are shown in Figure 1. Membranes rich in phosphatidylserine were not studied at the higher calcium concentrations due to apparent aggregation of the membranes (Nelsestuen and Lim, 1977; Papahadjopoulos et al., 1976). One of the most striking features in comparing these results for prothrombin and factor X is that factor X has decidedly lower K_4^{Ca*} values, especially at higher calcium concentrations. As calcium concentration decreases the value of K_4^{Ca*} increases in a regular manner. By multiplying K_4^{Ca*} by $[P'_{iCa}]/[P'_{iCa}] + [P_{iCa}]$ the effects of reaction 2 (eq 1) are eliminated and the resulting term, K_4^{Ca}, is a function of reaction 4 only (Nelsestuen and Lim, 1977). The $[P'_{iCa}]/[P'_{iCa}] + [P_{iCa}]$ values can be obtained from previous data on the protein transitions (Nelsestuen, 1976; Nelsestuen et al., 1976). By applying this correction to the values of K_4^{Ca*} in Figure 1, it was found that most of the upward curvature of these plots for membranes of very high phosphatidylserine content (>25%) is due to reaction 2 of eq 1. As the phosphatidylserine content decreases, the upward curvature of the K_4^{Ca*} plots is due to both reactions 2 and the changing value of m in reaction 4 of eq 1. This is consistent with tighter calcium binding to membranes of high phosphatidylserine content such that the molecularity of calcium in reaction 4 (m in eq 1) is very low at the calcium concentrations studied and increases at lower phosphatidylserine content.

In order to demonstrate more clearly the effect of membrane composition on K_4^{Ca*}, a plot of K_4^{Ca*} for prothrombin versus membrane composition at constant calcium concentration is shown in Figure 2. It is apparent that binding affinity falls off sharply below 15% phosphatidylserine content and that very little protein will bind to membranes of less than 5% phosphatidylserine. Similar plots for factor X give similar results except that the upward curvature occurs at lower phosphatidylserine content.

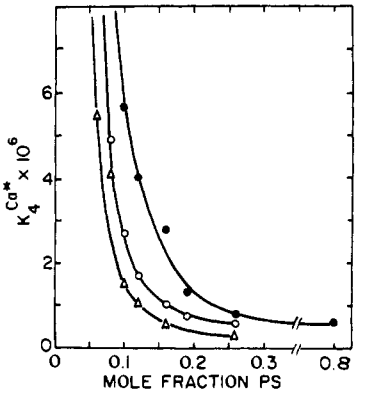

FIGURE 2: Prothrombin-membrane dissociation constants as a function of the phosphatidylserine content in the membrane. The values of K_4^{Ca*} from Figure 1 are plotted at 1 mM calcium (●), 2 mM calcium (○), and 5 mM calcium (△).

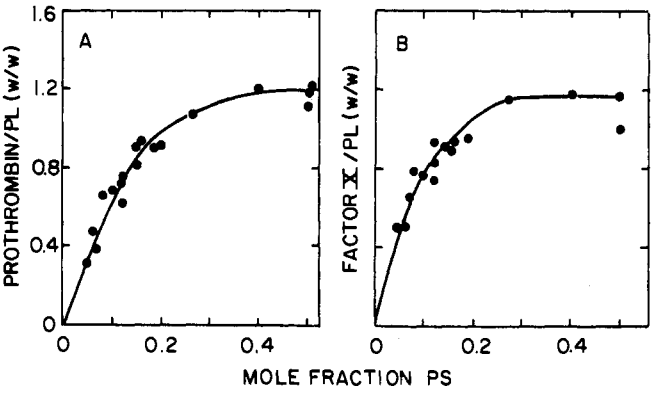

FIGURE 3: The maximum protein binding capacity of single bilayer membranes of various phosphatidylserine content. The maximum protein binding was determined from the intercept of double-reciprocal plots for prothrombin- and factor X-membrane binding as described in Materials and Methods.

