Factor Xa−Factor Va Complex Assembles in Two Dimensions with Unexpectedly High Affinity: An Experimental and Theoretical Approach†

Jia Ye‡ and Charles T. Esmon*,‡,§,∥

Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation Departments of Pathology, Biochemistry, and Molecular Biology, University of Oklahoma Health Sciences Center, and Howard Hughes Medical Institute, Oklahoma City, Oklahoma 73104

Received June 16, 1994; Revised Manuscript Received February 14, 1995⊗

ABSTRACT: The influence of phospholipid vesicle concentration and size on the affinity and the kinetics of assembly of the prothrombin activation complex are examined. Activation of prethrombin 1 was used to monitor complex formation between factors Va and Xa. When activation rates were measured immediately after the addition of the reactants, the rate of activation increased, and subsequently decreased, as a function of increasing vesicle concentration. Larger vesicles did not inhibit the reaction to a comparable extent until much higher phospholipid concentrations were present. The inhibition by high vesicle concentrations was significantly reduced by a prolonged incubation period. These results are interpreted as an initial step of factors Va and Xa binding independently to separate phospholipid vesicles, followed by a slow redistribution between vesicles to maximize complex formation. These experiments indicated that the $K_d \leq 25$ pM, much tighter than previously reported. Two-dimensional binding on the membrane surface was investigated under conditions where all of the proteins were membrane bound. The complex formation was independent of the surface density of the reactants, indicating a near complete complex formation at the lowest surface density of the reactants. Thus, we conclude that (i) the overall affinity of factor Va−factor Xa interaction in the presence of vesicles is higher than previously appreciated, and (ii) factor Va and factor Xa complex once they bind to the same vesicle.

It has been recognized for many years that coagulation factors assemble on membranes to accelerate subsequent reactions [reviewed in Mann et al. (1990) and Bevers et al. (1991)]. In the case of the prothrombin activation complex (prothrombinase), rapid activation involves the formation of a complex between factor Xa, the enzyme, factor Va, a regulatory protein, and prothrombin, the substrate, in the presence of negatively charged phospholipids and Ca^{2+}. Prothrombin (Nelsestuen & Lim, 1977), factor Xa (Nelsestuen & Lim, 1977), and factor Va (Krishnaswamy & Mann, 1988) have all been shown to bind to membranes independently, but the binding interactions with membrane surfaces are enhanced by protein−protein interactions between factor Va and factor Xa (Miletich et al., 1977; Tracy et al., 1992; Nesheim et al., 1981) and between prothrombin and factor Va (van de Waart et al., 1984). The kinetics of factor Va−factor Xa complex assembly has also been monitored and shown to be rapid (Krishnaswamy et al., 1988). Membrane-dependent conformational changes have been observed in factor Xa (Krishnaswamy et al., 1988; Husten et al., 1987) and prothrombin (Armstrong et al., 1990; Lentz et al., 1994). In principle, these conformational changes could enhance binding affinities for protein−protein interactions or contribute to the functional effects of the complex.

Since the overall process is governed by linked equilibria, analysis can be simplified by using saturating phospholipid to force all of the proteins to be surface bound. Under these conditions, the protein−protein interactions can be described as a two-dimensional process. Since the reactants could partition onto separate vesicles, there is the potential for a redistribution of reactants between vesicles before complex formation could occur. If this step were slow, it could cause a significant underestimate of the binding affinity of factors Va and Xa on the membrane surfaces. In the present study, we analyzed interaction with membranes and found that there was indeed a slow step in the establishment of binding equilibria at saturating phospholipid. In addition, the analysis of factor Va−factor Xa interaction on vesicles as a two-dimensional binding process suggests that when a factor Va and a factor Xa molecule bind to the same phospholipid vesicle, the proteins are virtually always in complex.

EXPERIMENTAL PROCEDURES

Proteins and Reagents. Bovine factor Va (Esmon, 1979), factor Xa (Guinto, 1983), and prethrombin 1 (Owen et al., 1974) were prepared as before. The following molecular weights and extinction coefficients ($E^{1\%}_{1cm}$) at 280 nm were used: 180 000 and 15.0 for factor Va (Guinto & Esmon, 1982), 45 300 and 12.4 for factor Xa (Jackson et al., 1968; Fujikawa & Davie, 1976), and 50 000 and 19.2 for prethrombin 1 (Owen et al., 1974). L-α-Phosphatidylcholine

† This research was supported by a grant awarded by the National Heart, Lung, and Blood Institute of the National Institutes of Health (Grant R01 HL 29807 to C.T.E.). C.T.E. is an investigator of the Howard Hughes Medical Institute.

