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Possible Basis for the Apparent Surface Selectivity of the Contact Activation of Human Blood Coagulation Factor XII†

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ABSTRACT: The activation of factor XII by the proteases factor XIIa and kallikrein is known to be greatly enhanced by certain negatively charged surfaces. Studies that compared factor XII surface binding to factor XII activation found that binding alone was insufficient to account for surface enhancement of the activation rate. The temperature dependence of the reaction showed unusual behavior that may be related to the conformational change of factor XII following binding; the rate of factor XII activation had a relatively low temperature optimum (0-47 °C) that was sensitive to choice of surface and salt concentration. In temperature studies, below 47 °C, the decrease in the activation rate was not related to the thermal denaturation of enzyme or substrate, nor to the choice of activator enzyme (factor XIIa or kallikrein), nor to the species of factor XII (human or bovine) but to a behavior, designated a thermal transition, associated with the surface or the protein–surface interaction. The previously reported surface selectivity of contact activation is possibly due to the temperature characteristics and other properties of the thermal transition; a surface that has a low-temperature thermal transition and that is highly sensitive to salt will be a "poor" contact surface under the usual choice of reaction conditions (~150 mM ionic strength and 37 °C). However, solution conditions were identified that allowed the following negatively charged surfaces to function, in nearly equal potency, in the activation of factor XII: phosphatidylserine, phosphatidylglycerol, phosphatidic acid, phosphatidylinositol 4-phosphate, heparin, and 5-kDa dextran sulfate, as well as the previously characterized sulfatide and 500-kDa dextran sulfate. The thermal transition may also explain the phenomenon of cold-promoted activation of plasma; plasma, or its storage containers, may contain "poor" contact surfaces that become active at low temperature. The surface property that is responsible for the thermal transition has not been identified but appears to account for several properties of contact activation.

Glass tubes, when compared to silicon-coated glass tubes, shorten the clotting time of normal platelet-poor plasma (Conley et al., 1949). This effect has been termed contact activation because a component of plasma becomes activated upon contact with glass. This observation led to the hypothesis that a plasma deficient in the contact protein could be identified by its long clotting time in either type of tube. Four contact proteins, factor XI (Rosenthal et al., 1953), factor XII[1] (Ratnoff & Colopy, 1955), prekallikrein (Wuepper, 1973), and high molecular weight kininogen (Colman et al., 1975; Saito et al., 1975; Wuepper et al., 1975), have since been identified. With the purified proteins and several different surfaces, the complex interactivations have been studied and a model for the in vitro contact activation scheme has been

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; factor XII, 78-kDa single-chain factor XII (596 residues); factor XIIa, 78-kDa two-chain disulfide-linked factor XII that is enzymatically active (596 residues with a heavy chain from Ile^{1} to Arg^{353} and a light chain from Val^{354} to Ser^{596}); factor XIIf, 28-kDa two-chain disulfide-bonded fragment of factor XII that is enzymatically active (251 residues with a heavy chain from Val^{354} to Ser^{596} and a light chain from Asn^{335} to Arg^{343}); HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; kDa, kilodalton(s); PA, egg phosphatidic acid; PG, egg phosphatidylglycerol; PIP, bovine brain phosphatidylinositol 4-phosphate; PS, bovine brain phosphatidylserine; Tris, tris(hydroxymethyl)aminomethane.

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worked out [for recent reviews, see Kaplan (1983) or Colman (1984)]. Of the four contact protein deficient types, only some of the factor XI deficient individuals have significant in vivo clotting abnormalities (Ragni et al., 1985). Thus, there is some conceptual difficulty in relating the in vitro and in vivo phenomena. However, this system may be activated during the following disease states: gout (Kellermeyer & Breckenridge, 1965, 1966); bacteremia (Morrison & Cochrane, 1974; Kalter et al., 1983); disseminated intravascular coagulation (Lämmle et al., 1984); synovitis (Moskowitz et al., 1970); cardiovascular disease (Becker et al., 1981); various disease states (Deutsch et al., 1983); others.

Activation of factor XII is achieved by several proteases, including factor XIIa (Silverberg et al., 1980) and kallikrein (Cochrane et al., 1973). The initial bond cleaved by either of these two proteases is within a disulfide bridge to form a two-chain activated enzyme, factor XIIa. The surface-binding domain is in the N-terminal heavy chain (Revak et al., 1974), while the active site is in the C-terminal light chain (Revak et al., 1977). The comlete amino acid sequence of factor XII has been determined (Fujikawa & McMullen, 1983; McMullen & Fujikawa, 1985) as well as nearly all of its cDNA sequence (Cool et al., 1985; Que & Davie, 1986).

