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Binding and Activation Properties of Human Factor XII, Prekallikrein, and Derived Peptides with Acidic Lipid Vesicles†

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ABSTRACT: The binding of human factor XII and prekallikrein to vesicles of various compositions and the relationship to activation of factor XII were studied. Factor XII, factor XII_a, and the 40-kilodalton binding fragment of factor XII bound tightly to all of the negatively charged lipids investigated, including sulfatide, phosphatidylserine, and phosphatidylethanolamine, but not to the neutral lipid phosphatidylcholine. Binding could be reversed by high salt, and the dissociation constant for binding to sulfatide vesicles was in the nanomolar range at an ionic strength of 0.15 M. Prekallikrein did not bind significantly to either sulfatide or phosphatidylethanolamine vesicles under the conditions used. Stopped-flow studies showed that the association rate for the factor XII–sulfatide interaction was biphasic and very rapid; the faster rate corresponded to about 30% collisional efficiency. The kinetics of activation of factor XII was investigated and was in agreement with previous studies; sulfatide promoted activation but phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine did not. Autoactivation rates correlated closely with the packing density of factor XII and factor XII_a on the vesicle surface. In contrast, kallikrein activation of factor XII correlated with the amount of sulfatide-bound factor XII and was relatively insensitive to the density of factor XII on the vesicle surface. When the concentration of factor XII was reduced to only several molecules per vesicle, the autoactivation rate dropped very low whereas kallikrein activation held relatively constant. These results indicated that the autoactivation and the kallikrein activation of factor XII were dependent on different properties of the surface component.

The human plasma proteins factor XII, factor XI, prekallikrein, and high molecular weight kininogen compose the contact activation system of blood coagulation [for a review, see Griffin & Cochrane (1979)]. The contact system is initiated when plasma in plastic tubes comes into contact with certain negatively charged surfaces such as kaolin, glass (Margolis, 1963), ellagic acid–metal complex (Bock et al., 1981), dextran sulfate (Kluft, 1978), and sulfatide vesicles (Fujikawa et al., 1980). Factor XII_a[1] appears to play the central activator role through limited proteolysis of prekal-

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[1] Abbreviations: factor XII, single-chain factor XII; factor XII_a, two-chain factor XII which is enzymatically active; factor XII_f, 28-kDa fragment of factor XII which is enzymatically active; factor XII_{bf}, 40-kDa N-terminal nonenzymatic binding fragment of factor XII; PC, egg phosphatidylcholine; PE, egg phosphatidylethanolamine; PS, bovine brain phosphatidylserine; DMPS, synthetic dimyristoyl-PS; dansyl, 8-(dimethylamino)-1-naphthalenesulfonyl; dansyl-PE, *N*-dansyl-PE; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; kDa, kilodalton(s); Tris, tris(hydroxymethyl)aminomethane.

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likrein to kallikrein (Kaplan & Austen, 1970; Wuepper & Cochrane, 1972) and factor XI to factor XI_a (Ratnoff et al., 1961), while high molecular weight kininogen is thought to act as a cofactor (Schiffman & Lee, 1975; Meier et al., 1977). Factor XI_a is involved in intrinsic coagulation (Rosenthal et al., 1953), whereas kallikrein is involved in kinin formation, fibrinolysis (Hathway et al., 1965), and prorenin activation (Derkx et al., 1979; Sealey et al., 1979). Whether initiation of blood coagulation via the contact system has physiological importance has been controversial since persons deficient in factor XII (Ratnoff & Colopy, 1955), prekallikrein (Wuepper, 1973), or high molecular weight kininogen (Colman et al., 1975; Saito et al., 1975; Wuepper et al., 1975) do not commonly exhibit bleeding disorders. Nevertheless, this plasma system has distinct characteristics in vitro and may be important in vivo for the systems mentioned above. Other important systems which appear to be initiated by contact activation in vitro are the complement cascade (Ghebrehiwet et al., 1981), the intrinsic coagulation pathway (Kisiel et al., 1977), and bacterial recognition (Morrison & Cochrane, 1974).

The limited proteolytic cleavage of factor XII to factor XII_a results in several active molecular species. The rates of production of these species vary depending on which enzyme is catalyzing the activation. Activation of factor XII by kallikrein leads to the formation of an 80-kDa factor XII_a (Cochrane et al., 1973), which consists of a 52-kDa heavy chain and a 28-kDa light chain held together by disulfide bond(s). The heavy chain contains the surface binding domain (Revak & Cochrane, 1976), and the light chain contains the catalytic domain (Revak et al., 1977). This initial cleavage is followed by cleavages in the heavy chain yielding 40-kDa factor XII_{bf}, 12-kDa fragment, and 28-kDa factor XII_f (Griffin & Cochrane, 1976). The rates of production of all of these species differ when factor XII is activated by factor XII_a during autoactivation (Dunn et al., 1982).

