Assembly of the Prothrombinase Complex on Lipid Vesicles Depends on the Stereochemical Configuration of the Polar Headgroup of Phosphatidylserine†

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ABSTRACT: The conversion of prothrombin into thrombin is an imperative step in the sequence of reactions leading to the formation of a hemostatic plug. This reaction is catalyzed by the prothrombinase complex, composed of factors Xa and Va, which is assembled on a phospholipid surface through Ca-mediated interactions with the lipid polar headgroups. In this paper we describe experiments indicative for a major role of the stereochemical configuration of phosphatidylserine in the binding of the prothrombinase complex to a phospholipid surface. Using two stereoisomers of phosphatidylserine, i.e., L-α-glycerophosphoryl-L-serine (PLS) and L-α-glycerophosphoryl-D-serine (PDS), we demonstrate that membranes containing PLS are appreciably more favorable than membranes containing PDS in promoting assembly of the prothrombinase complex and catalysis of prothrombin conversion. Ellipsometric analysis of the binding of factor Va and factor Xa to a surface composed of phosphatidylcholine and 10 mol % of either PLS or PDS reveals that the apparent K_d for factor Va increases about 25-fold when substituting PDS for PLS. For factor Xa a 5-fold increase in K_d was observed on replacing PDS for PLS. When PLS is replaced by phosphatidyl-β-lactate (PLac), a phospholipid resembling PS but lacking the amino group, a similar decrease in prothrombinase activity is found as observed with PDS, implicating the importance of both the amino group and the stereoconfiguration of the serine moiety for the assembly of the prothrombinase complex. The much higher efficiency in supporting prothrombinase of membranes containing PLS as compared to those containing PDS or PLac is further illustrated by their higher capacity to retain their catalytic properties upon changes in surface charge. Unlike PLS, the incorporation of positively charged stearylamine leads to a considerable reduction in prothrombinase activity when PDS or PLac are used as anionic phospholipid. Taken together, these findings demonstrate the important and unique character of phosphatidyl-L-serine as procoagulant phospholipid.

The prothrombinase complex is known to consist of a serine protease, factor Xa, and a protein cofactor, factor Va, assembled in a tight complex at phospholipid surfaces containing anionic phospholipids (Zwaal, 1978; Mann et al., 1990). Formation of the complex is strictly dependent on the presence of calcium ions. After assembly, the enzyme complex is able to convert prothrombin into thrombin with high catalytic efficiency [$k_{cat}/K_m \approx 2.5 \times 10^9$ M^{-1} s^{-1}, van Rijn et al., 1984)]. The high efficiency of the complete prothrombinase complex is explained by a decrease of the K_m for prothrombin upon binding of the proteins to the lipid surface and an increase of the k_{cat}, brought about by factor Va (Nesheim et al., 1979; Rosing et al., 1980). A chelate model has been suggested for the interaction of the vitamin K dependent proteins with a lipid surface containing phosphatidylserine (PS), in which calcium ions are thought to form a coordinate complex with γ-carboxyglutamic acid (gla) residues of the proteins and negatively charged headgroups of PS (Rosing et al., 1988). The presence of three fixed charges localized in the polar headgroup of PS would allow the formation of such a stable chelate complex and thereby explain the exceptional quality of PS as component of a procoagulant surface.

The aim of the present study was to explore in more detail the specific requirements of the serine moiety of PS to allow optimal binding and activity of the components of the prothrombinase complex. For that purpose we synthesized PS with either the L- or the D-isomer of serine in the polar headgroup, in order to probe for possible stereospecificity in the binding and activity of prothrombinase. To assess the importance of the amino group of the serine moiety of PS, the anionic phospholipid phosphatidyl-β-hydroxypropionate (phosphatidyl-β-lactate, PLac) was synthesized, and its procoagulant activity was compared with both stereoisomers of PS. The procoagulant activity of these lipids, i.e., dioleoylphosphatidyl-L-serine (PLS), dioleoylphosphatidyl-D-serine (PDS), and dioleoylphosphatidyl-β-lactate (PLac), was compared in a prothrombinase assay system with purified coagulation proteins under different reaction conditions. Also, using ellipsometry, direct binding of factors Va and Xa to planar phospholipid surfaces containing the above mentioned phospholipids was investigated, to assess the apparent dissociation constants of these proteins.