* To whom correspondence should be addressed: Howard Hughes Medical Institute, Oklahoma Medical Research Foundation, 825 N. E. 13th Street, Oklahoma City, OK 73104. Tel: (405) 271-7571. FAX: (405) 271-3137.

‡ Oklahoma Medical Research Foundation.

§ Departments of Pathology, Biochemistry, and Molecular Biology.

∥ Howard Hughes Medical Institute.

⊗ Abstract published in *Advance ACS Abstracts,* May 1, 1995.

0006-2960/95/0434-6448$09.00/0

(PC)[1] (egg yolk) and L-α-phosphatidylserine (PS) (bovine brain) were purchased from Sigma. Octyl β-D-glucopyranoside was from Calbiochem. Spectrozyme TH was from American Diagnostica. [^{14}C]PC was from NEN.

Preparation of Vesicles. Phospholipid vesicles containing 75% PC (w/w) and 25% PS (w/w) were prepared by either sonication (Barenholz et al., 1977) or dialysis (Mimms et al., 1981). The vesicles prepared by dialysis had a diameter of ≈200 nm with more than 70% of the vesicles between 150 and 250 nm on the basis of electron microscopy as described (Smirnov & Esmon, 1994). No evidence for multilammelar liposome formation was detected. Trace amounts of [^{14}C]PC were added to the PC/PS mixture to quantify the recovery of PC/PS and therefore determine final vesicle concentrations.

Prethrombin 1 Activation. Prethrombin 1 activation was used to assess factor Va−factor Xa complex formation. Activation was performed in 20 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 5 mM Ca^{2+}, and 1 mg/mL gelatin. The reaction was initiated by the addition of 10 μL of prethrombin 1 (1 μM) to a 96-well vinyl plate that contained 20 μL of factor Xa (with or without PC/PS vesicles) and factor Va. After the reaction was allowed to proceed for the indicated time, 10 μL of 50 mM EDTA and 0.20 M Tris-HCl (pH 7.5) was added to stop the reaction. Fifty microliters of 0.4 mM Spectrozyme TH in 20 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 5 mM EDTA, and 1 mg/mL gelatin was added to measure the thrombin concentration. Since, under these conditions, usually less than 10% of the prethrombin 1 was activated during the assay, the rate of thrombin formation was linearly dependent on time and, thus, the rate of thrombin formation was calculated by dividing the concentration of thrombin by the time allowed for the activation reaction. All reactions were performed at room temperature.

When this assay was used to determine the apparent dissociation constant ($K_{d(app)}$) between factor Va and factor Xa, the following equation was used:

$$\text{Rate} = [\text{Max}/(2[\text{Xa}])]\{K_{d(app)} + [\text{Xa}] + [(K_{d(app)} + [\text{Xa}] + [\text{Va}])^2 - 4[\text{Xa}][\text{Va}]]^{1/2}\} \quad (1)$$

where Rate is the rate of prethrombin 1 activation (the rate of thrombin formation), Max is the maximal rate of prethrombin 1 activation at saturating concentrations of factor Va, and [Xa] and [Va] are the concentrations of factor Xa and factor Va, respectively. The equation assumes a 1:1 stoichiometry between factor Xa and factor Va [reviewed in Mann et al. (1990)]. A nonlinear regression program (ENZFITTER, Elsevier-Biosoft, London) was used to fit the data.

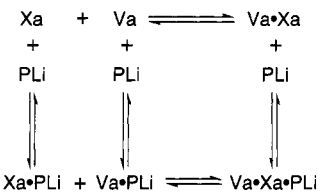
Conversion of K_d to K_{d2D}. In the presence of a saturating concentration of PC/PS vesicles, the binding interaction between factors Xa and Va takes place on a two-dimensional surface. Hence, the physical parameter (K_{d2D}) that describes the binding interaction should have units of surface density, such as mol/100 cm^2, instead of a concentrational unit, such as mol/liter. If only the loss of one degree of freedom in translational motion upon binding of Va or Xa to the membrane surface is considered, K_d and K_{d2D} will have the following relationship (see Appendix for derivation):

$$\ln K_{d2D} = \ln K_d - 26.45 \quad (2)$$

Equation 2 is the result of substituting the molecular masses of factor Xa (7.52×10^{-23} kg), factor Va (2.99×10^{-22} kg), and factor Va−factor Xa complex (3.74×10^{-22} kg) into eq A.15 in the Appendix. The units for K_d and K_{d2D} are M and mol/100 cm^2, respectively.

RESULTS

Vesicle Size Influences the Phospholipid Concentration Dependence of Prethrombin 1 Activation. Membrane-dependent assembly of protein−protein complexes presents special problems in analysis and interpretation of results. If both factor Xa and factor Va bind to one vesicle, then the following linked equilibria exist.