Sulfatide [a cerebroside sulfate; for a review, see Stoffyn (1966)] vesicles appear to provide the best surface identified to date for activating the contact system in plasma (Tans & Griffin, 1982; España & Ratnoff, 1983) and in solutions of purified proteins (Fujikawa et al., 1980; Kurachi et al., 1980; España & Ratnoff, 1983; Tans et al., 1983). Sulfatide is a negatively charged lipid which is found in biological membranes and has been identified in human erythrocyte membranes (Hansson et al., 1978) in low concentration (0.5 mg of sulfatide/kg wet cells).

The kinetics of autoactivation and kallikrein activation of factor XII have been studied recently by several groups. They have used different surfaces and different mathematical models for data analysis, including a computer iterative model with a glass surface (Silverberg et al., 1980), a strict, second-order model with a sulfatide surface (Tans et al., 1983), and a Michaelis–Menten-like model with a dextran sulfate surface (Tankersley & Finlayson, 1984). Some similarities between these systems can be observed. For example, the $k_{cat}\bar{y}$ of Tankersley & Finlayson (1984) appeared to be numerically equal to the $k_2[XII]_{total}$ of Tans et al. (1983) under many conditions, where k_{cat} was the first-order catalytic rate constant, $\bar{y}$ was the degree of saturation of enzyme by substrate, and k_2 was the second-order rate constant.

That factor XII binds to negatively charged surfaces has been inferred primarily from kinetic studies in which the surfaces greatly accelerated the reactions. One exception to this was a previous study (Kirby & McDevitt, 1983) which quantitated the binding of factor XII to kaolin, a nonphysiological surface that is often used when studying the contact system. The present study examined various aspects of the binding of factor XII and prekallikrein to vesicles of several naturally occurring lipids and how this binding correlated with activation reactions. The binding of factor XII_a to lipid vesicles was found to be tight and relatively nonspecific with respect to negatively charged lipids, whereas both autoactivation and kallikrein activation of factor XII appeared to be specific for sulfatide. Prekallikrein did not bind to sulfatide vesicles under the conditions used, and kallikrein activation of factor XII was affected by sulfatide concentration in a manner distinct from autoactivation of factor XII.

Experimental Procedures

Highly purified lipids were purchased from Sigma Chemical Co., except for DMPS which was purchased from Avanti Polar Lipids, Inc. Vesicles were prepared by sonication of the lipids in the appropriate buffer in an ice bath (Nelsestuen & Lim, 1977; Huang, 1969). The lipid compositions of the vesicles in this paper are indicated as the mole percent of the different lipid species which make up the vesicle. After sonication, the vesicle preparations were gel filtered on a Sepharose CL-2B column (1.7 × 23 cm) unless otherwise stated. Dansyl-PE was a gift from Dr. Marc Pusey [Pusey et al. (1982) as per Waggoner & Stryer (1970)]. The concentrations of the vesicles used in these experiments are given as the concentration of total lipid in solution. Phospholipids were quantitated for phosphorus by the method of Chen et al. (1956). Sulfatide was quantitated by the method of Kean (1968), and the molar concentration was calculated by using a molecular weight of 1000 for sulfatide. PE-containing vesicles are considered anionic because they often display more negative character than would be anticipated given the standard amino pK_a of 9.0 (Bangham, 1968); the negative charge could arise from impurities.

The diffusion coefficient (D) and the hydrodynamic radius (R) of the 100% sulfatide vesicles prepared with Sepharose CL-2B were determined by quasi-elastic light scattering; D^{20} was 6.23×10^{-8} cm^2/s (±5%), and R was 350 Å (±5%). Quasi-elastic light scattering [described by Bloomfield & Lim (1978)] was monitored by using a Langley Ford Instruments LSA2 and Model 1096 correlator.

Human factors XII and XII_a were prepared by the method previously described (Fujikawa & Davie, 1981). Factors XII_f and XII_{bf} were isolated from the tryptic digest of factor XII by chromatography on a DEAE-Sephacel column as described previously (Fujikawa & McMullen, 1983). The unadsorbed fraction from the DEAE-Sephacel column that contained factor XII_{bf} and 12-kDa fragment was concentrated and applied to a Sephadex G-100 column with 50 mM Tris and 150 mM NaCl (pH 7.5). The first peak was pooled for factor XII_{bf} and concentrated by Amicon ultrafiltration. Human plasma prekallikrein was isolated by the method of Heimark & Davie (1979). Kallikrein was prepared by the activation of human plasma prekallikrein with a catalytic amount of human factor XII_f. The protein preparations, including factor XII, factor XII_a, factor XII_f, prekallikrein, and kallikrein, were homogeneous as per sodium dodecyl sulfate–polyacrylamide gel electrophoresis, whereas factor XII_{bf} was observed to have a strong band at 40 kDa along with a small contamination of a 36-kDa band. However, isolated factor XII was found to have some factor XII_a as determined by S-2302 amidase activity; two different preparations of factor XII were used in the present study; one contained ~1.5% factor XII_a, and the other contained ~3.0% factor XII_a. These two preparations gave similar results in all experiments. The protein concen-

trations of the purified human plasma proteins were quantitated by absorbance using $E^{1\%}_{280nm}$ = 14.2 for factor XII and XII_a (Fujikawa & Davie, 1981), 10.9 for prekallikrein and kallikrein (Heimark & Davie, 1979), 13.0 for factor XII_f, and 12.0 for factor XII_{bf}.

