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ACCELERIN (FACTOR V), A CATABOLIC PRODUCT OF FIBRINOGEN

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A method of isolating a highly active and highly purified preparation of human Ac-globulin (factor V) is described. It is shown that factor V is part of the fibrinogen molecule and is an intermediate product of its conversion under the action of thrombin or of another enzyme with similar action.

KEY WORDS: *Fibrinogen; human Ac-globulin (factor V); purification and properties.*

Ac-globulin (factor V) is a plasma protein which accelerates the conversion of prothrombin in a reaction catalyzed by factor Xa, phospholipids, and Ca^{++}. Ac-globulin (accelerin), especially human, is labile and is inactivated during blood clotting. Difficulties accordingly arise in the isolation of accelerin and the study of its properties. Human factor V has so far hardly been investigated.

The object of this work was to obtain a highly active and highly purified preparation of human Ac-globulin and to study its physicochemical and immunological properties.

EXPERIMENTAL METHOD

Factor V was isolated from human plasma previously treated with $BaSO_4$ to remove the prothrombin complex. This plasma was adsorbed on DEAE-cellulose DE-52 in 0.05 M phosphate buffer, pH 7.0, the accelerin was eluted with 0.4 M phosphate buffer, pH 7.0, and the eluate was chromatographed on a column with Amberlite C G-50 [1]. The solution of factor V in 0.3 M phosphate buffer obtained after chromatography was fractionated with ammonium sulfate and the protein precipitated at 25-70% saturation with $(NH_4)_2SO_4$ was collected. The resulting precipitate was then subjected to gel filtration on a Sephadex G-200 column. The activity of the factor V was determined by a one-stage method [2].

EXPERIMENTAL RESULTS AND DISCUSSION

One of the basic conditions for obtaining a highly active preparation of Ac-globulin is that the whole procedure of purification must be carried out within 1 or 2 days. The

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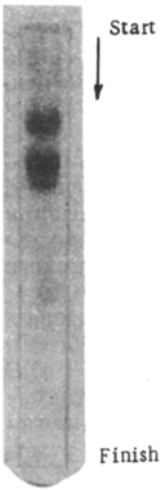

Fig. 1. Anode electrophoresis of Ac-globulin in 7% polyacrylamide gel in the presence of sodium dodecylsulfate.

TABLE 1. Changes in Ac-Globulin Activity in Preparations of Human and Bovine Fibrinogen during Keeping before (A) and after (B) Incubation with Thrombin (1 unit/ml)

Day of keeping at −20°C	Activity (in %)					
	bovine fibrinogen		human fibrinogen		human fibrinogen*	
	A	B	A	B	A	B
1-	10	300	<1	14	<1	12
3-	17	350	<1	9,6	1,8	168
5-	38	540	<1	5,4	2,6	186
7-	120	960	<1	4	<1	444
9-	22	240	<1	4	<1	132

*Sample kept in the presence of ammonium sulfate.

preparation obtained had a specific activity 5000 times greater than the accelerin activity of adsorbed plasma. The purified protein was stable. When kept for 1 month at −20°C its activity remained unchanged. Activation of factor V during keeping in the presence of ammonium sulfate was observed, and in some cases its activity increased a hundredfold.

The sample obtained was homogeneous on ultracentrifugation and it sedimented in one peak, but it separated into two fractions on disk electrophoresis in polyacrylamide gel (PAG). The sample was uncontaminated by other clotting factors, including fibrinogen, as was shown by biological and immunological methods.

Some of the physicochemical properties of human accelerin were studied: amino-acid composition, molecular weight, and N-terminal amino acids. The molecular weight of Ac-globulin was determined and its quaternary structure studied by electrophoresis in PAG in the presence of sodium dodecylsulfate after preliminary treatment of the protein with β-mercaptoethanol [10]. Four components with molecular weights of 100,000, 60,000, 50,000, and 30,000 were obtained by electrophoresis (Fig. 1).

N-terminal amino acids were determined by the dansylation method [4]. N-terminal glycine, tyrosine, and traces of N-terminal alanine and leucine were found in the accelerin sample.

Fractions 1-9 of fibrinogen are known to consist of the same components as were found in the course of this investigation of human accelerin and to have the same N-terminal amino acids (alanine, glycine, and tyrosine) [6]. The results of these experiments, together with

analysis of recent observations regarding the heterogeneity of fibrinogen [5-7], suggest that factor V is a part of the fibrinogen molecule formed by the action of thrombin during blood clotting.

To confirm this hypothesis a series of experiments was carried out. Purified specific Hyland antifibrinogen (Hyland Division, Travenol Laboratories Inc., Costa Mesa, California 92626, USA), in a dilution of 1:20, was found to reduce the activity of Ac-globulin solution by five times.

