RAT PLATELET FACTOR XIII AND ITS ROLE IN PLATELET AGGREGATION

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Factor XIII with an activity of 1430 units/mg protein was isolated from rat platelets. Experiments in vitro showed that on addition of platelet factor XIII (160-200 units/ml) to a suspension of washed platelets or of platelets purified by passage through a column with Sepharose 4B their aggregating power was increased. Addition of inactivated platelet factor XIII had no effect on platelet aggregation induced by ADP.

The role of plasma factor XIII in the stimulation of platelet aggregation has been demonstrated previously [1-3]. Factor XIII was shown to increase platelet aggregation both in vitro in platelet-enriched plasma and after intravenous injection of a preparation of factor XIII into animals. There is little information in the literature on the participation and role of platelet factor XIII in platelet aggregation and the data are contradictory. For instance, in patients with congenital deficiency of platelet factor XIII, a normal level of platelet aggregation was found [12]. These findings may be evidence that platelet factor XIII plays no part in aggregation. On the other hand, a decrease in platelet aggregation in various types of thrombocytopathies, when the platelet factor XIII level is low, points to its possible role in aggregation.

Preparations of factor XIII have been obtained from platelets of several species of animals and man [4, 5, 11] and its physicochemical properties and structure have been studied, but the physiological role of platelet factor XIII still remains unexplained.

The object of this investigation was to study the effect of a preparation of factor XIII obtained from rat blood platelets on platelet aggregation.

EXPERIMENTAL METHOD

Platelet factor XIII was obtained by the method in [13] with slight modifications from rat platelets isolated from 400 ml of citrated blood. The residue of platelets was first washed to remove traces of plasma with 0.85% NaCl solution and suspended in a small volume (20 ml) of the same solution. The platelets were then disintegrated by freezing the suspension to −20°C and thawing 3 times. The suspension of disintegrated platelets was centrifuged for 20 min at 2000 rpm and the fraction containing factor XIII was precipitated from the supernatant with $(NH_4)_2SO_4$ at 40% saturation. This fraction was dissolved in a small volume of 0.05M Tris-acetate buffer, containing 0.001 M EDTA solution, pH 7.3, and dialyzed against it. The dialysate (35-40 mg protein) was applied to a column (2.5 × 40 cm) with Sephadex G-150. Elution was carried out with Tris-acetate buffer. Fractions containing factor XIII were concentrated and used in the experiments or purified further on Sepharose 6B. The activity of the final preparation was 1430 units/mg protein.

Plasma factor XIII was obtained from bovine plasma [10]. The preparations of both plasma and platelet factor XIII were activated with small doses of thrombin without plasminogen (3 units/ml). Activity of factor XIII was determined with the aid of fibrin-monomer [7].

Platelet aggregation was studied in a suspension of washed rat platelets. The residue of platelets was washed 3 times with 0.85% NaCl solution. In some experiments a suspension of washed platelets, further purified on a column of Sepharose 4B [8] was used. The finally prepared platelets also were suspended in 0.85% NaCl solution. The character and level of aggregation in the two cases were the same. Platelet aggregation was determined by the method in [6] and expressed in indices (the ratio of the optical density of the test sample before addition of the aggregating agent to the optical density at the time of its greatest decrease during aggregation of platelets in suspension).

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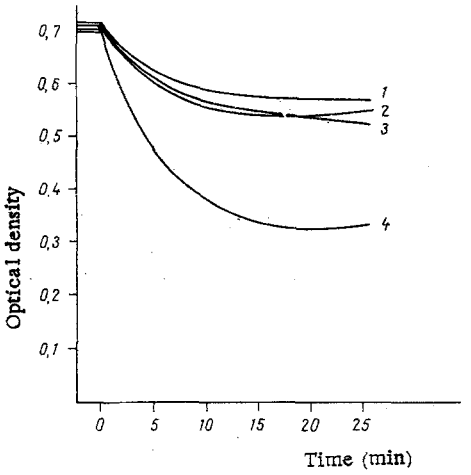

Fig. 1. Platelet aggregation induced by ADP in suspension of washed platelets in presence of platelet factor XIII (160-200 units/ml). Abscissa, time of testing (in min); ordinate, degree of platelet aggregation (in optical density units). Agents added: 1) 0.85% NaCl solution; 2) inactive factor XIII, 3) thrombin in dose used to activate factor XIII, 4) active factor XIII.

EXPERIMENTAL RESULTS

In the experiments of series I a preparation of factor XIII was obtained many times over from rat platelets in small portions (from 400 ml blood). After application of the fraction from the platelet suspension containing factor XIII to the column three protein peaks were eluted. All fractions were tested for factor XIII, antithrombin, and antiplasmin activity. The first protein peak was found to have factor XIII activity unaccompanied by antithrombin, antiplasmin, or procoagulant activity. In subsequent fractions, moreover, a peak with low antithrombin activity and a peak containing considerable antithrombin and antiplasmin activity were found.

