Autoactivation of human blood coagulation factor XII on Dextran derivatives of different molecular weight

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AUTOACTIVATION OF HUMAN BLOOD COAGULATION FACTOR XII ON DEXTRAN DERIVATIVES OF DIFFERENT MOLECULAR WEIGHT

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ABSTRACT

We prepared a derivative of dextran T40 (average Mr 43,000) from which fractions of different Mr but with equal charge density were obtained and tested for their ability to promote autoactivation of human blood coagulation factor XII. The mechanism of autoactivation appeared dependent upon the Mr of the polymer used. Thus, with polymers of 38,000 Mr or higher only α -factor XIIa was formed and the reaction could be completely described in terms of a simple second-order mechanism of autoactivation. With smaller polymer molecules β -factor XIIa became a major reaction product and as a result of this the autoactivation kinetics did not adhere to the second-order mechanisms thus far described.

INTRODUCTION

The activation of blood coagulation factor XII is one of the key events occurring during contact activation. Factor XII activation can be brought about by plasma kallikrein or through autoactivation of the zymogen factor XII by its own enzymatically active form α -factor XIIa (for a review see ref 1). Several forms of activated factor XII have been described, the most important ones being α -factor XIIa (80,000 Mr, also known as HFa) and β -factor XIIa (30,000 Mr, HFf). Both these enzymes contain a fully functional active site, however β -factor XIIa lacks the ability to bind to negatively charged surfaces (1). During autoactivation of factor XII, the product that is formed is mostly α -factor XIIa

Key words: contact autoactivation. Factor XII. Dextran derivatives.
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whereas during activation of factor XII by kallikrein both forms of activated factor XII are observed.

In an earlier paper, it was shown that the chemical nature of the charge carriers on derivatized dextran is an important determinant for contact activation in plasma as evidenced by the strong cooperative effects exhibited by carboxylate groups associated to sulfonate groups covalently linked to the same dextran backbone (2). However, the rate at which kallikrein is generated also depends on the molecular weight of the activator used (3). Little additional information exists as to the effect of variation in size of the activating particle or polymer on its contact activation promoting activity. The substances described are usually large polymers or particles of average Mr 300,000 or higher (1). Recently, Silverberg and Diehl (4), using dextran sulfate and heparin, reported that the rate of factor XII autoactivation decreased with decreasing size of the polymer and below a certain critical size of 8,000-10,000 Mr only very low rates of autoactivation remained.

In this paper, we present experiments that were aimed at gaining more insight in the mode of action via which well defined negatively charged dextran derivatives promote autoactivation of factor XII. To this end, a highly active derivative of dextran T40 (abbreviated CMDBS) was prepared by introduction of negatively charged groups and from this fractions, of different size but with equal charge density, were obtained. With the materials, we performed a kinetic and gel electrophoretic analysis of factor XII autoactivation.

METHODS

Materials. The chromogenic substrate H-D-Pro-Phe-Arg-p- nitroanilide (S2302) was from AB Kabi Diagnostica, Stockholm, Sweden. Nitrocellulose membranes were purchased from BioRad, Richmond, Ca (USA) and 3,3'-diaminobenzidine tetrahydrochloride from Fluka AG, Switzerland. Swine anti-rabbit IgG antibodies were from Nordic, Tilburg, The Netherlands. Dextran T40 was from Pharmacia Fine Chemicals. All other reagents were of the highest grade commercially available.

Methods. Factor XII was isolated from human plasma as described earlier (5) and was stored at - 80°C in 4 mM sodium acetate, 2 mM acetic acid, 150 mM NaCl, 1 mM EDTA and 0.02 % sodium azide (pH 5.3). Protein concentrations were determined according to Lowry et al. (6) using bovine serum albumin as a standard and factor XII concentrations were calculated assuming a Mr of 80,000 (7).