Schemes 1 and 2 in the Appendix refer to the upper and the lower equilibrium, respectively, in these linked equilibria. PLi indicates binding to the same vesicle. For the model in which the proteins are allowed to bind to separate vesicles and therefore to be physically partitioned, the following equilibria exist.

$$\text{Xa•PLi} + \text{Va•PLj} \rightleftharpoons \text{Xa} + \text{PLi} + \text{Va•PLj} \rightleftharpoons \text{Xa•PLj} + \text{Va•PLj} + \text{PLi} \rightleftharpoons \text{Xa•Va•PLj} + \text{PLi}$$

or

$$\text{Xa•PLi} + \text{Va•PLj} \rightleftharpoons \text{Xa•PLi} + \text{Va} + \text{PLj} \rightleftharpoons \text{Xa•PLi} + \text{Va•PLi} + \text{PLj} \rightleftharpoons \text{Xa•Va•PLi} + \text{PLj}$$

When factor Xa and factor Va bind to different vesicles, the situation is much more complicated. Previous studies have shown that each of the components of the prothrombin activation complex binds independently and rapidly to the membrane surface (Krishnaswamy et al., 1988). Binding is usually believed to increase the local concentrations of reactants, facilitating productive complex formation (Nesheim et al., 1984). High phospholipid concentrations not only guarantee that all of the factor Xa and factor Va are membrane bound but also could allow these proteins to interact with different vesicles. This would be detected as inhibition of prothrombin activation and reflect decreased complex formation. In our experiments to test the latter possibility, prethrombin 1 was employed as the substrate since it lacks a high-affinity membrane binding site (Gitel et al., 1973) and hence would not compete for membrane interaction. Activation of prethrombin 1 was used to monitor complex formation between factors Va and Xa. When activation rates were measured immediately after the addition of the reactants, the rate of activation increased, and subsequently decreased, as a function of increasing vesicle concentration (Figure 1). The ascending portion of the curves showed similar dependencies on phospholipid concentration for both large and small vesicles. Larger vesicles did not inhibit the reaction to a comparable extent until much higher phospholipid concentrations were present.

[1] Abbreviations: DEGR, dansyl-Glu-Gly-Arg chloromethyl ketone; PC, phosphatidylcholine; PS, phosphatylserine.

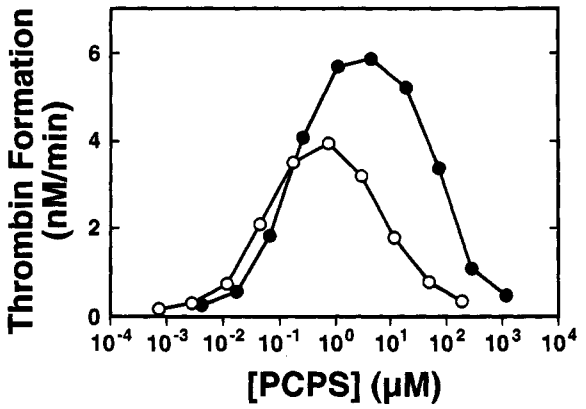


FIGURE 1: Phospholipid concentration dependence of factor Va–factor Xa complex formation. A 100-s prethrombin 1 activation was performed immediately after mixing factor Va and factor Xa with PC/PS vesicles to yield final concentrations of 0.05 nM factor Xa, 0.1 nM factor Va, and 0.33 μM prethrombin 1 and the indicated concentrations of PC/PS. The vesicles were prepared by either dialysis (●) or sonication (○)

Factor Va–Factor Xa Interaction Is Dependent on the Incubation Time of the Factor Xa–PCPS Complex with Factor Va. One interpretation of the above experiments is that, at high vesicle concentration, the vesicles inhibit prethrombin 1 activation by binding factor Va and factor Xa to separate vesicles and preventing complex formation. If the binding of the complex to the vesicles were much tighter than the independent binding interactions to the vesicles, there might be a time-dependent increase in complex formation. Factor Xa was first allowed to bind to vesicles, and factor Va was added subsequently and allowed to incubate for the times indicated. When either large (Figure 2A) or small (Figure 2B) vesicles were present in excess, there was a slow time-dependent increase in activity. When the vesicle concentration was lower, there was little or no time-dependent increase in activity with either type of vesicle.