The molecular weight of phospholipid vesicles and protein–vesicle complexes was determined from 90° relative light-scattering intensity by using the method described by Nelsestuen & Lim (1977). The protein to lipid ratios were plotted in weight to weight units to facilitate drawing the theoretical binding curve. The instrument used was a Perkin-Elmer MPF-44A fluorescence spectrophotometer in which both the excitation and emission wavelengths had been set at 320 nm. The buffer used was 50 mM HEPES and 100 mM NaCl (pH 7.4) at 25 °C unless otherwise stated.

Fluorescence energy transfer measurements were performed on a Perkin-Elmer MPF-44A fluorescence spectrophotometer in which the excitation wavelength was set at 285 nm and the emission wavelength was set at 490 nm with a 290-nm cutoff emission filter. The composition of the vesicles used for fluorescence energy transfer was 90% sulfatide–10% dansyl-PE. Transfer of fluorescence energy requires close association of donating and accepting moieties, so that only protein bound to the vesicle will participate in energy transfer. Fluorescence at 490 nm from direct excitation of the dansyl moiety at 285 nm (F_0) was proprtional to the concentration of vesicles present and thus served as a convenient internal standard with which to compare the transferred fluorescence intensity. When protein was added to a vesicle solution, an increase in fluorescence (F) indicated binding. The ratio F/F_0 provided an indication of the relative amount of energy transfer independent of concentration. The protein to lipid ratios were plotted in weight to weight units. The buffer used was 50 mM HEPES and 50 mM NaCl (pH 7.4) at 25 °C unless otherwise stated.

For light-scattering and energy transfer experiments, the 1-cm fluorescence quartz cuvettes were treated prior to use with a similar vesicle–protein mixture and then rinsed with filtered, distilled water. This was found to be all that was necessary to prevent protein adsorption to the cuvette walls under the experimental conditions used.

The association rate constant for factor XII_a–vesicle binding was obtained from an analysis of 90° relative light-scattering intensity changes following protein–vesicle mixing in a stopped-flow apparatus. The apparatus was described by Wei et al. (1982); all stopped-flow experiments were carried out at 14 °C using 100% sulfatide vesicles which had been gel filtered on a Sepharose 4B column (1.7 × 25 cm). The association was modeled as a bimolecular, irreversible binding interaction. The assumption of irreversibility will hold if the rate of dissociation is slow on the experimental time scale; binding affinity indicated that this assumption was valid. Rate constants were expressed on the basis of protein binding interactions with multiple interactions per lipid vesicle.

Autoactivation studies were carried out essentially according to Tans et al. (1983) except that no ovalbumin was added to the buffers and the generation of factor XII_a was monitored by the rate of hydrolysis of 390 μM S-2302 (H-D-prolyl-L-phenylalanyl-L-arginine-*p*-nitroanilide; Helena Laboratories, Beaumont, TX). Since S-2302 is also a substrate of kallikrein, the rate of its hydrolysis due to kallikrein was subtracted from those mixtures which included kallikrein. The kinetic model used to represent these data was similar to that of Tankersley & Finlayson (1984), except that the slope of a semilog plot of generated factor XII_a concentration vs. time was taken as k_{obsd}, where k_{obsd} was the observed first-order rate constant (see Figure 6B). This simple method of expression gives an experimental rate constant for autoactivation which will be valid as long as the substrate remains saturating or the substrate concentration does not change significantly. Experimentally, these plots were often linear up to 50% activation, but the data were usually gathered for only the first 20% of activation to ensure that the initial rate was being calculated.

The experimental conditions for the kallikrein activation of factor XII were the same as those described for autoactivation. However, kallikrein activation of factor XII was complicated by concomitant autoactivation of factor XII, so an iteration procedure was used to correct for it. The velocity of autoactivation was taken to be $k_{obsd}[XII_a]$ where k_{obsd} was the activation rate under identical conditions except for the absence of kallikrein. The concentration of XII_a generated was multiplied by k_{osbd}, and for every 5-s interval, this product, $v_{autoactivation}$, was subtracted from the average total activation velocity, v_{total}, during that 5-s interval to get the velocity of XII activation due to kallikrein only ($v_{total\ activation} - v_{autoactivation} = v_{activation\ by\ kallikrein\ only}$).

Results

Equilibrium Binding Measurements. To characterize the binding of factor XII_a to vesicles, the techniques of light scattering (Figure 1A) and fluorescence energy transfer (Figure 1B) were utilized. Binding was observed when 3.3 μM mixtures of 100% sulfatide or 90% sulfatide–10% dansyl-PE vesicles were titrated with factor XII_a. At saturation, the weight ratio of protein to lipid was 0.80, which corresponded to a molar ratio of sulfatide to factor XII_a of about 100, whether determined by light scattering (Figure 1A) or by fluorescence energy transfer (Figure 1B). There was no measurable difference between factor XII or factor XII_a when binding to lipids, and these proteins were considered interchangeable for binding experiments. Under presaturation conditions, all added protein bound to the vesicles, leaving a negligible level of free protein even when the concentration of vesicles was varied from 13 to 0.7 μM (Figure 1A). This result indicated tight binding, and if the limits of error of the experiment are considered, then the free protein concentration at half-saturation of the sulfatide vesicles was less than 7 nM.