Gel electrophoresis and blotting . Polyacrylamide gel electrophoresis was carried out in the presence of sodium dodecyl sulfate as described by Laemmli (8) on 10 % slabgels (5 % stacking gel) in a miniprotean II cell from BioRad followed by electrophoretic transfer to nitrocellulose according to Towbin et al. (9). Transfer was performed in the miniprotean II blot module according to the manufacturer's instruction at constant voltage (200 V) for 1 hr. Immunologic detection of factor XII and its activation products was performed essentially according to Towbin et al. (9) with rabbit anti-human factor XII as first and swine anti-rabbit IgG con-

jugated with horseradish peroxidase as second antibody. Staining was achieved by subsequently soaking the sheets in a freshly prepared and filtered solution containing 0.5 mg/ml 3,3'-diaminobenzidine tetrahydrochloride, 0.01 % H_2O_2 in 50 mM Tris (pH 7.5).

Factor XII activation. Activation of factor XII was carried out in 1.6 ml Sarsted snapcap centrifuge tubes. Protein adsorption was prevented by incubation overnight with reaction buffer containing 5 mg/ml ovalbumin after which the tubes were washed twice with distilled water and dried before use. This treatment was necessary to recover entirely factor XII (or XIIa) from the reaction tubes. In a typical experiment, factor XII was preincubated at 37°C for 5 min in reaction buffer after which activation was started by addition of 50 μl distilled water containing CMDBS. Final concentrations were : 70 mM Hepes (pH 7.5 at 37°C) , 50 mM NaCl, 0.5 mM EDTA, 1 mg/ml ovalbumin and appropriate amounts of factor XII and activator. At different time intervals, aliquots were removed from the reaction mixture for gel electrophoretic analysis and for quantitation of factor XIIa using the chromogenic substrate S2302 as described earlier (10). The amount of factor XIIa present in the reaction mixture was calculated from the rate of change in absorbance at 405 minus the absorbance at 500 nm measured using an Aminco DW2A spectrophotometer set in the dual mode and a calibration curve made with known amounts of fully activated factor XIIa. The autoactivation reaction was analyzed according to the second-order mechanism of autoactivation described earlier (10) in which

For such a mechanism, a plot of the logarithm of the ratio of XII/XIIa at each time point versus time should give a straight line, the slope of which equals the apparent second-order rate constant k_{obs} times the total factor XII concentration present in the reaction mixture (see also results).

Synthesis of dextran derivatives . Derivatives of dextran were prepared by modification of an earlier procedure (11) starting with dextran T40 (average Mr 40,000). 30 g Dextran T40 was dissolved in 120 ml H_2O and activated by addition of 130 ml 6 M NaOH. Activation was allowed to proceed for 20 min at 0°C while stirring, after which 25 ml water was added containing a 2.5-fold molar excess of acetic acid over dextran subunits (0.46 mole acetic acid/0.185 dextran subunits). Reaction was allowed to proceed at 50°C for 40 min after which the pH was brought to pH 7.0 with concentrated HCl. The product was precipitated with 1.8 l methanol while stirring overnight, filtered and dried under vacuum at 40°C. This procedure was repeated twice to achieve a sufficiently high number of subunits with a methylcarboxyl group attached. Benzylamination of the methylcarboxyl groups was achieved with the aid of the coupling agent N-ethoxycarbonyl-2-ethoxy 1,2-dihydroquinone (EEDQ) as follows. 12 g of methylcarboxylated dextran was dissolved in 80 ml H_2O and the pH was adjusted to 3.5 with concentrated HCl. Under stirring, 24.5 g EEDQ in 195 ml absolute ethanol was added and after 30 min 10.5 ml benzylamine was added dropwise while stirring. Most of the reaction mixture was evaporated under vacuum after which the final product was completely precipitated by addition of 0.5 l methanol followed by drying under vacuum at

40°C. Finally, the benzylamine groups thus introduced were sulfonated under mild conditions : 4 g of methylbenzylamide derivatized dextran was dispersed in 80 ml dichloromethan. After 30 min, chlorosulfonic acid was slowly added under vigorous stirring. To avoid acid hydrolysis or sulfonation of other groups, chlorosulfonic acid was added in a threefold molar excess over benzylamide groups present. The reaction was allowed to proceed for 3 hr at room temperature after which the reaction product was filtered, extensively washed with ethanol and dried under vacuum. Quantitation of the groups introduced was performed as described earlier (11) and the percentage of dextran units derivatized was calculated assuming introduction of only one carboxymethyl group per dextran subunit.