Although factor Xa was not studied extensively, its membrane binding characteristics appeared to be similar to those of factor X. The K_4^{Ca*} values for factor Xa and factor X binding to membranes of 40% Folch fraction III (or about 30% phosphatidylserine) were substantially the same. The conversion of factor X to Xa does not appear to alter the membrane-binding site significantly. This is consistent with our previous report on the calcium dependence of factor X- and factor Xa-membrane binding (Nelsestuen et al., 1976).

The Membrane Capacity for Protein Binding as a Function of Phosphatidylserine Content. The maximum protein-binding capacity of the single bilayer membranes shown in Figure 1 along with a number of others was determined as described previously (Nelsestuen and Lim, 1977) from the y intercepts of the double-reciprocal plots. The results plotted in Figure 3 show that total binding capacity increases for prothrombin up to about 20% phosphatidylserine and a ratio of 1.2/1.0 (w/w, protein/phospholipid) and is then relatively stable to at least 50% phosphatidylserine. At high phosphatidylserine content the limiting factor in protein binding is probably the packing of protein on the membrane surface (Lim et al., 1977). Below about 15% phosphatidylserine the protein-binding capacity appears to be directly proportional to phosphatidylserine content and the factor limiting protein binding is assumed to be the availability of phosphatidylserine.

The number of phosphatidylserine residues required per prothrombin molecule was estimated as follows: For single

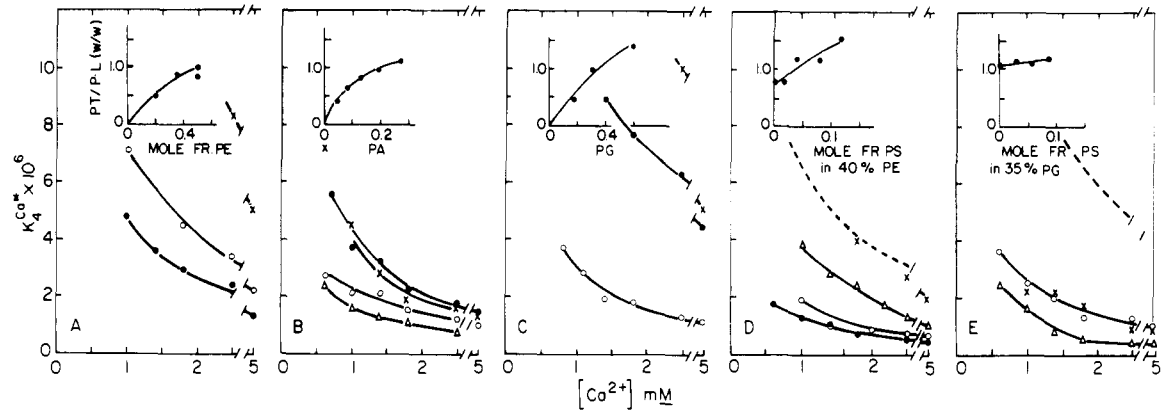

FIGURE 4: The characteristics of prothrombin binding to single bilayer vesicles of various acidic phospholipid composition. The values of $K_4^{Ca^*}$ and maximum protein binding capacity (insets) of these membranes were determined as outlined in Materials and Methods. A gives the values for membranes of 0.20 (X), 0.35 (○), and 0.50 (●) mol fraction of phosphatidylethanolamine. B gives the values for membranes of 0.045 (●), 0.09 (X), 0.13 (○), and 0.20 (△) mol fraction of phosphatidic acid. C gives the values for membranes of 0.19 (X), 0.30 (●), and 0.60 (○) mol fraction of phosphatidylglycerol. D gives the values for membranes which all contain 40% phosphatidylethanolamine with additional phosphatidylserine added: 0.02 (X), 0.04 (△), 0.08 (○), and 0.12 (●) mol fraction of phosphatidylserine. The dotted line gives the values expected for membranes containing no phosphatidylserine. E gives the values for membranes which all contain 35% phosphatidylglycerol with varying additions of phosphatidyl serine: 0.03 (X), 0.06 (○), and 0.09 (△) mol fraction phosphatidylserine. The dotted line gives the values expected for membranes containing no phosphatidylserine.