Cold-promoted activation of citrated plasma is the result of activation of factor VII upon storage and occurs in plasmas from about 15% of untreated adults and at a higher frequency in females taking oral contraceptives or in the third trimester of pregnancy (Gjønnaess, 1972a). Several groups (Laake & Ellingsen, 1974; Laake & Österud, 1974; Saito & Ratnoff, 1975) later showed that plasmin, factor IXa, and factor XIIa could activate factor VII at low temperatures while plasma kallikrein, factor XIa, and factor XIIf were not as effective. In the bovine system, factor XIIa was shown to be effective in activating bovine factor VII (Kisiel et al., 1977). Consequently, contact activation could lead to the activation factor VII. Even though the actual cause of cold-promoted activation has yet to be determined, Czendlik et al. (1985) hypothesized that it may be due to the decrease of C1-inhibitor efficiency at low temperatures (van der Graaf et al., 1983); C1 inhibitor is the major inhibitor of factor XIIa in plasma (Schreiber et al., 1973).

Many different materials have been tested for their ability to promote the factor XII dependent activation of the contact proteins. Of those surfaces that promote contact activation, the only common features seem to be a negative charge and a high molecular mass, either as crystals, soluble polysaccharides, or colloids. Commonly used surfaces are kaolin (Margolis, 1958), 500-kDa dextran sulfate (Kluft, 1978), and sulfatide (Fujikawa et al., 1980). There are many comparable surfaces that share these two features and yet do not promote contact activation. However, since several different assays have been used in these experiments, it is difficult to compare the relative abilities of the various surfaces and to determine whether contact activation is a specific interaction or a general surface phenomenon. The binding of factor XII and factor XIIa to a surface was insufficient to account for the action of a surface in autoactivation (Griep et al., 1985).

The studies reported here were initiated to better understand the role of the surface and protein–surface binding in the contact activation reaction as well as the basis for the surface selectivity displayed by this reaction. Contact activation appeared to be due to a general surface property, highly dense negative charges, rather than to specific surface functional groups, such as a sulfate moiety. The reaction displayed unusual thermodynamic properties, which possibly provided an explanation for phenomena as diverse as cold-promoted activation of plasma and contact activation surface selectivity.

Experimental Procedures

Preparation of Contact Surfaces. Highly purified lipids were purchased from Sigma Chemical Co. (St. Louis, MO). Vesicles were prepared either by the sonication technique (Nelsestuen & Lim, 1977; Huang, 1969) or by a slight modification of the extrusion technique (Hope et al., 1985). When the sonication technique was used, the vesicle preparation was gel-filtered on a Sepharose CL-2B column (1.7 × 2.3 cm) to obtain unilammelar vesicles. A separate column was used for each lipid type to avoid cross-contamination of the vesicles. Briefly, the extrusion technique consisted of drying the lipid in a test tube under a stream of N_2 and suspending the lipid in buffer. The solution was vigorously agitated and freeze-thawed 6 times in methanol–dry ice. It was then extruded 6 times, with 75 psi N_2, through a Nuclepore PC membrane (0.2-μm filter) in an Amicon pressure filtration apparatus. The concentrations of the various vesicle preparations were determined as the concentration of total lipid in solution after gel filtration or extrusion. Sulfatide was quantitated by the method of Kean (1968), and phospholipids were quantitated by the method of Chen et al. (1956; Nelsestuen & Lim, 1977). The two different molecular weight dextran sulfates, 500-kDa and 5-kDa, were purchased from Sigma (St. Louis, MO), and the fractionated pork mucosal heparin (161 units/mg; 13-kDa average molecular mass) was kindly donated by Riker Laboratories (3M Co., Northridge, CA). The stock solutions of the contact surfaces were buffered with 50 mM HEPES (pH 7.5 at 25 °C; Sigma Chemical Co., St. Louis, MO), 1 mM EDTA, and 50 mM NaCl.

Proteins. Human factor XII (~3% factor XIIa as determined by S-2302 amidase activity) was prepared and quantitated ($E_{280}^{1\%} = 14.2$) by the method of Fujikawa and Davie (1981), bovine factor XII ($E_{280}^{1\%} = 14.2$) by the method of Fujikawa et al. (1977), and human plasma prekallikrein ($E_{280}^{1\%} = 10.9$) by the method of Heimark and Davie (1981). Kallikrein was prepared by the activation of prekallikrein with a catalytic amount of human factor XIIf, as previously described (Griep et al., 1985).

