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OCCLUSION OF BASIC PROTEINS BY FIBRIN: STUDIES WITH ^{14}C-ARGININE LABELLED PROTEINS

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SUMMARY: With the use of ^{14}C-arginine labelled basic proteins isolated from cytoplasma of Ehrlich ascites tumor cells it has been shown that these proteins are occluded by fibrin clots under the influence of thrombin. The occlusion of these proteins depends on their concentration. The experiments indicated that 2.5 ug of these proteins were occluded per one mg of fibrinogen in the presence of thrombin. The basic proteins occluded by fibrin make the clots resistant to the fibrinolityc action of plasmin. The clinical role of arginine-rich basic proteins appearing in circulation in malignancy have been discussed briefly.

INTRODUCTION

The retention of plasma proteins during the process of fibrin clot formation was defined by Morrison as "occlusion"/1/. By means of radioactive techniques Regoeczi observed occlusion of 13 different plasma proteins by human fibrin. Among plasma proteins plasminogen showed the highest occlusion by fibrin. Furthermore, myeloma proteins and Bence Jones proteins were also occluded by fibrin /2/.

In comparison to the control samples, increased values of arginine in the fibrin clot from the blood of patients with neoplasms were observed earlier. Similar changes in rats with Guérin tumor were noted /3/.

It seemed advisable to study in vitro the effect of ^{14}C-arginine labelled basic proteins isolated from the cytoplasma of Ehrlich ascites tumor cells on the thrombin induced fibrin clot.

MATERIALS AND METHODS

Bovine fibrinogen was prepared according to Kekwick et al. /4/. Thrombin was produced by Warsaw Serum and

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Vaccine Plant, Poland and expressed in NIH units /5/ and plasmin by Novo Industri A/S, Copenhagen, Denmark.

^{14}C-arginine labelled basic proteins were isolated from cytoplasma of Ehrlich ascites tumor cells by fractionation on the CM-cellulose column chromatography described in detail elsewhere /6/. These proteins contained 14 % of arginine and exhibited specific radioactivity about 14387 cpm/mg.

Occlusion of ^{14}C-arginine labelled basic proteins by fibrin clot was estimated by means of total radioactivity measured in the clot in β-liquid scintillation spectrometer. For this purpose a system composed of 0.25 ml of fibrinogen /0.5%/, 0.25 ml of basic proteins /various concentration/ and 0.5 ml of thrombin /20 U/ml/ was incubated at 37^{o} for 30 min. The clot was separated from the solution by means of a silk filter, washed three times with 0.9% NaCl and, subsequently, with distilled water. The clot was solubilized in 1 ml 2N NaOH by heating at 100^{o} for 5 min. Samples /0.3 ml/ neutralized with HCl were added to 3 ml of Bray's solution /7/ and ^{14}C-radioactivity in all samples was measured /Nuclear Chicago/.

The effect of ^{14}C-arginine labelled basic proteins upon the fibrinolytic activity of plasmin in fibrin clots was evaluated in a system containing: 0.25 ml of plasmin /0.1%/ + 0.25 ml of basic proteins /various concentrations/. After a 5 min incubation 0.25 ml of fibrinogen /0.5%/ and 0.25 ml of thrombin /40 U/ml/ were added and from that moment the clot lysis time were measured.

RESULTS

Table illustrates the effect of ^{14}C-arginine labelled cytoplasma basic proteins on the clot fibrin formation under the influence of thrombin at constant concentration of fibrinogen 1.25 mg/ml. All clots formed in the presence of labelled proteins showed radioactivity dependent on the concentration of basic proteins and their specific radioactivity. The proteins at concentration lower than 3.1 /ug/ml in samples were fully occluded by fibrin. The concentration of basic proteins above 25 /ug per 1.25 mg

Table. The occlusion of ^{14}C-arginine labelled basic proteins by fibrin[X]

Basic proteins		Total radioactivity of basic proteins in fibrin clot cpm/min,ml	Fibrinolysis time sec.
Final concentration /ug/ml	Radioactivity cpm/min,ml		
50	1628	186	1130
25	813	181	960
12.5	407	153	900
6.25	203	133	810
3.125	101	100	750
0.000	-	-	720

[X]Results represent the mean values of three experiments for each of the protein concentration.

of fibrinogen did not increase the fibrin clots radioactivity. It was calculated that per one mg of fibrin not more than 2.5 /ug of arginine labelled basic proteins can be occluded.

Arginine-rich basic proteins occluded by fibrin even in a very low concentration showed an antifibrinolytic action related to the concentration of these proteins. In final concentration of 50 /ug these proteins prolonged fibrinolysis time from 720 sec to 1130 sec.

DISCUSSION

The results obtained with ^{14}C-arginine labelled basic proteins isolated from cytoplasma of Ehrlich ascites tumor cells indicate that these proteins are occluded by fibrin clots. They can not be removed from the clots by repeated washings. Thus it is to be assumed that these proteins are strongly built-up into the structure of a thrombin induced fibrin clots. After adding higher concentration of basic proteins than 50 /ug to fibrinogen, fibrin-like clot is formed without thrombin.

The basic proteins occluded by fibrin clots possibly alter their structure and act antifibrinolytically. The

occlusion of basic proteins by fibrin clots makes the clot resistant to the fibrinolytic action of plasmin. It has been found previously that if the fibrin clot is formed in the presence of calf thymus histones or arginine-rich basic proteins derived from Guérin epothelioma, these proteins are occluded by fibrin and exhibit an anti-fibrinolytic action /8/.

It should be stressed that the basic proteins which are abundant in the neoplastic cells /9,10/ may be released into the extracellular spaces and, further, into the circulation /11/. In final stages of tumor growth the liberation of these proteins or of their degradation products can be connected with the disintegration of tumor cells. In patients with malignancy, possibly as a consequence of this fact, the increase of plasma tolerance to heparin has been observed /12/. In such cases basic proteins may be an important factor contributing both to the occlusion by fibrin, particularly when coagulation abnormalities are present, and to the formation and deposition of fibrin-like material /13/.

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