EXPERIMENTAL PROCEDURES

Materials. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), phospholipase D from Streptomyces *species*, *n*-octyl-β-D-glucopyranoside, L- and D-serine, 3-hydroxypropionitrile, and stearylamine were all purchased from Sigma Chem. Co. (St. Louis, MO). The thrombin-specific chromogenic substrate S2238 was obtained from AB Kabi Diagnostica, Stockholm, Sweden.

Proteins. Bovine prothrombin was purified as described by Owen et al. (1974). Bovine factor Xa was a kind gift of Dr. Rosing (Department of Biochemistry, University of Limburg) and was prepared according to Fujikawa et al. (1972). Bovine factor Va was obtained from Dr. Wagenvoord (Department of Biochemistry, University of Limburg) and was purified according to Lindhout et al. (1982).

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Lipids. β-Hydroxypropionic acid (β-lactate) was prepared from the propionitrile as described by Read (1927). Briefly, in a round-bottom flask with mechanical stirrer, 1.75 mol of 3-hydroxypropionitrile was added slowly to 250 mL of 8 M NaOH under continuous stirring while keeping the temperature below 30 °C. The mixture was left overnight at room temperature. After refluxing for 4 h, 200 mL of H_2O was added followed by slow addition of 125 mL of 50% (v/v) H_2SO_4 while keeping the temperature below 35 °C. The 3-hydroxypropionic acid was extracted from the acidic solution with six portions of 150 mL of ether. The combined ethereal extracts were dried under reduced pressure until a sirupy liquid remained which contains approximately 75% β-lactate, the remainder being mainly water. PLS, PDS, and PLac were synthesized from DOPC by phospholipase D catalyzed base exchange using the bacterial enzyme from Streptomyces *species*, which results in higher yields as compared to cabbage phospholipase D (Juneja et al., 1989). Synthesis was carried out as described before (Comfurius et al., 1990). Briefly, DOPC was dried in a glass tube. L-serine, D-serine, or β-lactate was dissolved in a concentration of 40% (w/v) in a solution containing 0.1 M $CaCl_2$ and 0.1 M sodium acetate (final concentrations) and added to the dried DOPC. β-D-Octyl glucoside (2% w/v) was added to disperse the lipid. Thereto the mixture was stirred for approximately 5 min. Then phospholipase D was added to a final concentration of 5 μg/mL. The mixture was stirred for 2 h at 45 °C. Extraction of the lipid, purification, and quantification were carried out as described before (Comfurius et al., 1990). Overall yields amount to at least 75% for all lipids. The lipids were found to be more than 99% pure by two-dimensional thin layer chromatography using chloroform/methanol/ammonia/water (95:50:5.5:5.5, v/v/v/v) in the first, and chloroform/methanol/acetic acid/water (90:40:12:2, v/v/v/v) in the second direction. Moreover, migration of PDS in these solvent systems was found to be indistinguishable from that of PLS.

Preparation of Phospholipid Vesicles. Phospholipid vesicles were prepared by mixing appropriate amounts of stock solutions in chloroform/methanol 1:1 (v/v) and drying the lipid under a stream of nitrogen. The dried lipids were suspended in Tris/NaCl buffer (50 mM Tris, 120 mM NaCl, pH 7.5) at a concentration of 250 μM and sonicated for 10 min at 4 °C with a MSE sonicator set at 6-μm amplitude.

