MECHANISM OF INTERACTION OF FACTOR XII WITH SURFACES HAVING DIFFERENT PHYSICOCHEMICAL PROPERTIES

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Interaction between factor XII and surfaces having different physicochemical properties, and the effect of surfactants, urea, EDTA, and an increased ionic strength on its desorption were studied. The results showed that factor XII can be activated by interaction of the blood plasma with surfaces having negative and positive charges, and its desorption was induced by all agents to a different degree. The most important condition for activation of factor XII was shown to be electrostatic interaction with a surface having the highest charge density. The formation of hydrogen bonds of protein protons with the frequently distributed oxygen atoms on the surface of silica provides the most favorable conditions for manifestation of the enzyme activity.

KEY WORDS: athrombogenic substances; factor XII and its adsorption; rabbit plasma.

For the choice of athrombogenic substances as one aspect of the problem of creating an artificial intima, the mechanism of contact between factor XII and surfaces with different physicochemical properties and the desorption of factor XII as a result of weakening of ionic, hydrogen, and hydrophobic interactions were studied.

EXPERIMENTAL METHOD

Platelet-free rabbit plasma [2] was stabilized by passing it through an ion-exchange column with Dowex 50B × 10. Adsorption of factor XII from the stabilized intact plasma was carried out by the procedure described earlier [2]. The activity of the adsorbed factor XII was determined by correction of the coagulation defect of the substrate plasma in which factor XII was previously inhibited by the addition of ribonuclease in a concentration of 3 mg/ml plasma. For this purpose 1 ml of substrate was added to the washed absorbent and incubation was carried out for 10 min. As a result the factor XII adsorbed on the surface interacted with the factor XI of the inhibited plasma and formed a contact activation product. Next, 0.2 ml of 0.025 M $CaCl_2$ solution was added to 0.2 ml of this plasma and the clotting time was determined at 37°C. In control experiments absorption was carried out by means of inhibited plasma.

To study the desorption of factor XII from the surface of quartz powder, it was adsorbed with the use of intact citrated rabbit plasma [2], after which the adsorbent was incubated for 1 h in 10 ml of the desorbing agent, washed 10 times with water, and the residual activity of the enzyme was determined. In the control series adsorption was carried out from plasma inhibited by ribonuclease. The experimental results were analyzed statistically by Fischer's method [3].

EXPERIMENTAL RESULTS AND DISCUSSION

The effect of charged surfaces on the activation of factor XII in normal intact plasma was studied in the experiments of series I. The strongest activator of factor XII was quartz powder, which led to the

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TABLE 1. Activity of Factor XII Adsorbed on Various Surfaces (in sec)

Adsorbent	Ionic form	Clotting time		P
		expts.	control	
Dowex				
1× 10	Cl	279	289	>0,2
2× 10	Cl	322	317	>0,05
50 B× 10	Na	307	340	<0,001
Amberlite IRA-400	Cl	298	309	<0,05
Quartz powder	Na	225	363	<0,001
Sephadex				
CM C-50	Na	285	276	>0,1
SE-C-50	Na	245	255	>0,1
DEAE-A-50	Cl	442	420	>0,2
I-50	—	372	361	>0,05

TABLE 2. Desorption of Factor XII (in %) from Quartz Surface

Desorbent	Concentration	Decrease in activity	P
Water	—	5,2±1,2	<0,001
EDTA-Na_2	10 mM	31,3±2,4	<0,001
Urea	8 M	34,0±2,5	<0,001
Tween-80	1%	34,6±2,3	<0,001
Triton X-100	1%	34,7±2,0	<0,001
Na deoxycholate	1%	39,0±1,7	<0,001
Na dodecylsulfate	0,4%	39,5±2,0	<0,001
NaCl	2,6 M	42,8±2,4	<0,001
KCl	2,6 M	42,2±1,8	<0,001

maximal shortening of the clotting time compared with the control (Table 1). The activity of the enzyme was lower after the use of Dowex 50B × 10 and also of Amberlite IRA-400. The remaining adsorbents did not activate the factor XII, although adsorption did take place [2]. In quartz glass the distance between the negatively charged groups is about 3.2 Å and in Dowex 50B × 10 it is about 7 Å. Considering that the distance between two H-groups in the peptide chain is about 4 Å, the denser distribution of the charges of the quartz evidently creates more favorable conditions for unformational change in the factor XII molecule, and this is responsible for its activation.

In the experiments of series II, the activity of factor XII adsorbed from intact plasma was reduced by a varied degree under the influence of the various desorbing agents (Table 2). The greatest decrease in activity was observed after treatment with Na dodecylsulfate and an increased ionic strength. The decrease in activity from the action of EDTA, urea, and other detergents was less marked. In control experiments with water as the desorbing agent the activity of factor XII was reduced much less than by the other agents. Since the activity of the adsorbed enzyme was reduced by the action of EDTA, the binding of bivalent ions presumably weakened interaction between factor XII and the activating surface, although this was not ruled out completely.

The action of urea is ascribed to its ability to weaken hydrogen, hydrophobic [5-7], and ionic [4] bonds. This combined action evidently leads to the desorption of the protein from the surface of the quartz and to a decrease in enzyme activity. Since anionic detergents (Na deoxycholate and Na dodecylsulfate) increase the negative charge on a protein, and the quartz surface also is negatively charged, powerful electrostatic repulsion takes place and, consequently, desorption of the protein from the surface is greatest and its activity is considerably reduced. Nonionic detergents (Tween-80 and Triton X-100), which do not have this mechanism of action, reduce the activity of factor XII by a lesser degree. An increase in ionic strength weakens the salt bonds between the protein and the quartz surface. The ionic strengths make a greater contribution of energy than other bonds, and for that reason an increase in the salt concentration caused the greatest loss of activity of factor XII. As an agent weakening hydrogen bonds, water also reduced the activity of factor XII, but the effect was less marked than that of the contact activation product [1]. Consequently, factor XII is more firmly adsorbed than the contact activation product.

The most important condition for activation of factor XII is thus electrostatic interaction with a surface having a high charge density. The formation of hydrogen bonds of the protein protons with the frequently distributed oxygen atoms on the surface of the quartz must evidently also favor exhibition of the activity of the enzyme.

LITERATURE CITED

1. D. M. Zubairov, Z. Z. Asadullina, and L. G. Popova, Abstracts of Sectional Proceedings of the 4th International Biophysical Congress [in Russian], Book 4, Moscow (1972), p.235.
2. D. M. Zubairov, Z. Z. Asadullina, and L. G. Popova, Probl. Gematol., No. 10, 49 (1971).
3. R. A. Fisher, Statistical Methods for Research Workers [Russian translation], Moscow (1958).
4. G. Colaccico, Nature, 198, 583 (1963).
5. Y. Nozaki and C. Tanford, J. Biol. Chem., 238, 4074 (1963).
6. D. B. Wetlaufer, S. K. Malik, J. Stoller, et al., J. Am. Chem. Soc., 86, 508 (1964).
7. P. Whitney and C. Tanford, J. Biol. Chem., 237, 1735 (1962).