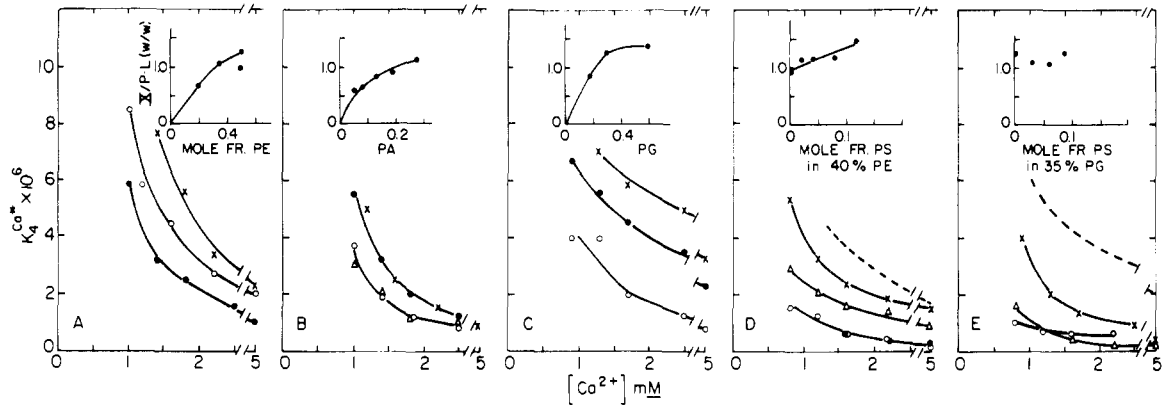

FIGURE 5: The characteristics of factor X binding to single bilayer membranes of various acidic phospholipid composition. The symbols and membrane compositions are the same as for prothrombin in Figure 4.

bilayer vesicles of about 180 Å radius, the average size of the vesicles used in these studies (Nelsestuen and Lim, 1977; Lim et al., 1977), and 50 Å membrane thickness, the external surface accounts for 64% of the total membrane surface and 64% of the phosphatidylserine present in these vesicles is assumed to be available for protein binding. From the molecular weights of these phospholipids (775) and prothrombin (72 000) and the results shown in Figure 3 (below 15% phosphatidylserine), an average value of 9.3 ± 1.5 phosphatidylserine residues per prothrombin is obtained. Since phosphatidylserine is found in higher concentrations on the inner surface of these mixed vesicles than on the outer surface, this value calculated assuming random distribution represents a maximum value. Based on one estimate for the distribution of phosphatidylserine in small vesicles (Berden et al., 1975), the actual ratio of exposed phosphatidylserine per prothrombin binding site would be about 15% lower than the value reported. The same potential correction would also apply to the factor X–phosphatidylserine ratios.

The number of phosphatidylserine residues required per factor X molecule was estimated by comparison of the prothrombin and factor X binding capacities of membranes containing less than 15% phosphatidylserine. In this way a number of membrane preparations consisting of crude phosphatidylserine (not shown) could be included. On a molar basis, these membranes all displayed a higher capacity for factor X than for prothrombin and the average value of factor X/prothrombin was 1.79 ± 0.37. If only those membranes below 10% phosphatidylserine were included, this ratio was 1.82 ± 0.4. The value of phosphatidylserine/factor X obtained from these data is 5.2 ± 1.5. The lower number of acidic phospholipids but the tighter binding of factor X to the membrane indicates a substantial difference in the mode of factor X- and prothrombin-membrane binding. A difference is also evident from the observation that factor X requires higher calcium concentration for membrane binding (Nelsestuen et al., 1976; Nelsestuen and Lim, 1977).