RESULTS

From the inactive non-charged dextran T-40 starting material, a highly negatively charged polymer was obtained by chemical derivatization as described in the Methods section. Table I shows the

Table I
Chemical Modification of Dextran T40

Modification Step	Cycles	Chemical Group	% Moieties Substituted	Mr
Methylcarboxylation	3	-COONa	96	39,000
Benzylamination	2	-COONa	67	40,000
		-benzylamide	21	
Sulfonation	1	-COONa	62	43,000
		-benzylamide	0	
		-SO ₃ Na	20	

different steps in the synthesis. After three cycles of carboxymethylation, 96 % of the dextran moieties were substituted. Two successive benzylamination steps resulted in a material with 67 % of the dextran moieties still containing a carboxylate group and 21 % containing a methylcarboxybenzylamide group (Table I). Sulfonation of these benzylamide groups finally yielded the polymer used in this study in which 62 % of the dextran moieties contained a methylcarboxylate group, 20 % a methylcarboxybenzylamide sulfonate group and 18 % remained unsubstituted. The size distribution of the polymer changed only slightly during the stepwise substitutions as judged from the small increase in average Mr (Table I). However, in order to obtain a material better defined with respect to size, the derivatized dextran was subjected to gel permeation chromatography on Sephacryl S300. From the eluted material, three fractions were chosen of which the average Mr was determined using a Superose 12 column on an HPLC system calibrated with polystyrenesulfonate molecular weight markers. The average Mr

of the fractions was determined to be 200,000, 38,000 and 15,000 respectively (Table II). Elemental analysis showed that these fractions had the same chemical composition, comparable with respect to charge density and differed only in Mr (Table II).

Autoactivation with unfractionated CMDBS

Incubation of 330 nM factor XII with 50 $\mu\text{g/ml}$ derivatized dextran T40 resulted in complete autoactivation of factor XII as judged by the appearance of amidolytic activity towards the chro-

TABLE II
Characteristics of Fractionated CMDBS

	% Moieties Substituted With			Mr
	COONa	Benzyl- amide	Benzyl- amide SO ₃ Na	
Starting Material	62	0	20	43,000
Fraction 1	62	0	20	200,000
Fraction 2	62	0	20	38,000
Fraction 3	60	0	20	15,000

FIG. 1

CMDBS-dependent activation of Factor XII (330 nM) in the presence of unfractionated polymer (50 $\mu\text{g/ml}$, Mr = 43,000)

- S2302 amidolytic activity as a function of the incubation time.
- Plot of the integrated kinetic data versus the incubation time according to Equation (1) (see text).

FIG. 2

Immunoblots of aliquots taken in the time-course of factor XII autoactivation (330 nM) shown in Figure 1.

- A. Samples reduced with β -mercaptoethanol
- B. Non-reduced samples.

mogenic substrate S2302 (Fig. 1A). In agreement with earlier findings (4,10), the observed activation showed an absolute requirement for the presence of the derivatized dextran T40 since in the absence of CMDBS no activation of factor XII occurred (data not shown). Earlier studies (10) have shown that autoactivation of factor XII can be described in terms of a second-order reaction mechanism, in which the zymogen factor XII is activated by its own enzymatically active form, α -factor XIIa. Indeed, the plot of $\ln(\text{XII}/\text{XIIa})$ versus time gave a straight line according to the second-order mechanism (equation 1)(Fig. 1B). However, deviation from this kinetics was observed after some 50-60 % of the available factor XII had been activated.

Fig. 2 shows immunoblots of aliquots of the reaction mixture taken at the same time intervals. The immunoblots showed that uncleaved unactivated factor XII migrated as a very closely spaced doublet under non-reducing conditions. This doublet represents two slightly different forms of factor XII since both bands are recognized by monoclonal antibodies against factor XII (J. Nuijens, personal communication), and both bands are cleaved during activation of factor XII by kallikrein (data not shown). Reduced factor XII showed a single band on the immunoblots. During the time course of activation, factor XII became cleaved and the characteristic heavy and light chain of α -factor XIIa (52,000 and 28,000 Mr) can be seen on the blots of the reduced gel (Fig. 2A). Inspection of the non-reduced gels showed that after some 30 min α -factor XIIa (30,000 Mr) became clearly visible on the blots as

well as a 50,000 Mr fragment and other cleavage products. β -Factor XIIa has no capacity to bind to negatively charged surfaces and cannot participate in the autoactivation reaction (12,10) which

FIG. 3

CMDBS-dependent autoactivation of Factor XII (330 nM) in the presence of 50 $\mu\text{g/ml}$ high Mr (200,000) fractionated polymer.

