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ACTIVATION OF DUCK PROTHROMBIN BY FACTOR Xa AND THROMBIN*

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Prothrombin isolated from duck sodium citrate plasma was activated in a system containing duck factor Xa and calcium ions. Polyacrylamide gel electrophoresis showed that intermediates and the final product, thrombin, of *Mr* in the range 21 500 - 52 000 were present in the incubation mixture Serine and isoleucine were found to be the *N*-terminal amino acids of the intermediate form 1 and thrombin, respectively.

Both human and bovine prothrombins are glycoproteins consisting each of a single polypeptide chain of molecular weight of about 70 000. At the final stages of blood coagulation this protein converts fibrinogen into fibrin. Activation of prothrombin by factor Xa complexed with factor Va, phospholipids and calcium ions results in formation of thrombin and two activation products: fragment 1 and fragment 2 (Owen *et al.*, 1974; Magnusson *et al.*, 1975; Grant & Suttie, 1976; Seegers *et al.*, 1975, 1981). Factor Xa is responsible for splitting of two polypeptide bonds: Arg_{274}-Thr_{275} and Arg_{323}-Ile_{324} to form fragment 1·2 and thrombin via intermediate 2. The generated thrombin cleaves the bonds Arg_{156}-Ser_{157} both in the prothrombin and fragment 1·2, releasing fragment 1 and intermediate form 1 from the former and fragment 1 and fragment 2 from the latter (Esmon *et al.*, 1974).

In this paper activation of duck prothrombin by duck factor Xa and thrombin was demonstrated and intermediate product appearing during this process was identified.

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MATERIALS AND METHODS

Reagents. DEAE-Sephadex A-50 was from Pharmacia Fine Chemicals (Uppsala, Sweden); *N,N*-dimethylformamide, imidazole, DC-Fertigplatten Kieselgel 60 F-252, pyridine and ammonia solution from Merck (Darmstadt, F.R.G.); 1-dimethylaminoaphthalene-5-sulphonyl chloride and toluene from Koch-Light Labs. Ltd (Colnbrook, Bucks., England); soybean trypsin inhibitor from POCh (Gliwice, Poland); phenylmethanesulphonyl fluoride (PMSF) from Sigma Chem. Comp. (St. Louis, MO., U.S.A.); heparin from Polfa (Warsaw, Poland); dansyl-amino acids from Serva (Heidelberg, F.R.G.); ethylene chlorohydrin from VEB Jenapharm-Laborchemie Apolda (G.D.R.); Stypven Russell's viper venom protease from Wellcome Reagents Ltd (England). All other reagents were of analytical grade.

Prothrombin was isolated from fresh duck plasma according to Grant & Suttie (1976).

Factor X was isolated from duck blood by the method of Esnouf *et al.*, (1973) and converted to the active form (Xa) with Stypven Russell's viper venom protease (Fujikawa *et al.*, 1974).

Prothrombin was activated by factor Xa according to Grant & Suttie (1976) at 37°C.

N-Terminal amino acids were identified as described by Gros & Labouesse (1969).

Electrophoresis in 7.5% polyacrylamide gel containing sodium dodecyl sulphate (SDS) was performed according to Weber & Osborn (1969).

RESULTS AND DISCUSSION

Activation of duck prothrombin in the system containing factor Xa and calcium ions led to the appearance of intermediates: fragment 1, fragment 2, fragment 1·2, intermediate form 1 and thrombin (Fig. 1A), of M_r ranging from 21500 to 52000 (Table 1). When the generated thrombin was inhibited by diisopropyl fluorophosphate (DFP) only fragment 1·2 and thrombin were present in the incubation mixture (Fig. 1B). Serine and isoleucine were found to be the *N*-terminal residues of the intermediate form 1 and thrombin, respectively. On the basis of these results we can assume that duck factor Xa, like mammalian factor Xa (Magnusson *et al.*, 1976; Seegers *et al.*, 1975; Grant & Suttie, 1976) cleaves probably the same binding sites in prothrombin: Arg_{274}-Thr(Ile)$_{275}$ and Arg_{323}-Ile_{324} to form the active enzyme, i.e. thrombin. However, this suggestion still requires confirmation.