In the experiments of series II the effect of the platelet factor XIII on platelet aggregation was studied. Preparations of factor XIII purified on Sephadex G-150 and Sepharose 6B were used. Since thrombin was used to activate the factor XIII, the aggregating agent was added to control samples of the suspension in conjunction with the equivalent quantity of thrombin when aggregation was determined.

The effect of platelet factor XIII on platelet aggregation in the suspension is illustrated in Fig. 1. In the control the platelet suspension (addition of 0.85% NaCl solution) aggregation took place slowly and reached a maximum only after 20-25 min of observation. This is in agreement with experimental data showing that aggregation induced by ADP takes place more slowly and at a lower level in a suspension of washed platelets of certain species of animals or of platelets isolated by the gel-filtration method [9]. On the addition of 160-200 units active platelet factor XIII to the test samples of platelet suspension (2 ml) the aggregating power of the platelets was increased. This increase ($P < 0.001$) took the form of an increased degree of aggregation at the time of its maximal manifestation (20 min after addition of the aggregating agent; Fig. 1). Inactive factor XIII had no such effect and aggregation of platelets took place just as in the control. In the second control sample, to which thrombin was added in a dose equivalent to that used to activate factor XIII, aggregation likewise was indistinguishable from the normal level. On addition of the preparation of factor $XIII_a$, increased aggregation took place in the platelet suspension whether obtained by washing 3 times or by purification of the platelets on a column with Sepharose 4B.

The writers showed previously that plasma factor XIII increased platelet aggregation induced by ADP in experiments in which platelet-enriched rat plasma was used [2]. In the present investigation similar experiments were carried out with the addition of a preparation of plasma factor XIII to the platelet suspension. As Table 1 shows, the preparation of plasma factor XIII caused an increase in aggregation induced by ADP in the platelet suspension also. The degree of increase in aggregation after the addition of both plasma and platelet factor $XIII_a$ was the same.

TABLE 1. Aggregation of Platelets Induced by ADP in Suspension After Addition of Plasma Factor $XIII_a$ (200 units/ml)

Statistical index	Components added			
	0.85% NaCl solution	factor $XIII_a$	inactive factor XIII	thrombin in dose used to activate factor XIII
$M \pm m$	1,78±0,14	2,27±0,09	1,88±0,15	1,85±0,19
n	6	6	6	6
P		<0,01	>0,01	>0,01

Note. P calculated by comparison with control (addition of 0.85% NaCl solution).

The experiments described above thus yielded evidence to show that platelet factor XIII, like plasma factor XIII, increases the degree of platelet aggregation induced by ADP.

LITERATURE CITED

1. V. P. Baluda, G. N. Sushkevich, N. A. Zhukova, et al., in: Problems in Neurohumoral Regulation of Blood Clotting Under Normal and Pathological Conditions [in Russian], Chita (1971), pp. 18-25.
2. V. E. Pastorova and B. A. Umarova, Vestn. Moskovsk. Univ., No. 5, 60 (1974).
3. V. E. Pastorova and B. A. Umarova, Byull. Éksp. Biol. Med., No. 7, 14 (1975).
4. H. Bohn, Thrombos. Diathes. Haemorrh. (Stuttgart), 23, 455 (1970).
5. H. Bohn, Ann. N.Y. Acad. Sci., 202, 256 (1972).
6. G. V. R. Born, Nature, 194, 927 (1962).
7. T. Buluk, T. Januszko, and J. Olbrowski, Nature, 191, 1093 (1961).
8. H. J. Day, H. Holmsen, and M. B. Zucker, Thrombos. Haemostas., 33, 648 (1975).
9. K. A. Hutton, M. A. Howard, D. Deykin, et al., Thrombos. Diathes. Haemorrh. (Stuttgart), 31, 119 (1974).
10. A. G. Loevy, K. Dunathan, R. Krel, et al., J. Biol. Chem., 236, 125 (1961).
11. J. McDonagh and R. H. Wagner, Am. J. Physiol., 219, 1555 (1970).
12. S. Özsoylu and G. Hicsönmez, Acta Haemat. (Basel), 56, 314 (1976).
13. M. L. Schwartz, S. V. Pizzo, R. L. Hill, et al., J. Biol. Chem., 248, 1395 (1973).

USE OF A LASER PHOTOMETER TO STUDY PLATELET SHAPE AND AGGREGATION IN A CONTINUOUS-FLOW SYSTEM

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An original apparatus is described for photometric investigation of the shape of platelets during their aggregation and disaggregation in a continuous flow system. Experiments on a model of stenosed blood vessel showed that in the presence of ADP (1 μM) stenosis potentiates platelet aggregation in platelet-enriched rabbit plasma.

A change in the shape of platelets and in their aggregation and disaggregation may play an essential role in thrombus formation [1]. Methods of investigation of the functional properties of platelets used at the present time enable the behavior of a platelet suspension in plasma to be studied in a test tube, but not in a continuous-flow system, although hemodynamic forces can have a significant effect on the functional properties and behavior of platelets during thrombus formation [2]. The optical density of a suspension of platelets in plasma has been shown to depend on their shape and orientation [3], for the scatter diagram of an ellipsoid of rotation is determined by its position relative to the direction of incidence of light. The investigation described below

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