The autoactivation of Factor XII (Hageman factor) induced by low- M_r heparin and dextran sulphate

The effect of the M_r of the activating polyanion

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Human Factor XII is known to undergo autoactivation in the presence of dextran sulphate of M_r 500 000. We have now studied the dependence of this reaction on the M_r of the dextran sulphate by using fractions resolved by gel filtration. We have found that autoactivation can be induced by dextran sulphate fractions with M_r as low as 3000, and there is a marked dependence of the rate constant of autoactivation on the M_r value. Fractions with M_r below 8000 gave very low rates of autoactivation; there was a sharp increase in the rate obtained when the M_r of the dextran sulphate was greater than 10 000. Various preparations of heparin were also able to support the autoactivation of Factor XII and gave a very similar relationship between molecular size and reaction rate. The data provide support for the hypothesis that the mechanism by which the 'surface' acts in contact activation involves the presence, on the same particle, of multiple binding sites for the proteins.

INTRODUCTION

Contact activation occurs when blood is allowed to come into contact with a variety of negatively charged surfaces (Margolis, 1956; Nossel, 1964). As a result, the intrinsic coagulation pathway and the Factor XII-dependent pathways of kinin formation and fibrinolysis are initiated. The proteins involved in the initial reactions are Factor XII (Hageman factor), prekallikrein and high- M_r kininogen (Kaplan & Silverberg, 1987). Since none of these circulates as an active enzyme, but both prekallikrein and Factor XII become active proteinases upon contact with a surface (Kaplan & Silverberg, 1987), the mechanism of the initiation of contact activation is the subject of considerable interest.

The study of these interactions is complicated by the positive feedback involved in the reciprocal activations of Factor XII and prekallikrein. This feedback is responsible for an extremely rapid acceleration of the rates of activation of either component when the two zymogens are incubated together in the presence of a surface (Silverberg & Kaplan, 1982; Tankersley & Finlayson, 1984). As a result the events responsible for the initiation of the process become obscured and have been difficult to elucidate.

In recent years the autoactivation of human Factor XII has been demonstrated (Silverberg *et al.*, 1980; Tans *et al.*, 1983; Tankersley & Finlayson, 1984; Griep *et al.*, 1985), and this reaction provides us with an experimental tool with which to study the interaction between Factor XII and the surface in the absence of kalikrein. It has long been known that particulate materials bearing fixed negative charges, such as Celite and kaolin, are active in promoting contact activation (Nossel, 1964). Among soluble compounds that have been investigated, various natural or synthetic sulphated polysaccharides have been identified that were able to support contact activation in

plasma (Kellermeyer & Kellermeyer, 1969; Schwartz & Kellermeyer, 1969; Moskowitz *et al.*, 1970; Kluft, 1978). Of these, high- M_r dextran sulphate is an efficient and reproducible activator (Kluft, 1978; Van der Graaf *et al.*, 1982). Natural analogues, especially heparins, have also been studied (Moskowitz *et al.*, 1970; Hojima *et al.*, 1984). Earlier studies were conducted in whole plasma with relatively crude methods of assaying activation, but more recently studies with purified components with kinetic assays have become feasible (Fujikawa *et al.*, 1980; Silverberg & Kaplan, 1982; Tankersley & Finlayson, 1984; Hojima *et al.*, 1984; Shimada *et al.*, 1985).

In the study reported in the present paper we have taken advantage of the availability of dextran and dextran sulphate of a wide range of M_r to examine the effect of the molecular size of the activating compound. This is the first step in a study of the number and nature of the binding sites for Factor XII that are required to support contact activation, conducted with the aim of providing evidence for the mechanism by which the surface is able to initiate contact activation.

EXPERIMENTAL

Materials

Dextran of nominal M_r 15 000–20 000 was obtained from Polysciences, Warrington, PA, U.S.A. Dextran sulphate preparations of M_r 5000 and 500 000 were from Sigma Chemical Co., St. Louis, MO, U.S.A., as were anthrone, thiourea, orcinol and Triton X-100. Heparin (from pig mucosa; M_r 13 500–15 000) and low- M_r heparin (prepared by depolymerization of heparin; M_r 5000) were from Calbiochem, La Jolla, CA, U.S.A. Polystyrene-sulphonates of defined M_r values were from Scientific Polymer Products, Ontario, NY, U.S.A. Chloro-

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sulphonic acid was from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Prolylphenylalanylarginine *p*-nitroanilide (S2302) was made by Kabi and obtained from Helena Laboratories, Beaumont, TX, U.S.A. Standard sulphate solution was BANCO from Anderson Laboratories, obtained from VWR Scientific, South Plain Field, NJ, U.S.A. Other chemicals were of reagent grade and obtained from Sigma Chemical Co., Fisher Scientific Co., Fair Lawn, NJ, U.S.A., or J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A.