Light-Scattering Measurements. A Perkin-Elmer MPF-44A fluorescence spectrophotometer, in which both the excitation and emission wavelengths had been set to 320 nm and slit widths to 5 nm, was used to measure 90° relative light scattering intensity. For a review of light scattering by small particles, see Doty and Edsall (1951). When the scattering particle is small relative to the wavelength of light, when the second virial coefficient is negligible, and when the number concentration of particles is constant, then light scattering intensity measurements can be used to calculate surface-bound protein by the following relationship (Nelsestuen & Lim, 1977):

$$(I_{s2}/I_{s1})^{1/2} = \frac{\partial n/\partial c_2}{\partial n/\partial c_1} M_{w2}/M_{w1}$$

where I_s is the intensity of the sample's scattered light in arbitrary units, M_w is the weight-average molecular mass of the light scattering particle, c is its weight concentration, $\partial n/\partial c$ is the refractive index increment of a particle, subscript 1 represents the surface particle, and subscript 2 represents the protein–surface complex. Light scattering intensity was measured for surface alone (I_{s1}) and, as aliquots of protein were added, for the protein–surface complex (I_{s2}). For details of this approach, see Nelsestuen and Lim (1977). To prevent protein adsorption to the cuvette walls, the 10-mm fluorescence

quartz cuvettes (NSG Precision Cells, Inc., Farmington, NY) were treated prior to use with an ethanol wash followed by incubation with a mixture containing the surface and protein components. The cuvettes were then rinsed with filtered, distilled water.

Since the 500-kDa dextran sulfate solution did not scatter much light in the absence of bound protein, it was necessary to determine its light scattering intensity (I_{s1}) in a separate experiment at higher polysaccharide concentrations; a linear increase in intensity was observed when a buffer solution was titrated with 500-kDa dextran sulfate. Since the particle size of the factor XII–dextran sulfate complex probably approached the wavelength of light, direct calculation of protein bound by the method described above was not appropriate. In this case, the data were represented as $(I_{s2}/I_{s1})^{1/2}$. Nevertheless, saturation of binding will still correspond to the saturation of signal change. Furthermore, if tight binding of the protein to the polysaccharide is assumed, the protein to surface weight ratio in the complex at any point in the titration, prior to saturation, will equal the protein to surface weight ratio added.

Activation Studies. Activation was carried out with a procedure modified (Griep et al., 1985) from Tans et al. (1983). To unwashed Sarstedt 1.5-mL snap-cap centrifuge tubes factor XII and buffer were added and allowed to preincubate for 200 s at 22 °C. Autoactivation was initiated by the addition of a contact surface. If the kallikrein activation of factor XII was measured, 3 nM kallikrein was added prior to the surface. The standard conditions were 200 nM factor XII, 22 °C, 50 mM HEPES (pH 7.5), and 1 mM EDTA. The NaCl concentrations indicated are the sum from all added solutions. At various times after the addition of surface, the activation sample was assayed for amidase activity.

Factor XIIa activity was assayed by diluting 55 μL of the activation solution into 195 μL of assay buffer consisting of 100 mM Tris (pH 8.3)–150 mM NaCl at 22 °C. These buffer conditions cause complete dissociation of factor XII and factor XIIa from most surfaces. No further activation was observed after mixing of the activation sample in the assay buffer. The chromogenic substrate in the factor XIIa assay was 385 μM S-2302 (H-D-propyl-L-phenylalanyl-L-arginine-*p*-nitroanilide; Helena Laboratories, Beaumont, TX). The hydrolysis of S-2302 was measured on a Beckman DU-8, which was programmed to continuously calculate ΔA_{405}/min. When kallikrein was in the activation sample, the background ΔA_{405}/min due to kallikrein was subtracted. With a standard activity curve, ΔA_{405}/min could be related to a corresponding concentration of generated factor XIIa.

The rates of autoactivation of factor XII and kallikrein activation of factor XII were determined as per Griep et al. (1985). The factor XII autoactivation rate k_{obsd} was the slope from a plot of the natural log of factor XIIa generated vs. time. In those reactions containing kallikrein, the rate v_{kall} was the slope from a linear plot of factor XIIa generated by kallikrein vs. time. In the activation experiments, standard error was obtained from triplicate experiments.

RESULTS

Temperature Dependence of Surface-Promoted Activation of Factor XII. Previous studies had shown that the binding of factor XII to PS was not enough to promote its activation by either factor XIIa or kallikrein activation (Griep et al., 1985). Furthermore, the temperature dependence of autoactivation by sulfatide was unusual in that it had a relatively low temperature optimum at 25 °C. The unusual aspects of temperature dependence extend to PS and to kallikrein activation as detailed below.

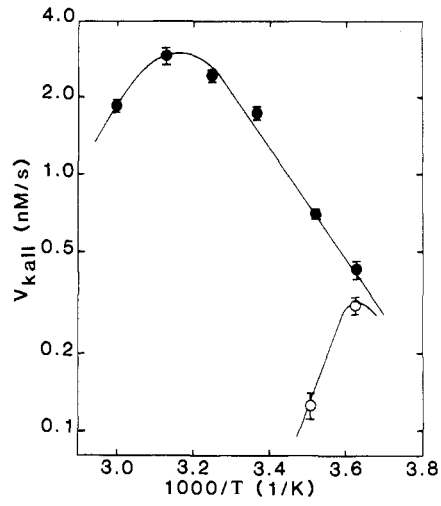

FIGURE 1: Effect of temperature on rate of kallikrein activation of human factor XII as promoted by PS (32 μg/mL). The activation experiments were carried out in buffer of 50 mM HEPES (pH 7.5), 1 mM EDTA, and either 0 mM NaCl (●) or 25 mM NaCl (○). The data are presented in an Arrhenius plot. The rates were obtained as under Experimental Procedures.