In order to further confirm the membrane capacities for factor X vs. prothrombin, all of the values obtained from membranes containing phosphatidylethanolamine, phosphatidic acid, and phosphatidylglycerol (see below) which were below maximum protein packing were averaged separately and gave a molar ratio of factor X:prothrombin of 1.93 ± 0.34. These observations are all consistent with prothrombin interacting with about twice as many acidic phospholipid molecules as factor X.

The Interaction of Prothrombin and Factor X with Other Acidic Phospholipids. The characteristics of prothrombin and factor X binding to membranes containing varying amounts of phosphatidylethanolamine, phosphatidic acid, and phosphatidylglycerol are shown in Figures 4 and 5. The maximum protein-binding capacities of these membranes are given in the

insets. The results for the single bilayer membranes containing phosphatidic acid are similar to those given above for phosphatidylserine except that the values of K_4^{Ca*} are somewhat higher, especially for factor X (Figure 5B). There are considerable differences observed when comparing phosphatidylserine with phosphatidylethanolamine and phosphatidylglycerol. First of all, for the membranes where protein packing density is not limiting (below a protein/phospholipid ratio of about 1.2, Figure 4), the protein bound per acidic phospholipid residue is much lower for phosphatidylethanolamine and phosphatidylglycerol. A second difference is that phosphatidylethanolamine and phosphatidylglycerol display much lower affinity for the proteins. This is true both at similar percentages of these acidic phospholipids and at similar membrane capacities. This low affinity undoubtedly accounts for the failure of prothrombin to co-chromatograph with membranes of phosphatidylethanolamine on gel filtration (Bull et al., 1972).

Figures 4D, 4E, 5D, and 5E show an interesting affect of phosphatidylserine on membranes containing phosphatidylethanolamine and phosphatidylglycerol. Inclusion of small amounts of phosphatidylserine appears to increase the protein-binding affinities in more than a simple additive fashion. This result may indicate different types of protein–acidic phospholipid interactions with phosphatidylserine functioning more effectively in one type of interaction but all acidic phospholipids functioning equally in another. In this way a membrane of 10% phosphatidylserine plus 30% phosphatidylglycerol could be as effective in binding these proteins as a membrane of 40% phosphatidylserine. This is nearly the case for prothrombin–membrane binding (compare 35% PG–9% PS, Figure 4E with Figure 1A). The double reciprocal plots for these combined phospholipids were linear indicating a single class of protein-binding sites. This observation eliminates the possibility that a few tight binding sites are created by lateral concentration of the phosphatidylserine in these membranes.

Calcium Titration of Protein–Membrane Binding. The calcium dependence of prothrombin and factor X–membrane binding (reactions 3 and 4 of eq 1) has been reported for membranes of high phosphatidylserine content using methods of fluorescence energy transfer (Nelsestuen et al., 1976) and light scattering (Nelsestuen and Lim, 1977). It was observed that factor X has a higher calcium requirement than does prothrombin. These studies were extended to the other phospholipid compositions with the results given in Figure 6. Manganous ion was included to eliminate effects of reaction 2 (eq 1) on this titration. The value reported is the midpoint of the cooperative binding reaction as determined from a Hill plot with the protein bound at 1 mM calcium assigned 100% reaction (Nelsestuen et al., 1976; Nelsestuen and Lim, 1977). This titration is believed to measure the formation of a minimum calcium–membrane complex (reaction 3, eq 1) referred to as a protein-binding-site "nucleus" (Nelsestuen and Lim, 1977) to which the protein adheres and, with addition of further calcium ions, the protein–membrane complex is formed (reaction 4, eq 1). As shown in Figure 6, the calcium dependence of protein–phospholipid binding increases as acidic phospholipid content decreases for all the types of phospholipids studied. This is probably due to a combination of a lower calcium-binding affinity (reaction 3 of eq 1) and a lower protein–membrane affinity (reaction 4) for membranes of lower charge density. In agreement with our previous reports, factor X has a higher calcium requirement than prothrombin, and the relative difference is quite consistent for all membrane compositions. As was the case for protein binding capacity and

