ACTIVATION OF FACTOR IX BY ACTIVATED FACTOR X: A LINK BETWEEN THE EXTRINSIC AND INTRINSIC COAGULATION SYSTEMS*

Frantisek KALOUSEK, William KONIGSBERG and Yale NEMERSON
Department of Molecular Biophysics and Biochemistry and the Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut, 06510, USA

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1. Introduction

Blood coagulation is initiated by two pathways which converge at a point where factor X is converted to factor X_a. In the intrinsic pathway, this reaction is catalyzed by activated factor IX; in the extrinsic, or tissue factor pathway, the conversion is catalyzed by a complex of tissue factor and factor VII [1–3]. Until recently, it had been thought that X_a acted only on prothrombin, but data from this laboratory have shown that factors X, X_a and VII all have bonds which are cleaved by X_a [4,5]. In view of the fact that X_a acts on three homologous proteins, prothrombin and factors VII and X [6], we studied its effect on a fourth homologous protein, factor IX. We now report that factor X_a converts factor IX to an active form. By examining the reaction products by electrophoresis in acrylamide gels containing sodium dodecylsulfate, we conclude that activation of the zymogen is accompanied by cleavage of at least two peptide bonds.

2. Materials and methods

Prothrombin and factors VII, X and X_a were prepared as previously described [4]. Factor IX was purified from the descending limb of the prothrombin peak obtained by DEAE-Sephadex chromatography of a barium citrate eluate of bovine plasma [5]. Factor VII was removed from the mixture by affinity chromatography on benzamidine coupled to agarose [7]. Prothrombin was removed by chromatography on heparin-agarose [8]; factor IX was freed of residual contaminants by preparative acrylamide gel electrophoresis as previously described [9]. The final preparation was purified about 20 000-fold, in good agreement with the reported value [8].

Factors IX and IX_a were assayed as described by Fujikawa et al. [11], except that factor IX-deficient canine plasma was used as the substrate.

Electrophoresis was performed in acrylamide gel slabs and stained for protein as previously described [4]. Gels were also stained for carbohydrate [10].

3. Results

Incubation of factor IX (3.0 mg/ml) with factor X_a resulted in the rapid appearance of coagulant activity (fig.1). At the highest enzyme concentration used (a ratio of 1:20, w/w) maximum activity was obtained in 30 min. Examination of the electrophoretic gel (fig.2), however, shows some factor IX to be present at this time suggesting that degradation is proceeding at about the same rate as is activation. In the absence of either calcium ions or phospholipids, no activation was observed (fig.1). Thus, the same cofactors are required for the factor X_a-catalyzed activation of factor IX as are required for X_a to cleave the susceptible bonds in factor X and X_a [4] and factor VII [5]. For activation of prothrombin, a plasma protein cofactor, factor V, is also required for optimal rates.

As noted in fig.1, with lower enzyme concentrations the reaction did not go to completion as judged by bioassay and by electrophoresis (not shown). Activated factor IX requires factor VIII, a non-enzymatic plasma protein, to accelerate the coagulation of plasma [1].

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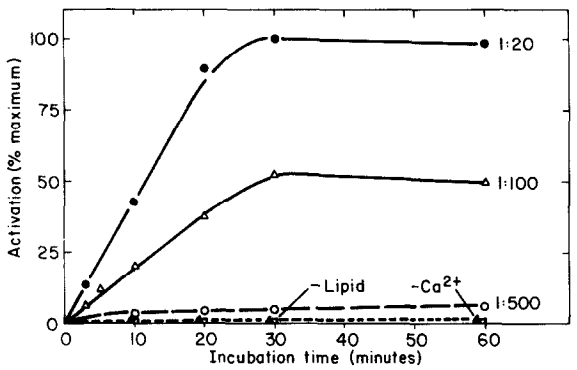

Fig.1. Time course of activation of factor IX by activated factor X. The incubation contained 1.5 mg factor IX (estimated from the A_{280}, assuming $A^{1\%}_{280} = 10$), 0.05 mg mixed brain phospholipids [4], 12 μmoles $CaCl_2$ and the indicated amount of X_a in 0.5 ml 0.05 M Tris, 0.1 M NaCl, pH 7.4. After incubation at 37°C for the indicated intervals, aliquots were removed, diluted in the same buffer containing 0.2% ovalbumin and assayed for activated factor IX. In the experiments in which $CaCl_2$ or phospholipids were omitted, the X_a to IX ratio was 1:20.