Kallikrein activation of factor XII promoted by PS in the absence of added NaCl (Figure 1) was affected by temperature in a manner typical of reactions involving enzymes. The Arrhenius activation energy was about 10.2 kcal/mol, and the temperature at the maximum rate was about 47 °C ($1/T$ = 0.003 12 K^{-1}). At temperatures above 47 °C, the velocity decreased. The final amount of factor XIIa produced at 60 °C ($1/T$ = 0.003 00 K^{-1}) was about half of that produced at temperatures below 47 °C, indicating substrate denaturation.

When 25 mM NaCl was included in the activation buffer, the temperature of maximum activation rate was about 0 °C (Figure 1). The decreased activation rate above 0 °C was not due to protein thermal denaturation, since all factor XII became activated to factor XIIa.

Similar temperature-dependence experiments were performed on bovine factor XII (data not shown). At all temperatures in the presence of PS (32 μg/mL), 3 nM human kallikrein activated 200 nM bovine factor XII at one-fortieth the rate that it activated the human protein. The Arrhenius plot for bovine factor XII activation had similar shape, slope, and NaCl effect to that shown in Figure 1. At 0 °C, bovine factor XII activation was the same at 0 and 25 mM NaCl. At higher temperature, the velocity increased in the absence of added salt but decreased when 25 mM NaCl was included in the buffer. Thus, even though the velocity for activation of bovine factor XII was much slower, the same thermal behavior was observed.

The temperature dependence of the sulfatide-promoted autoactivation of human factor XII was also affected by NaCl (Figure 2, circles). At 75 and 200 mM NaCl, the Arrhenius activation energies appeared similar at about 13.0 kcal/mol. As with the kallikrein activation of factor XII, an inhibitory thermal transition was observed for autoactivation that was very dependent on NaCl. The thermal transition was initiated at aboout 20 °C for 200 mM NaCl, about 30 °C for 75 mM NaCl, and about 35 °C for 25 mM NaCl. At temperatures below the thermal transitions, the autoactivation rates were unaffected by NaCl. At temperatures above the thermal transition but below 50 °C, full factor XIIa activity was attained so that substrate denaturation did not account for the different activation rates.

The temperature dependence of autoactivation by PS was also monitored (Figure 2, triangles). With no added salt in

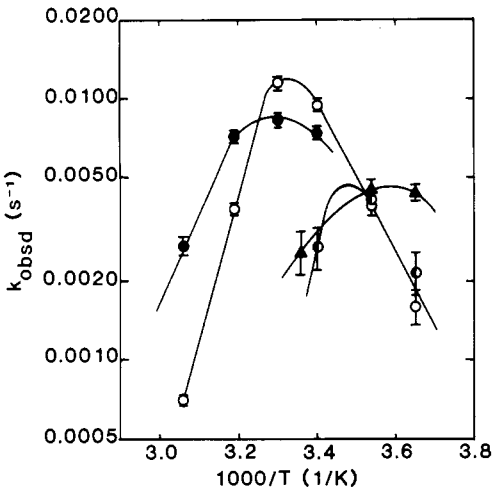

FIGURE 2: Effect of temperature on rate of autoactivation of factor XII as promoted by PS [16 μg/mL (▲)] or sulfatide [32 μg/mL (●, ○, ◐)]. The data are presented in an Arrhenius plot, and the rates were obtained as under Experimental Procedures. The sulfatide-promoted rates were performed at three NaCl concentrations: 25 mM NaCl (●); 75 mM NaCl (○); 200 mM NaCl (◐). The PS-promoted rates were at a single NaCl concentration [0 mM NaCl (▲)]. The activation experiments were carried out in 50 mM HEPES (pH 7.5), NaCl as indicated, and 1 mM EDTA.

Table I: Autoactivation Rates Observed with Various Surfaces

surface	k_{obsd} (s^{-1})[a]	[surface] (μg/mL)[a]	[NaCl] (mM)[a]	$[NaCl]_{50}$ (mM)[b]
sulfatide	0.0091 ± 0.0008	32	75	145
PG	0.0062 ± 0.0003	24	1	20
PA	0.0055 ± 0.0002	8	50	130
500-kDa dextran sulfate	0.0055 ± 0.0006	1.6	(100)[c]	
PIP	0.0025 ± 0.0004	24	26	50
PS	0.0020 ± 0.0001	24	2	15

[a] The first-order autoactivation rates, k_{obsd}, were determined as outlined under Experimental Procedures. The NaCl and surface concentrations were alternately varied until a maximum rate was found at 23 ± 1 °C. [b] $[NaCl]_{50}$ was the concentration of added NaCl that reduced the maximum activation rate by 50%. [c] The ability of this surface to promote autoactivation was measured at only one NaCl concentration.

the buffer, a thermal transition was observed at about 5 °C ($1/T = 0.00360\ K^{-1}$). Clearly, PS would be considered a poor contact activation surface at room temperature or 37 °C, the usual conditions for these studies. However, below the transition at 0 °C, PS was actually slightly more effective than sulfatide in promoting autoactivation. Since the NaCl effect was different for the various surfaces, the thermal transition seemed to be related to a property of the surface.

