EFFECT OF FIBRIN-PEPTIDES A AND B ON BLOOD-CLOT RETRACTION

T. A. Borisova and G. I. Edokova

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Fibrin-peptides A and B isolated from bovine fibrinogen influence clot retraction. If injected intravenously the peptides lower the degree of retraction, and peptide B also prolongs the retraction time. In vitro both peptides accelerate the beginning, lengthen the time, and reduce the degree of retraction.
KEY WORDS: fibrinogen; fibrin-peptides; clot retraction.

Most workers consider that blood-clot retraction takes place only if intact platelets are present [2, 8] and that they act in the early stages of clotting as centers of approximation of fibrin strands [13, 14]. Retraction has a definite dependence not only on the number and state of the platelets, but also on many other factors and, in particular, on the ratio between the concentrations of fibrinogen and thrombin [12]. During conversion of the molecule of fibrinogen into fibrin (through the action of thrombin) two peptides, A and B, with physiological activity are removed from its N-terminal part [4, 10, 11]. Peptide B potentiates and prolongs the contracting effect of bradykinin [10], possesses vasoconstrictor activity in sympathectomized rats [11], and increases the pressure in the pulmonary artery of the rabbit [4]. Peptide A is also biologically active. It has been shown [6] that removal of peptide A only from the fibrinogen molecule is an essential condition for the beginning of polymerization. Peptide A also affects the rate of action of thrombin [5].

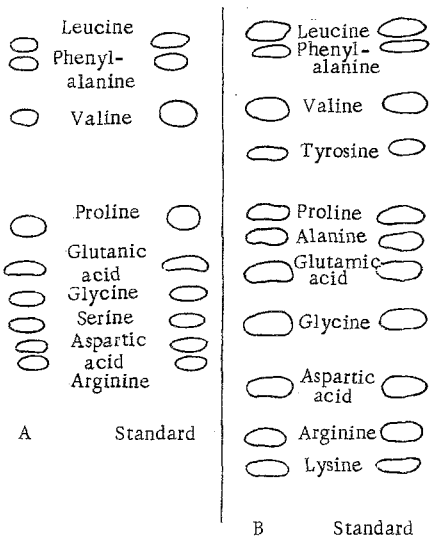

Fig. 1. Amino-acid composition of fibrin-peptides A and B isolated from bovine fibrinogen.

In this investigation the effect of peptides A and B, isolated from bovine fibrinogen, on the process of clot retraction was studied.

EXPERIMENTAL METHOD

Peptides A and B were isolated from bovine fibrinogen and purified by the method described previously [1]. The isolated peptides were hydrolyzed in sealed ampules in 6 N HCl for 24 h at 110°C. Next, 10-20 μl of the hydrolyzed material was chromatographed on No. 1 paper (VEB Niederschlag) in a system of butanol, acetic acid, and water (4:1:5). To study the process of retraction, a conductometric method was used and changes in the electrical resistance of the blood and the clot were recorded on an electroretractograph [3]. The retraction process was characterized by the time to the beginning of retraction (in min), the retraction time (in min), and the initial (R_1) and final (R_2) resistance of the clot (in Ω). The degree of retraction also was calculated by the formula $R_2/R_1 \times 100$ (in %).

The effect of the peptides on retraction was investigated in vivo and in vitro. In the experiments in vitro, 0.1 ml of a solution

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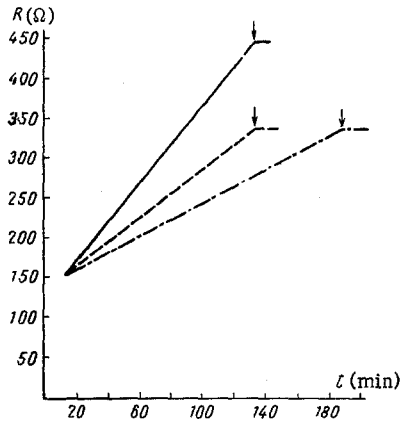