Methods

Factor XII was purified by using published procedures (Silverberg *et al.*, 1980).

Dextran of M_r 15000–22000 (Polysciences) was sulphated by an adaptation of the method of Ricketts (1952). A 40 ml volume of dry pyridine was cooled in a solid- CO_2 /propan-2-ol mixture, and 8.8 ml of chlorosulphonic acid was slowly added. The mixture was warmed to 65 °C and more pyridine added as necessary to dissolve all of the pyridinium salts. Then 6 g of solid dextran was added and the mixture maintained at 65–70 °C for 4 h. Although the mixture was stirred constantly the dextran swelled to form a gummy mass that is not really amenable to agitation. At the end of the incubation, the pyridine solution was poured off, and a mixture of distilled water and crushed ice (made from distilled water) was added and the dextran dissolved in a final volume of 200 ml. Approx. 25 ml of 10 M-NaOH was added to turn the solution dark red and cause remaining pyridine to separate. The aqueous phase was treated with an equal volume of ethanol at 40 °C to precipitate the dextran as a syrup. This was repeated once, and the precipitated dextran was then dialysed against water and freeze-dried.

Dextran concentrations were determined by the anthrone reaction (Spiro, 1966). Samples (200 μl) were each mixed with 1 ml of anthrone reagent prepared by mixing 28 ml of water and 72 ml of conc. H_2SO_4 and dissolving 50 mg of anthrone and 1 g of thiourea. The reaction mixture was boiled for 15 min in a water bath, and the colour was read at 620 nm. Non-sulphated dextran was used to construct a standard curve.

Heparin concentrations were determined by the orcinol reaction (Davidson, 1966). Samples (250 μl) were each mixed with 750 μl of orcinol reagent (62 ml of conc. HCl, 4 ml of 1.5% FeCl_3 in 0.1 M-HCl and 160 mg of orcinol) and heated in a boiling-water bath for 40 min. The absorbance at 660 nm was then read; a standard curve was constructed with U.S.P. heparin.

Sulphate was determined by the barium chloranilate reaction (Lloyd, 1966) after acid hydrolysis. Samples (200 μl) of dextran sulphate in water were each placed into a 10 mm $\times$ 75 mm glass tube and several tubes were placed together in a pressure vessel with 20 ml of 6 M-HCl. The vessel was placed in an oven at 110 °C for 6 h, after which 1 ml of distilled water was added to each tube, which was then frozen and freeze-dried to remove traces of HCl. Then 200 μl of water was added to each tube, followed by 750 μl of ethanol, 200 μl of 0.05 M-potassium phthalate, pH 4.01, and 500 μl of barium chloranilate suspension (1 mg/ml in 0.25 M-sodium acetate/acetic acid buffer, pH 4.0). After 20 min shaking, the tubes were spun for 10 min at approx. 3000 g and the absorbance of the supernatant at 350 nm was determined. A standard

sulphate solution was used to construct a standard curve.

Factor XIIa activity was assayed by adding 5 μl of enzyme solution to 400 μl of 50 mM-Tris/HCl buffer, pH 7.8, containing 500 μM -S2302 and 0.001 % Triton X-100 in semi-micro cuvettes at 37 °C. The change of absorbance at 405 nm was determined with a Gilford recording spectrophotometer interfaced with an Apple IIe microcomputer.

Autoactivation of Factor XII was determined by incubating Factor XII at 50–70 $\mu\text{g}/\text{ml}$ with dextran sulphate at approx. 5 $\mu\text{g}/\text{ml}$ at 37 °C in 50 mM-Tris/HCl buffer, pH 7.5, containing 0.001 % Triton X-100 and 1 mM-EDTA. When column fractions were compared, the actual concentration of activator was standardized relative to dextran by the anthrone assay, and the measured concentrations of dextran are reported in the Figure legends. Samples (5 μl) were removed at intervals and assayed for Factor XIIa activity.