Prothrombinase Assay. The prothrombinase assay was performed at 37 °C in a system containing Tris/NaCl buffer (50/120 mM, pH 7.5), 3 mM $CaCl_2$, and 0.5 mg/mL human serum albumin. Phospholipid vesicles, coagulation factors Xa, Va, and prothrombin were present in concentrations indicated in the legends to the figures. After an incubation of 5 min to allow assembly of the factor Xa/Va complex, reactions were started by the addition of prothrombin. At different time intervals samples were transferred to a cuvette containing Tris/NaCl/EDTA buffer (50/120/2 mM, pH 7.9). The amount of thrombin formed was determined by measuring the change in absorbance per minute after addition of 200 μM S2238. The ΔA/min was converted to nM thrombin using a calibration curve made with known amounts of active-site-titrated thrombin.

Ellipsometric Measurement of Protein Binding to Planar Phospholipid Bilayers. Planar bilayers were deposited on silicon slides (Wacker Chemie) as described (Giesen et al., 1991). Briefly, silicon slides were thoroughly cleaned, treated with chromic sulfuric acid for 24 h, and rinsed extensively with water before use. The planar phospholipid bilayer was deposited on the slide by immersion in a stirred suspension of sonicated phospholipid vesicles (30 μM). Binding of blood coagulation factors Va and Xa to these planar bilayers was measured as described before (Corsel et al., 1986). The experiments were performed in Tris/NaCl buffer (50/120 mM, pH 7.5) containing 3 mM $CaCl_2$ and 0.5 mg/mL bovine serum albumin. Protein adsorption was started by addition of protein to the trapezoidal cuvette (5mL), and the protein was allowed to adsorb until equilibrium was attained (approximately 10 min for factor Va and 5 min for factor Xa on a surface of 10 mol % PLS in PC). At regular time intervals (20 min for factor Va and 10 min for factor Xa) the protein concentration in the cuvette was increased and the protein adsorption at the end of the interval was measured. For factor Va the concentrations used were 1, 2, 4, 8, 16, and 32 nM, and for factor Xa 100, 200, 400, 600, and 1000 nM. The experiments were analyzed using the relation:

$$\Gamma_{eq} = \Gamma_{max} C_{bulk}/(K_d + C_{bulk}) \quad (1)$$

which, for independent binding sites, gives the amount of bound protein (Γ_{eq}) as function of the concentration of unbound protein (C_{bulk}), the maximal protein adsorption (Γ_{max}), and the dissociation constant (K_d). For PLS/PC (10/90) the adsorption at the highest protein concentration approached Γ_{max}, allowing values for K_d and Γ_{max} to be estimated from experimental data of Γ_{eq} versus C_{bulk} using a least-squares fit. For PDS and PLac, however, the adsorption at the highest protein concentration remained too far below Γ_{max} to estimate this parameter from the data. Therefore, K_d was estimated by a least-squares fit of eq 1 to the experimental data using the value of Γ_{max} obtained from PLS/PC.

RESULTS

Prothrombinase Activity of Stereoisomers of PS. To compare the two stereoisomers of PS, a series of prothrombinase measurements were carried out using lipid mixtures composed of dioleoyl-PC and increasing amounts of either of the isomers of dioleoyl-PS (PLS or PDS). Since the total amount of PS in blood platelets, which are the most relevant cells for providing a procoagulant surface under physiological conditions, approximates 10 mol % (Perret et al., 1979, Bevers et al., 1983), this amount of PS was taken as a maximum for the artificial lipid vesicles used in this study. First, prothrombinase activity was measured in the absence of factor Va. This required both a high lipid concentration (25 μM) and a high factor Xa concentration (10 nM) in order to have sufficient thrombin formation to allow accurate measurements. As shown in Figure 1A, in the absence of factor Va there is a clear difference between both stereoisomers of PS: at 10 mol % an almost 5-fold difference in rate of thrombin formation is observed in favor of the naturally occurring L-form.

