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Initiation of contact system activation in plasma is dependent on factor XII autoactivation and not on enhanced susceptibility of factor XII for kallikrein cleavage

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Summary. Various mechanisms have been hypothesized to explain the initiation of contact system activation in plasma. We investigated the capability of dextran sulphate (DS) of different molecular weights to initiate contact system activation in normal human plasma, and compared this with their capability to support factor XII autoactivation and to enhance factor XII susceptibility for cleavage by kallikrein. Dextran sulphate of Mr 500 000 (DS500) and 50 000 (DS50) was able to initiate contact system activation in plasma (determined by measuring the amount of factor XIIa–C1-inhibitor, kallikrein–C1-inhibitor and factor XIa–C1-inhibitor complexes generated) as well as to support factor XII autoactivation and to enhance factor XII susceptibility for cleavage by kallikrein. In contrast, dextran sulphate of Mr 15 000 (DS15) and 5000 (DS5) neither induced contact system activation in plasma, nor supported autoactivation of factor XII, although both of these DS species enhanced the rate of activation of factor XII by kallikrein in the purified system. Based on these properties (i.e. binding of factor XII without inducing autoactivation), DS15 and DS5 were predicted to be inhibitors of contact system activation induced in plasma by DS500, which indeed was observed.

We conclude that enhanced factor XII susceptibility for kallikrein activation and factor XII autoactivation are distinct phenomena, the latter being necessary to support activation of the contact system in plasma.

Keywords: contact system, dextran sulphate, factor XII autoactivation.

Four proteins constitute the contact system in plasma: the proenzymes factor XII (FXII), prekallikrein, factor XI and the cofactor high molecular weight kininogen (HK). The contact system can be activated by exposure of plasma to negatively charged surfaces and subsequent binding of FXII to the surface which induces activation of this contact protein. Prekallikrein and factor XI, which both circulate in plasma complexed to HK, assemble onto the negatively charged surfaces via HK. Surface-bound activated factor XII (FXIIa) activates prekallikrein to yield kallikrein, which in turn further activates surface-bound FXII (reciprocal activation). FXIIa also activates factor XI yielding factor Xla. Activated contact system proteases trigger the intrinsic pathways of fibrinolysis and coagulation, the complement system and kinin production (Cochrane & Griffin, 1982; Colman, 1984; Griffin & Bouna, 1987; Kaplan & Silverberg, 1987).

Negatively charged surfaces influence contact system activation in at least two ways: they serve as a site on which the proteins are assembled, thus allowing efficient activation due to high local concentrations; furthermore, they may induce conformational changes in contact proteins upon binding. It has been postulated that the conformational changes induced in FXII upon binding constitute the key event to initiate contact activation by inducing autoactivation of FXII (Silverberg et al, 1980; Dunn et al, 1982; Espana & Ratnoff, 1983; Tans et al, 1983; Tankersley & Finlayson, 1984). However, conclusive experimental evidence for this ‘autoactivation’ hypothesis has not been reported due to the difficulty in obtaining FXII preparations free of traces of two-chain FXIIa, which may function as the initial enzyme to convert surface-bound single-chain FXII into a two-chain active molecule. Alternatively, FXII bound to a surface does
not autoactivate but rather undergoes a conformational change that enhances its susceptibility for cleavage by kallikrein (Griffin, 1978; Rosing et al., 1985). According to this ‘susceptibility’ hypothesis, initiation of the contact system is dependent on the capability of a surface to induce conformational changes in FXII, as well as on the presence of traces of active kallikrein in plasma, possibly resulting from autoactivation of prekallikrein (Tans et al., 1987).