Gel filtration of polysaccharide sulphates was performed on a Sephadex G-75 (superfine grade) column (1.0 cm $\times$ 120 cm) equilibrated in 0.1 M-sodium phosphate buffer, pH 7.0, containing 0.1 M- Na_2SO_4 , 1 mM-EDTA and 0.001 % Triton X-100. Fractions (1.3–1.5 ml) were collected and alternate fractions were assayed for dextran by the anthrone reaction. A series of five to ten of those fractions were selected from different regions of the peak for detailed study of their ability to support the autoactivation of Factor XII. The column was calibrated with polystyrenesulphonates of known M_r values, which were detected in the eluate by their absorbance at 240 nm.

RESULTS

Use of dextran sulphates

The autoactivation of Factor XII was studied with the use of soluble sulphated polysaccharides as activators. Initially, commercially available preparations of dextran sulphate were used that had nominal M_r values of 5000 and 500000. Subsequently we wished to examine the effects of dextran sulphate at an M_r in between these two extremes. This involved the synthesis of a suitable species by sulphation of dextran; accordingly, a dextran fraction of nominal M_r 15000–20000 was sulphated by the method of Ricketts (1952). The sulphate incorporation was 50.9%, which was similar to that of the commercial preparations of M_r 5000 and 500000 (see Table 1).

Previous studies with sulphatide (España & Ratnoff, 1983; Tans *et al.*, 1983; Griep *et al.*, 1985) have shown

Table 1. Characteristics of dextran sulphates

Nominal M_r	Sulphate (% by wt.)*	Apparent rate constant of auto-activation (min^{-1})†
500000	48.2	0.435
15000–20000	50.9	0.294
5000	50.2	0.089

* Percentage of total recovery of sulphate and dextran.

† Assays conducted at 53.2 μg of Factor XII/ml and 1.925 μg of dextran/ml (see Fig. 1).

Fig. 1. Autoactivation of Factor XII with dextran sulphates

Factor XII ($53.2 \mu\text{g/ml}$) was incubated with dextran sulphates of M_r 5000 ($\square$), M_r 15000–20000 ($\triangle$) and M_r 500000 ($+$) and, as a control, with no dextran sulphate ($\circ$). In each case the concentration of dextran in the incubation mixture was $1.925 \mu\text{g/ml}$, as determined by the anthrone assay.

that the rate of autoactivation of Factor XII is a function of the concentration of activator used. In those studies populations of sulphatide micelles with uniform size were used; thus the variation of concentration was equivalent to a variation in the number of binding sites present. In our studies the question arises as to what constitutes equivalent concentrations of polysaccharide when the M_r is varied. Since the question that we are asking concerns the number of binding sites per particle, it is important to keep the total number of sites constant to avoid having two variables from one determination to another. Therefore we decided to standardize by the weight of dextran as determined by assay with the anthrone reagent as described in the Experimental section. The effect of this is to make the sugar monomer concentration the same: the variation in M_r then becomes equivalent to a variation in the arrangement of the same number of monomers into particles of different size.

Fig. 1 shows the results of incubating Factor XII with the same concentration (by weight) of dextran sulphate of M_r 5000, 15000–20000 and 500000. Autoactivation is clearly seen in each case. The rate of the autoactivation reaction decreases as the M_r of the dextran sulphate decreases. This rate may be expressed as an apparent rate constant for the initial, exponential, part of the reaction obtained as the slope of plots of $\ln(\Delta A/\text{min})$ versus time as described by Tankersley & Finlayson (1984). The apparent rate constants for the three curves are shown in Table 1 along with the sulphate content of each preparation. Although there is a decrease in the rate constant as the M_r decreases, the rate obtained with the 5000- M_r dextran sulphate is still significant compared with that obtained in the absence of any activator. There is an apparent variation of the plateau value of activated Factor XII reached that appears to correlate with the initial rate of the autoactivation reaction. This phenomenon has been observed repeatedly, but the cause is unknown; a possible explanation may lie in the accumulation of the second cleavage product of Factor XII, Factor XII_f, at the lower rates of surface reaction. Unlike Factor XII_a, Factor XII_f does not catalyse the activation of Factor XII (Silverberg *et al.*, 1980).