In the presence of factor Va, thrombin formation is strongly increased, requiring different reaction conditions to prevent substrate depletion. Therefore, prothrombinase measurements were performed at 2.5 μM total phospholipid and 0.5 nM factor Xa in the presence of 1 nM factor Va. Under these conditions, the difference in catalytic properties between PLS and PDS is even more pronounced (Figure 1B). Up to 5 mol %, prothrombinase activity of vesicles containing PLS is at least 10-fold higher than the activity observed with PDS containing vesicles. Above 5 mol % of PLS no further increase in rate of thrombin formation is found. In contrast, for vesicles with PDS, prothrombinase activity still increases up to 10 mol %. At 20 mol %, the prothrombinase activity of vesicles with PDS amounts to approximately 80% of the activity of PLS

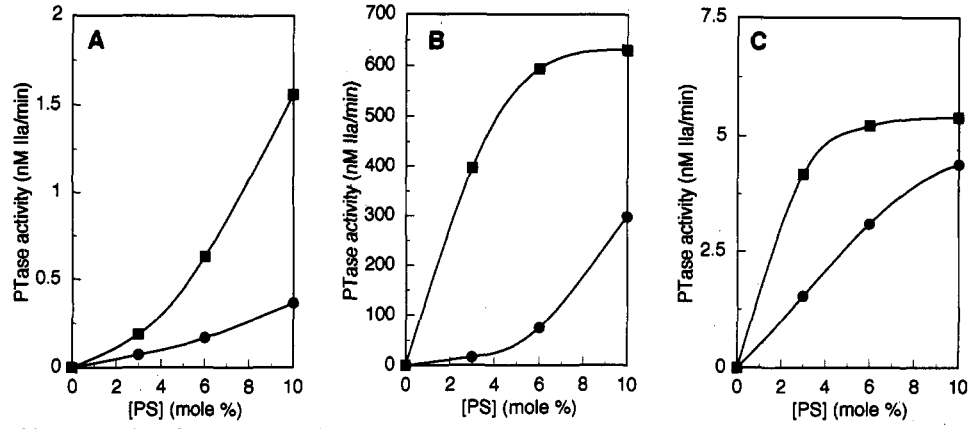

FIGURE 1: Prothrombinase activity of a phospholipid surface as a function of the mole fraction anionic phospholipid: (■) PLS, (●) PDS. Data shown are from a representative experiment out of four independently so performed. Reaction conditions differ between panels A, B, and C as follows:

panel	[PL]	[Xa]	[Va]	[PT]
A	25 μM	10 nM		4 μM
B	2.5 μM	0.5 nM	1 nM	4 μM
C	25 μM	2 pM	2 nM	4 μM

Table 1: For Lipid Mixtures Containing 5 mol % PLS or PDS, K_m and V_{max} Values Were Estimated from Prothrombin Titrations[a]

	A		B		C	
lipid	K_m (μM)	V_{max} (nM/min)	K_m (μM)	V_{max} (nM/min)	K_m (μM)	V_{max} (nM/min)
PLS	0.31	0.4	1.20	523	0.26	6.6
PDS	0.24	0.1	0.79	25	0.22	4.1

[a] The characters above the columns refer to the reaction conditions as described in the legend to Figure 1. K_m values are expressed as μM PT and V_{max} as nM IIa/min.

containing vesicles (data not shown). In the experiment depicted in Figure 1C, a 1000-fold excess of factor Va (2 nM) over factor Xa (2 pM) was used in the presence of a high lipid concentration (25 μM) to ensure maximal binding of factor Xa to the lipid surface. Even under those conditions the difference between the two isomeric forms of PS remains, albeit to a lesser extent than observed under the conditions used in Figure 1B. Similar differences were obtained between vesicles containing PLS and PLac (data not shown).