Other types of lipid vesicles were tested by light scattering for factor XII_a binding (Figure 1A). Factor XII_a bound to 100% PE and 100% PS vesicles but not to 100% PC or 20% PS–80% PC vesicles. When saturated with factor XII_a, the molar ratios of lipid to protein in the complexes were similar for 100% PE, 100% PS, and 100% sulfatide vesicles (110, 150, and 100, respectively). Under presaturation conditions, the binding of factor XII_a to 100% PE and 100% PS did not appear quantitative, suggesting the presence of free protein due to weaker binding interactions.

The pH dependence of the binding of factor XII_a and prekallikrein to sulfatide was investigated by light scattering (Figure 2). The data for factor XII_a indicated that from approximately pH 6.8 to 7.8 virtually all added protein was surface bound at a protein to lipid weight ratio of 0.50. The binding capacity of the vesicles at a saturating protein to lipid weight ratio did appear to be affected by pH in the range shown. The binding capacity decreased somewhat from pH 6.8 to 7.8 and then remained level to pH 8.2. Prekallikrein did not appear to bind to 100% sulfatide between pH 6.8 and 8.2 (Figure 2), nor was it observed to bind to 100% PE at pH 7.4 (data not shown).

Reversibility of binding was shown by eluting the protein from the vesicles with NaCl (Figure 3). Fluorescence energy

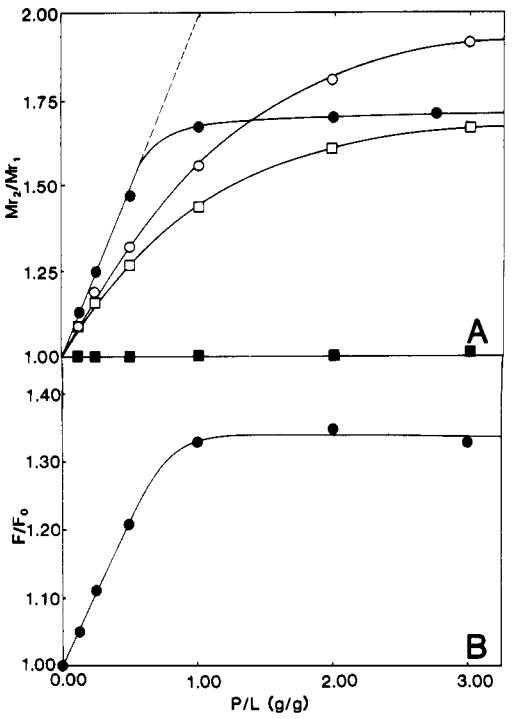

FIGURE 1: Measurement of factor XII_a–vesicle binding by light scattering (A) and fluorescence energy transfer (B). (A) Lipid vesicles were assigned a relative molecular weight of 1 (M_1), and the relative molecular weight of the protein–vesicle complex (M_2) was obtained from analysis of light-scattering intensity measurements. The data are plotted vs. the weight ratio of protein to lipid (P/L). A 1.5-mL solution containing one of the following lipids was monitored: 20, 5, 2, or 1 μg of 100% sulfatide (●); 5 μg of 100% PS (○); 5 μg of 100% PE (□); 5 μg of 100% PC (■). The dashed line shows the theoretical curve if all of the added protein were bound to the lipid. (B) The fluorescence intensity of emission at 490 nm (excitation at 285 nm) of the 90% sulfatide–10% dansyl-PE vesicles (F_0) was assigned the value of 1. The increase in fluorescence intensity when factor XII was added to 5 μg of these vesicles (F) is plotted as F/F_0 to represent the increase of signal due to protein binding. Light-scattering experiments similar to those in part A showed that the dansyl-labeled vesicles gave a binding curve similar to that shown for 100% sulfatide in part A. All experiments were carried out in 50 mM HEPES and 100 mM NaCl (pH 7.4) at 25 °C.