Until now it has been unclear whether contact activation in vivo is initiated via FXII autoactivation or via FXII enhanced susceptibility for cleavage by kallikrein. In part this is because the nature of the physiological surface involved in contact activation in vivo is unknown (Lawson et al., 1994) and this has prevented studies elucidating the molecular mechanism underlying contact activation under physiological conditions. These conditions may be approximated by inducing contact activation in plasma using various artificial compounds. Compounds commonly used such as glass, kaolin, ellagic acid, sulphatide micelles, and high molecular weight dextran sulphate (Revak et al., 1982; Nuijens et al., 1987; Lewin et al., 1983; van der Graaf et al., 1982; Bock et al., 1981), are all able to support FXIII autoactivation as well as to enhance activation of FXII by kallikrein (Dunn et al., 1982; Heimark et al., 1980; Griep et al., 1985; Espana & Ratnoff, 1983; van der Graaf et al., 1982). To gain insight into the initial event of contact system activation we investigated the potency of various artificial compounds commonly used such as glass, kaolin, ellagic acid, sulphatide micelles, and high molecular weight dextran sulphate (Revak et al., 1982; Bock et al., 1981), are all able to support FXIII autoactivation as well as to enhance activation of FXII by kallikrein (Dunn et al., 1982; Heimark et al., 1980; Griep et al., 1985; Espana & Ratnoff, 1983; van der Graaf et al., 1982). Our results indicate that in order to initiate contact system activation in plasma, a surface must be able to efficiently support autoactivation of FXII.

MATERIAL AND METHODS

General. Dextran sulphates of nominal m.w. 500000, 50000, 15000 (DS500, DS50, DS15, DS5), trypsin (13 000–20 000 BAEE units/mg) and soybean trypsin inhibitor (SBTI) were obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A.). Hexadimethrine bromide (Polybrene) was from Janssen Chimica (Beerse, Belgium), the chromogenic substrate H-D-Pro-Phe-Arg-p-nitroanilide (S-2302) was from Chromogenix AB (Möln达尔, Sweden), and CNBr-activated Sepharose 4B and benzamidine–Sepharose from Pharmacia Fine Chemicals AB (Uppsala, Sweden). Prekallikrein- and FXII-deficient plasma were obtained from George King Biomedical Inc. (Overland Park, Kansas). Prekallikrein and FXII-deficient plasma were obtained from George King Biomedical Inc. (Overland Park, Kansas).

Proteins. FXII and prekallikrein were immunopurified from 150 ml of human citrated plasma as described (Dors et al., 1992; Ravon et al., 1995). FXII preparation was applied onto a benzamidine-Sepharose column to remove FXIIa. The amount of FXIIa present in the final preparation was 0·3% of the total amount of protein as determined by measuring the conversion of the chromogenic substrate S-2302 by the preparation. β-factor XIa (β-FXIa) and kallikrein were prepared as described (Ravon et al., 1995). Protein concentrations were determined by radioimmunoassays (Nuijens et al., 1988). All the protein preparations used were >95% homogenous as determined by SDS-PAGE.

Activation of contact system in plasma. Blood from five healthy donors was collected in siliconized Vacutainer tubes (Becton Dickinson, Plymouth, U.K.) containing EDTA (10 μmol final concentration) or sodium citrate (0·38% final concentration) and immediately transferred to polystyrene tubes (Becton Dickinson, Plymouth, U.K.). The tubes were centrifuged at room temperature for 15 min at 1300g. Plasma samples were then aliquoted in polypropylene tubes and stored at −70°C. Contact activation in plasma was induced as follows: 40 μl of EDTA-plasma were preincubated, in Dynatech (Plochingen, Germany) microplates (96 wells), for 5 min at 37°C, after which 40 μl of prewarmed (serial dilutions of) DS in phosphate-buffered saline (PBS: sodium phosphate 10 mm, NaCl 140 mm, pH 7·4) were added. The microplate was shaken for 3 min at room temperature and then incubated for 20 min at 37°C, and the activation was stopped by adding 120 μl of stop buffer (PBS, 0·1 mg/ml SBTI, polybrene 0·05%, wt/vol).