$K_{d(app)}$ of Factor Va–Factor Xa Interaction Is Dependent on Both Phospholipid Concentration and Incubation Time. The observation that the binding of factors Va and Xa is both PC/PS concentration and time dependent suggests that the formation of the protein–protein complex on vesicles involves an initial rapid and random interaction of either factor Va or factor Xa with vesicles, followed by a slow redistribution of the proteins to form additional productive complexes. If the number of vesicles is larger than the number of factor Va and factor Xa molecules, only a fraction of the protein molecules bind to the same vesicles initially to form productive complexes. Subsequent slow redistribution of these proteins between vesicles would increase productive complex formation. If the above reasoning were true, the $K_{d(app)}$ of factor Va–factor Xa interaction would be both vesicle concentration and incubation time dependent. As expected, when factor Xa was incubated with vesicles first and prethrombin 1 activation was performed immediately after mixing factor Va with the factor Xa (Figure 3A), the presence of excess large vesicles shifted the saturation curve to the right ($K_{d(app)} = 0.6 \pm 0.05$ nM) relative to that observed at a lower vesicle concentration ($K_{d(app)} = 0.12 \pm 0.01$ nM). When factor Va and factor Xa were allowed to incubate for 90 min, both saturation curves shifted to the left (Figure 3B). The $K_{d(app)}$s at the high and low concentrations of vesicles were now 100 ± 6 and 25 ± 3 pM, respectively. At saturating factor Va concentrations,

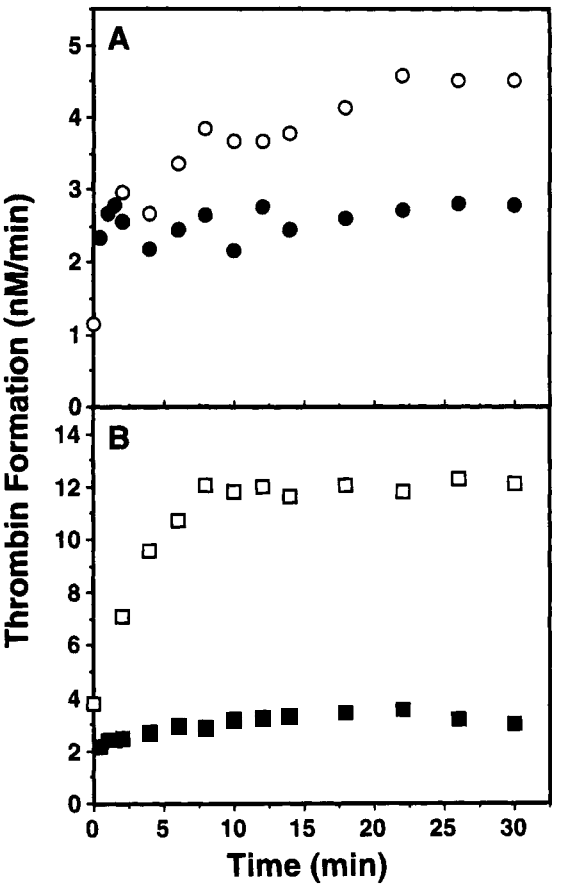


FIGURE 2: Time dependence of factor Va–factor Xa complex formation. A 2-min prethrombin 1 activation assay was performed after factor Va had been preincubated with factor Xa–PC/PS for the indicated periods of time. (A) Vesicles prepared by dialysis were used at nonsaturating (0.065 μM, ●) and above saturating (200 μM, ○) concentrations. The final concentrations of factors Xa and Va and PC/PS were 0.02 nM, 0.125 nM, and 200 μM. (B) Vesicles prepared by sonication were used at nonsaturating (0.008 μM, ■) and above saturating (100 μM, □) concentrations. The final concentrations of factors Xa and Va and PC/PS were 0.1 nM, 0.4 nM, and 100 μM.

the rate of prethrombin 1 activation was virtually identical whether or not the samples were preincubated or a large excess of vesicles was present. This is consistent with the concept that when the factor Va concentration is much greater than the vesicle concentration, every vesicle will contain factor Va and, therefore, factor Xa–factor Va vesicle segregation will not occur. Since the factor Va–factor Xa complex is much more active than free factor Xa, the results suggest that at high factor Va concentrations the same amount of factor Xa is in complex with or without excess vesicles or preincubation.

Binding of Factor Va to Factor Xa on Vesicles Is Independent of the Surface Density of Factor Xa and Factor Va. Since factor Va and factor Xa interaction in the presence of PC/PS is governed by linked equilibria involving first the binding of both factors Va and Xa to the phospholipid surface followed by the formation of the complex on the membrane surface (Krishnaswamy et al., 1988), a two-dimensional dissociation constant can describe the latter process. To simplify the analysis, saturating concentrations of vesicles were used to ensure near complete membrane binding by both proteins [based on published dissociation constants reviewed in Mann et al. (1990)], and the experiment was performed in the absence of unoccupied vesicles. Specifi-