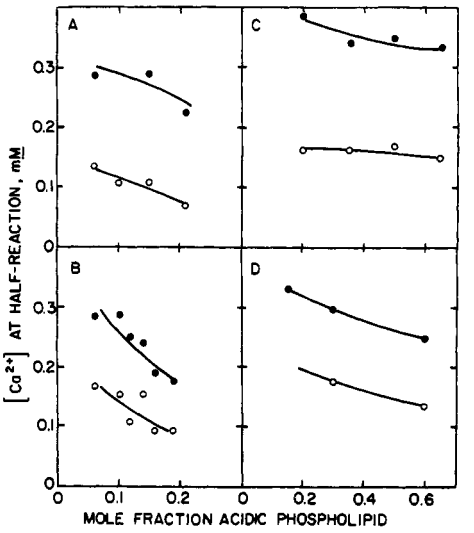

FIGURE 6: The midpoints for calcium titration of prothrombin- and factor X–membrane binding. Titrations were conducted in 0.2 mM manganous ion to catalyze the protein transitions. Hill plots for calcium titration of protein–membrane binding were constructed as described previously (Nelsestuen and Lim, 1977) by assigning 100% reaction at 1.0 mM calcium. The calcium concentration at half-reaction is plotted for membranes of varying acidic phospholipid composition. The acidic phospholipids are: phosphatidic acid (A), phosphatidylserine (B), phosphatidylethanolamine (C), and phosphatidylglycerol (D). The values for prothrombin (○) and factor X (●) are given in each case. The protein: phospholipid ratio was selected so that 40% of the protein-binding sites would be saturated at 1 mM calcium.

the values of K_4^{Ca*} reported above, the results for phosphatidic acid and phosphatidylserine are very similar. This indicates that the calcium-binding properties of these two phospholipids are similar, an observation consistent with other reports (Barton, 1968). The requirement for higher calcium concentrations by membranes containing phosphatidylethanolamine and phosphatidylglycerol is probably due to higher values of K_4^{Ca} and the lower calcium-binding affinity of these phospholipids.

The Hill coefficients for the titrations in Figure 6 were similar for all types of phospholipids and gave average values of 1.74 ± 0.4 for prothrombin and 2.24 ± 0.4 for factor X. These values are somewhat lower than reported previously (Nelsestuen, 1976; Nelsestuen et al., 1976) but were obtained at protein to phospholipid ratios of 1:1 (w:w) or greater while the previous studies were conducted at protein to phospholipid ratios of 1:4. High protein to membrane ratios generally appeared to give lower Hill coefficients.

Discussion

Earlier studies have indicated that prothrombin–membrane binding occurs with essentially any acidic phospholipid but the amount of binding varies greatly with the type of phospholipid (Bull et al., 1972). The method used to determine protein–membrane binding in most previous studies, coelution of protein and membrane from gel filtration columns, is dependent on many parameters. For proteins where an equilibrium exists between free and bound protein (as the case with prothrombin and factor X) coelution will not be observed with any membrane composition if the solutions are sufficiently dilute or the columns are sufficiently long. For this reason the failure to observe coelution of the protein and phospholipid from gel filtration does not establish that protein–membrane binding does not occur. Other reports have indicated that a small amount of prothrombin binds very tightly to vesicles of high phosphatidic acid content and that the bound protein exhibits

a reduced activity in the blood-clotting reactions. This protein remains bound to the membrane even after addition of EDTA[1] to remove calcium from the complex (Bull et al., 1972). This is not consistent with the observations reported in these communications where considerable similarity between phosphatidic acid and phosphatidylserine is observed and protein-membrane binding is reversible in all cases. It seems possible that the earlier reports were affected by the use of very high calcium concentrations which caused aggregation of the vesicles and entrapment of prothrombin. The entrapped protein would coelute with the membrane regardless of the presence of calcium and would not be available for activation by the prothrombinase complex. The result would be a low prothrombin activity. Our studies have indicated that membranes of high phosphatidic acid content are very susceptible to aggregation by calcium (Nelsestuen and Lim, 1977).

