Effect of Lupus Anticoagulant on the Interaction of Prothrombin with Platelets

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 6, pp. 614-617, June, 2004
Original article submitted March 26, 2003

We studied binding of ^{125}I-labeled prothrombin to platelets in the presence of circulating endogenous lupus anticoagulant. Lupus anticoagulant modulated the interaction and increased affinity of prothrombin for calcium-independent binding sites on platelets. The number of these sites decreased, while the total number of calcium-dependent binding sites increased. Our results indicate that lupus anticoagulant plays a role in the binding of prothrombin to nonactivated platelets.

Key Words: *prothrombin; platelets; lupus anticoagulant*

The presence of circulating lupus anticoagulant lengthens phospholipid-dependent clotting tests (activated partial thromboplastin time and prothrombin time). Deceleration of thrombin formation is associated with disturbances in the interaction between plasma coagulants and cell membrane phospholipids. The phenomenon of hypocoagulation observed *in vitro* is accompanied *in vivo* by microthromboses, thromboembolism, and local microcirculatory disorders in vitally important organs. Dysregulation of homeostasis is closely related to pathological changes in the immune response during systemic connective tissue diseases, collagenosis, leukemias, and malignant neoplasms. Progression of the pathological process results from accumulation of immunoglobulins involved in autoimmune aggression. Lupus anticoagulant is a subset of antiphospholipid antibodies. It was described for the first time as pathological antithrombin IV-V in secondary antiphospholipid syndrome complicating systemic lupus erythematous. Recent studies with enzyme immunoassay kits revealed primary antiphospholipid syndrome in various patients and healthy volunteers. Accumulation of autoantibodies against blood cells and endothelium and changes in lipid asymmetry of cell membranes impair activation of procoagulants and lead to the appearance of circulating anticoagulant inhibiting thrombin generation. Moreover, circulating anticoagulants acting as antibodies against plasma procoagulant factors were recently described [7]. For example, inhibitor of intrinsic thrombin generation pathway complicates the course of hemophilia A. Our previous studies showed that the inhibitor present in 36% patients with hemophilia A is similar to lupus anticoagulant, since it blocks both the intrinsic and extrinsic pathways of thrombin generation [10]. The molecular mechanism of this process remains unclear. Probably, accumulation of autoantibodies in the blood violates homeostasis due to disturbances in physiological binding of coagulation factors to the surface of cell membranes. Here we studied the effect of lupus anticoagulant on the binding of prothrombin, the source of the major coagulation factor thrombin, to platelets.

MATERIALS AND METHODS

Prothrombin isolated from citrate-stabilized human plasma [2] was labeled with ^{125}I-chloramine using (^{125}I)Na without a carrier. Platelet-rich plasma was obtained from citrate-stabilized venous blood of healthy donors. Centrifugation was performed in Teflon tubes at 450*g* and 20°C for 10 min. Platelets were pelleted from the plasma by centrifugation at 1000*g*

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0007-4888/04/1376-0538

and 20°C for 10 min. Nonactivated platelets were resuspended in 0.5 ml plasma from patients with circulating lupus inhibitor and healthy donors. The cell suspension was maintained at room temperature for 60 min. The total volume was brought to 10 ml with 0.01 M Tris-HCl buffer (pH 7.4) containing 0.137 M NaCl, 0.004 M KCl, and 0.011 M glucose. Plasma samples from healthy donors and patients were heated at 56°C for 20 min to prevent platelet activation with residual thrombin. Lupus anticoagulant retained activity in the activated partial thromboplastin time (APTT) test. The index of APTT (lengthening of the period for plasma coagulation) with soybean reagent was 1.7 [5]. Binding of radioactive prothrombin was studied in the incubation mixture with 2×10^6 platelets per 1 ml buffer solution containing 1% human albumin and 0.002 M $CaCl_2$ or 0.002 M ethylenediaminetetraacetic acid (EDTA). Mixtures were kept at room temperature for 1 h and centrifuged at 8000*g* for 30 min. Radioactivity of aliquots from supernatants and pellets was measured on a Minigamma counter (LKB Wallac). The amount of free and bound proteins was expressed in Scatchard coordinates. Calculation was performed using special software [4]. We conducted 6 series of experiments with binding of prothrombin to platelets. The plasma was taken from a patient with hemophilia A and disseminated osteoarthrosis examined at the Russian Research Institute of Hematology and Transfusiology (St. Petersburg) since 1995. The blood contained circulating immune complexes (67 arb. units). The amount of IgG, IgM, and IgA in the blood increased to 21.1, 4.9, and 6.0 g/liter, respectively. The blood coagulation time was 23 min. Study of platelet-rich plasma allowed us to derive the following indexes: APTT 1.7; prothrombin time 73%; factor VIII 4%; factor VIII inhibitor 7.5 BU (Bethesda unit); tissue thromboplastin test 2.2 (thromboplastin dilution 1:500, normal <1.3).