The influence of DS15 or DS5 on the activation of the contact system by DS500 was investigated in an analogous way: 30 μl of serial dilutions of DS15 or DS5 (500–1·5 μg/ml in PBS) were mixed with 10 μl of DS500 (125 μg/ml) and added to 40 μl of normal pooled EDTA-plasma preincubated in Dynatech microplates for 5 min at 37°C. The microplate was shaken for 3 min at room temperature and then incubated for 20 min at 37°C after which the reaction was stopped by adding 120 μl of stop buffer.

Assays for contact system activation in plasma. Contact system activation in plasma was assessed by measuring the generation of FXIIa–C1-inhibitor and kallikrein–C1-inhibitor complexes using radioimmunoassays which could detect 0·05% activation of prekallikrein or FXII in normal plasma (Nuijens et al., 1988). Sample dilutions were tested in duplicate and the concentrations of FXIIa–C1-inhibitor and kallikrein–C1-inhibitor complexes generated were calculated by comparison with normal pooled human plasma fully activated by DS500 (50 μg/ml) (Nuijens et al., 1988). Generation of factor Xa–C1-inhibitor complexes was determined by an enzyme-linked immunosorbent assay (ELISA) using kaolin-activated normal plasma as an in-house standard (Wuillemin et al., 1995).

Factor XII activation by kallikrein. Activation of FXII by kallikrein was carried out in a 96-well microtitre plate (Nunc, Roskilde, Denmark): 25 μl of prewarmed FXII (125 mU), 25 μl of serial dilutions of DS, 25 μl of prewarmed kallikrein (25 mU) and 25 μl of buffer yielding a final concentration of 50 mm Tris-HCl, 50 mm NaCl, 0·1% (wt/vol) Tween-20, pH 7·8, were incubated for 20 min at 37°C. The amount of FXIIa formed was determined from the rate of hydrolysis of the chromogenic substrate S-2302: 50 μl of...
assay mixture (S-2302 1 mM, SBTI 100 μg/ml in Tris 50 mM, NaCl 150 mM, pH 7-8) were added to the wells and the increase in absorbance at 405 nm was recorded at time intervals of 2 min by a Bio-Kinetics Reader (Bio-tek Instruments Inc.) set at 37°C. Under these conditions, the rates of increase in absorbance were constant for at least 10 min and were used to calculate the amount of FXIIa present in the wells on the basis of a calibration curve obtained with known amounts of activated FXII. Results were expressed as percentage of the total amount of FXII present in the mixture. In each experiment this amount was assessed by activating FXII with trypsin under limited proteolytic conditions. Variation of FXIIa generated under these conditions was <10% in all experiments and agreed with the concentration of FXII known to be present in the wells. In control experiments it was established that the amount of SBTI added was sufficient to prevent conversion of the chromogenic substrate by either kallikrein or trypsin, up to a concentration of 100 nM.

The kinetics of FXII activation by kallikrein in the presence of different DS species was determined at 37°C. 25 μl of FXII (125 nM) were added to 25 μl of DS (200 μg/ml). After 5 min at 37°C, 50 μl of prewarmed kallikrein (12.5 nM) were added to start the reaction. After various time intervals, 50 μl of assay mixture were added and the increase in absorbance at 405 nm was immediately recorded.

Factor XII autoactivation. Autoactivation of FXII was studied by incubating 25 μl of FXII (500 nM), 25 μl of twofold serial dilutions of DS of different molecular weight and 50 μl of buffer yielding a final concentration of 50 mM Tris-HCl, 50 mM NaCl, 0.1% (wt/vol) Tween-20, pH 7-8, in a 96-well microtitre plate for 60 min at 37°C. Thereafter, 50 μl of assay mixture (S-2302 1 mM, SBTI 100 μg/ml in Tris 50 mM, NaCl 150 mM, pH 7-8) were added to the wells and the increase in absorbance at 405 nm was recorded at time intervals of 2 min. Under these conditions, the rates of increase in absorbance were constant for at least 10 min and were used to calculate the amount of FXIIa present in the wells as described above.