The results of the present studies allow the calculation of the amount of prothrombin and factor X which is bound to membranes of almost any composition regardless of membrane concentration. The most interesting membrane compositions are those which might be found in the systems where these proteins are located. Based on the results of Figure 2, the binding capacities and binding constants for all membranes containing greater than 20% phosphatidylserine are approximately the same. Such membrane compositions would be found on the interior surface of plasma membranes. In the case of the erythrocyte membrane, the interior surface contains essentially all of the phosphatidyserine and two-thirds of the phosphatidylethanolamine (Gordesky and Marinetti, 1973). Based on the reported composition of the platelet plasma membrane (Douste-Blazy et al., 1973) and a similar distribution, the interior surface of the platelet membrane should be about 12 to 20% phosphatidylserine and 35% phosphatidylethanolamine. The low levels of phosphatidylethanolamine or phosphatidylglycerol which are found on the exterior surface of plasma membranes bind a negligible quantity of these proteins under normal physiological conditions (about 2 μM prothrombin, 0.2 μM factor X and 1.2 mM calcium). The large difference in the phospholipid composition of the interior vs. the exterior plasma membrane surfaces may constitute an important control mechanism in blood coagulation. An appropriate membrane component for blood coagulation is available only under the abnormal conditions where interior plasma membrane, or perhaps other interior membranes of similar composition, are exposed. This would be expected in the case of damaged tissue or when platelets undergo aggregation, a process which severely changes their cellular morphology (Murer and Day, 1972).

One value of the studies on membranes of intermediate acidic phospholipid content is in determination of the number of acidic phospholipids which are necessary for protein-membrane interaction. The values obtained are 9.3 ± 1.5 phosphatidylserine residues per prothrombin molecule and 5.2 ± 1.5 per factor X molecule. Since the maximum calcium-binding stoichiometry under the conditions used is 1 calcium ion per two phosphatidylserine residues, it is tempting to speculate that an even number of phosphatidylserine residues are involved in these protein-membrane interactions. In this case, the number would be 8 or 10 phosphatidylserine residues per prothrombin molecule and 4 or 6 per factor X molecule.

The observation that membrane-bound protein is proportional to phosphatidylserine content for membranes containing less than 15% phosphatidylserine indicates that the protein causes clustering or lateral phase separation of the acidic phospholipid molecules in the membrane. Prothrombin would require a cluster of 8 or 10 and factor X a cluster of 4 or 6 phosphatidylserine residues. At protein saturation these membranes of low phosphatidylserine content would have essentially no phosphatidylserine in the membrane area between the bound protein molecules. This lateral phase separation would be accompanied by a positive free energy and is probably a major factor for the increasing values of $K_4^{Ca^*}$ as the membrane concentration of phosphatidylserine decreases.

The requirement of a larger number of phosphatidylethanolamine residues per molecule of bound prothrombin and factor X is undoubtedly due to the presence of a majority of these residues in their protonated or zwitterion state (Bangham, 1968). The actual concentration of acidic phospholipid is much lower than the concentration of phosphatidylethanolamine. The explanation is not as clear, however, for the requirement of a larger number of phosphatidylglycerol molecules per bound protein. Phosphatidylglycerol should have a net charge of minus one under all conditions used in these studies. It is possible that the isolated single bilayer vesicles do not contain a representative concentration of the added phosphatidylglycerol but further studies will be required to clarify this point.

The studies presented here indicate that there are substantial differences between prothrombin–membrane interaction and factor X–membrane interaction. While these vitamin K dependent proteins both rely on γ-carboxyglutamic acid for calcium and membrane binding (Nelsestuen and Suttie, 1972; Stenflo and Ganrot, 1973; Esmon et al., 1975) and appear to be descendent from a common ancestral gene (Fujikawa et al., 1974), the actual structure of the protein–membrane interface has some variation. It follows that, if γ-carboxyglutamic acid actually functions directly in this interaction, it must be capable of participating in at least two different protein–membrane bonding structures. It will be interesting to determine whether there are only two or many possible variations by which γ-carboxyglutamic acid containing proteins can participate in membrane binding. If only two structures are possible, factors VII and IX would display membrane-binding characteristics identical with either factor X or prothrombin and, if many variations are possible, these proteins may display entirely unique protein–membrane binding characteristics.