Kinetic Parameters of Prothrombin Activation. To evaluate possible changes in the binding parameters of the γ-carboxyglutamic acid (gla) containing coagulation factors, prothrombin titrations were performed with vesicles containing 5 mol % of PS using the reaction conditions described in each of the three panels of Figure 1. The results are summarized in Table 1. No appreciable differences in the apparent K_m for prothrombin were observed between a lipid surface containing either PLS or PDS, when prothrombinase activity was measured in the absence or presence of factor Va. The somewhat higher K_m's observed under conditions used in Figure 1B possibly reflect competition between the Xa/Va complex and prothrombin for the limited number of binding sites. In contrast, the apparent V_{max} seems to be dependent on the stereochemical configuration of PS; in all cases a higher V_{max} is found for vesicles with PLS, being most pronounced (some 20-fold) under conditions used in Figure 1B. However, when excess of factor Va over factor Xa is employed, there is only 33% difference in the apparant V_{max} value between PDS- and PLS-containing vesicles, suggesting that the observed differences in the apparent V_{max} reflect changes in the amount of factors Xa/Va bound, rather than an actual difference in k_{cat}.

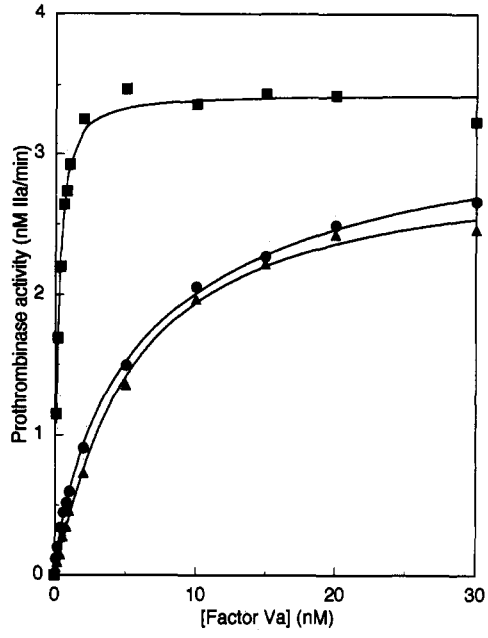

FIGURE 2: Prothrombinase activity as a function of the factor Va concentration using 5 mol % anionic phospholipid in DOPC. Activities were measured with 25 μM PL, 2 pM factor Xa, and 4 μM prothrombin. Data shown are from a representative experiment out of five independently so performed. (■) PLS; (●) PDS; (▲) PLac.

Factor Va Titrations. In order to evaluate whether extent of binding of factors Xa/Va depends on the nature of the polar headgroup of the anionic lipid, titrations with factor Va were carried out using vesicles with 5 mol % PLS or PDS. In addition, prothrombinase activity as a function of the concentration of factor Va was measured using vesicles in which PS was replaced by PLac, in order to obtain information on the importance of the amino group of serine. The results are given in Figure 2. Lines shown were fitted to the data using the formula of a rectangular hyperbola, i.e., $y = ax/(x + b)$, with the parameters a and b representing the maximal prothrombinase activity (A_{max}) attainable under these conditions and the concentration of factor Va required to approach half A_{max}, respectively. From these fits it can be calculated that there is no appreciable difference between the A_{max} using the different lipids: 3.5 nM thrombin formed per minute for

Table 2: Determination by Ellipsometry of the Binding of Factor Va and Factor Xa to a Phospholipid Surface Containing 10 Mol % PLS, PDS, or PLac in PC[a]

protein	PLS	PDS	PLac
factor Va	6.7 nM	152 nM	75 nM
factor Xa	0.7 μM	3.5 μM	11.5 μM

[a] Apparent K_d's were estimated from a titration curve of the different proteins and fitting of the observed data to a hyperbola.

PLS versus 3.1 and 3.0 nM per minute for PDS and PLac, respectively. In contrast, the concentration of factor Va at which half-maximal activity is reached is clearly dissimilar for the various lipid surfaces. This concentration, which is 0.2 nM factor Va for a PLS containing surface, increases to 4.4 and 6.0 nM, respectively, when PDS or PLac are used as anionic phospholipid, suggesting differential binding of factors Va and Xa.