RESULTS

Activation of factor XII, prekallikrein and factor XI in plasma by dextran sulphates of different molecular weights

EDTA-plasma samples from five healthy donors were incubated with increasing amounts of DS500, DS50, DS15 and DS5, and the amount of generated FXIIa–C1-inhibitor, kallikrein–C1-inhibitor and factor Xla–C1-inhibitor complexes was determined. Results were expressed as a percentage of the maximum amount of each complex generated in each plasma sample by DS500, which was arbitrarily set at 100%. As shown in Fig 1, protease–C1-inhibitor complexes were generated only when plasma was incubated with either DS500 or DS50. Maximum activation was observed in the presence of DS500. In the presence of DS50, the amount of FXIIa– and factor Xla–C1-inhibitor complexes was 60% and 33%, respectively, of that generated by DS500. In contrast, the amount of kallikrein–C1-inhibitor complexes generated by DS500 or DS50 was similar. The amounts of complexes formed when plasma was incubated with DS15 or DS5 were not different from the values observed in plasma incubated with PBS alone, thus indicating that neither DS15 nor DS5 were able to induce activation of the contact system in

Fig 1. Activation of contact system in plasma by dextran sulphate of different molecular weights. 40 μl EDTA-plasma were incubated with 40 μl serial dilutions of DS in PBS as described in Material and Methods. The reaction was stopped by adding 120 μl stop buffer, and the amount of FXIIa–C1-inhibitor (A), kallikrein–C1-inhibitor (B) and factor Xla–C1-inhibitor (C) complexes generated was measured. Results, expressed as a percentage of the maximum amount of complexes generated in each plasma sample, represent the mean values ±SD obtained with plasma samples from five healthy donors. Symbols: DS500 (+), DS50 (●), DS15 (○), DS5 (▲).
plasma. Similar results were obtained with citrated plasma (data not shown) thus ruling out the possibility that the lack of contact system activation in EDTA-plasma incubated with DS15 or DS5 was due to insufficient Zn\(^{2+}\) levels.

The amount of complexes generated was dependent on the concentration of activator (Fig 1). The maximum amount of kallikrein–C1-inhibitor and factor XIa–C1-inhibitor complexes was generated in the presence of 25 mg/ml dextran sulphate whereas that of FXIIa–C1-inhibitor complexes was obtained in the presence of 125 mg/ml dextran sulphate.

To rule out that the lack of generation of complexes observed with DS15 and DS5 was due to a too short incubation time, the time course of activation was studied. The generation of FXIIa– and kallikrein–C1-inhibitor complexes by DS500 and DS50 at a final concentration of 125 mg/ml appeared to be similar. After a lag phase of 45 s the amount of complexes rapidly increased to maximum values within 4 min in the presence of DS500. The lag time for the generation of factor Xlla–C1-inhibitor complexes was longer and the maximum amount of complexes was not formed until 9 min (Fig 2A). In the presence of DS50 the generation of each complex was slightly slower (Fig 2B).

Since no protease–C1-inhibitor complexes were formed in plasma after a 20 min incubation with DS15 and DS5 at concentration of 125 \(\mu\)g/ml (Fig 1), the incubation time was prolonged. However, no complexes were generated in plasma incubated with DS15 or DS5 (5–500 \(\mu\)g/ml) for up to 4 h (data not shown).