RESULTS

Binding of prothrombin was described by straight lines in the Scatchard plot. In the presence of lupus anticoagulant these lines gained a bell shape (Fig. 1). The shape of these curves reflected the existence of only 1 type of binding sites for prothrombin, which interacted with platelets in the presence of lupus anticoagulant with a positive cooperative effect. Indexes for binding of prothrombin were calculated from the specific points on descending lines in Scatchard plots (K_d, observed dissociation constant; Q, number of sites; Table 1). Prothrombin was characterized by low affinity for platelets ($K_d=6.5\times10^{-6}$ M). Affinity of prothrombin for these sites decreased in the absence of free Ca^{2+} ($K_d=19.1\times10^{-6}$ M). However, the number of binding sites increased by 4 times. Total binding in the absence of Ca^{2+} became more intensive due to an increase in the number of binding sites. In the presence of lupus anticoagulant, the number of prothrombin binding sites on platelets increased by 2 times, while affinity for these sites remained unchanged. The increase in the number of binding sites did not depend on the presence of free Ca^{2+}. The value of Q did not change in the presence of EDTA (Table 1). Affinity of prothrombin for platelets in the presence of EDTA and lupus anticoagulant increased by 2 times, which was accompanied by a decrease in the number of binding sites. Therefore, lupus anticoagulant plays a role

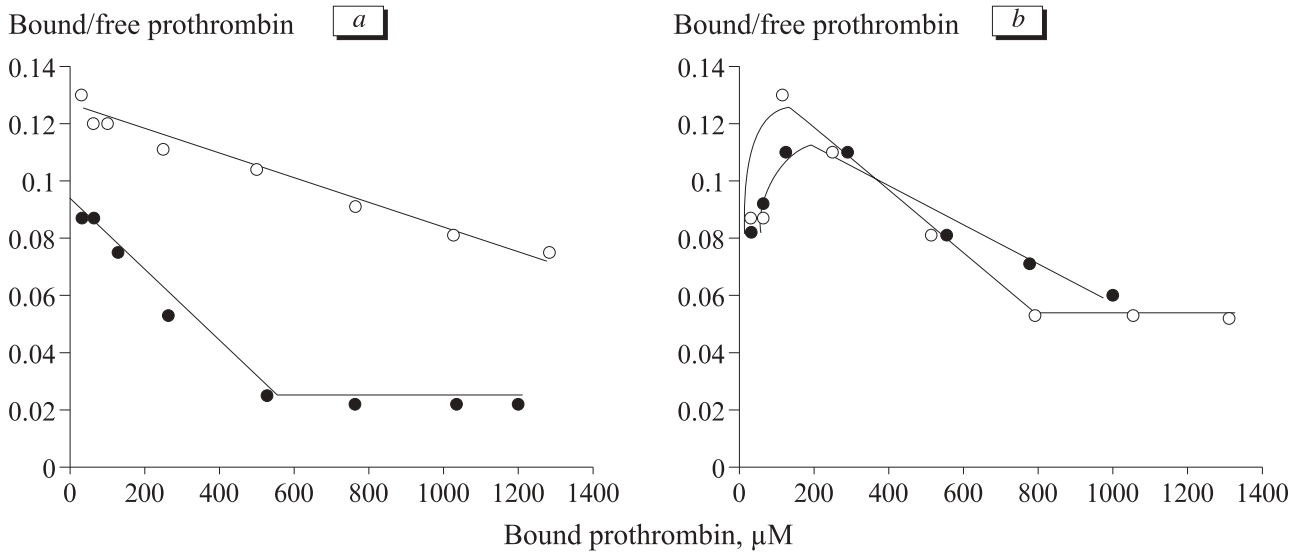

Fig. 1. Scatchard plots for binding of prothrombin to platelets. Binding in the absence (*a*) and presence of lupus anticoagulant (*b*). Dark circles: in the presence of Ca^{2+}. Light circles: in the presence of EDTA.