Binding Experiments Using Ellipsometry. In the experiments described above, enzymatic activities have been measured which reflect the binding properties of all the components of the prothrombinase complex. With the purpose to compare the binding of the individual factors Va and Xa to the different anionic phospholipid surfaces, apparent K_d's were determined using ellipsometry. To obtain meaningful values for the comparison of the binding parameters, in particular for factor Xa, these experiments were carried out with phospholipid mixtures containing 10 mol % of anionic phospholipid. The results presented in Table 2 indicate that mainly factor Va, but to a lesser extent also factor Xa, is sensitive to the stereochemical configuration of the polar headgroup of PS and also to the presence of the amino group which is lacking in PLac. An increase in the apparent K_d of factor Va of more than one order of magnitude was found when PLS is replaced by either PDS or PLac. A similar but much smaller change in apparent K_d is observed for the binding of factor Xa. It is unlikely that the differences between PLS- and PDS-containing surfaces are caused by putative differences in surface charge, since the electrophoretic mobilities in agarose gels of vesicles containing equal amounts of PLS or PDS, carried out according to Rosing et al. (1988), are indistinguishable over a wide pH range (data not shown).

Effect of Surface Charge on Prothrombinase Activity. The binding of proteins to various phospholipid surfaces was further evaluated by measuring the effect of a change in the overall surface charge of the lipid vesicles on the prothrombinase activity. As previously shown, addition of increasing amounts of stearylamine, a positively charged lipid compound, will cause a change in the net surface charge of phospholipid mixtures containing anionic phospholipid (Rosing et al., 1988). Figure 3 shows the effect of various concentrations of stearylamine on the prothrombin converting activity of lipid mixtures containing 5 mol % of anionic phospholipid. Unlike vesicles containing PLS, vesicles containing PDS or PLac undergo a gradual decrease in their capacity to stimulate prothrombinase activity upon increasing the stearylamine concentration. The effect on 5 mol % PDS is more pronounced than on 5 mol % PLac, possibly reflecting the difference in net charge between the two molecules. Since PLac has two negative charges at pH 7.5, it requires 10 mol % of stearylamine to reach electroneutrality, while 5 mol % of stearylamine is sufficient to neutralize the PDS which has only one negative charge. As can be seen from Figure 3, the extent of inhibition at electroneutrality appears to be very similar for PDS- or PLac-containing vesicles.

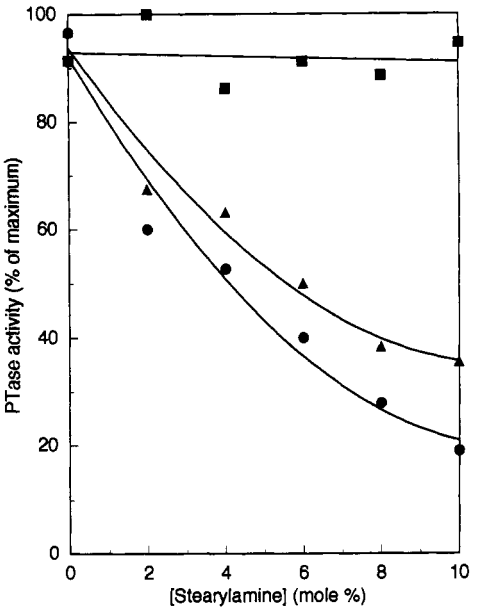

FIGURE 3: Influence of addition of different amounts of stearylamine to a phospholipid surface composed of 5 mol % of various anionic phospholipids in PC. Activities were measured using 25 μM PL, 2 pM factor Xa, 2 nM factor Va, and 1 μM PT. Data shown are from a representative experiment out of three independently so performed. Activities are expressed as percentage of the reaction rate without stearylamine. Rates measured as 100% are 150, 60, and 55 nM IIa formed in 30 min, respectively, for PLS, PDS and PLac. (■) PLS; (●) PDS; (▲) PLac.