- A. S2302 amidolytic activity as a function of the incubation time.
- B. Plot of the integrated kinetic data at varying time intervals according to Equation (1) (see text).
- C. Variation of the slope of the preceding relation (kobs) with the amount of active material.

explains the deviation from second-order observed in the latter part of the reaction time-course. The minor bands in between the characteristic bands at 52,000 and 28,000 represent an additional cleavage in the heavy-chain domain of factor XII (α -factor XIIa) upon which some 40,000 Da are removed from the aminoterminal part of factor XII (XIIa) and this also results in the loss of binding capacity to negatively charged surfaces (1,13). The minor bands observed at approximately 60 to 65,000 Mr in the reduced gels were not further identified but most likely represent forms of factor XII (XIIa) in which an additional cleavage has occurred at a site located near the aminoterminal portion of the molecule (13).

Autoactivation with CMDBS of defined molecular weight

Fig. 3A shows the time course of autoactivation with 50 $\mu\text{g/ml}$ CMDBS of 200,000 Mr. As can be seen, factor XII became readily and completely activated and the second-order plot of factor XII autoactivation yielded a straight line over the whole time course of activation (Fig. 3B). Immunoblots of gel samples taken at the same time intervals revealed that factor XII was quantitatively converted into α -factor XIIa and no other products were visible (not shown). The rate of autoactivation as judged by the slope of the second-order plots was dependent upon the amount of CMDBS present and increased with decreasing concentration of material (Fig. 3C) until at low concentrations ($<5 \mu\text{g/ml}$) the initial rate constant of autoactivation became constant. With these low amounts of

FIG. 4

CMDBS-dependent activation of Factor XII (330 nM) in the presence of low-Mr (15,000) fractionated polymer. S2302 amidolytic activity as a function of the incubation time at (Δ) 25 µg/ml ; (o) 50 µg/ml ; (●) 400 µg/ml fractionated polymer.

FIG. 5

Immunoblots of aliquots of the reaction mixture containing factor XII (330 nM) in the presence of (50 µg/ml) low-Mr (15,000) fractionated CMDBS, taken at varying time intervals of incubation (0; 5; 10; 15; 20; 25; 35 and 75 min).
A : non-reduced and B : reduced samples.

activator present, however, not all available factor XII became activated as judged by a decrease in the final plateau of amidolytic activity reached. Further addition of activator at the end of the reaction readily yielded activation of the remaining amount of unactivated factor XII, a result that could also be achieved by addition of kallikrein (data not shown). Thus, the deviation from linearity was neither caused by inactivation of factor XII nor by β -factor XIIa formation since under all conditions only α -factor XIIa was observed (data not shown). Therefore, the decrease in the final plateau observed at low CMDBS concentrations was presumably due to saturation of the activating polymer material. Autoactivation experiments with the polymer fraction of 38,000 Mr gave similar results as those obtained with the 200,000 Mr material (data not shown). However, results differed when the polymer fraction of 15,000 Mr was used.

Fig. 4 shows an experiment in which factor XII was allowed to autoactivate in the presence of varying amounts of 15,000 Mr material. From the time course of activation, it is obvious that the reaction kinetics were completely different from those obtained with the higher Mr materials. The reaction did not adhere to the simple second-order reaction mechanism since the second-order plots of $\ln(XII/XIIa)$ vs time were non-linear at any tested concentration. Consistently with the results obtained with the material of higher Mr, the initial velocity of factor XIIa formation was dependent on the amount of material present and was the highest at low concentrations (Fig. 4). At higher concentrations however, not all factor XII became activated. For instance, at 50 $\mu\text{g/ml}$ CMDBS of 15,000 Mr, only 75 % of the available factor XII became activated and only 50 % at 400 $\mu\text{g/ml}$ (Fig. 4). This decrease in the amount of factor XIIa reached in the final plateau was not due to inactivation of factor XII since addition of kallikrein resulted in activation of the remaining factor XII (data not shown). However, in contrast with the results obtained with the polymers of higher Mr, gel electrophoretic analysis indicated that substantial β -factor XIIa formation occurred. Figure 5 shows immunoblots of non-reduced (Fig. 5A) and reduced (Fig. 5B) gels of samples obtained during the time course of autoactivation of factor XII with 50 $\mu\text{g/ml}$ CMDBS of 15,000 Mr (cf. also Fig. 4).