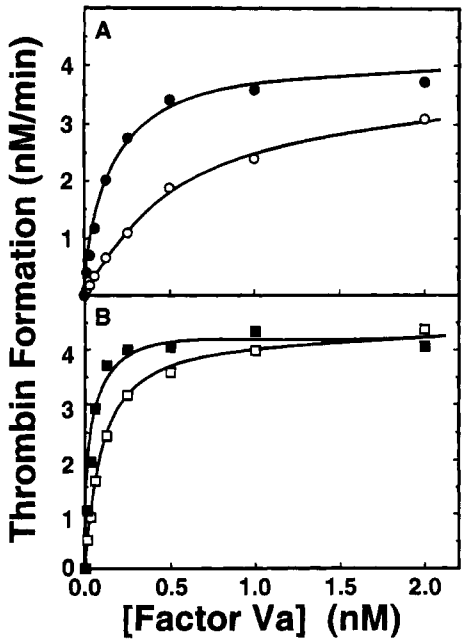


FIGURE 3: Dependence of $K_{d(app)}$ of factor Va−factor Xa interaction on both phospholipid concentration and incubation time. A 90-s prethrombin 1 activation assay was performed either immediately following the mixing of factor Va with factor Xa−PC/PS (A) or after a 90-min incubation of factor Va with factor Xa−PC/PS (B). The vesicles were prepared by dialysis. The final concentration of factor Xa was 0.02 nM, and the final concentration of PC/PS vesicles was either 20 μM (●, ■) or 200 μM (○, □). The rate of thrombin formation was plotted versus the concentration of factor Va.

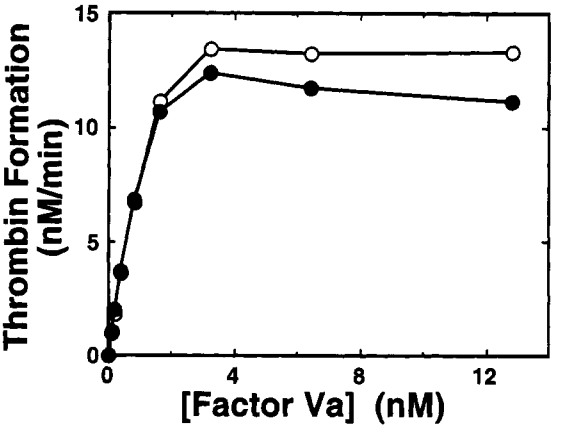


FIGURE 4: Surface density independence of factor Va−Xa complex formation on the surface of PC/PS vesicles. Prethrombin 1 activation was performed in the presence of either 20 (●) or 200 μM (○) PC/PS vesicles prepared by dialysis. The final concentration of factor Xa was 1 nM. The reaction mixture contained a final concentration of 4 mM benzamidine to decrease the rate of prethrombin 1 activation.

cally, 1 nM factor Xa bound to either 20 or 200 μM PC/PS and various concentrations of factor Va was used in the experiment. Assuming that (i) the average diameter of a vesicle prepared by dialysis is approximately 200 nm (Ueno et al., 1984), which we confirmed independently by electron microscopy (Smirnov & Esmon, 1994), (ii) the head group of each phospholipid molecule occupies 0.7 nm^2 (Small, 1967), and (iii) approximately half of the phospholipid molecules have their head groups facing the exterior surface of the vesicles, 1 nM factor Xa−200 μM PC/PS and 1 nM factor Xa−20 μM PC/PS represent on average 1.8 and 18 molecules of factor Xa per vesicle, respectively. When the rate of thrombin generation is plotted versus the bulk concentration of factor Va following a 20-min preincubation, the two curves are very similar (Figure 4). This indicates that although the surface density of factor Xa differed by 10-fold, the amounts of factor Va−factor Xa complex are indistinguishable. This implies nearly complete complex formation whenever a molecule of factor Xa and factor Va are bound to the same vesicle. This corresponds to a surface density of factor Va or factor Xa of 2.3×10^{-13} mol/100 cm^2. The two-dimensional K_d should be at least 100 times lower than this density to observe the experimental data presented in Figure 4, that is, less than 2.3×10^{-15} mol/100 cm^2. Since we have used the lowest surface density attainable using vesicles prepared by dialysis, the exact K_{d2D} cannot be measured experimentally using our conditions.

