A C T A U N I V E R S I T A T I S L O D Z I E N S I S
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ACTIVATION OF GOOSE PROTHROMBIN BY THROMBIN

Prothrombin isolated from goose sodium citrate plasma was activated in a system containing goose thrombin and calcium ions. Polyacrylamide gel electrophoresis showed two intermediates of molecular weight of 21 000 and 57 000 in the digest. Ser was found to be the N-terminal amino acid residue for intermediate form 1.

INTRODUCTION

Prothrombin is converted into thrombin by action of factor Xa. It can be also activated by direct action of thrombin which splits Arg_{156} - Ser_{157} bond in prothrombin releasing fragment 1 and intermediate form 1 [8-10]. The presence of a non active intermediate form 1 is a very important factor of the mechanism regulating thrombin concentration in plasma since this form is not sensitive to thrombin action. Intermediate form 1 does not contain a vitamin K dependent fragment with binding sites for phospholipids and calcium ions. It cannot, therefore, be activated in phospholipid-dependent system.

In this paper we examined goose prothrombin activation process using thrombin and characterized intermediate products.

MATERIALS AND METHODS

Prothrombin was isolated from fresh goose sodium citrate plasma (9 : 1) according to the method of E s n o u f, L l o y d and J e s t y [4]. Electrophoretically homogeneous prothrombin preparations were additionally purified using the method of

Benarous, Labie and Josso [3]. The goose thrombin preparations used to activate prothrombin were obtained according to Fenton et al. method [5]. Prothrombin activation process in the presence of homologeneous thrombin and calcium ions was performed by Grant and Suttie method [6]. Prothrombin samples (200 µg) were incubated with thrombin (10 µg) in the presence of calcium ions (0.01 M) for 0, 1, 5, 10, 30 and 60 min. at 37°C. The reaction was stopped by adding sodium dodecylsulphate (SDS) to a final concentration of 1%, and heating to 70°C (water bath) for 5-10 min. Identification of N-terminal amino acid was carried out by the method of Gros and Labouesse [7].

Prothrombin activation products (fragment 1 and intermediate form 1) were examined by SDS polyacrylamide gel electrophoresis [11].

RESULTS AND DISCUSSION

Geese prothrombin and thrombin preparations used in experiment appeared to be electrophoretically homologeneous since during the electrophoresis in 7.5% polyacrylamide gel they migrated as a single bond (not shown). It was found that prothrombin activation process in a system containing thrombin and calcium ions causes the appearance of intermediate degradation products already after 10 min. SDS polyacrylamide gel electrophoresis showed the presence of two protein fragments of molecular weights 21 000 and 57 000 (Tab. 1, Fig. 1). Chromatographic separation of dansyl derivatives showed that serine is the terminal residue for intermedia-

Table 1

Molecular weights of prothrombin and prothrombin activation products
Masa cząsteczkowa protrombiny i produktów aktywacji protrombiny

Protein	Mol. weight	Incubation time (min)					
		0	1	5	10	30	60
Prothrombin (goose)	79 000	+	+	+	+	±	-
Intermediate form 1	57 000	-	-	±	+	+	+
Fragment 1	21 000	-	-	-	+	+	+