As can be seen, β -factor XIIa formation was already visible at early time-points during the reaction. Since β -factor XIIa cannot support autoactivation (10,12), this will lead to a substantial decrease in the reaction rate: the more β -factor XIIa is formed during the reaction, the more the reaction rate will decrease which may explain why the reaction does not go to completion. At higher amounts of material used β -factor XIIa formation was even more prominent (data not shown), consistent with the observation that the final plateau reached decreased with increasing concentrations of 15,000 Mr material used.

DISCUSSION

In this paper we report a kinetic and gel electrophoretic analysis of factor XII autoactivation with a well defined, highly charged dextran derivative. It should be emphasized that the autoactivation reaction reported here was in all cases completely

dependent upon the addition of the derivatized dextrans since in the absence of added material no factor XIIa formation occurred (cf. also refs. 4 and 10). Our data confirm and extend the observations of Silverberg and Diehl (4) who reported that autoactivation of factor XII can occur on dextran sulfate or heparin polymers of low Mr. The results shown here indicate that the actual mechanism via which autoactivation occurs depends on the size of the polymer molecules. It appears that the polymer that we used (average Mr 43,000) shows a complex kinetic behaviour when it is tested for its ability to promote factor XII autoactivation (Fig. 1). This is caused by an heterogeneity in size of the activating polymer particles. With polymer molecules of 38,000 or 200,000 Mr, autoactivation can be completely described in terms of the simple second-order mechanism described earlier (10), in which the zymogen factor XII is activated by its own enzymatically active form α -factor XIIa and α -factor XIIa is the only product formed. In this mechanism it is supposed that the polymer acts by promoting the formation of productive enzyme-substrate complexes through binding (presumably in close proximity i.e. on the same particle) of the enzyme (α -factor XIIa), factor XII and the substrate (10). The fact that the rate of autoactivation decreases with increasing concentrations of polymer supports this concept, since at higher polymer concentrations the chances of binding both reactants to the same particle become reduced. A similar finding was reported by Silverberg and Diehl (4). These authors also reported that at lower concentrations of dextran sulfate the overall rate of autoactivation decreased. The result presented in Fig. 3C seems in contrast with this finding. At low concentrations of material ($<5 \mu\text{g/ml}$), we observed that not all factor XII became activated and a concomitant deviation from linearity occurred at the end of the reaction. The amount of factor XIIa at which this deviation occurred became less and less with decreasing amounts of polymer present but the apparent rate constant with which the autoactivation started (deduced from the initial slope in the second-order plots) was constant irrespective of the actual concentrations of polymer present. Under all circumstances, no β -factor XIIa formation was observed. These findings strongly suggest that under these conditions the polymer was saturated and that once the bound factor XII is activated it is only slowly replaced with unbound protein. Thus although the apparent rate constant of autoactivation is constant under these conditions (the small amount of polymer is saturated the overall factor XIIa generation decreases at such low polymer concentrations and this may explain why Silverberg and Diehl reported an apparent decrease in the rate of autoactivation at very low dextran sulfate concentrations.

The results presented here indicate that, in the case where we used CMDBS polymer molecules of 38,000 Mr or higher, the reaction can be completely understood in terms of a second-order mechanism of activation provided the binding equilibria involved are taken into account. In agreement with the findings of Silverberg and Diehl (4) slower rates of factor XIIa generation were observed with the 15,000 Mr material as compared to the rates obtained with the high Mr material. However, in addition, our results also indicate that in this case the reaction does no longer adhere to the mechanism of autoactivation as outlined in the Methods section. Moreover, the gel electrophoretic analysis shows

not only the formation of α -factor XIIa which is the enzyme responsible for the autoactivation reaction, but the gels also show that β -factor XIIa becomes a major reaction product (Fig. 5). A linear second-order plot of autoactivation can only be obtained if all the factor XIIa that is formed can further participate in the reaction (cf. eq. 1 in the Methods section). The fact that a major part of the enzymatic reaction product formed is β -factor XIIa (which cannot participate further in the reaction), even at early time-points during the activation, explains why in this case the second-order plots are no longer linear. Furthermore, with more and more β -factor XIIa formed, the reaction will slow down more and more and this, in addition to the slow reaction rates already observed in the presence of the 15,000 Mr material (cf. Figs. 3 and 4), may also explain why the reaction fails to go to completion at higher amounts of 15,000 Mr material used (cf. Fig. 4).