As an alternative, we considered a theoretical approach. A model was constructed in which the degrees of translational motion were restricted from three dimensions (the solution case) to two dimensions (the membrane surface case). Thus, the K_d between factors Va and Xa in solution was converted to K_{d2D} by eliminating one degree of freedom (see the Appendix for the derivation). The $K_{d(app)}$ for the factor Va−factor Xa complex formation was determined to be 0.85 ± 0.19 μM (n = 3) in the absence of phospholipid and using 0.33 μM prethrombin 1 as the substrate. This agrees well with the value obtained by others using centrifugation, K_d = 0.8 μM (Pryzdial & Mann, 1991). With this three-dimensional K_d, a K_{d2D} of 2.8×10^{-18} mol/100 cm^2 is calculated using eq 2. This value is 3 orders of magnitude lower than the highest K_{d2D} that is compatible with the data in Figure 4. Therefore, the theoretical calculation is consistent with our experimental data.

DISCUSSION

These studies are consistent with a model in which, when the vesicle numbers are higher than the factor Xa or factor Va concentration, binding to the vesicles occurs in a rather random process, and then a slow redistribution step occurs that ultimately leads to the formation of factor Va−factor Xa complexes on the surface of the same vesicle. Taken together, the results from Figures 2 and 3 suggest that equilibration is very slow. The $K_{d(app)}$ decreases with time, and even the 90-min preincubation is probably not sufficient to allow complete equilibrium to be reached. This latter conclusion is based on the observation that $K_{d(app)}$ is still lower at lower vesicle concentration even after the 90-min incubation (Figure 3B). Alternatively, this direct binding to the excess vesicles may reduce the concentration of free factor Xa and factor Va so greatly that even the very high affinity interaction of the vesicle surface cannot "pull" the system sufficiently to allow complete complex formation. Interpretation of the results is further complicated by the fact that the $K_{d(app)}$ is on the same order as the affinity of the factor Va subunits within the heterodimer ($K_{d(app)}$ = 5.9 nM) (Krishnaswamy et al., 1989). Thus, subunit dissociation over time may partially mask the benefits of prolonged incubation. Although previous experiments used to determine the K_d may

have been performed under conditions where there were no excess vesicles (Krishnaswamy, 1990), uneven distribution of the proteins on different vesicles could still have contributed to an overestimation of the K_d.

A two-dimensional K_d is perhaps a more accurate description of the actual equilibrium situation on the membrane surface. Experimentally, the K_{d2D} was determined to be $\leq 2.3 \times 10^{-15}$ mol/100 cm^2. Theoretically, if only the loss of one degree of freedom in translational motion is considered, K_{d2D} is calculated to be 2.8×10^{-18} mol/100 cm^2 based on a K_d of 0.85 μM for binding between factors Va and Xa in solution. Since this binding constant is tighter than can be examined experimentally, and since the limit of the vesicle size that we can prepare prevents us from reducing the surface density further without introducing unoccupied vesicles, it is not possible to determine whether membrane-induced conformational changes or restriction of rotational freedom influences the binding interaction between factors Va and Xa on the membrane surface. From a theoretical point of view, it would appear that even a 100-fold reduction in protein−protein binding affinity would fail to alter the amount of complex once both proteins bind to the same membrane surface. Thus, while membrane-dependent structural changes in these proteins do occur, they are not required for the high-affinity interaction observed here.

The above model considers that the proteins bind to the membrane and then diffuse in two dimensions to form a complex. An alternative model would also be consistent with this data. In this model, the amount of complex is essentially independent of the concentration of bound factor Va or factor Xa. Complex formation may occur when the incoming protein comes from the bulk phase and initially binds near the other component. This model would be consistent with inhibition at high membrane concentration (Figure 1), since high phospholipid concentrations would deplete free reactant concentrations in the solution phase, and it would also be essentially independent of the surface density (Figure 4). This model would appear, however, to be inconsistent with the observation that much higher phospholipid concentrations are required to inhibit the reaction when detergent-dialyzed vesicles are employed.

The surprisingly slow reequilibration between factor Va and factor Xa when vesicle numbers exceed the protein concentrations could have potential physiological relevance. In blood coagulation *in vitro*, the concentration of functional membrane surface is probably limiting (Wiedmer et al., 1986). Under some circumstances *in vivo*, significant tissue disruption at sites with low tissue factor concentration, joints for instance (Drake et al., 1989), could lead to generation of more lipid particles than *activated* coagulation factors. In the case of factors Xa and Va this could result in a rather long occupancy on separate membranes before complex formation. In this situation, factor Xa is susceptible to antithrombin, and factor Va is susceptible to activated protein C, but both proteins are relatively resistant to these inhibitors when in complex (Teitel & Rosenberg, 1983; Walker et al., 1979; Nesheim et al., 1982). Whether such situations actually arise *in vivo* remains to be determined.

ACKNOWLEDGMENT

The authors would like to thank Dr. Sherril D. Christian for helpful discussions on the derivation of the equation converting K_d to K_{d2D}, Subramanian Yegneswaran for preparing the small vesicles and for helpful discussion, Drs. Naomi Esmon and James Morrissey for helpful discussion, and Karen Deatherage and Julie Wiseman for help in the final stages of the manuscript preparation.