DISCUSSION

Although negatively charged phospholipids in general greatly accelerate the activation of prothrombin by the serine protease factor Xa, there is a clear difference in catalytic potency between the various anionic phospholipids, phosphatidylserine being by far the most favorable. The relative contributions of the various parts of the phosphatidylserine polar headgroup to the assembly of the prothrombinase complex have been investigated earlier. The poor ability of phosphatidylethanolamine to promote prothrombinase activity (Gerads et al., 1990) illustrates the essential requirement of the carboxyl group of the serine moiety. Direct information on the importance of the amino group was obtained from experiments with PLac, a phospholipid that differs from PS only by lack of the amino group (Rosing et al., 1988). Despite the fact that this phospholipid contains two negative charges at physiological pH, its procoagulant properties were shown to be rather poor in comparison to PS. A possible explanation for the unique position of PS among the procoagulant phospholipids was given by the suggestion that this lipid allows the formation of a coordination complex in which calcium is chelated by gla residues of the proteins and the polar headgroup of PS (Resnick & Nelsestuen, 1980; Rosing et al., 1988; Gerads et al., 1990).

While this model suggests contributions of both the amino and carboxyl group of the serine moiety of PS to the binding and catalytic properties of prothrombinase, the present data provide evidence that the three-dimensional orientation of the amino and carboxyl group of PS is also of crucial importance. In this respect, it is important to point out that PLS and PDS employed in this study are related as diastereomers and are not true mirror images of each other (i.e., enantiomers). However, there are reasons to assume that the actual polar, hydrophilic surface of lipid vesicles composed of L-α-glycerophosphorylcholine and either L-α-glycerophosphoryl-L-serine (PLS) or L-α-glycerophosphoryl-D-serine (PDS)

can be considered as true mirror images, since the chiral center of the α-carbon atom of the glycerol backbone is not directly exposed to the outer lipid surface. Moreover, insertion of the D-α form of PS into the L-α form of PC might result in packing differences that disturb the mirror image. Ideally, this will be overcome by using the D-α form of PC as well. However, this could affect possible hydrophobic interactions that may occur upon binding of factor Va to the membrane, which make it ambiguous to attribute binding differences only to the stereochemical configuration of the polar headgroup of PS. It is well known that the pK's of the amino and carboxyl group of PS differ from those of free serine, most likely due to the vicinity of the phosphate group in the polar headgroup of PS. While it could still be argued that the physicochemical properties of the two diastereomers of PS containing either D- or L-serine may be different, these differences (if existing) are unlikely to play a role. The chromatographic behavior of PLS and PDS using conventional acidic and basic solvent systems is identical, and, more relevant, the electrophoretic mobility of PC-PLS and PC-PDS vesicles is indistinguishable, which strongly suggests that possible charge differences (if present) are unrelated to the observed differences in binding and catalytic properties of the prothrombinase complex.

Comparison of the apparent dissociation constants obtained with ellipsometry show clear differences in the interaction of coagulation factors Va and Xa with a lipid surface containing PLS or PDS (or PLac). The relative differences in binding found by this technique are in good agreement with the functional differences observed in the prothrombinase assay. The approximately 5-fold lower prothrombinase activity on vesicles with 10 mol % PDS in comparison to PLS measured in the absence of factor Va is consistent with the decreased affinity of factor Xa for this surface. In view of the proposed chelate complex model, this finding strongly suggests that the mutual position of the amino and carboxyl group of PS is an important ingredient for the proper formation of the calcium-mediated complex of PS with the gla residues of factor Xa. Although this would also predict a difference in affinity of the substrate prothrombin between a PDS- or PLS-containing lipid surface, this is not reflected in the apparent K_m for prothrombin.