Surface-Promoted Activation of Factor XII. A variety of negatively charged surfaces were tested for their ability to promote the activation of factor XII at room temperature. The importance of added NaCl has been shown above, and the need to optimize surface concentration has been documented in a previous paper (Griep et al., 1985). At a constant factor XII concentration, surface and NaCl concentration were alternately varied until the rates were optimized for each surface and each reaction: autoactivation (Table I) and kallikrein activation (Table II).

It can be seen in Table I that, compared to sulfatide, the surfaces PA, PG, and 500-kDa dextran sulfate promoted the autoactivation of factor XII about 70% as well, while PIP and PS were about 25% as effective. The decreased efficacy of PS and PIP at 22 °C could be attributed to the temperature-dependent behavior of this system (see Figure 2). In

Table II: Kallikrein Activation Rates Observed with Various Surfaces

surface	V_{kall} (nM/s)[a]	[surface] (μg/mL)[a]	[NaCl] (mM)[a]	$[NaCl]_{50}$ (mM)[b]
sulfatide	1.97 ± 0.12	67	(100)[c]	
PS	1.72 ± 0.16	32	2	11
heparin	0.98 ± 0.12	8	2	11
5-kDa dextran sulfate	0.73 ± 0.05	8	2	25
500-kDa dextran sulfate	0.31 ± 0.04	3.2	(100)[c]	

[a] The kallikrein activation rates, V_{kall}, were determined as outlined under Experimental Procedures. The NaCl and surface concentrations were alternately varied until a maximum rate was found at 23 ± 1 °C. [b] $[NaCl]_{50}$ was the concentration of added NaCl that reduced the maximum activation rate by 50%. [c] The ability of this surface to promote activation was measured at only one NaCl concentration.

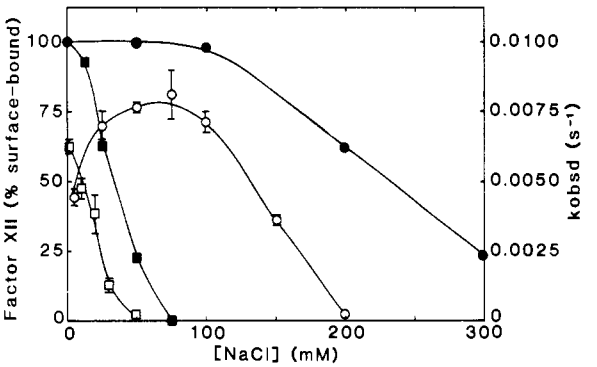

FIGURE 3: Effect of NaCl on factor XII–lipid vesicle binding (closed symbols) and on autoactivation rate (open symbols). Factor XII (200 nM) in 1.5 mL of 50 mM HEPES (pH 7.5)–1 mM EDTA buffer was mixed with sulfatide [32 μg/mL (●, ○)] or PG [24 μg/mL (■, □)]. These surface concentrations were chosen to give optimum activation rates. Binding and autoactivation were monitored as under Experimental Procedures. For binding, initially all protein was surface-bound, and aliquots of NaCl were added that eluted the protein. Minor adjustment were made for NaCl-induced changes in the light scattering of vesicles alone.

contrast, neither heparin nor 5-kDa dextran sulfate promoted autoactivation under any conditions tested (data not shown; $k_{obsd} < 0.0002\ s^{-1}$).

Under conditions similar to those shown in Table I, bovine factor XII did not exhibit detectable autoactivation (data not shown; $k_{obsd} < 0.0002\ s^{-1}$). The conditions used were 200 nM bovine factor XII with 32 μg/mL sulfatide at 0, 50, and 100 mM NaCl and with 4, 16, 32, and 80 μg/mL sulfatide at 50 mM NaCl (24 °C). The buffer included 50 mM HEPES (pH 7.5) and 1 mM EDTA.

Several surfaces were tested for their ability to promote kallikrein activation of factor XII (Table II). Sulfatide and PS had similar maximum velocites, while heparin and 5-kDa dextran sulfate only achieved about half of that velocity. Other properties of these activations are given in Table II.

