11. H. J. Seitz and W. Tarnowski, Hoppe-Seylers Z. Physiol. Chem., 349, 1800 (1968).
12. G. Schmidt and S. S. Thannhauser, J. Biol. Chem., 161, 83 (1945).

LIBERATION OF β-LIPOPROTEIN, ALKALINE PHOSPHATASE, AND 5'-NUCLEOTIDASE FROM PLATELETS IN RESPONSE TO AGGREGATION INDUCED BY ADRENALIN

A. L. Storozhev and D. M. Zubairov

UDC 612.111.7.015.1-06:612.452.018

During aggregation induced by adrenalin, β-lipoprotein, alkaline phosphatase, 5'-nucleotidase, and factor 3 are liberated from the platelets. The electrophoretic mobility of platelet alkaline phosphatase is the same as that of the β-lipoprotein. This suggests that the β-lipoprotein, alkaline phosphatase, and 5'-nucleotidase are structural components of platelet material carrying the factor 3 activity.

KEY WORDS: platelets; adrenalin; β-lipoprotein; alkaline phosphatase; 5'-nucleotidase.

One of the most important consequences of reactions associated with emergence in neurohumoral regulation of blood clotting is the secretion of adrenalin by the adrenals [4]. The increased liberation of adrenalin into the blood stream causes the development of a hypercoagulemic reaction, the time of which coincides with that of increased activity of alkaline phosphatase and 5'-nucleotidase in the blood plasma [2, 3]. During adrenalin-induced aggregation of platelets, platelet factor 3 is liberated [13], whereas 5'-nucleotidase and 80% of the alkaline phosphatase are bound with membrane structures which possess factor 3 activity [7].

It is possible that 5'-nucleotidase and alkaline phosphatase are indicators of the appearance of fragments of platelet membranes in the blood stream during the reaction of adrenalin-induced aggregation of platelets. This paper describes an attempt to verify this hypothesis experimentally.

EXPERIMENTAL METHOD

Blood taken from the femoral artery of dogs was stabilized with 3.8% of sodium citrate (9 : 1) to study the platelet release reaction in the presence of plasma, or with EDTA solution (7.5 ml of 0.077 M EDTA + 92.5 ml blood) to study the reaction in buffer solution, pH 7.4. Platelet-rich plasma was obtained from blood by centrifugation at 12°C (280 g, 25 min). A suspension of washed platelets was prepared by Dechavanne's method [11]. Platelet-rich plasma, stabilized with EDTA, was centrifuged at 12°C (500 g, 30 min) and the residue of platelets was rinsed twice with a solution containing 139 mM NaCl, 5 mM KCl, 5 mM glucose, 15 mM Tris-HCl, pH 7.4, and 0.5% bovine albumin, and mixed with 77 mM EDTA in the ratio of 49 : 1. The washed platelets were suspended in the same buffer without EDTA. The morphological control and counting the platelets in the suspensions were carried out by phase-contrast microscopy in a Goryaev's counting chamber.

The platelet release reaction was induced by a $5.4 \cdot 10^{-4}$ M (final concentration) solution of adrenalin hydrochloride in the cell of an aggregometer at 37°C, with constant mixing for 15 min. To reproduce the release reaction in buffer at pH 7.4, fibrinogen and $CaCl_2$ up to final concentrations of 3.75 mg/ml and $4 \cdot 10^{-4}$ M respectively were added to the platelet suspension in addition to adrenalin. Aggregation was recorded automatically by the nephelometric aggregometer. At the end of 15 min the contents of the cells were centrifuged at 4°C (5000g, 30 min). Activity of alkaline p-nitrophenylphosphatase [10], alkaline β-glycerophosphatase [1], 5'-nucleotidase, and platelet factor 3 [5] was determined in the supernatant. The results were subjected to statistical analysis [8].

Department of Biochemistry, S. V. Kurashov Medical Institute, Kazan'. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No, 5, pp. 545-547, May, 1976. Original article submitted November 5, 1975.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $7.50.

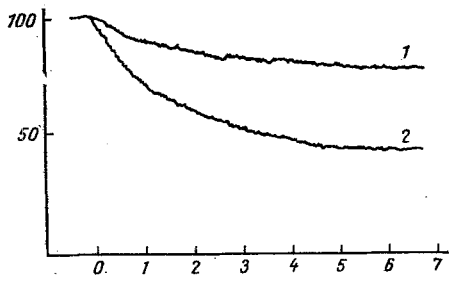

Fig. 1

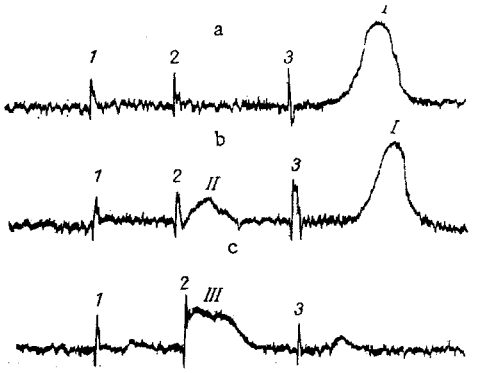

Fig. 2

Fig. 1. Platelet aggregation induced by adrenalin: 1) in buffer solution, pH 7.4; 2) in citrated plasma. Abscissa, time (in min); ordinate, optical density (in %).