It has been extensively documented that α -factor XIIa is the major (if not the only) product of autoactivation whereas in the case of kallikrein-dependent factor XII activation both α and β -factor XIIa are readily observed (cf. ref. 1). To our knowledge, this is the first time that it is described that the relative amounts in which these products are formed can be influenced by a change in size of the contact activating surface.

REFERENCES

1. TANS, G. and ROSING, J. Structural and functional characterization of factor XII. Sem. Thrombosis and Hemostasis, 13, 1-14, 1987.
2. CORRETGE, E., JOZEFOWICZ, M. and NIGRETTO, J-M. Contribution of negatively charged chemical groups to the surface-dependent activation of human plasma by soluble dextran derivatives. Biomaterials, 10, 449-454, 1989.
3. CORRETGE, E. and NIGRETTO, J-M. Molecular weight-dependent contact activation of plasma induced by soluble polystyrene and dextran derivatives. Thrombosis Res. 59, 463-473, 1990.
4. SILVERBERG, M. and DIEHL, S.V. The autoactivation of factor XII (Hageman factor) induced by low-Mr heparin and dextran sulphate. The effect of Mr of the activating polyanion. Biochem. J., 248, 715-720, 1987.
5. VAN DER GRAAF, F., TANS, G., BOUMA, B.N. and GRIFFIN, J.H. Isolation and functional properties of the heavy and light chains of human plasma kallikrein. J. Biol. Chem. 257, 14300-14305, 1982.
6. LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. J. Biol. Chem. 193, 265-275, 1951.
7. FUJIKAWA, K. and DAVIE, E.W. Human factor XII (Hageman factor). Methods Enzymol. 80, 198-211, 1981.
8. LAEMMLI, U.K. Nature 227, 680-685, 1970.

9. TOWBIN, H., STAEBELIN, T. and GORDON. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. *J. Proc. Natl. Acad. Sci. USA*, 76, 4350-4354, 1979.
10. TANS, G., ROSING, J. and GRIFFIN, J.H. Sulfatide-dependent autoactivation of human blood coagulation factor XII (Hageman factor). *J. Biol. Chem.* 258, 8215-8222, 1983.
11. BOUTTEMY, M., Contribution à l'étude des carboxyméthylcelluloses. Préparation des carboxyméthylcelluloses de hauts degrés de substitution. *Bull. Soc. Chim.*, 1750-1754, 1969 and SUNDARAM, P.V., A new method of coupling proteins to insoluble polymers. *Biochem. Biophys. Res. Commun.*, 61, 717-722, 1974.
12. SILVERBERG, M., DUNN, J.T., GAREN, L. and KAPLAN, A.P. Autoactivation of human Hageman factor. Demonstration utilizing a synthetic substrate. *J. Biol. Chem.* 255, 7281-7286, 1980.
13. GRIFFIN, J.H. and COCHRANE, C.G. Recent advances in the understanding of contact activation reactions. *Sem. Thrombosis and Hemostasis*, 4, 254-273, 1979.

INTRODUCTION

The activation of blood coagulation factor XII is one of the key events occurring during contact activation. Factor XII activation can be brought about by plasma kallikrein or through autoactivation of the zymogen factor XII by the zymogen itself. Active form of factor XII (120,000 Mr) has been described as being α -factor XIIa (80,000 Mr) and β -factor XIIa (40,000 Mr). Both these factors possess a functional active site, however β -factor XIIa alone is unable to bind to negatively charged surfaces. It is the autoactivation of factor XII, the product that is formed is α -factor XIIa.

Key words: contact autoactivation, Factor XII, Hageman factor, dextran derivatives.