APPENDIX

Relating the Binding Equilibrium in Three-Dimensional Solution to a Two-Dimensional Surface. The reversible binding of A and B in a three-dimensional solution (Scheme, defined in Results) is governed by a dissociation constant, K_d:

$$K_d = [A][B]/[AB] \quad (A.1)$$

where [A], [B], and [AB] represent the concentrations of A, B, and complex AB.

Consider a hypothetical situation in which A and B bind reversibly with each other on a two-dimensional surface (Scheme 2, defined in Results). This binding interaction is governed by a two-dimensional dissociation constant, K_{d2D}:

$$K_{d2D} = \{A\}\{B\}/\{AB\} \quad (A.2)$$

where {A}, {B} and {AB} represent the surface density of A, B and complex AB.

If the standard state of Scheme 1 is defined as 1 mol/L for both substance A and B at 25 °C and 1 atm of pressure, then the standard Gibbs free energy of complex formation is

$$\Delta G^\circ = RT \ln K_d \quad (A.3)$$

where R is the gas constant (8.314 J K^{-1} mol^{-1}), T is 298.2 K, and K_d is the dissociation constant in units of M.

Similarly, if we define the standard state for Scheme 2 as 1 mol/100 cm^2 for both A and B at 25 °C and 1 atm of pressure, then the standard Gibbs free energy of complex formation under the two-dimensional situation is

$$\Delta G^\circ_{2D} = RT \ln K_{d2D} \quad (A.4)$$

where K_{d2D} is the two-dimensional dissociation constant in units of mol/100 cm^2.

$\Delta S^\circ_{A,2D}$ is defined as the change of standard entropy when the state of substance A is changed from the standard state in Scheme 1 to that of Scheme 2. $\Delta S^\circ_{B,2D}$ and $\Delta S^\circ_{AB,2D}$ are defined similarly.

If the only difference between Schemes 2 and 1 is the loss of one degree of freedom in translational motion, then

$$\Delta G^\circ_{2D} - \Delta G^\circ = T\Delta S^\circ_{A,2D} + T\Delta S^\circ_{B,2D} - T\Delta S^\circ_{AB,2D} \quad (A.5)$$

When a mole of substance with particle mass m (kg) is confined to a cube with sides of length a (m), there are three degrees of translational freedom. According to statistical mechanics (Adamson, 1990; Moore, 1972), the partition function per mole for translational motion is

$$Q_{3D,tran} = [(2\pi mkT)^{3/2}a^3/h^3]^N/N! \quad (A.6)$$

where k is the Boltzmann constant (1.381×10^{-23} J K^{-1}), h is the Planck constant (6.626×10^{-34} J s), and N is Avogadro's number (6.022×10^{23} mol^{-1}).

The molar entropy from the translational component is

$$S_{3D,trans} = R \ln[e^{5/2}a^3(2\pi mkT)^{3/2}/(Nh^3)] \quad (A.7)$$

Similarly, when the dimension is changed from a three-dimensional cube with sides of length a to a two-dimensional square with sides of length a,

$$Q_{2D,tran} = [(2\pi mkT)a^2/h^2]^N/N! \quad (A.8)$$

and

$$S_{2D,trans} = R \ln[e^2a^2(2\pi mkT)/(Nh^2)] \quad (A.9)$$

Therefore, when substance A changes from the standard state in Scheme 1 to that of Scheme 2, the change of standard molar entropy is

$$\Delta S^\circ_{A2D} = S^\circ_{A,2D,trans} - S^\circ_{A,3D,trans}$$

$$= R \ln[e^2a^2(2\pi m_A kT)/(Nh^2)] - R \ln[e^{5/2}a^3(2\pi m_A kT)^{3/2}/(Nh^3)]$$

$$= -R \ln(m_A)^{1/2} - R \ln[(2e\pi kT)^{1/2}a/h] \quad (A.10)$$

where m_A is the particle mass for substance A and a is 0.1 m.

Equation A.10 is simplified by substituting the actual values of e, π, k, T, a and h into the expression

$$\Delta S^\circ_{A,2D} = -R \ln(m_A)^{1/2} - 52.04R \quad (A.11)$$

Similarly,

$$\Delta S^\circ_{B,2D} = -R \ln(m_B)^{1/2} - 52.04R \quad (A.12)$$

$$\Delta S^\circ_{AB,2D} = -R \ln(m_{AB})^{1/2} - 52.04R \quad (A.13)$$

where m_B and m_{AB} are particle masses for B and complex AB, respectively.