Fig. 2. Electrophoresis of lipoproteins and alkaline phosphatase of extrathrombocytic medium: a, b) lipoproteinogram before and after action of adrenalin on platelets, respectively; c) alkaline phosphatase after action of adrenalin on platelets. 1) Boundary between 3% and 3% gels with different buffers; 2) boundary between 3% and 5% gels; 3) boundary between 5% and 10% gels. I) Complex of albumin with nonesterified fatty acids from bovine albumin preparation; II) β-LP; III) alkaline phosphatase. Arrow indicates direction toward finish.

TABLE 1. Secretion of Phosphohydrolases and Factor 3 of Platelets during Adrenalin-Induced Aggregation (M ± m)

Enzymes	Activity released from $1 \cdot 10^7$ platelets (in units)	
	in citrated plasma	in buffer soln., pH 7.4
Alkaline p-nitrophenyl-phosphatase	0,27±0,09	0,20±0,04
Alkaline β-glycerophosphatase	0,40±0,07	0,47±0,06
5'-Nucleotidase	0,53±0,03	0,39±0,13
Activity of factor 3	23,4±2,4*	5,91±0,4*

*In % of initial activity.

To detect lipoproteins (LP) the supernatant was stained with Sudan Black B [12] and subjected to disk electrophoresis with a rod [17] in a four-layered polyacrylamide gel [6] with a current of 5 mA applied to the tube (4°C, 1 h). The rods were then removed from the gels and 0.1% iodine solution in 7% acetic acid was poured into the resulting hole. Lipoproteins stained with Sudan Black B not clearly distinguishable after electrophoresis were fixed with iodine solution and stained [15]. Chylomicrons remained at the starting line, next followed pre-β-LP; β-LP were found on the boundary between the 3% and 5% gels, α-LP at the boundary between the 5% and 10% gels, and the lowest band of all consisted of a complex of albumin with nonesterified fatty acids. In parallel tests of the supernatant by disk electrophoresis, alkaline phosphatase activity was subsequently detected by the azo-coupling method [9]. The disks after electrophoresis were examined by direct densitography in the MF-4 microphotometer.

EXPERIMENTAL RESULTS AND DISCUSSION

Aggregation of platelets induced by adrenalin (1) was accompanied by release of factor 3, parallel with the liberation of alkaline phosphatase and of 5'-nucleotidase in both citrated plasma and buffer solution, pH 7.4 (Table 1). The ability of platelets suspended in buffer, pH 7.4, to aggregate was much lower than in citrated plasma (Fig. 1). In the buffer solution, moreover, less of the factor 3 and 5'-nucleotidase was liberated. The release reaction in buffer solution was accompanied by the appearance of an additional fraction in the β-LP

zone of the electrolipoproteinograms (Fig. 2a, b). Alkaline phosphatase was discovered by electrophoresis after the original material had been concentrated 20-fold by lyopholization, and its mobility was the same as that of β-LP (Fig. 2c).

It was also shown previously [13, 16] that the quantity of factor 3 released into a medium containing platelets is proportional to the intensity of aggregation. The released β-LP is evidently the carrier of activity of platelet factor 3, which is known to be phospholipoprotein in nature. The identical electrophoretic mobility of β-LP and alkaline phosphatase indicates that these are structurally connected components, probably of the platelet plasma membrane.

Platelet aggregation induced by adrenalin is thus accompanied by liberation of β-LP, alkaline phosphatase, and 5'-nucleotidase into the blood stream. The hypercoagulemic reaction and increased activity of alkaline phosphatase and 5'-nucleotidase in the blood plasma after intravenous injection of adrenalin may perhaps be partly the result of the action of adrenalin on the platelets.

LITERATURE CITED

1. M. M. Alimova, Lab. Delo, No. 6, 346 (1964).
2. I. A. Andrushko, Byull. Éksp. Biol. Med., No. 10, 64 (1973).
3. D. M. Zubairov, I. A. Andrushko, and A. L. Storozhev, Kardiologiya, No. 11, 75 (1974).
4. W. Cannon, The Physiology of Emotions [Russian translation], Leningrad (1927).
5. M. A. Kotovshchikova, Technical Instructions for the Investigation of the Blood Clotting System [in Russian], Leningrad (1966).
6. E. Ya. Magracheva, Vopr. Med. Khim., No. 6, 561 (1973).
7. A. L. Storozhev, Tsitologiya, No. 2, 212 (1975).
8. R. A. Fisher (editor), Statistical Methods for Research Workers, 14th edition, Hafner, New York (1973).
9. J. M. Allen and G. Hyncik, J. Histochem. Cytochem., 11, 169 (1963).
10. O. H. Bessey, O. H. Lowry, and M. J. Brock, J. Biol. Chem., 164, 321 (1946).
11. E. Dechavanne, Coagulation, 4, 55 (1971).
12. M. Gros and T. Jurman-Gros, Clin. Chim. Acta, 45, 165 (1973).
13. R. M. Hardisty, Brit. J. Haemat., 11, 258 (1965).
14. P. Kubisz, I. Pinkhas, and J. R. Caen, Israel J. Med. Sci., 6, 494 (1970).
15. K. A. Narayan and F. A. Kummerow, Clin. Chim. Acta, 13, 532 (1966).
16. T. M. Spaet and J. Cintron, Brit. J. Haemat., 11, 269 (1965).
17. R. A. Zeineh, E. M. Mbawa, B. J. Fiorella, et al., Analyt. Chem., 45, 2159 (1973).