Absence of metal ion effect

The efficacy of low- M_r dextran sulphate in supporting the autoactivation of Factor XII is an unexpected result, since the concept of an activating 'surface' is not apparently compatible with an M_r of only 5000. Ellagic acid (Ratnoff & Crum, 1964) is a low- M_r compound that is used as an activator, but this is apparently active only as microparticulate material formed from complexes with heavy-metal ions (Bock *et al.*, 1981). To determine if a similar mechanism was present with low- M_r dextran sulphate we investigated the effect of metal ion chelators on the autoactivation reaction. A portion of 5000- M_r dextran sulphate was dissolved in 10 mM-EDTA or 5 mM-*o*-phenanthroline, and then samples were removed and incubated with Factor XII in the presence of 1 mM-EDTA. We found that $5 \mu\text{g}$ of dextran sulphate/ml gave the same rate of autoactivation whether treated with chelator or not. Thus there is no indication that a metal ion complex is responsible for the observed activity.

Fractionation of dextran sulphates

We would expect that the preparations of dextran and dextran sulphate obtained are heterogeneous mixtures in terms of molecular size. To define further the effect of M_r of the dextran sulphate, we chromatographed the 5000- M_r preparation on Sephadex G-75. The column was calibrated with a series of polystyrenesulphonates, chosen because they are linear negatively charged polymers and should therefore be a reliable guide to the behaviour of the sulphated polysaccharides. The eluted polysaccharide formed a broad peak encompassing 26 fractions of apparent M_r values 18000 to 2000; the K_{av} at the centre of the peak corresponded to an apparent M_r of 4600. Rechromatographed fractions were eluted in narrow peaks at the expected volumes (results not shown, but see Fig. 4), indicating that material was indeed resolved into fractions of different average M_r values.

Fig. 2. Concentration-dependence of dextran sulphate-induced autoactivation of Factor XII

A 10 mg sample of 5000- M_r dextran sulphate was chromatographed on Sephadex G-75 as described in the Experimental section. Column fractions were assayed, at the indicated concentrations, with Factor XII at $53.1 \mu\text{g/ml}$. The estimated M_r values of the fractions were 11100 ($\square$), 9400 ($\triangle$), 8000 ($\blacklozenge$), 4900 ($\bullet$) and 3000 ($\circ$).

Fig. 3. Sephadex G-75 elution profile of dextran sulphate

Samples (10 mg) of 5000- M_r dextran sulphate ($\circ$ — $\circ$) and of 15000–20000- M_r dextran sulphate ($\triangle$ — $\triangle$) were chromatographed on Sephadex G-75. The continuous curve (—) and $\bullet$ and $\blacktriangle$ symbols show apparent rate constants determined as described in the text. The elution positions of the M_r markers are shown at the top of the figure.

Five individual fractions of dextran sulphate were assayed for their ability to support the autoactivation of Factor XII. The concentration of the fractions was determined by the anthrone reaction, and we determined the rate constants for autoactivation as a function of dextran concentration for the selected fractions eluted from the Sephadex G-75. The results are shown in Fig. 2. For each fraction the rate of activation rose to a peak and then declined with further increases in concentration. Significantly, the maximum rate of autoactivation obtained with different fractions, having M_r values of 4800 and above, all occurred at about $1.5 \mu\text{g}$ of dextran/ml, equivalent to $3.0 \mu\text{g}$ of dextran sulphate/ml. The very smallest fraction examined, having an M_r of approx. 3000, gave very low rates of autoactivation, with an apparent peak in the region of $5 \mu\text{g}$ of dextran/ml. This indicates that the activity in the fractions is not due to contamination with traces of higher- M_r material, since that would lead to a progressive increase in the concentration at which the maximum rate occurs. Thus it appears that the maximum rate occurred at the same concentration of binding sites for each fraction, but that the value of that maximum increased as those sites were arranged into particles with a larger average number of binding sites per particle. At M_r between 3000 and 4000 it appears that the limit is reached below which no activation of Factor XII can occur.