Binding and Activation Measurements. Light scattering was used to characterize the binding of factor XII to vesicles of sulfatide, PG, and PA. When these vesicles were titrated with protein under the conditions listed in Figure 3, negligible free factor XII was observed prior to saturation of vesicle binding sites [data not shown; see Griep et al. (1985) for sulfatide studies]. This indicated that the protein concentrations used were well above the dissociation constants for the protein–lipid interaction and that the K_D must be less than about 5 nM. The binding of factor XII to sulfatide was not influenced by temperature from 10 to 30 °C (data not shown), indicating that the thermal transition detailed above was not related to protein–surface dissociation. For several surfaces

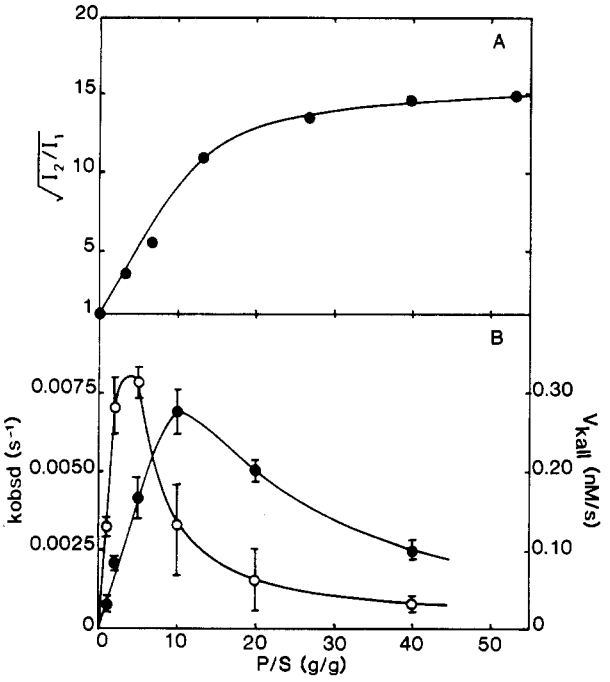

FIGURE 4: Effect of 500-kDa dextran sulfate on binding of factor XII (A) and on rates of autoactivation and kallikrein activation of factor XII (B). (A) The light-scattering signal from 500-kDa dextran sulfate (I_1) was assigned an intensity of 1. The relative intensity of the protein–surface complex (I_2) was obtained after adding protein. The data are plotted vs. the protein to surface weight ratio (P/S). The 500-kDa dextran sulfate (0.375 μg in 1.5 mL) was titrated with factor XII. (B) Autoactivation rates [k_{obsd} (●)] and kallikrein activation rates [V_{kall} (○)] were determined as under Experimental Procedures. The experiments were carried out in 50 mM HEPES (pH 7.5), 100 mM NaCl, and 1 mM EDTA at 22 °C.

it was found that maximum autoactivation occurred when the surface was about 70% saturated with protein: sulfatide (Griep et al., 1985); 500-kDa dextran sulfate (Figure 4); PG, PS, and PA (data not shown).

Previous reports had indicated an important role of NaCl in the steps involved in contact activation [reciprocal activation, Sugo et al. (1985); factor XIIa–surface binding, Griep et al. (1985)]. The NaCl needed to dissociate the factor XII from the surface (sulfatide or PG) was consistently higher than that needed to inhibit the activation reaction. This was also found to be true for PA and PS (data not shown). Consequently, protein–surface binding did not correlate directly with activation. Factor XII binds to a surface but then requires an additional step (possibly a protein conformational change) before it can be activated by factor XIIa or kallikrein.

In Figure 3, the observed decrease in the activation rate for sulfatides below 50 mM NaCl was not due to a loss of maximum velocity. Other experiments showed that at lower salt the surface concentration needed to achieve maximum activation was decreased and that the sulfatide concentration used in Figure 3 was no longer optimal. For example, at 11 mM NaCl, the optimal sulfatide concentration was 24 μg/mL.

Binding and Activation of Factor XII with 500-kDa Dextran Sulfate. A solution of 500-kDa dextran sulfate was titrated with factor XII, and binding was monitored by light scattering (Figure 4A). The dextran sulfate became saturated with factor XII at a protein to surface weight ratio of about 14 g/g. Figure 4B shows that maximum autoactivation of factor XII occurred at a protein to surface weight ratio of about 10 g/g, while maximal kallikrein activation of factor XII occurred at about 5 g/g. These properties were qualitatively similar to the trends shown with sulfatide [see Figure 8 of Griep et al. (1985)] and other surfaces (see above). The maximum autoactivation rates with 500-kDa dextran sulfate were similar to those obtained with sulfatide.

Table III: Autoactivation Ion Effect

salt	$[salt]_{50}$ (mM)[a]	salt	$[salt]_{50}$ (mM)[a]
KCl	42	NaCl	100
NaI	60	NH_4Cl	132
NaBr	85	NaF	142
$NaNO_3$	85	LiCl	400

[a] The rate of sulfatide-promoted autoactivation was measured at different salt concentrations and then plotted as in Figure 5. $[Salt]_{50}$ was the added salt that decreased the rate to about half-maximum, $k_{obsd} = 0.010\ s^{-1}$.