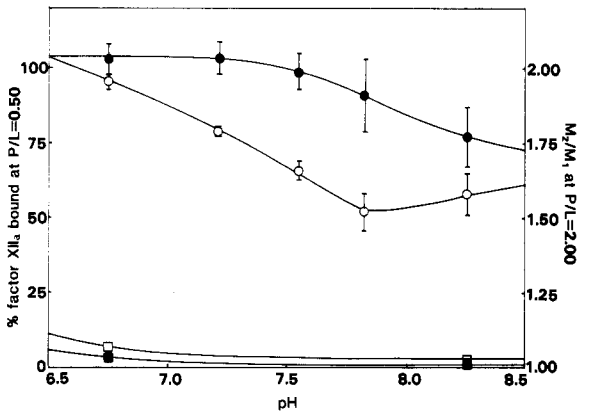

FIGURE 2: Effect of pH on the parameters of factor XII_a– and prekallikrein–100% sulfatide vesicle binding as determined by light scattering. The data for factor XII_a are represented by circles and those for prekallikrein by squares. The percent protein bound at a protein to lipid weight ratio of 0.50 (●, ■) and M_2/M_1 (see Figure 1A for identification) at a protein to lipid weight ratio of 2.00 (○, □) were used as indicators of the strength of binding and the maximum number of binding sites, respectively. All experiments were carried out with a 1.5-mL solution containing 5 μg of 100% sulfatide, 20 mM HEPES, and 100 mM NaCl (variable pH) at 25 °C. Experiments were performed in triplicate with mean values plotted and standard error shown.

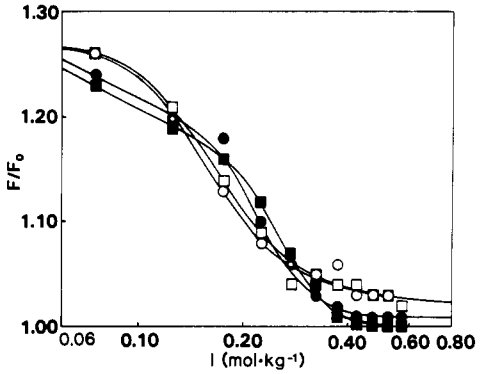

FIGURE 3: Effect of ionic strength on factor XII_a–sulfatide binding as determined by fluorescence energy transfer. Aliquots of NaCl were added to protein–vesicle mixtures in 1.5 mL of 50 mM HEPES and 50 mM NaCl (pH 7.4) at 25 °C. The weight ratio of protein to lipid was held constant at 0.50 while the amounts of lipid were varied as follows: 2 (○), 5 (□), 20 (●), and 100 μg (■) of vesicles.

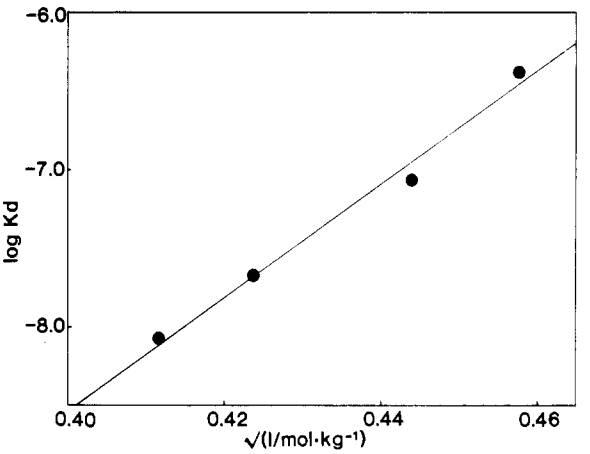

FIGURE 4: Effect of salt on the free protein concentration at half-maximum saturation plotted per the modified Debye–Hückel reaction rate relationship (see Results). The data in Figure 3 were analyzed as described under Results. The ionic strength was calculated from the buffer and added salts.

transfer was used to monitor the amount of protein bound to the 90% sulfatide–10% dansyl-PE vesicles, and the weight ratio of protein to lipid was maintained at 0.50; at this ratio, all added protein is surface bound under the starting ionic strength conditions (Figure 1). Even though the rato of protein to lipid was kept constant, the concentrations of protein and lipid were varied. At the initial ionic molality of 0.075, F/F_0 was constant from 420 to 8.4 nM factor XII_a. Since the proportion of protein bound remained constant, this indicated that the dissociation constant for the protein and lipid was less than the lowest protein concentration (8.4 nM).

To obtain an estimate of the approximate dissociation constant for factor XII_a and sulfatide vesicles at physiological ionic strength, the Debye–Hückel reaction rate equation was adapted to K_D values: $\log K_D = \log K_D^0 - 1.02 z_A z_B I^{1/2}$ mol kg^{-1} where K_D^0 is the dissociation constant at zero ionic strength, 1.02 is a constant derived from the Debye–Hückel limiting law and is valid for an aqueous solution at 25 °C, I is the molal ionic strength of the solution, and z_A and z_B are the charges of the reactants A and B. The midpoint for salt dissociation occurs when half of the protein is free, and therefore, the concentration of free protein at half-saturation should be directly related to the K_D. Figure 4 shows the plot of the log of free protein concentration at half-dissociation vs. ionic strength; least-squares analysis indicated an r^2 value of 0.98. This plot was extrapolated to the approximate physio-