TABLE 1. Binding of Prothrombin to Platelets (*M*±*m*)

Index of binding, mixture		Plasma	
		control	with anticoagulant
K_d, μM	$CaCl_2$	6.5±2.1	9.3±2.4
	EDTA	19.1±3.8+	10.6±3.5*
Q, μM	$CaCl_2$	0.58±0.22	1.22±0.23*
	EDTA	2.44±0.51+	1.16±0.4*
Mean percent of total prothrombin binding, %			
	$CaCl_2$	6.0±1.4	8.2±1.2
	EDTA	9.1±1.1+	7.6±2.5

Note. $p<0.05$: *compared to the control plasma, +compared to $CaCl_2$.

in the binding of prothrombin to platelets. We revealed changes in binding curves in Scatchard plots, number of binding sites, and affinity. This interaction produces a positive cooperative effect and does not depend on the presence of Ca^{2+}.

Prothrombin is converted into thrombin in the prothrombinase complex on the surface of activated platelets. Activation is accompanied by changes in the composition of phospholipids in the outer layer of cell membranes. Prothrombin binds to membrane phosphatidylserine through calcium bridges. Affinity of this interaction directly depends on the amount of prothrombin [8]. In previous experiments affinity of prothrombin to activated platelets was higher than that observed in our study ($K_d=4.7\times10^{-7}$ and $K_d=6.5\times10^{-6}$ M, respectively) [11]. Low affinity of prothrombin for binding sites on platelets is probably related to small amount of phosphatidylserine on the external surface of cell membranes. The amount of phosphatidylserine in activated platelets is higher by 3 times [6]. This hypothesis is confirmed by low number of binding sites ($Q=0.58\times10^{-6}$ M). Low affinity of prothrombin and small number of binding sites indicate that platelets obtained in Teflon tubes were inactive. However, the percent of prothrombin binding was high (6%) and increased in the presence of EDTA (9.1%). These data attest to the existence of not only calcium-dependent, but also another mechanism for prothrombinbinding. Similar results were obtained in studies of prothrombin binding to erythrocytes and lung macrophages [3].

It was hypothesized that lupus anticoagulant binds to epitopes of the antigen, which are presented by regions of the cell membrane with adsorbed prothrombin and/or β_2-glycoprotein I [9]. However, Fc receptor on platelets binds to Fc domain of IgG [6]. Therefore, lupus anticoagulant can interact with platelets via adsorbed prothrombin and Fc receptor. We observed a 2-fold increase in the number of binding sites for prothrombin in the presence of lupus anticoagulant. The data suggest that these antibodies act as true bivalent immunoglobulins binding two molecules of prothrombin. In our experiments the interaction of prothrombin in low concentrations with immunoglobulins had a positive cooperative effect. Thus, binding of the first molecule facilitated binding of the second molecule in prothrombin. Lupus anticoagulant interacts with the adsorbed molecule of prothrombin and promotes binding of an additional molecule from the environment. Moreover, lupus anticoagulant can organize new binding sites by interacting with Fc receptor on platelets. The latter possibility seems unlikely, since binding of the Fc domain in IgG to the Fc receptor on platelets depends on the presence of Ca^{2+} [6]. These peculiarities were not observed in our experiments. Values of K_d and Q remained unchanged in the presence of EDTA.

Our results illustrate the molecular mechanism for thrombus formation in the presence of circulating lupus inhibitor. Lupus antibodies bind to prothrombin on the surface of nonactivated platelets. The antigen—antibody complex can activate platelets [6] and initiate blood coagulation. However, *in vitro* coagulation assays showed that these antibodies bind to prothrombin and suppress thrombus formation. These changes result in lengthening of plasma coagulation time in phospholipid-dependent tests.

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