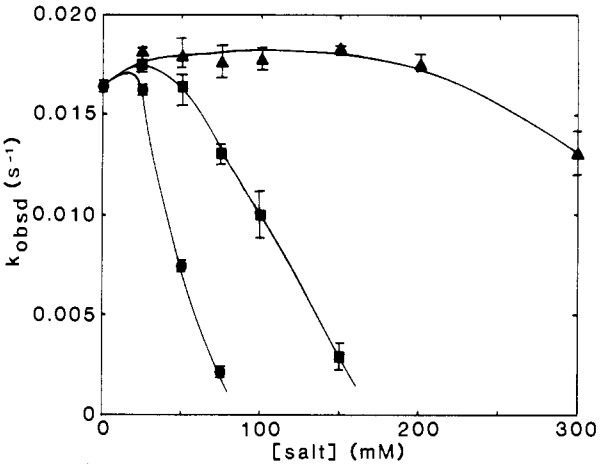

FIGURE 5: Effect of monovalent cations on rate of sulfatide-enhanced autoactivation. Factor XII (200 nM) and sulfatide (16 μg/mL) were added to buffer [1.5 mL final volume, 50 mM HEPES (pH 7.5), 1 mM EDTA] containing the indicated concentrations of KCl (●), NaCl (■), or LiCl (▲) at 22 °C. Autoactivation rates were then determined as under Experimental Procedures.

Since the 500-kDa dextran sulfate had a sulfur content of 17% by weight (about two sulfates per monosaccharide), the average monosaccharide molecular mass was about 340 daltons. Saturable binding occurred when one factor XII molecule was bound to about 17 sugar residues or 88 factor XII molecules bound per 500-kDa dextran sulfate molecule. Given similar binding properties for heparin and 5-kDa dextran sulfate, there would be about two and one factor XII binding sites per polysaccharide molecule, respectively.

Specific Ion Effects on Autoactivation Rate. Abramson et al. (1967) have shown that sulfatide coulombically binds to cations in a selective manner, $K^+ > Na^+ > Li^+$. Several neutral salts were compared for their ability to inhibit the sulfatide-promoted autoactivation of factor XII. The cation effect (Figure 5; Table III) had a selective order for inhibition of $K^+ > Na^+ > NH_4^+ > Li^+$, which paralleled the order of cation binding to sulfatide. For both cation binding to sulfatide (Abramson et al., 1967) and cation inhibition of activation, approximately twice as much NaCl as KCl was required to cause the same effect. In light of these results, the NaCl effect on factor XII–sulfatide binding was due to direct competition of Na^+ and protein for binding to the anionic surface rather than to ionic shielding as was previously proposed (Griep et al., 1985).

For inhibition of the PS-promoted autoactivation rate, all three cations were equally effective and required only 20 mM concentration for complete inhibition. This agreed with the observation that at low concentration these monovalent cations show little selectivity for PS (Hauser & Shipley, 1983).

The anion inhibition effect (Table III) was less dramatic than the cation effect and had a selective order of $I^- > NO_3^-$

> Br^- > Cl^- > F^-, which followed the Hofmeister series (von Hippel & Schleich, 1969). These results suggested that the anions primarily interacted with the protein and played a minor role in the inhibition of sulfatide-promoted autoactivation of factor XII.

Discussion

The results obtained in this study provide further insight into the process of contact activation and related phenomena in blood coagulation. Contact activation showed an unusual thermal behavior that appeared to account for the apparent surface selectivity previously observed in this system at room temperature or 37 °C. This thermal behavior may also explain the mechanism of cold-promoted activation.

Cold-promoted activation occurs when plasma from certain individuals is stored at temperatures between 4 and −10 °C (Schrogie et al., 1967; Gjønnaess, 1972a,b). This cold-promoted activation involves factor VII to factor VIIa activation and is inhibited by NaCl added to the plasma before cold storage (Gjønnaess, 1972b), is preceded by contact activation (Gjønnaess, 1972c,d), and may be catalyzed by factor XIIa (Lake & Ellingsen, 1974; Laake & Österud, 1974; Saito & Ratnoff, 1975). The physical properties of cold-promoted activation appear to correlate well with the in vitro properties of surface-promoted activation in the present study. Some surfaces, such as PS (Figures 1 and 2), which appeared to be "poor" contact surfaces at room temperature, actually became relatively active at low-temperatures due to the thermal transition discussed below. If any given plasma contained a poor surface displaying such behavior, that surface might function during low-temperature storage. The presence of these surfaces might be related to possible thrombotic disease states (Czendlik et al., 1985).