[1] Abbreviation used: EDTA, ethylenediaminetetraacetic acid.

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Structure of the Prothrombin- and Blood Clotting Factor X-Membrane Complexes†

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ABSTRACT: The configuration of the prothrombin- and factor X-membrane complexes was investigated by the technique of quasielastic light scattering. It is concluded that the fragment 1 region of prothrombin is located at one end of the prothrombin molecule and that the membrane binding site is at the tip of the fragment 1 region. Prothrombin binds to the surface of the membrane with no detected penetration into the lipophilic region of the membrane. The remainder of the prothrombin molecule extends radially from the membrane surface with maximum protrusion into solution. Factor X also binds to the membrane at one end of the molecule and extends into solution. Based on the evidence presented here and in other communications [Nelsestuen, G. L., and Lim, T. K. (1977), *Biochemistry 16,* and Nelsestuen, G. L., and Broderius, M. (1977), *Biochemistry 16* (respectively the first and second in a series of three papers in this issue)] a model for prothrombin-membrane interaction is given. Quasielastic light scattering appears to be a valuable new method for studying protein-membrane interactions.

Membrane-associated proteins are often referred to as intrinsic or extrinsic membrane proteins. A more recent terminology which appears closely related is the terms "integral" and "peripheral" membrane proteins (Singer, 1974). Proteins are categorized largely on the ease by which they can be separated from the membrane. Proteins which bind tightly to the membrane and require detergents for release are considered integral or intrinsic proteins and are thought to be buried in the lipid region of the membrane. Those which are released by chelating agents or high salt concentrations are considered extrinsic or peripheral proteins and are thought to bind to the membrane through ionic forces or to integral membrane proteins. While the available evidence supports these conclusions, they have not been established for a large number of proteins.

Since prothrombin and factor X require calcium ions and acidic phospholipid for membrane interaction (Papahadjopoulos and Hanahan, 1964) and are always in rapid equilibrium between the bound and free state (Nelsestuen et al., 1976; Nelsestuen and Lim, 1977), the current model for protein-membrane interaction would predict that these proteins bind to the membrane surface through ionic forces only. This is merely an expectation, however, and does not eliminate considerable penetration of these proteins into the lipophilic region of the membrane. Direct experimental evidence is required to support this expectation.

The technique of quasielastic light scattering (Berne and Pecora, 1976) can be used to rapidly determine the diffusion constant of a molecule or complex in solution. From the diffusion constant and knowledge of the molecular shape, the dimensions of the particle can be calculated. This communication utilizes quasielastic light scattering for determination of the configuration of the prothrombin- and factor X-membrane complexes. The results provide evidence about the structure of the protein as well as that of the protein-membrane complex. When combined with results presented in other communications (Nelsestuen and Lim, 1977; Nelsestuen and Broderius, 1977), a model containing considerable detail about the prothrombin- and factor X-membrane complexes can be drawn. The technique of quasielastic light scattering appears to be a valuable new tool in the study of protein-membrane structure.

Materials and Methods

Diffusion Constant Measurement. Quasielastic laser light scattering (QLS) spectroscopy was used to determine the diffusion coefficients for the phospholipid vesicles and protein-phospholipid vesicle complexes. The QLS apparatus used in this experiment has been described previously by Benbasat and Bloomfield (1975). The concentration of sample was kept at 2 to 5 mg/mL, giving an average distance between the particles of ca. 1600 to 1200 Å. With an ionic strength 0.13 for all samples (Debye shielding length < 100 Å), charge interaction between the particles was negligible. No dependence

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