SELF-INDUCED CROSSLINKING OF FACTOR XIII

Takashi Takagi and R. F. Doolittle

Department of Chemistry, University of California, San Diego

La Jolla, California 92037

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SUMMARY: Activated plasma factor XIII has been found to autopolymerize as a result of self-induced crosslinking. Only the "a" subunits are involved in the crosslinking process, an a_2 dimer being produced upon urea-SDS dissociation of the polymer. The dimerization can take place between a subunits whether or not these chains have been converted to a^* by thrombin. Taken in context with other properties of factor XIII, the autopolymerization phenomenon suggests interesting possibilities for its evolution and participation in vertebrate hemostasis.

Factor XIII is a precursor enzyme found in vertebrate blood plasma and platelets, the activated form of which introduces ε-(γ-glutamyl)lysine crosslinks into fibrin (1-4). Under physiological conditions, the enzyme is activated by thrombin (5,6), peptide material being released from one of its two constituent subunit polypeptide chains (7):

$$a_2b_2 \xrightarrow{\text{thrombin}} a^*_2b_2 + \text{peptide material}$$

Although calcium ions are not necessary for the thrombin-catalyzed release of the peptide material (7), they are essential for the transamidase activity of factor XIII*. In this article we report that both factor XIII and XIII* are substrates capable of being crosslinked by factor XIII*. The autopolymerization process ensues as a result of the introduction of crosslinks between a chains only; furthermore, only covalently bound a_2 dimers are formed, higher polymers not being present. These results suggest that portions of the a chains are located on the exterior of factor XIII molecules in a symmetrical manner such that a reciprocal dimerization can take place. Apart from the structural implications, the phenomenon has interesting connotations with regard to the evolution of factor XIII and its involvement with the vertebrate clotting process.

EXPERIMENTAL

Factor XIII was prepared from freshly drawn ox blood plasma which had been decalcified with trisodium citrate and from human Blood Bank ACD plasma according to previously published procedures (8,9). Mixtures of factor XIII and thrombin were incubated in the presence and absence of calcium ions and aliquots examined by SDS gel electrophoresis in the presence and absence of mercaptoethanol (10).

Bovine factor XIII, before the addition of catalytic amounts of thrombin, exhibited two well-defined bands upon staining with Coomassie Blue, corresponding to the a and b subunits. Both bands developed pink color during the PAS-staining procedure for carbohydrate (11), but the a band lost most of its color during the destaining operation. After activation with thrombin, the a chain disappeared and was replaced by the smaller molecular weight a^* band at a position corresponding to a loss of about 5000 daltons. The b chain remained at its original position (Fig. 1). On the other hand, if calcium ions were present in the activation mixture, the a^* band did not appear, being replaced instead by a band corresponding to an a^*_2 dimer, indicating that the activated a chains had been covalently crosslinked. The patterns were more or less the same in the presence and absence of mercaptoethanol, especially when allowances were made for the relative compactness of subunits with intact disulfide bonds. The results with human factor XIII were similar, although the b chains of human factor XIII are slightly larger than their bovine counterparts.

Crosslinks could also be introduced between the a subunits of factor XIII molecules which had not yet been activated by thrombin. This was shown by examining mixtures in which only minute traces of thrombin were present, leading to only catalytic amounts of factor XIII*. It was also possible to demonstrate the effect by adding thrombin-free factor XIII*, prepared by gel filtration on BioGel A 1.5M, to unactivated factor XIII preparations. In these cases the a_2 dimers formed were readily distinguished on SDS gels from the smaller a^*_2 dimers made under the conditions described in the previous paragraph.

That the crosslinking of factor XIII resulted in an autopolymerization was

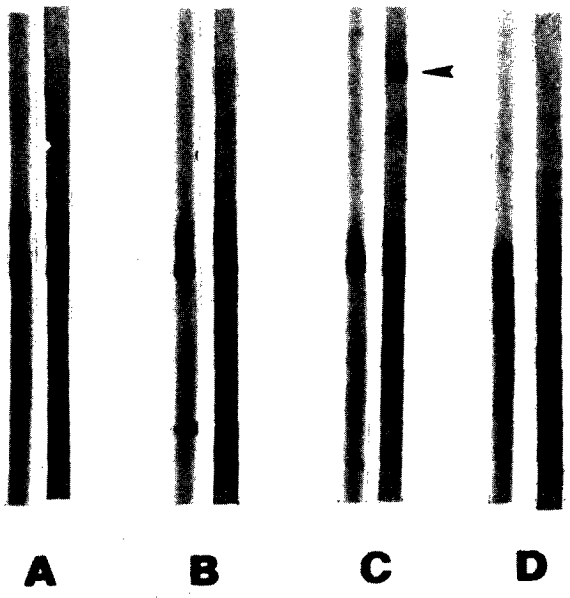

Fig. 1. SDS-gel electrophoresis of bovine factor XIII and XIII*. In each set of two, the left gel was stained for carbohydrate (PAS) and the right gel for protein (Coomassie blue). A, factor XIII; B, 100 μg factor XIII and 4 U thrombin incubated for 30 min. before being stopped by addition of urea-SDS; C, 100 μg factor XIII, 4 U thrombin in the presence of 20 mM $CaCl_2$ incubated for 30 min. (arrow indicates a^* dimer); D, reduced bovine fibrinogen run as markers.

readily demonstrable by monitoring the turbidity of a mixture of factor XIII, thrombin and calcium ions (Fig. 2). Under these conditions, there was a typical lag period followed by a logarithmic development of turbidity. Mixtures of factor XIII and thrombin alone did not become turbid, nor did mixtures of calcium ions and factor XIII. These observations are in complete accordance with a polymerization process developing as a direct result of the introduction of covalent bonds, presumably of the ε-(γ-glutamyl)lysine type. A schematic illustration of these events is depicted in Fig. 3.