We extended these observations by fractionating the 15000–20000- M_r dextran sulphate in the same fashion. The 15000–20000- M_r dextran sulphate preparation was eluted at lower values of K_{av} than the 5000- M_r preparation, with the peak at a value corresponding to M_r 18000. Fractions were assayed in the autoactivation reaction at a dextran concentration of $1.43 \mu\text{g}/\text{ml}$. We compared the rate constants obtained with those interpolated from the data in Fig. 2 for the same dextran concentration. The results are shown in Fig. 3, where the elution profiles for the two dextran sulphate fractions are plotted along with the rate constants obtained. The plot of rate constant versus elution volume was described by

Fig. 4. Sephadex G-75 elution profile of heparins

Samples (10–15 mg) of various heparins were chromatographed on a Sephadex G-75 column (1 cm $\times$ 110 cm) equilibrated in 0.1 M-sodium phosphate buffer, pH 7.0, containing 0.001% Triton X-100 and 1 mM-EDTA. Heparin concentrations were determined by the orcinol method (b). Autoactivation assays were done with $10 \mu\text{g}/\text{ml}$ samples of the column fractions and Factor XII at $53.1 \mu\text{g}/\text{ml}$ (a). Heparin fractions were 5000- M_r preparation ($\square$), 13000–15000- M_r preparation ($\diamond$) and U.S.P. heparin ($\triangle$); +, rechromatography of fractions 21–23 and 39–41 of the 5000- M_r heparin.

a single curve for both preparations, which showed a marked dependence of the efficiency of autoactivation on the M_r of the dextran sulphate with a sharp increase occurring as an M_r value of 10000 is reached. Above an M_r of approx. 12000 the efficiency appears to increase smoothly, but further data are required to determine the shape of the curve.

Action of heparin

Since it appeared that low- M_r species can support Factor XII autoactivation, we also examined three preparations of heparin. One of these was U.S.P. heparin for clinical use of unspecified M_r , one of the others was specified as having M_r 13000–15000 and the third preparation, obtained by nitrous acid depolymerization, had an M_r of 5000. These were all chromatographed on Sephadex G-75, and the eluted fractions were tested for their abilities to promote the autoactivation of Factor XII. We did not investigate the dependence of autoactivation on heparin concentration in detail, but preliminary measurements showed that maximal efficiency occurred at a higher concentration of heparin than of dextran sulphate. Accordingly these experiments were conducted with a higher concentration of heparin in the assays. As before, however, each assay was performed

with the same concentration of heparin, measured by the orcinol reaction.

The results are shown in Fig. 4 as plots of fraction number versus heparin concentration or apparent rate constant. The U.S.P. heparin gave a similar elution profile to that of the 13000–15000- M_r heparin. In both cases the apparent rate constant of autoactivation decreased with elution position in the same manner as the 5000- M_r dextran sulphate. Although the elution peak of the 5000- M_r heparin is found at a later fraction than that of the larger heparins, its curve of rate constant versus fraction number is superimposable on the other two. Thus it appears that with heparin there is a reproducible relationship between molecular size and the ability to support the autoactivation of Factor XII, just as with synthetic dextran sulphate. We confirmed that the heparin was truly fractionated by size by rechromatographing fractions of 5000- M_r heparin that were eluted from either early or late from the Sephadex G-75. As shown in Fig. 4, these fractions were eluted in the same position upon rechromatography as they had been obtained from originally. Furthermore, their ability to support autoactivation remains characteristic of the fractions in the original chromatogram.

It is remarkable that the data obtained for heparin show the same steep increase in autoactivation rate in the same region of the chromatogram. The data suggest that a change in the mechanism of autoactivation occurs when the size of the polyanion is greater than M_r 10000 and that this is related more closely to size than to the precise chemical nature of the polyanion.

DISCUSSION

The rate of autoactivation of Factor XII has been shown previously to be a function of the concentration of the activating surface (Tans *et al.*, 1983); the rate rises to a maximum and then declines with further increases in activator concentration (Espana & Ratnoff, 1983; Griep *et al.*, 1985). This inhibition of activation by excess surface may be intuitively understood in terms of the inability of molecules of Factor XII to interact with each other when bound to different particles. However, it is not known whether the mechanism of activation of surface-bound Factor XII is accomplished by an enzyme molecule (Factor XIIa or kallikrein) acting out of solution or by a second molecule bound to the surface at a neighbouring binding site.

Previous experiments were done with sulphatide micelles of narrowly defined size ranges as the activating surface. In the present study we have used dextran sulphate preparations of various M_r values to determine the effect of the arrangement of binding sites on the activating particles. As in the previous studies, we find that the excess surface is inhibitory. However, the maximum rate of activation attained was a pronounced function of the molecular size of the particle, and presumably, therefore, of the number of binding sites per polysaccharide molecule.