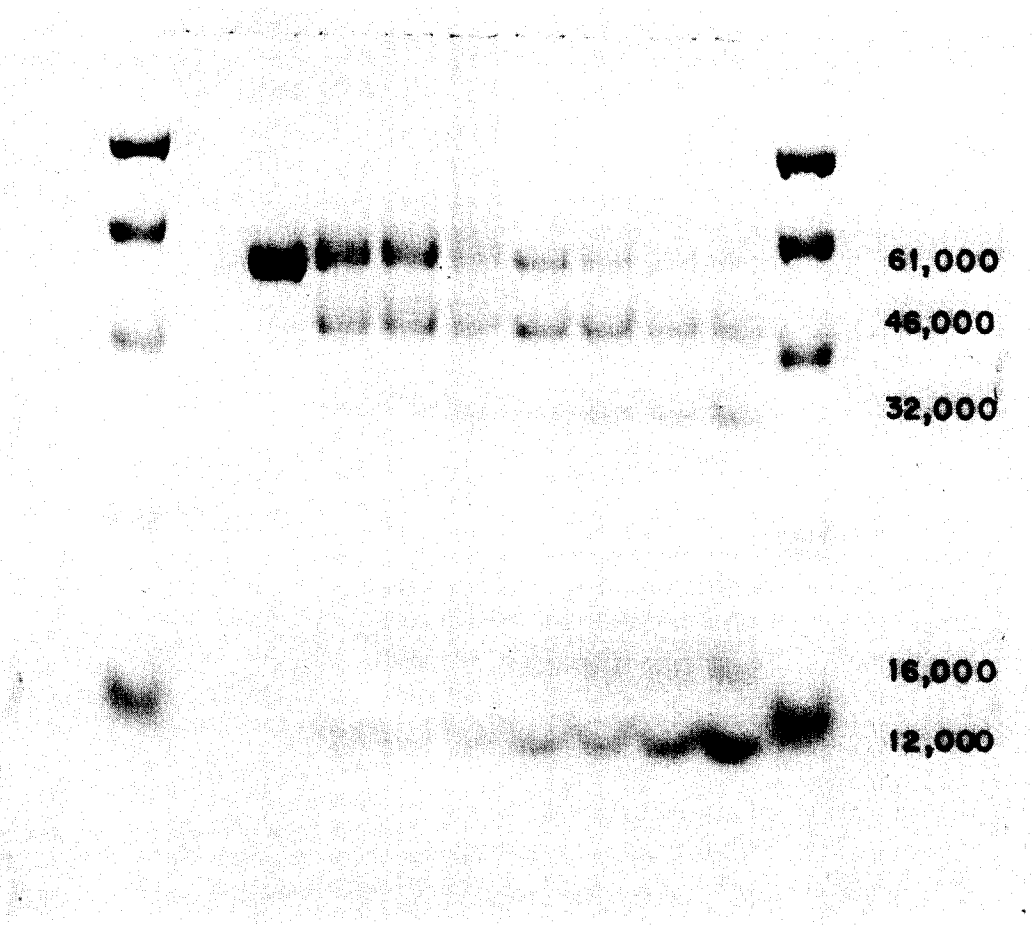

Fig.2. Sodium dodecylsulfate polyacrylamide gel electrophoresis of the activation of factor IX by activated factor X. Conditions the same as for fig.1. Activation was accomplished with a 1:20 ratio of X_a to IX. The outer channels contain molecular weight standards: phosphorylase, bovine serum albumin, ovalbumin, chymotrypsinogen and pancreatic ribonuclease. At 0, 3, 5, 10, 15, 20, 30 and 60 min. 30 μg of protein were removed and added to an equal volume of 4% sodium dodecylsufate–5% 2-mercaptoethanol and heated in a boiling water bath for 3 min. The molecular weights estimated by comparing the mobility of the various bands to the standards are indicated on the figure.

Our product was shown to be factor IX_a by substituting factor VIII-deficient plasma for factor IX-deficient plasma in the assay. Under these conditions there was no shortening of the clotting time even when factor IX was fully activated.

The formation of activity was accompanied by the appearance of several new bands on the gel (fig.2). A new band with an apparent mol. wt of 46 000 appears first. Subsequently, bands at mol. wt 32 000, 16 000 and 12 000 are noted. When a similar gel was stained for carbohydrate, all bands except the one at 16 000 were positive. The bands at mol. wt 32 000 and 16 000 apparently correspond to the heavy and light chains of factor IX_a reported by Fujikawa et al. [11]. The presence of carbohydrate may explain the different estimates of molecular weights.

4. Discussion

When blood clots spontaneously, factor IX is converted to an active form, factor IX_a, by the action of activated factor XI [12]. Activated factor IX, in turn, catalyzes the conversion of factor X to X_a [1]. In the tissue factor pathway, a complex of tissue factor and factor VII promotes this conversion [2,3]. In this paper we show that factor X_a can activate factor IX; thus, we have established that the tissue factor pathway can activate the intrinsic system by activating factor X which, in turn, converts factor IX to IX_a. A link between the intrinsic and extrinsic coagulation pathways has been suggested [12,13], but the enzyme involved has not been previously identified.

The amount of coagulant activity formed when factor IX is incubated with activated factor X is a direct function of the concentration of factor X_a (fig.1). This same phenomenon is observed when factor X is activated by the tissue factor–factor VII complex [5,7] and when prothrombin is activated by a complex of X_a, factor V, calcium ions and phospholipids [15]. In the former case, this damped activation has been ascribed to proteolytic inactivation of factor VII by X_a [5,7]. In the latter, inactivation of the non-proteolytic cofactor, factor V, limits prothrombin activation [15]. The mechanism of the damping of the X_a-catalyzed activation of factor IX is not known, but may involve the inactivation of X_a by activated factor IX.

Fujikawa et al. [11] investigated the mechanism of activation of factor IX by factor XI_a, and proposed that the first event is a cleavage of one peptide bond converting a single chain zymogen into a two chain intermediate. A subsequent cleavage releases an activation peptide yielding the active enzyme. The data we present suggest the same activation mechanism pertains when the reaction is catalyzed by activated factor X. Chemical confirmation of the identity of the cleavage sites awaits further study.

In view of the fact that patients with a deficiency of factor XI have only a mild hemorrhagic disorder, an alternative mechanism for the activation of factor IX has often been suggested. With the identification of factor X_a as an enzyme which can convert factor IX to IX_a, we propose that the tissue factor pathway can generate sufficient activated factor X to activate factor IX, by-passing factors XII and XI. Owing to the fact that factor XI-deficient patients *do* have a hemorrhagic tendency, we surmise that this alternate activation of factor IX is less efficient than activation catalyzed by factor XI_a. Indeed, comparing the data presented by Fujikawa et al. [11] to those presented in fig.1 of this paper, it appears that activated factor X catalyzes the conversion of factor IX at about 20% the rate obtained with activated factor XI.

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