DISCUSSION

The autopolymerization of factor XIII seems significant on several grounds. First, there are structural implications about the arrangement of chains in the

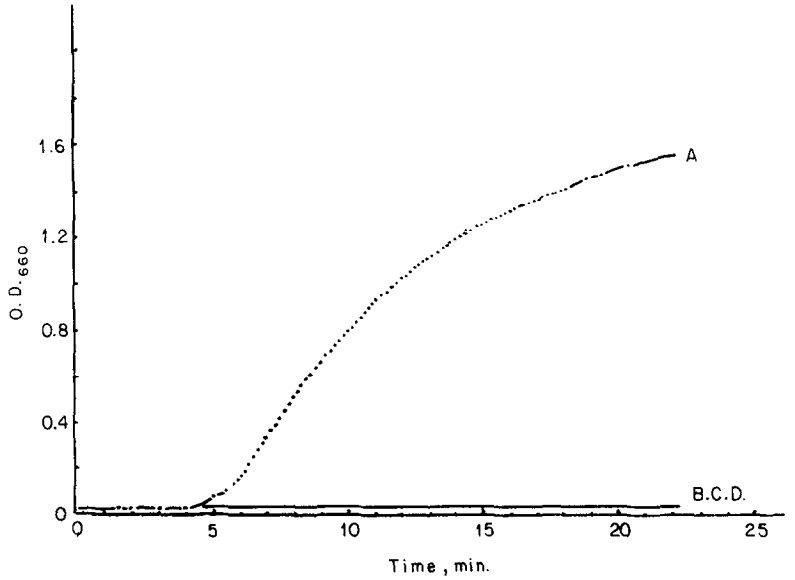

Fig. 2. Polymerization of factor XIII followed by turbidity measurement at λ = 660 mμ. A, thrombin (20 U/ml) and factor XIII (1 mg/ml) in the presence of 20 mM $CaCl_2$; B, thrombin (20 U/ml) and factor XIII (1 mg/ml) only; C, factor XIII (1 mg/ml) in the presence of 20 mM $CaCl_2$; D, thrombin (20 U/ml) and $CaCl_2$ only. In all cases the pH was maintained at 7.5 by a 0.05 M Tris buffer containing 0.1 M NaCl.

factor XIII molecule. Secondly, there are functional aspects which can be considered, among others, from the point of view of evolution, especially by comparison with the generation of other crosslinked fiber systems involved in hemostasis.

On the first count, of the two types of subunit found in plasma factor XIII, only a-chains are involved in the crosslinking process, indicating a high degree of specificity. Furthermore, the a-chain crosslinking is limited to the formation of dimers--as opposed to higher multimers--implying a rigid spatial order in the growth of the molecular chains (Fig. 3). Schwartz and co-workers (7) have reported that platelet factor XIII does not have b chains, only the a chain being essential for the transamidase function. It is likely that b-chains are environmental necessities for maintaining a-chains extra-cellularly. Whatever their role, it is clear that they do not have accessible crosslinking sites in either factor XIII or XIII*.

Functionally, it is interesting to compare factor XIII and fibrinogen.

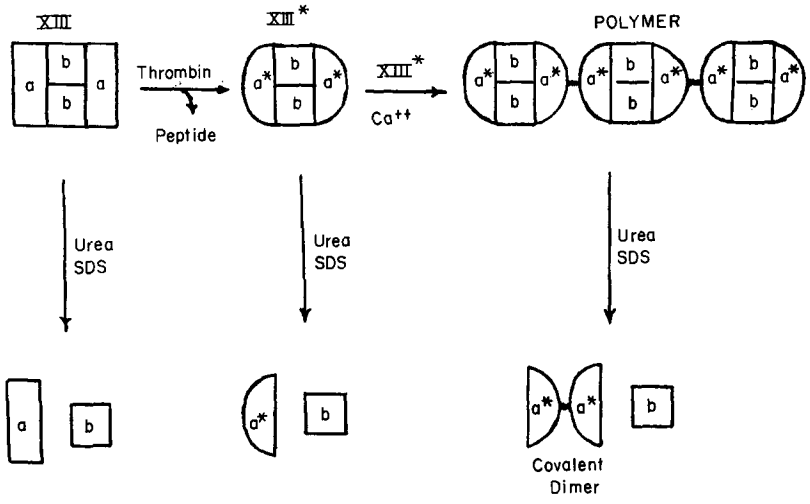

Fig. 3. Schematic depiction of events leading to autopolymerization of factor XIII.

Both proteins are substrates for thrombin, the same peptide linkages (arginyl-glycine) being cleaved in both kinds of molecule (Takagi and Doolittle, unpublished). Also, in both molecules the thrombin cleavage results in the release of peptide material from the amino-terminal end of at least one of the constituent chains. Now we find that the products of thrombin cleavage can be crosslinked in both instances by factor XIII*, the initial product being the dimerization of one particular kind of subunit chain. It will be of considerable interest to find if the factor XIII crosslinking site exists near the carboxy-terminal of a-chains in a comparable situation to the reciprocal crosslinking of γ-chains in fibrin (4).

The autopolymerization of factor XIII may be an evolutionary vestige of a primitive hemostatic scheme, perhaps involving the clumping of platelets. Alternatively, it may represent a regulatory mechanism for keeping the amount of active factor XIII* under control, since it is reasonable to presume that the polymerized material will be more readily localized and/or removed from the circulation by phagocytosing cells.

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