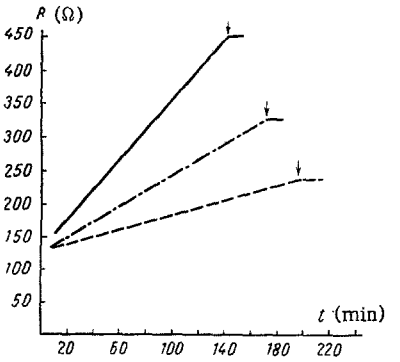

Fig. 1

Fig. 2

Fig. 2. Retraction indices before (1) and 10 min after intravenous injection of fibrin-peptide A (2) and B (3). Here and in Fig. 3 the end of retraction is marked by an arrow. Absicissa, time of beginning of retraction and total retraction time (in min); ordinate, resistance of clot (in Ω).

Fig. 3. Retraction indices in control (1) and under the influence of fibrin-peptides A (2) and B (3) in vitro.

of peptide A or B (40-50 μg of the substance) was added to 0.9 ml blood taken by cardiac puncture from the rabbit with a silicone-treated syringe. The control sample was treated with 0.1 ml physiological saline. In the experiments in vivo 20 mg of peptide A or B in 2 ml physiological saline was injected into the marginal vein of the rabbit's ear. Blood for investigation was taken 10 and 60 min after the injection. Altogether 40 animals were used. The results were subjected to statistical analysis by the small sample method.

EXPERIMENTAL RESULTS

Photographs of chromatograms of the isolated peptides A and B are shown schematically in Fig. 1. These peptides corresponded to the standards in their complement of amino acids [7, 9].

It will be clear from Fig. 2 that in blood taken 10 min after intravenous injection of peptide A there was a decrease in the final resistance (from 440 ± 22.6 to 335.5 ± 15 Ω; $P < 0.02$) and in the degree of retraction (by 60%; $P < 0.05$) compared with the control. The time of beginning of refraction and its total time were unchanged. The final resistance in blood taken after 60 min was normal. More marked changes in the course of retraction were observed after injection of peptide B into the animals. Although the time of the beginning of retraction was unchanged, its duration was lengthened. In blood taken after 10 min it was 55 min longer than normal ($P < 0.001$). The final resistance also was lowered (to 332 ± 13.2 Ω; $P < 0.01$); this led to a decrease in the degree of retraction by 61%; $P < 0.05$. In blood taken after 60 min the retraction time still remained increased (by 24 min; $P < 0.001$), whereas the final resistance and the degree of retraction were reduced ($P < 0.05$). Peptide B thus not only lowers the degree of retraction, but unlike peptide A, it also retards the course of clot retraction itself. Peptide B also has a more prolonged action in vivo than peptide A.

By contrast with the results obtained after intravenous injection of the peptides, in vitro (Fig. 3) peptide A accelerated the beginning of retraction by 3.4 min ($P < 0.01$) and lengthened the total retraction time by 55 min ($P < 0.01$). The final resistance fell more sharply than in vivo (by 214 Ω; $P < 0.001$), as also did the degree of retraction (by 111%; $P < 0.001$). Peptide B had a similar action; it accelerated the beginning of retraction (by 4.2 min; $P < 0.01$), lengthened the total retraction time (by 30 min; $P < 0.01$), and lowered the final resistance (by 123 Ω; $P < 0.001$) and the degree of retraction (by 46%; $P < 0.01$). It follows from these results that peptide A was approximately twice as active as peptide B in vitro.

It can thus be concluded that fibrin-peptides A and B affect the course of retraction both in vivo and in vitro. The process is retarded in both cases; the degree of retraction also is reduced (loosening of the clot), as reflected in a decrease in the final resistance. It is interesting to note that under the influence of

the peptides in vivo there was no decrease in the time before the beginning of retraction. Since this index reflects the time of clotting before the beginning of retraction rather than characterizes the process itself, it can be concluded that the peptides in vivo affect the formation of the blood clot both directly and indirectly, through other systems.

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