The present study indicated that virtually any negatively charged surface could promote contact activation. This process is a general surface phenomenon rather than biological recognition of certain functional groups. At temperatures below the unusual thermal transition, all surfaces appeared to be equally effective in stimulating the activation of factor XII. The thermal transition was characterized by a decrease in the activation rate at higher temperatures that was not due to the denaturation of substrate or enzyme. Both the kallikrein-catalyzed and factor XIIa catalyzed activations showed this behavior.

Hojima et al. (1984) reported that heparin and 500-kDa dextran sulfate are activators of factor XII in plasma. The present study suggested that these two surfaces might act by different mechanisms. Autoactivation occurred readily with 500-kDa dextran sulfate (Figure 4) but not with either of the smaller sulfated polysaccharides, 13-kDa heparin and 5-kDa dextran sulfate. However, activation of factor XII by kallikrein did occur in the presence of the smaller polysaccharides (Table II). The smaller polysaccharides would not provide the high local density of surface-bound enzyme and substrate required for autoactivation (Griep et al., 1985).

Both activation and protein–surface binding were influenced by NaCl (Figure 3). The NaCl required to dissociate half of the protein from the vesicle caused complete inhibition of autoactivation, which would not be predicted with a simple surface density model. Consequently, surface binding alone was inadequate to trigger activation, and a secondary process must be hypothesized. One hypothesis put forward to explain the surface effect is a factor XII conformational change following binding that makes it susceptible to limited proteolytic cleavage (Griffin, 1978).

The ionic binding of monovalent cations with sulfatide occurs with a stoichiometry of one sulfate moiety per cation (Abramson et al., 1967), whereas factor XII binding covers a larger number of sulfatides (Griep et al., 1985). The selective uptake of cations by sulfatide parallels sulfonic acid resins (Bregman, 1953) with an order of selectivity K > Na > Li. In fact, K^+ binds to sulfatide 2 times tighter than does Na^+, whereas Li^+ binds very weakly. This selectivity would predict the observed cation inhibition order in Figure 5; K^+ inhibited autoactivation about twice as well as Na^+ did. Factor XII activation may have a strict requirement for a large, dense area of negative charge, which is decreased by cation binding.

The actual physical property that correlates the thermal transition of contact activation with the salt effect is not known but possible candidates can be considered. Studies using synthetic sulfatides (Koshy & Boggs, 1983; Boggs et al., 1984) found two thermotropic mesophases by differential scanning calorimetry. The presence of NaCl or KCl significantly raised the temperature at which these phases occurred. The higher temperature mesophase occurred at about 55 °C and represented the gel-to-liquid crystalline phase transition. The lower temperature mesophase occurred at about 44 °C and can be ascribed either to interdigitation of the side chains or to changes in hydration [Boggs et al. (1984) and references cited therein]; these causes are not mutually exclusive. Both mesophases had an order for cation selectivity of K > Na ≫ Li, indicating that cation binding stabilized the gel phase relative to the liquid crystalline. However, both of these changes occurred at temperatures above the thermal transition of activation.

Bovine factor XII does not autoactivate in the presence of either kaolin (Heimark et al., 1980) or quartz (Sugo et al., 1982), and our results extended these observations to sulfatide. However, bovine factor XII bound to sulfatide (Sugo et al., 1985) and could be activated by human kallikrein (see Results). Activation of the bovine protein also displayed a thermal transition. In this respect, the human and bovine proteins were similar, which would be expected if the NaCl-dependent thermal transition primarily involved the surface and was not strictly a property of the proteins.

While thorough, detailed analysis of each surface with respect to solution and temperature conditions for each combination of contact activation proteins would require a prohibitive number of assays, the present studies have identified some general characteristics of contact activation that appear to apply to all surfaces. It was found that all surfaces tested did function in contact activation if solution conditions were adjusted appropriately. Two major criteria applied in the selection of contact surfaces were high negative charge and, for autoactivation, a surface large enough to have sufficient adjacent binding sites. Kallikrein activation did not require this latter property. The thermal transition behavior appears to be a general feature of contact activation that may have practical importance. For example, testing of synthetic materials for compatibility with blood contact might best be conducted at lower temperature where the tendency for contact activation is accentuated; compatible synthetic materials will remain inert even at lower temperatures. While the physical basis of the thermal transition is not known, this phenomenon appears to be the feature responsible for the different efficiencies of contact surfaces at 37 °C in plasma. It may also provide a basis for identifying a hypothetical surface component involved in cold-promoted activation.

Registry No. K, 7440-09-7; Na, 7440-23-5; NH_4^+, 14798-03-9; Li, 7439-93-2; I^-, 20461-54-5; NO_3^-, 14797-55-8; Br^-, 24959-67-9; Cl^-, 16887-00-6; F^-, 16984-48-8; blood coagulation factor XII, 9001-30-3; blood coagulation factor XIIa, 37203-62-6; kallikrein,

9001-01-8; dextran sulfate, 9042-14-2; heparin, 9005-49-6.

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