Fibrinogen was isolated from bovine and human plasma by Blombäck's method [3]. The clotting power of the resulting preparations was 91%. Bovine fibrinogen possessed 10% of the activity of factor V, which was increased to 300% in the presence of thrombin (Table 1). If this activity were due to the presence of Ac-globulin as an impurity in the fibrinogen preparation, it ought to decrease during keeping of the fibrinogen. Yet it is known that during prolonged keeping at a temperature of −20°C fibrinogen undergoes degradation, and on the assumption that factor V is a breakdown product of fibrinogen, its activity ought then to increase. Bovine and human fibrinogen were kept at −20°C and Ac-globulin activity was determined every 2 days. As Table 1 shows, during keeping of bovine fibrinogen activation of accelerin was observed and its activity reached a maximum on the seventh day. It then started to become inactivated, evidently because of the simultaneous formation and accumulation of fibrinogen breakdown products which inhibited the thrombin activation reaction, thereby interfering with the detection of Ac-globulin [9].

As regards human fibrinogen, it in fact possessed no accelerin activity (Table 1). Accelerin activity likewise was not found in the fibrinogen at any time during keeping at −20°C. In view of the experimental data on activation of factor V by ammonium sulfate solution, human fibrinogen was kept in a 50% solution of $(NH_4)_2SO_4$ and kept at −20°C. The accelerin activity, which was detectable only in incubation of human fibrinogen with thrombin, increased during keeping. Without thrombin the activity was very low. These conditions evidently favor deaggregation of the fibrinogen molecule and facilitate the liberation of its breakdown products, especially of factor V. Activity of Ac-globulin was thus found in both bovine and human fibrinogen, and it increased under conditions causing degradation of fibrinogen.

According to data in the literature [6], soluble fraction 1-9 of fibrinogen is catabolized most intensively. This fibrinogen fraction contains hardly any intact α chain but only the products of its decomposition, evidently by thrombin. This fraction, in accordance with our hypothesis, ought to contain the highest percentage of accelerin. Fibrinogen fractions 1-4, 1-8, and 1-9 were obtained from human plasma by Mosesson's method [8]. Fraction 1-4 possessed no accelerin activity without preliminary incubation with thrombin, and after activation with thrombin it had 5% of activity, fraction 1-8 had 1% of activity of factor V without thrombin and 10% in the presence of thrombin, whereas fraction 1-9 had 12% of accelerin activity, which increased to 400% after activation by thrombin. Consequently, fibrinogen fraction 1-9 possessed maximal activity, and our hypothesis was confirmed.

The results suggest that during blood clotting and, specifically, in the process of conversion of fibrinogen into fibrin under the influence of thrombin, Ac-globulin is formed at the same time as an intermediate product of this conversion. The factor V thus formed considerably accelerates the process of thrombin activation and this, in turn, stimulates the liberation of Ac-globulin until the moment of clot formation, when activation of thrombin ceases and mechanisms inhibiting it come into operation.

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EFFECT OF EXOGENOUS DNA ON CONTENT AND BIOSYNTHESIS OF ENDOGENOUS DNA IN RAT BONE MARROW

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The effect of exogenous DNA on the content and rate of biosynthesis of endogenous DNA in rat bone marrow was studied. After injection of high-polymer homologous DNA into intact rats the endogenous DNA content per gram bone marrow was found to be reduced for the first 3 days (the decrease was most marked after 3 days, namely by 36%) and it returned to normal by the 6th day. The rate of DNA biosynthesis in rat bone marrow was increased after 18 h (when it was twice as high as in the control) and after 6 days (by 58%), and it was close to normal again 1-3 days after injection of DNA.

KEY WORDS: *Exogenous DNA; bone marrow; rate of DNA biosynthesis.*

Data showing that homologous high-polymer exogenous DNA, if injected parenterally into rats, causes an increase in proliferative activity of the bone marrow blast cells [3] and differentiation of hematopoietic stem cells [1]. Since the molecular basis of these processes is nucleic acid metabolism, in order to understand the action of exogenous DNA information is required on its effect on endogenous DNA metabolism.

The object of this investigation was to study the content and rate of biosynthesis of DNA in rat bone marrow at various times after administration of exogenous DNA.

EXPERIMENTAL METHOD

Male Wistar rats weighing 170-180 g were used. Thymidine-^{3}H was used as the label. The experimental animals were given DNA, obtained by a modified Kay's method [7], by intraperitoneal injection in a dose of 5 mg per rat in SSC solution in a concentration of 2-2.25 mg/ml. The molecular weight of the DNA was 20-25 million daltons, protein impurities did not exceed 0.5%, and RNA 1%. Control rats received the corresponding volume of solvent; thymidine-^{3}H was injected in a dose of 200 μCi/100 g body weight 1 h before sacrifice. The rats were decapitated 18 h and 1, 3, and 6 days after injection of DNA. Bone marrow for investigation was taken from the femur and tibia. The DNA content in the bone marrow was determined by the method of Schmidt and Thannhauser [8] followed by spectrophotometry by Spirin's method [6]. Radioactivity in a neutralized aliquot of DNA digest was determined in a liquid scintillation counter. The specific radioactivity of DNA (in counts/min/mg DNA) was calculated.

The experimental data were subjected to statistical analysis with calculation of the arithmetic mean values and the errors of the means. The significance of differences was determined by Student's criterion [4].

EXPERIMENTAL RESULTS

The results in Table 1 show that the DNA content per gram bone marrow of the experimental rats fell during the 3 days after injection of DNA by comparison with its level in the control. The decrease in the DNA content was greater after 3 days (by 36% com-

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