It has been suggested that addition of factor Va to the prothrombinase system tends to decrease differences between anionic lipids, presumably because of increased binding of both factor Xa and prothrombin in the presence of factor Va (Nesheim, 1979; Lindhout, 1982; van de Waart, 1984). Nevertheless, our data show that at low mol % PS and nanomolar concentration of factors Va and Xa considerable differences in prothrombin converting potential between PLS and PDS are present, which can at least in part be overcome by conditions that favor binding of coagulation proteins. Thus, increasing the lipid concentration (thus the number of binding sites) and lowering the factor Xa concentration while keeping the factor Va concentration constant (thus increasing the fraction of added factor Xa bound to the vesicles) tends to decrease the difference between PLS and PDS. Also, increasing the number of binding sites for factors Xa/Va by increasing the PS mol % decreases the difference between PLS and PDS.

This strongly suggests that also the binding of factor Va is affected by the stereochemistry of the polar headgroup of PS. The experiments in which prothrombinase activity is measured as a function of the factor Va concentration (see Figure 2) lend further support to the notion that the affinity of factor Va for PLS is much higher than for PDS or PLac. This was confirmed by direct binding measurements using ellipsometry. Comparison of the binding data obtained with ellipsometry actually indicates that the binding of factor Va to PS is much more affected by the stereochemical configuration of the serine moiety of the polar headgroup than the binding of factor Xa. In thermodynamic terms, a 25-fold difference in K_d of factor Va between lipid surfaces containing either 10 mol % of PLS or PDS may be rather small, especially when it is realized that this effect is contributed by many PS molecules. However, in physiological terms it seems much more important when one considers that blood platelets contain approximately 10 mol % PS, and that the plasma concentration of factor V (the precursor of factor Va) is about 20 nM, i.e., above the K_d for naturally occurring PLS-containing membranes and considerably below the K_d for membranes containing PDS. In addition, the weaker binding and activity of prothrombinase in the presence of PDS-containing membranes approximates that of other negatively charged phospholipids, particularly to that of PLac that compared to PS lacks the amino group and, therefore, has two net negative charges in stead of one.

Interaction of factor Va with anionic phospholipid surfaces has been proposed to involve both nonionic (Bloom et al., 1979; Pusey et al., 1982) and ionic forces (Pusey et al., 1982; van de Waart et al., 1983). This binding, which is mediated by the light chain of factor Va, does not require calcium ions. The much higher affinity of factor Va for PLS relative to PDS- or PLac-containing membranes presumably implies a specific three point charge organization in the binding region of the factor Va light chain which contributes to a better interaction with the phosphate, amino, and carboxyl groups of the polar headgroup of naturally occurring PLS. The specificity of this interaction may be further illustrated by the observation that even a change in the net charge of the lipid surface from negative to positive, by incorporation of excess stearylamine, hardly influences the activity of PLS while strongly lowering the procoagulant activity of PDS- or PLac-containing vesicles.

In conclusion, we have obtained evidence that the prothrombinase reaction is sensitive to the stereochemical configuration of the serine moiety of PS. PS molecules that have L-serine as part of their polar headgroup bind factors Va and Xa more favorably than PDS (or PLac), which is reflected by differences in the catalytic properties of membranes containing these lipids. It is likely that the observed differences in the apparent V_{max} are solely due to differences in enzyme bound, rather than to changes in k_{cat}. Recently, Gilbert and Drinkwater (1993) have presented evidence that binding of coagulation factor VIII to phospholipids is stereospecific with respect to PS. Although these authors did not compare actual rates of factor X activation on PLS- and PDS-containing lipid surfaces, it is to be expected that also the tenase activity is stereoselective with respect to the serine headgroup of PS. Together, these results illustrate the unique position of the naturally occurring phosphatidyl-L-serine as procoagulant lipid in biological membranes. It can be conjectured that if naturally occurring coagulation proteins would have been built from D-amino acids in stead of L-amino acids, a biological membrane containing phosphatidyl-D-serine would have been the most favorable procoagulant surface. In this respect it is of interest to mention that a functional D-amino acid protease has been synthesized with reciprocal chiral specificity (Del Milton et al., 1992).

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