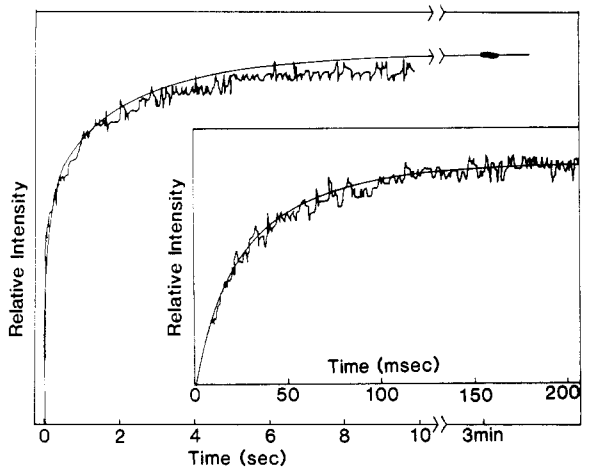

FIGURE 5: Chart tracing of the light-scattering intensity when 2 μM factor XII_a and 226 μM 100% sulfatide were mixed in a stopped-flow apparatus. The above concentrations were for the individual solutions before equivolume mixing. The intensity from the solution at full equilibrium (3 min) is also shown. The curve was found to be biphasic, and the inset shows the observed pseudosaturation of the faster binding sites. The curves generated by using the derived rate binding constants are indicated by the smooth lines.

logical ionic strength of 0.15 mol/kg, and the K_D obtained was 1.2 nM. This procedure was repeated for factor XII_{bf} (also at a protein to lipid weight ratio of 0.50), and the K_D obtained at 0.15 mol/kg was 2.4 nM (data not shown).

Dynamic Binding Measurements. The association rate for the factor XII_a and sulfatide interaction was determined by using stopped-flow light scattering with solutions of 2 μM factor XII_a and 226 μM 100% sulfatide vesicles at 14 °C (Figure 5). The results were fit reasonably well by assuming biphasic binding with about 55% of the intensity due to proteins having an association rate constant of 4.8×10^7 M^{-1} s^{-1} and the remaining intensity due to proteins having an association rate constant of 7.6×10^5 M^{-1} s^{-1}; these rates were treated as though there were 100 sulfatides per binding site (see Figure 1). This first association rate corresponded to about 30% collisional efficiency when it was compared to the theoretical collision rate (Smoluchowski, 1915). The theoretical rate was calculated by using typical diffusion constants ($D^{14} = 5.0 \times 10^{-7}$ and 1.6×10^{-7} cm^2/s) and radii (43 and 140 Å) for the protein and vesicle species, respectively.

Activation Kinetics. The kinetics of autoactivation can be analyzed in a number of ways (Figure 6A–C). The two published methods for obtaining rate constants from these data [(Figure 6B) Tankersley & Finlayson (1984) and (Figure 6C) Tans et al. (1983)] give values which are proportional to each other. The k_{obsd} obtained from the slope of the line in Figure 6B was used for further analysis.

An Arrhenius experiment indicated that the optimal temperature for autoactivation was approximately 25 °C (Figure 7) and that the energy of autoactivation was 8.44 kcal/mol.

Autoactivation studies with 200 nM factor XII at 22 °C and varying concentrations of sulfatide vesicles indicated that the rate of activation was dependent upon this variable (Figure 8A). At low sulfatide concentration, the rate increased proportionally, paralleling the increase in the percent of total bound factor XII (compare with Figure 8B). As the sulfatide concentration was further increased, the rate reached a maximum at about 20 μM sulfatide and then decreased rapidly, showing a close correlation with the density of factor XII on the vesicle surface (compare with Figure 8B). Results which could be interpreted as showing an autoactivation rate dependence on sulfatide concentration have been presented

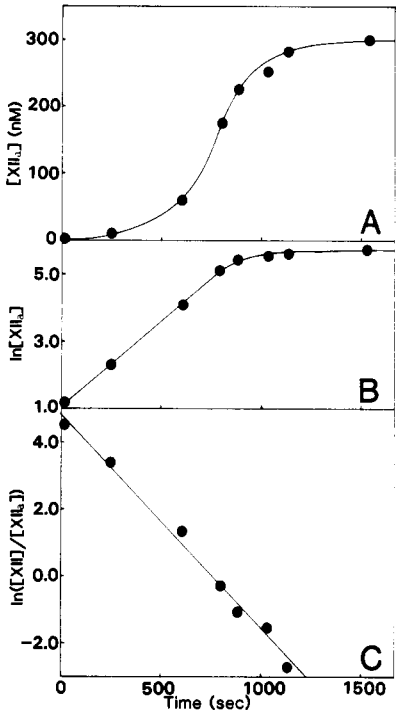

FIGURE 6: Assay of the autoactivation of factor XII by sulfatide vesicles. (A) A mixture containing 300 nM factor XII (1.5% factor XII_a initial) and 200 μM sulfatide in 50 mM HEPES and 100 mM NaCl (pH 7.4) was incubated at 37 °C for the times indicated and then an aliquot assayed for factor XII_a amidase activity as described under Experimental Procedures. (B) Semilogarithmic plot of the data from part A linearized the initial 50% of the autoactivation reaction. The rate obtained for these data was $k_{obsd} = 0.0067$ s^{-1} and was similar to the k_{cat}/y of Tankersley & Finlayson (1984). (C) Semilogarithmic plot of the ratio $[XII]/[XII_a]$ from the data in part A linearized up to 95% of the reaction as previously observed (Tans et al., 1983). The rate obtained from this plot was $k_{2_{app}} = 2.1 \times 10^4$ M^{-1} s^{-1}. This value times $[XII]_{total}$ was approximately equal to the k_{obsd} from part B. Several trial experiments indicated that the two different plots yielded approximately proportional rate constants under various conditions.