**Effects of various DS on factor XII susceptibility for cleavage by kallikrein**

Previous studies have indicated that binding of FXII to negatively charged surfaces enhances the susceptibility of bound FXII for cleavage by kallikrein. We therefore investigated the activation of purified FXII by kallikrein in the presence of DS of different molecular weight. As shown in Fig 3, all DS were able to enhance FXII susceptibility for cleavage by kallikrein. The rate of FXII activation was dependent on DS concentration. The optimal enhancement was observed at the same final concentration (10–50 \(\mu\)g/ml) for all DS species (Fig 3), thus indicating dependency on the concentration of the binding sites (sulphate groups); higher DS concentrations appeared to be inhibitory (Fig 3). The activation kinetics were further determined at a DS concentration (50 \(\mu\)g/ml) which was optimal for FXII activation by kallikrein and was too high to support FXII autoactivation (see below). As shown in Fig 4 and Table I, the initial rate of activation of FXII by kallikrein was increased 150-fold in the presence of DS500 and DS50, and 30- and 15-fold in the presence of DS15 and DS5.
respectively. The observed lower rate of FXII activation in the presence of DS15 and DS5 was not due to a direct inhibitory effect of these compounds on the amidolytic (enzymatic) activity of either FXIIa or kallikrein, since none of the DS species interfered with the amidolytic activity of FXIIa or kallikrein up to a final concentration of 50 μg/ml. However, at a final concentration of 250 μg/ml all DS species inhibited 50% of FXIIa amidolytic activity and 90% of kallikrein amidolytic activity (data not shown).

**Factor XII autoactivation in the presence of various DS**

The data thus far indicated that, in contrast to DS500 or DS50, neither DS15 nor DS5 were able to induce contact system activation in plasma. To determine the relationship between the activating properties of different DS in plasma and their capability to support FXII autoactivation, we re-examined FXII autoactivation in the presence of dextran sulphate of different molecular weights. Autoactivation of purified FXII after 60 min incubation at 37°C only occurred in the presence of DS500 and DS50 (Fig 5). The efficiency of autoactivation was dependent upon the concentration of DS, it reached an optimum at a final concentration of 1–2 μg/ml for both DS500 (FXII/DS molar ratio: 6.2–3.1:1) and DS50 (FXII/DS molar ratio 6.2–3.1:1), and decreased at higher concentrations of DS (Fig 5). The rate of autoactivation was significantly higher with DS500 than with DS50: after 60 min incubation at 37°C the maximum amount of FXIIa found in the presence of DS500 was 60.8 ± 4.9%, whereas in the presence of DS50 it was only 20.8 ± 1.8% of the amount of FXII added (Fig 5). In the presence of DS15 and DS5, autoactivation was negligible: 1% and 0.5% respectively, of the total amount of FXII was converted to FXIIa. After a prolonged incubation time of up to 24 h at room temperature and under the conditions used, 93% of

<table>
<thead>
<tr>
<th>DS</th>
<th>k' (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS500</td>
<td>0.126</td>
</tr>
<tr>
<td>DS50</td>
<td>0.118</td>
</tr>
<tr>
<td>DS15</td>
<td>0.023</td>
</tr>
<tr>
<td>DS5</td>
<td>0.012</td>
</tr>
<tr>
<td>No DS</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

k' represents the rate constant calculated from the equation: ln(FXIIa_max/FXIIa_max – FXIIa_t) = k't, where FXIIa_max is the total amount of factor XIIa (determined by limited proteolytic digestion with trypsin) and FXIIa_t is the amount of factor XIIa generated at time t by kallikrein (25 ns) (Scott & Colman, 1992).
FXII was activated in the presence of DS500 or DS50, in contrast to 13.2% and 4.6% in the presence of DS15 and DS5 respectively, and 3.4% in the absence of DS. As the presence of zinc ions may influence autoactivation of FXII by some activators (Schousboe, 1993), we also tested the effect of zinc ions on the autoactivation of FXII by DS15 or DS5. Zinc at concentrations of 0.005–0.5 mM, however, did not enhance this autoactivation (results not shown).