A new finding of this study is that very-low- M_r polysaccharides were able to induce autoactivation of Factor XII, although at a low rate. As the molecular size of the polysaccharide was increased above M_r 10000 we found a dramatic increase in the rate of autoactivation; very similar results were obtained with dextran sulphate and heparin preparations. We postulate that this rise is

based on the presence of multiple binding sites on the larger polysaccharide species. Although this hypothesis can only be proved by direct binding measurements, in advance of those there are theoretical considerations that lend support. Thus we can estimate the lengths of the sulphated polysaccharide molecules by comparison with the results of studies of chondroitin sulphate (Atkins *et al.*, 1972; Arnott *et al.*, 1973) and heparin (Stone, 1981). These have linear structure that occupies 1.6–1.8 nm per tetrasaccharide and thus give us an approximate scale of 1.3 nm per 1000 M_r . This may be compared with a diameter of 5–6 nm for Factor XII (M_r 80000), which is calculated on the assumption that the protein molecule is approximately spherical. Although approximate, these calculations are consistent with the notion that two Factor XII molecules can bind only to polysaccharide molecules with M_r values greater than about 10000 and that the marked increase in activation efficiency found in that range is due to the transition from one to two binding sites per molecule.

The mechanism by which polymers with single binding sites can support autoactivation of Factor XII is indicated by the results reported by Griffin (1978), who showed that Factor XII bound to the surface was cleaved more efficiently by a number of different proteinases. Apparently Factor XII, when bound to an activating surface, undergoes a conformational change that makes it more susceptible to activation by fluid-phase proteinases. Our results show that this phenomenon can account for only part of the surface effect, since the rate obtained with low- M_r fractions is low. Efficient activation clearly requires there be multiple binding sites on each activating particle. Under those conditions, the surface is able to create a zone where the local concentration of reversibly binding components is much greater than the concentration in the bulk fluid phase. The combination of this effect with the enhanced susceptibility of surface-bound Factor XII to activation is able to produce much the greater rates of autoactivation observed when the larger polysaccharide fractions are used.

A second interesting finding of this study was that the maximum rate occurred at the same monomer concentration for each fraction tested. This means that the maximum rate occurred at the same ratio of free to bound Factor XII, regardless of the arrangement of binding sites on the particles, and that excess sites, leading to an increase in the amount of bound Factor XII, led to lower rates. However, the rate of Factor XII autoactivation at the peak continued to increase quite steeply as the molecular size of the dextran sulphate fractions increased from M_r 10000 to M_r 18000. Thus the greater the number of binding sites present on a single particle the more efficient the autoactivation reaction. This suggests, but does not prove, that the activation of Factor XII is accomplished mainly by enzyme molecules present at high concentration in the fluid phase contiguous to the activating surface.

The results that we obtained with low- M_r dextran sulphate indicated that very large molecules are not necessary for Factor XII autoactivation or, by extrapolation, for contact activation. This prompted us to examine the action of different forms of heparin. There have been reports that heparin could induce the formation of bradykinin in plasma (Moskowitz *et al.*, 1970) and that heparin, of undefined M_r , could support the autoactivation of Factor XII (Hojima *et al.*, 1984).

Our results show that heparin is able to support the autoactivation of Factor XII and that the dependence of this reaction on molecular size is quite similar to that found with dextran sulphate. There has been considerable interest in the use of low- M_r heparin as an anticoagulant, since species containing two to four tetrasaccharide units are effective in the inhibition of Factor Xa by anti-thrombin III but not of thrombin (Holmer *et al.*, 1981; Rosenberg, 1982). Factor XIIa is also inhibited in the presence of low- M_r heparin. The results presented here show that heparins of M_r below 10000 are less likely to cause activation of Factor XII, which may be a competing reaction when larger species are added to plasma. Thus the molecular-size-dependence of Factor XII-dependent kinin formation induced by heparin in plasma is an interesting question that deserves further study.

The observations described in this paper provide support for a simple physicochemical mechanism for the contact activation of human plasma. Further studies are required to determine the binding parameters of Factor XII to dextran sulphates of different molecular sizes and correlate these with the efficiency of autoactivation and of activation of prekallikrein.

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