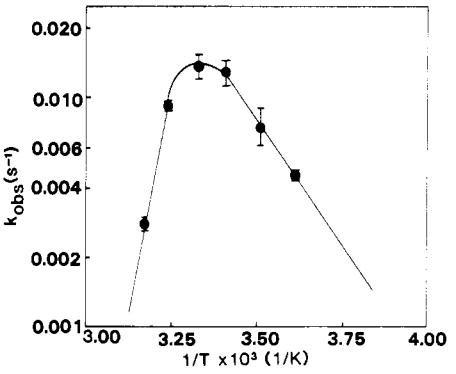

FIGURE 7: Temperature dependence of the autoactivation reaction. The protein to lipid weight ratio was 0.50, and 200 nM factor XII (3% factor XII_a initial) was assayed. The data are presented in an Arrhenius plot, and the rates were quantitated as per figure 6B. The experiments were carried out in 50 mM HEPES and 100 mM NaCl (pH 7.4) and were performed in triplicate with mean values plotted and standard error shown.

by other workers [e.g., see Figure 1 of España & Ratnoff (1984)].

The velocity of kallikrein activation of factor XII at different sulfatide concentrations (Figure 8A) was determined under the conditions of 200 nM factor XII and 3 nM kallikrein at 22 °C. The velocity approached an apparent maximum at about 75 μM sulfatide and then decreased gradually at higher sulfatide concentrations. A comparison with Figure 8B in-

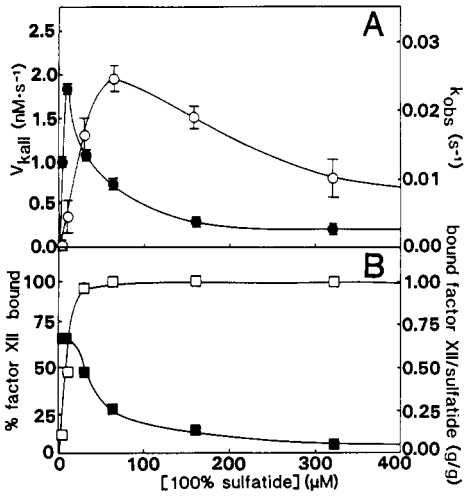

FIGURE 8: Effect of sulfatide concentration on the rates of autoactivation and kallikrein activation of factor XII (A) and their relationship to two surface interaction properties of factor XII (B). (A) The autoactivation rates (k_{obsd}, ●) were quantitated as per Figure 6B and the kallikrein activation rates (V_{kall}, ○) as per Experimental Procedures. All experiments were carried out with 200 nM factor XII (3% factor XII_a initial) in 50 mM HEPES and 100 mM NaCl (pH 7.4) at 22 °C. The kallikrein activitions contained 3 nM kallikrein. The experiments were performed in triplicate with mean values plotted and standard error shown. (B) The percent of factor XII bound to the vesicles (□) and the ratio of grams of bound factor XII to grams of sulfatide (■) are shown to aid comparison of the binding and the activation effects of sulfatide and factor XII. The data from Figure 1B are replotted here as though that experiment had been performed with 200 nM factor XII and titrated with 100% sulfatide vesicles.

Table I: Effect of Various Vesicle Compositions and Temperature on the Rate of Autoactivation[a]

lipid composition	T (°C)	protein to lipid ratios tested (w/w)	k_{obsd} (s^{-1})
100% sulfatide	37	0.10, 0.50, 2.00	0.0072, 0.0092, 0.0081
42% sulfatide–58% PE	37	0.15, 0.30, 0.60	0.0077, 0.0061, 0.0028
100% PC	37	0.10, 0.50, 2.00	*b*
100% PE	37	0.10, 0.50, 2.00	*b*
100% PS	37	0.10, 0.50, 2.00	*b*
100% DMPS	37	0.10, 0.50, 2.00	*b*
100% sulfatide	24	0.13, 0.50, 2.00	0.0080, 0.0168, 0.0111
100% DMPS	22	0.10, 0.50, 2.00	*b*

[a] The reaction conditions are the same as in Figure 8, except that the rates were measured at the temperatures shown. [b] The rates were indistinguishable from the background error rate of k_{obsd} = 0.0002 s^{-1}.

dicated that kallikrein activation correlated closely with the total amount of factor XII bound but did not correlate with the density of factor XII on the surface (see bound factor XII/sulfatide in Figure 8B).

Other lipid vesicles were tested for their ability to promote the autoactivation of 300 nM factor XII at 37 °C. The only vesicles which promoted significant autoactivation were 42% sulfatide–58% PE (Table I). Phosphatidylserine vesicles were tested for their ability to promote the kallikrein activation of factor XII, and it was found that no activation occurred under conditions similar to those used with sulfatide vesicles (data not shown).