Combining expressions A.11, A.12, and A.13 yields

$$T\delta S^\circ_{A,2D} = T\Delta S^\circ_{B,2D} - T\Delta S^\circ_{AB,2D} = -RT \ln(m_A m_B/m_{AB})^{1/2} - 52.04RT \quad (A.14)$$

Combining expressions A.3, A.4, A.5 and A.14 yields

$$\ln K_{d2D} = \ln K_d - \ln(m_A m_B/m_{AB})^{1/2} - 52.04 \quad (A.15)$$

REFERENCES

Adamson, A. W. (1990) in *Physical Chemistry of Surfaces*, Wiley & Sons, New York.

Armstrong, S. A., Husten, E. J., Esmon, C. T., & Johnson, A. E. (1990) *J. Biol. Chem. 265*, 6210.

Barenholz, Y., Gibbes, D., Litman, B. J., Goll, J., Thompson, T. E., & Carlson, F. D. (1977) *Biochemistry 16*, 2806.

Bevers, E. M., Comfurius, P., & Zwaal, R. F. A. (1991) *Blood Rev. 5*, 146.

Drake, T. A., Morrissey, J. H., & Edgington, T. S. (1989) *Am. J. Pathol. 134*, 1087.

Esmon, C. T. (1979) *J. Biol. Chem. 254*, 964.

Fujikawa, K., & Davie, E. W. (1976) *Methods Enzymol. 45*, 89.

Gitel, S. N., Owen, W. G., Esmon, C. T., & Jackson, C. M. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 1344.

Guinto, E. R. (1983) Ph.D. Dissertation, University of Oklahoma Health Science Center.

Guinto, E. R., & Esmon, C. T. (1982) *J. Biol. Chem. 257*, 10038.

Husten, E. J., Esmon, C. T., & Johnson, A. E. (1987) *J. Biol. Chem. 262*, 12953.

Jackson, C. M., Johnson, T. F., & Hanahan, D. J. (1968) *Biochemistry 7*, 4492.

Krishnaswamy, S. (1990) *J. Biol. Chem. 265*, 3708.

Krishnaswamy, S., & Mann, K. G. (1988) *J. Biol. Chem. 263*, 5714.

Krishnaswamy, S., Jones, K. C., & Mann, K. G. (1988) *J. Biol. Chem. 263*, 3823.

Krishnaswamy, S., Russell, G. D., & Mann, K. G. (1989) *J. Biol. Chem. 264*, 3160.

Lentz, B. R., Zhou, C.-M., & Wu, J. R (1994) *Biochemistry 33*, 5460.

Mann, K. G., Nesheim, M. E., Church, W. R., Haley, P., & Krishnaswamy, S. (1990) *Blood 76*, 1.

Miletich, J. P., Jackson, C. M., & Majerus, P. W. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4033.

Mimms, L. T., Zampighi, G., Nozaki, Y., Tanford, C., & Reynolds, J. A. (1981) *Biochemistry 20*, 833.

Moore, W. J. (1972) in *Physical Chemistry*, Prentice-Hall, Inc., Englewood Cliffs, NJ.

Nelsestuen, G. L., & Lim, T. K. (1977) *Biochemistry 16*, 4164.

Nesheim, M. E., Kettner, C., Shaw, E., & Mann, K. G. (1981) *J. Biol. Chem. 256*, 6537.

Nesheim, M. E., Canfield, W. M., Kisiel, W., & Mann, K. G. (1982) *J. Biol. Chem. 257*, 1443.

Nesheim, M. E., Tracy, R. P., & Mann, K. G. (1984) *J. Biol. Chem. 259*, 1447.

Owen, W. G., Esmon, C. T., & Jackson, C. M. (1974) *J. Biol. Chem. 249*, 594.

Pryzdial, E. L. C., & Mann, K. G. (1991) *J. Biol. Chem. 266*, 8969.

Small, D. M. (1967) *J. Lipid Res. 8*, 551.

Smirnov, M. D., & Esmon, C. T. (1994) *J. Biol. Chem. 269*, 816.

Teitel, J. M., & Rosenberg, R. D. (1983) *J. Clin. Invest. 71*, 1383.

Tracy, P. B., Nesheim, M. E., & Mann, K. G. (1992) *Methods Enzymol. 215*, 329.

Ueno, M., Tanford, C., & Reynolds, J. A. (1984) *Biochemistry 23*, 3070.

van de Waart, P., Hemker, H. C., & Lindhout, T. (1984) *Biochemistry 23*, 2838.

Walker, F. J., Sexton, P. W., & Esmon, C. T. (1979) *Biochem. Biophys. Acta 571*, 333.

Wiedmer, T., Esmon, C. T., & Sims, P. J. (1986) *Blood 68*, 875.

BI941333+