Effects of DS15 and DS5 on contact system activation induced in plasma

Two of the DS, DS15 and DS5, were able to enhance FXII susceptibility for cleavage by kallikrein but could not support FXII autoactivation in the purified system (Fig 3 and Fig 5). Since neither DS induced contact system activation in plasma (Fig 1), we performed further experiments to ascertain that enhanced susceptibility of FXII for cleavage by kallikrein \emph{per se} is not sufficient to induce contact activation in plasma. In these experiments we took advantage of the fact that our RIA for FXIIa–C1-inhibitor complexes hardly detects β-FXIIa–C1-inhibitor complexes due to the poor reaction of the anti-FXII rabbit antibodies with the light-chain region of FXII. Exogenous β-FXIIa was added to EDTA-plasma preincubated with PBS alone, or with PBS containing 125 μg/ml of DS15 or DS500 and the amount of kallikrein–C1-inhibitor complexes (reflecting the amount of kallikrein generated by β-FXIIa) and FXIIa–C1-inhibitor complexes (mainly reflecting the activation of endogenous FXII) was assessed. As expected, only prekallikrein, and not endogenous FXII, was activated by β-FXIIa in the absence of DS (76% of kallikrein–C1-inhibitor complexes, 0–5% of FXIIa–C1-inhibitor complexes; Table I). In the absence of β-FXIIa, DS15 was not able to induce activation of FXII or prekallikrein in plasma (Table I), confirming the results described above. Interestingly, in the presence of DS15, the amount of kallikrein–C1-inhibitor complexes formed upon addition of exogenous β-FXIIa was higher than that generated by β-FXIIa alone, whereas a significant amount of endogenous FXIIa–C1-inhibitor complexes was generated, i.e. 6% of the maximum amount generated in the presence of DS500 (Table I). Similar experiments were performed to ascertain that enhanced susceptibility of FXII for cleavage by kallikrein \emph{per se} is not sufficient to induce contact activation in plasma.

Table II. Activation of the contact system by exogenous β-FXIIa in plasma.

<table>
<thead>
<tr>
<th></th>
<th>EDTA-plasma</th>
<th>DS15</th>
<th>DS500</th>
</tr>
</thead>
<tbody>
<tr>
<td>FXIIa–C1-inh.</td>
<td>β-FXIIa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.2</td>
<td>0.1</td>
<td>88.9</td>
</tr>
<tr>
<td>10 μM</td>
<td>0.4</td>
<td>2.4</td>
<td>96.1</td>
</tr>
<tr>
<td>50 μM</td>
<td>0.5</td>
<td>76.1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>97.7</td>
<td></td>
</tr>
</tbody>
</table>

40 μl of plasma were incubated with 40 μl of PBS or PBS containing DS (125 μg/ml) in the presence of varying amounts of β-FXIIa for 20 min at 37°C. The reaction was stopped by adding 120 μl of stop solution and the amount of C1-inhibitor complexes generated was measured as described in Material and Methods. The results expressed as percentage of the maximum amount generated in plasma by DS500, are means of two experiments. Note that β-FXIIa–C1-inhibitor complexes are hardly detected in the RIA for FXIIa–C1-inhibitor complexes (the antibodies used in the assay mainly bind the heavy-chain region of FXII).