Discussion

Several attempts to analyze factor XII autoactivation by mathematical models have been published (Silverberg et al., 1980; Tankersley & Finlayson, 1984; Tans et al., 1983). These studies have produced rate constants which were dependent on the surface concentration (Tankersley & Finlayson, 1984; Tans et al., 1983) and were therefore valid for only one surface concentration. More general predictions of these kinetics require that the surface component be incorporated into the rate expression, and this in turn requires knowledge of the protein–surface binding interactions and their effect on reaction velocity. The current studies provide some of this information along with a mechanistic interpretation of how the activation rates could be affected by binding interactions.

There are many negatively charged surfaces which have been shown to greatly enhance the reactions of the contact activation system, and to date, sulfatides have been identified as the best surface for this function. Even though binding is often invoked as a prominent feature of the "contact" activation system, only one study has been concerned with quantitating the binding of factor XII to surfaces (Kirby & McDevitt, 1983), and it was found that factor XII binding to kaolin was largely irreversible. The results presented here are consistent with rapid, tight, reversible binding of factor XII to several negatively charged lipids, including lipids which do not promote activation.

Factor XII_a was observed to bind to sulfatide, PS, and PE, but not to PC (Figure 1). In keeping with these observations, factor XII_a also bound to 42% sulfatide–58% PE, 21% sulfatide–79% PE, and 8% sulfatide–92% PE but not to 8% sulfatide–92% PC or 20% PS–80% PC (data not shown). The two latter observations suggested that PC inhibited the binding of factor XII_a to adjacent negatively charged lipids if they were present in too low a percentage of the total lipid.

The binding interaction between factor XII_a and sulfatide was reversible as shown by dissociating the protein from the vesicles with high salt (Figure 3); this observation also suggested that the interaction was strongly ionic. These dissociation studies indicated a low dissociation constant of approximately nanomolar values under physiological conditions. Binding studies in the pH range from 7 to 8 (Figure 2) did not show a close correlation with previous studies on the pH dependence of autoactivation (Tans et al., 1983), suggesting that the binding and catalytic domains are acting somewhat independently and that the binding interaction does not account for the pH dependence of autoactivation. Through this pH range, the binding capacity of the sulfatide vesicles changed somewhat, but the dissociation constant remained in the low nanomolar range.

Activation of factor XII had a specific lipid requirement with only sulfatide–containing vesicles functioning as contact activation surfaces, which was in agreement with the observations of Fujikawa et al. (1980). In the present studies, 100% sulfatide, 90% sulfatide–10% dansyl-PE, and 42% sulfatide–58% PE vesicles were capable of promoting autoactivation, but 100% PS, 100% PE, and 100% PC vesicles were not (Table I). Kallikrein activation of factor XII was observed to occur in the presence of 100% sulfatide but not 100% PS vesicles. Autoactivation and kallikrein activation of factor XII had different dependences on sulfatide concentration (Figure 8A). The rate of autoactivation could be brought to a very low rate at high sulfatide concentrations which is predicted by a model in which the proteins act on an intrasurface basis and the density of bound protein is important. The rate of kallikrein activation of factor XII did not have such a sharp dependence on sulfatide concentration, but it did appear to correlate with the total amount of bound factor XII, suggesting that only bound factor XII is capable of being activated. These results were consistent with a reaction model in which kallikrein is not bound to the vesicles but binds to individual factor XII molecules which in turn are bound to the surface; this model

has been hypothesized previously (Cochrane et al., 1973). The decline in velocity at higher sulfatide concentrations could be due to a weak ionic binding of kallikrein and sulfatide, that we could not detect, which reduces the enzyme available from solution for factor XII activation; whether this possible binding of kallikrein to sulfatide also enhances kallikrein's interaction with factor XII cannot be determined from our studies.

It is possible that when factor XII binds to sulfatide it undergoes the hypothesized conformational change which exposes its activation site (Griffin, 1978) or that it initially leads to a substrate–induced catalysis by the zymogen (previously hypothesized for the activation of factor XI by factor XII; Kurachi et a., 1980) followed by autoactivation. In any case, the selective basis of the activation reactions for certain surfaces is unknown. An effective surface may provide some physical feature, in addition to its negative charge, which is absent from an ineffective surface. Glass, dextran sulfate, and kaolin all have relatively static surfaces, and at room temperature, sulfatide is in the gel phase [T_m is 86 °C (Abrahamsson et al., 1972)]. Our experiments show that the explanation may not be this simple because DMPS (T_m is 37 °C) was in the gel phase at 22 °C but it was ineffective for autoactivation (Table I). The curvature or geometry of the surface might also be an important feature for the bound proteins. Another alternative could be that the binding of enzyme to substrate only becomes significant when both are bound adjacently and tightly to a particular surface. Our results are not able to distinguish between these possibilities.

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Registry No. Blood coagulation factor XII, 9001-30-3; blood coagulation factor XIIa, 37203-62-6; prekallikrein, 9055-02-1; kallikrein, 9001-01-8.

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