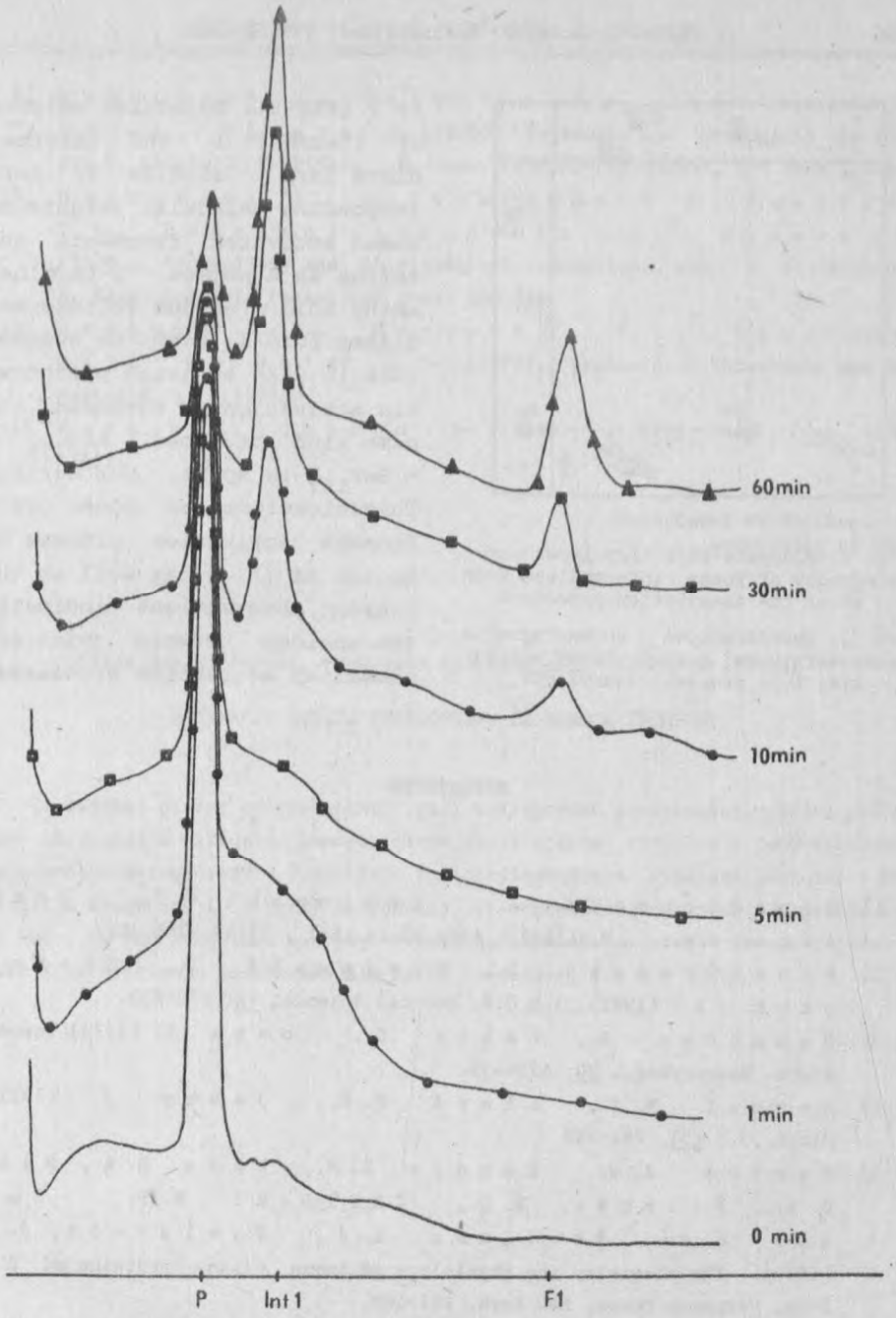

Fig. 1. Densitometer scans of stained polyacrylamide gel electrophoresis of the time course of the activation of goose prothrombin by thrombin (incubation time 0, 1, 5, 10, 30 and 60 min.), Int 1 - intermediate form 1, F 1 - fragment 1, P - prothrombin

Rys. 1. Densytogramy rozdziałów elektroforetycznych protrombiny i produktów aktywacji trombinowej (czas inkubacji 0, 1, 5, 10, 30, 60 min), Int 1 - forma pośrednia 1, F 1 - fragment 1, P - protrombina

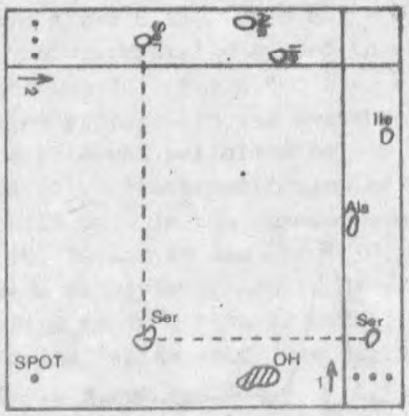

Fig. 2. Bidimensional thin layer chromatography of goose intermediate form 1 after the dansylation procedure

Rys. 2. Dwukierunkowa chromatografia cienkowarstwowa gęsiej formy pośredniej 1 po procesie dansylacji

te 1 (Fig 2). Molecular weights of fragment 1 and intermediate form 1 similar to corresponding molecular weights of human activated fragments and serine as a common N-terminal amino acid residue in intermediates form 1 seem to suggest that in case of avian prothrombin activation by thrombin, the same kind of bond Arg_{156} - Ser_{157} is split. Our earlier investigations on goose prothrombin activation process by factor Xa [1, 2] as well as the present observations indicated the analogy between avian and mammalian activation processes.

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AKTYWACJA GĘSIEJ PROTROMBINY ZA POMOCĄ TROMBINY

Ze świeżej plazmy cytrynianowej gęsi wyizolowano protrombinę, którą poddawano aktywacji w układzie homologicznym zawierającym trombinę i jony wapniowe. Za pomocą elektroforazy w 7,5% żelu poliakryloamidowym wykazano obecność w hydrolizie fragmentu 1 i formy pośredniej 1, odpowiednio o masach cząsteczkowych 21 000 i 57 000. Analiza końcowych reszt aminokwasów wykazała obecność seryny jako N-terminalnego aminokwasu dla formy pośredniej 1.