Effects of DS15 and DS5 on contact system activation induced in plasma

Two of the DS, DS15 and DS5, were able to enhance FXII susceptibility for cleavage by kallikrein but could not support FXII autoactivation in the purified system (Fig 3 and Fig 5). Since neither DS induced contact system activation in plasma (Fig 1), we performed further experiments to ascertain that enhanced susceptibility of FXII for cleavage by kallikrein \emph{per se} is not sufficient to induce contact activation in plasma. In these experiments we took advantage of the fact that our RIA for FXIIa–C1-inhibitor complexes hardly detects β-FXIIa–C1-inhibitor complexes due to the poor reaction of the anti-FXII rabbit antibodies with the light-chain region of FXII. Exogenous β-FXIIa was added to EDTA-plasma preincubated with PBS alone, or with PBS containing 125 μg/ml of DS15 or DS500 and the amount of kallikrein–C1-inhibitor complexes (reflecting the amount of kallikrein generated by β-FXIIa) and FXIIa–C1-inhibitor complexes (mainly reflecting the activation of endogenous FXII) was assessed. As expected, only prekallikrein, and not endogenous FXII, was activated by β-FXIIa in the absence of DS (76% of kallikrein–C1-inhibitor complexes, 0–5% of FXIIa–C1-inhibitor complexes; Table I). In the absence of β-FXIIa, DS15 was not able to induce activation of FXII or prekallikrein in plasma (Table I), confirming the results described above. Interestingly, in the presence of DS15, the amount of kallikrein–C1-inhibitor complexes formed upon addition of exogenous β-FXIIa was higher than that generated by β-FXIIa alone, whereas a significant amount of endogenous FXIIa–C1-inhibitor complexes was generated, i.e. 6% of the maximum amount generated in the presence of DS500 (Table I). Similar experiments were performed to ascertain that enhanced susceptibility of FXII for cleavage by kallikrein \emph{per se} is not sufficient to induce contact activation in plasma.

Contact Activation in Plasma Requires FXII Autoactivation

In this study we demonstrated that only those DS species which supported autoactivation of FXII, induced contact activation in normal human plasma, whereas DS species that did not support autoactivation, although able to enhance FXII susceptibility for cleavage by kallikrein, did not activate the contact system in plasma. These data point to autoactivation of FXII, rather than the enhanced susceptibility for kallikrein cleavage, as the predominant mechanism for contact activation in plasma.

Previous studies have used the generation of kallikrein amidolytic activity to assess contact system activation in plasma (Tankersley et al., 1983; Klut, 1978; Fisher et al., 1982). This approach, however, requires manipulation of plasma in order to avoid interference by plasma protease inhibitors. We measured activation of the contact system in plasma in a way that did not require plasma manipulation, i.e. by assessment of the generation of complexes of C1-inhibitor and activated FXIIa, factor Xla and kallikrein. Since C1-inhibitor is the major inhibitor in plasma of all three proteases of the contact system (Schapira et al., 1982; Pixley et al., 1985; Wuillemin et al., 1995), the generation of protease–C1-inhibitor complexes reflected the contact system activation in plasma (Nuijens et al., 1987; Lewin et al., 1983; Kaplan et al., 1985; Wuillemin et al., 1995).

Contact system activation in plasma by DS appeared to be dependent on the molecular weight of the DS, and was only observed with DS500 and DS50, but not with DS15 and DS5 (Fig. 1), confirming earlier observations (Corretge & Nigretto, 1990). Contact activation induced by DS50 yielded only 60% of the amount of FXIIa–C1-inhibitor complexes generated by DS500. DS50 also supported FXII autoactivation less efficiently than DS500 (Fig 5). These results emphasized the relevance of FXII autoactivation in the initiation of contact system activation in plasma. The fact that prekallikrein became fully activated in the presence of DS50 is explained, as one molecule of FXIIa can activate several molecules of prekallikrein (Lewin et al., 1983; van der Graaf et al., 1982; Nuijens et al., 1988).

The finding that all the DS used could render FXII susceptible for activation by kallikrein, whereas only DS500 and DS50 initiated contact system activation in plasma, strongly argues against the hypothesis that contact activation in plasma is started by traces of kallikrein that cleave FXII bound to an activating surface. Also, the lack of contact activation by DS15 or DS5 was not due to the fact that these DS were not able to induce a conformational change of FXII in plasma, since endogenous kallikrein, generated by the addition of β-FXIIa, activated some endogenous FXII in the presence of DS15 which was not observed in the absence of dextran sulphate (Table II).