The appearance of fragment 1 and fragment 2 as well as fragment 1 and intermediate form 1 observed on activation of duck prothrombin (Fig. 1A) could be due to presumable splitting by the generated thrombin

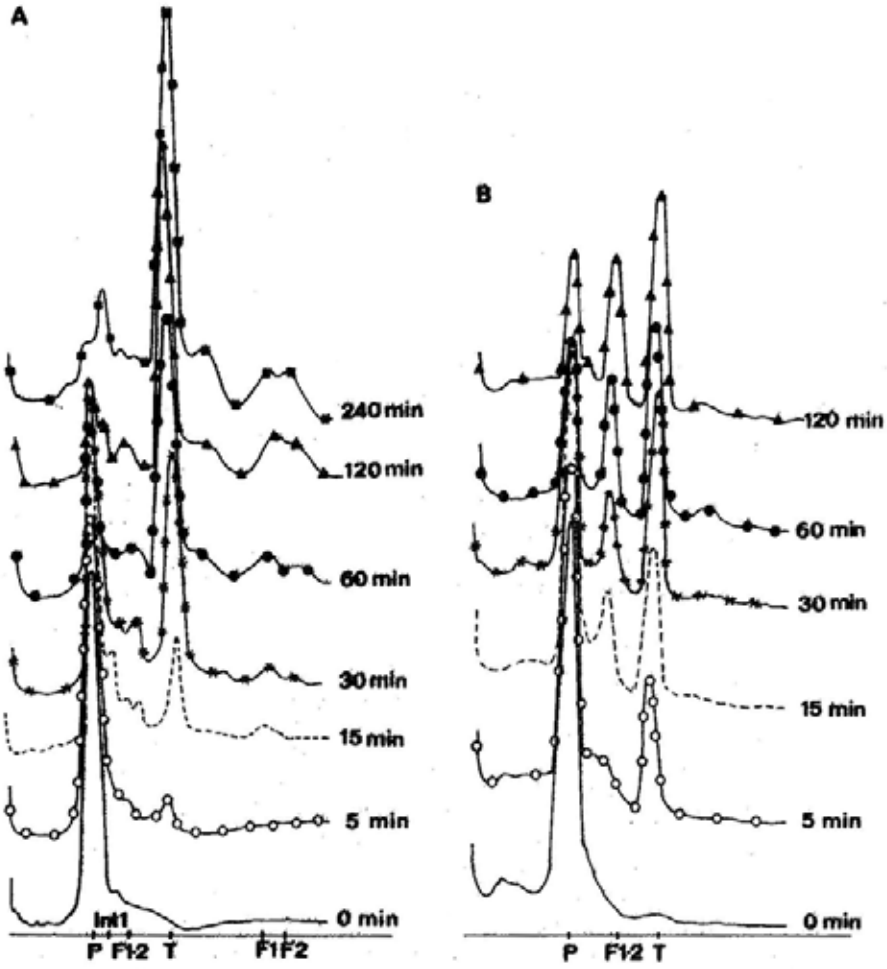

Fig. 1. Densitometric scans of SDS-polyacrylamide gel electrophoregrams of the products formed during activation of duck prothrombin by factor Xa. A, in the absence and B, in the presence of diisopropyl fluorophosphate (inhibition of thrombin action). P, Prothrombin; T, thrombin; Int1, intermediate form 1; F1·2, fragment 1·2; F1, fragment 1; F2, fragment 2

Table 1

Molecular weight of prothrombin and prothrombin activation products

Protein	M_r	Incubation time (min)						
		0	5	15	30	60	120	240
Prothrombin (duck)	77000	+	+	+	+	±	±	−
Intermediate form 1	52000	−	−	−	±	+	+	+
Fragment 1·2	45000	−	−	±	+	+	+	+
Thrombin	33000	−	±	+	+	+	+	+
Fragment 1	23500	−	−	+	+	+	+	+
Fragment 2	21500	−	−	−	±	+	+	+

+, Presence; −, absence; ±, trace amount.

of the bond between Arg_{156} and Ser_{157} in the proenzyme. A similar mechanism of goose protrombin activation by factor Xa has been suggested by Banaś-Gruszka *et al.* (1982). It should be noted that inactivated thrombin does not split the Arg_{156}-Ser_{157} bond.

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