An alternative hypothesis claims that contact activation is initiated by FXII autoactivation (Silverberg et al., 1980; Dunn et al., 1982; España & Ratnoff, 1983; Tans et al., 1983; Silverberg & Kaplan, 1982). We therefore investigated the relationship between the molecular weight of dextran sulphate and the capability to support FXII autoactivation. We found that DS500 and DS50 both supported autoactivation of purified FXII whereas DS15 and DS5 did not. This suggested that autoactivation and enhanced susceptibility for kallikrein cleavage were separate phenomena, since the latter was also observed with DS15 and DS5. It is also to be noted that the optimal dextran sulphate concentrations for autoactivation were different from those for enhanced susceptibility for kallikrein cleavage, which again suggested these phenomena to be different.

In our experiments neither DS15 nor DS5 promoted autoactivation of FXII. These results are in disagreement with the observations of Silverberg & Diehl (1987) who showed that FXII autoactivation occurred in the presence of low molecular weight heparin and dextran sulphate. The lower ionic strength in their incubation buffers could probably explain this discrepancy since ionic strength affects autoactivation of FXII (Griep et al., 1986). Indeed, we also observed an increased rate of autoactivation at lower concentrations of NaCl (data not shown). On the other hand, our data are in agreement with the results of Griep et al. (1986) who suggested that activating surfaces with low molecular weight would not provide the high local density of surface-bound enzyme and substrate required for autoactivation.

FXII autoactivation appears to be a cooperative mechanism, the efficiency of which is increased by the tight positioning of a number of FXII molecules on a surface. The surface must have a high density of negative charges and be large enough to allow the binding of more than two molecules of FXII. Samuel et al. (1992) showed that binding of FXII to DS5 induced a conformational change accompanied by a negative difference of the UV spectrum, whereas binding to DS500 was associated with a positive difference of the UV spectrum. The latter effect is the sum of two different phenomena: the conformational change induced by binding (which yielded a negative difference) and the aggregation of FXII molecules (which yielded a positive difference).
difference). Aggregation is probably important for FXII autoactivation. In agreement with this, binding and aggregation could occur on both DS500 and DS50 molecules, where 62.4 or 6.2 FXII molecules, respectively, could be positioned, as calculated from the relative concentrations of DS and FXII which supported maximum autoactivation. In contrast, in the presence of low molecular weight DS only the binding event occurred: there was no aggregation and consequently, no autoactivation.

The observation that DS15 and DS5 were able to inhibit activation of the contact system induced in plasma by DS500 indicated that the binding of FXII to negatively charged surfaces, which enhanced susceptibility for kallikrein cleavage but which did not support autoactivation, yielded enzyme–substrate complexes that were less able to propagate the contact system activation, even when traces of kallikrein were present. A similar correlation between FXII autoactivation and activation of the contact system in plasma can be deduced from the results obtained by Pixley et al (1991), who demonstrated that heparin enhanced the rate of FXII activation by kallikrein, but did not support FXII autoactivation or contact system activation in plasma at 37°C.

Our studies were carried out using DS as an activator. Various other substances, which with or without the presence of zinc ions activate the contact system in vitro, have been identified, such as kaolin, ellagic acid and various sulphatides (Cochrane & Griffin, 1982; Colman 1984; Espana & Ratnoff, 1983; Griffin & Bouma, 1987; Schousboe, 1993). In addition, we have recently described a monoclonal antibody, mAb F1 which, upon addition to plasma, activated the contact system (Rayon et al, 1995). There is no evidence which argues against the fact that all these activators, except for mAb F1, which activate the molecule by binding to the kringle domain of the heavy chain, induce activation of FXII via a similar mechanism. Hence we assume that our findings may apply to other activators than DS.

In conclusion, we observed that high molecular weight DS induced contact system activation in plasma and supported FXII autoactivation, whereas low molecular weight DS neither, although these DS enhanced the rate of FXII activation by kallikrein. These data imply that in plasma, autoactivation of FXII, rather than enhanced susceptibility for cleavage by kallikrein, is the triggering event for the activation of contact system by negatively charged surfaces.

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REFERENCES


Contact Activation in Plasma Requires FXII Autoactivation


