EFFECT OF THROMBOMODULIN ON SPECIFIC FUNCTIONS OF THROMBIN: FIBRINOGEN COAGULATION AND PLATELET AGGREGATION

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UDC 612.115.12.06

KEY WORDS: thrombin; thrombomodulin; fibrinogen coagulation; platelet aggregation.

Thrombin (EC 3.4.21.5), a key serum proteinase of the blood clotting system, exerts regulatory functions in hemostasis and in coupled reactions with high specificity. The unique specificity of thrombin is due to the presence of a special, additional recognition (or binding) site for high molecular weight compounds, separate from the active center [2, 4, 10]. It is through this recognition center that high-affinity binding of thrombin with specific protein substrates and blood cells as well as other tissue cells is realized. Blockade of the active center of thrombin does not disturb the characteristics of this binding. Moreover, inactivated thrombin, on binding with cell membranes, can give rise to definite effects: excite a reaction of the anticlotting system and stimulate chemotaxis and aggregation of leukocytes, proliferation of macrophage-like cells, etc. [2, 6]. This is due to the presence of specific thrombin receptors in the cell membrane, complementary to the recognition center for high molecular weight compounds. Thrombomodulin, an endotheliocyte membrane protein with high affinity for thrombin, for which it serves as coenzyme during activation of protein C — an inhibitor of thrombin formation and an activator of fibrinolysis, is a possible candidate for the role of such a receptor [8, 9].

This paper gives data on the effect of thrombomodulin on thrombin activity relative to fibrinogen and platelets.

EXPERIMENTAL METHOD

Thrombomodulin was obtained from rabbit lungs as in [9] with certain modifications of the conditions of affinity chromatography on thrombin-sepharose. The α-thrombin was obtained from

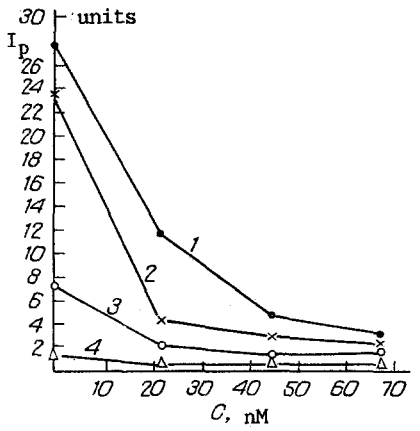

Fig. 1. Dependence of fibrinogen-clotting activity of thrombin (in I_p, relative units) on thrombomodulin concentration (in nM). α-Thrombin concentration: 1) 15 nM, 2) 11.25 nM, 3) 7.5 nM, 4) 3.75 nM.

Institute of Physiologically Active Substances, Academy of Sciences of the USSR. Laboratory of Physiology and Biochemistry of Blood Clotting, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 517-519, November, 1988. Original article submitted September 8, 1987.

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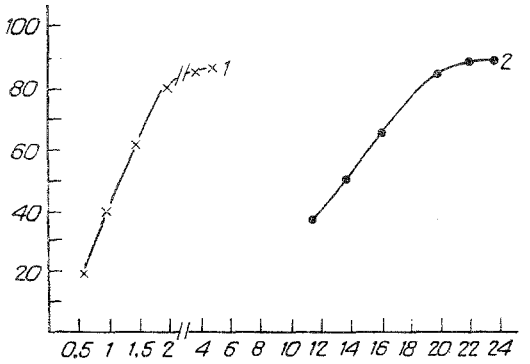

Fig. 2. Inhibition of fibrinogen-clotting activity of thrombin (1) and thrombin-induced platelet aggregation (2) by thrombomodulin. Abscissa, molar ratio of thrombomodulin and thrombin concentrations; ordinate, % inhibition of thrombin activity.

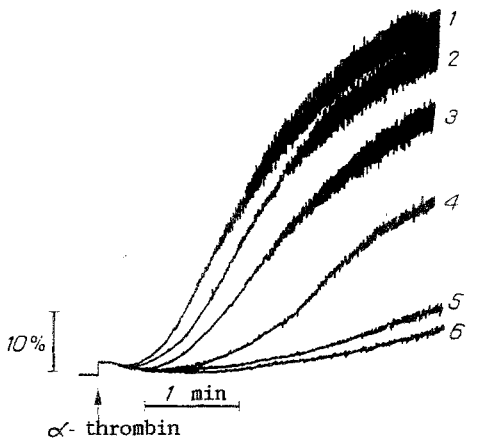

Fig. 3. Inhibition of thrombin-induced platelet aggregation by thrombomodulin. Concentration of thrombomodulin: 1) control, 2) 6.8 nM, 3) 17 nM, 4) 23.8 nM, 5) 30.6 nM, 6) 34.0 nM.

a commercial preparation of bovine thrombin by the method in [5]. The enzyme preparations thus obtained were homogeneous on PAG electrophoresis in the presence of SDS and possessed fibrinogen-clotting activity of 1500-2000 NIH units/mg protein. The fibrinogen-clotting activity of thrombin was determined spectrophotometrically as in [12], with estimation of the value of I_p, which characterizes the rate of accumulation of fibrin monomers in the system. The esterase activity of thrombin was determined spectrophotometrically, as the initial velocity of hydrolysis of the methyl ester of N-benzoyl-L-arginine (BAME), by the method in [5], and amidase activity was determined spectrophotometrically relative to H-D-phenylalanyl-L-pipecolyl-L-arginine paranitroanilide by the method recommended by the firm "Kabi Diagnostica." The effect of thrombomodulin on thrombin-induced platelet aggregation was investigated in cells isolated from donated human blood by the method in [3]. Platelet aggregation was recorded by Born's optical method on the Elvi-840 aggregometer.

EXPERIMENTAL RESULTS

Thrombomodulin was found to significantly reduce the ability of thrombin to clot fibrinogen (Figs. 1 and 2). This effect depended on the relative molar concentrations of thrombomodulin and thrombin: a 50% decrease in the fibrinogen-coagulating activity of thrombin was observed if the thrombomodulin/thrombin ratio was 1:2. The inhibition constant, calculated by the equation $K_i = IC_{50}/(1 + l/K_d)$, where IC_{50} is the concentration of inhibitor inducing 50% inhibition of enzyme activity, K_d the dissociation constant for binding sites of α-thrombin with high affinity, l the fraction of the unbound form of the enzyme [1], was 14.7 ± 1.24 nM.

One of the most important functions of thrombin as an activator of the blood clotting system is induction of platelet aggregation. Thrombomodulin reduces the ability of thrombin to induce platelet aggregation (Fig. 3). This is in agreement with results in [11]. We also have shown that this effect depends on the ratio of the molar concentrations of thrombomodulin and thrombin. Inhibition of platelet aggregation by 50% was observed if the thrombomodulin/thrombin ratio was 14:1 (Fig. 2). It was further discovered that thrombomodulin does not affect platelet aggregation induced by ADP or by β/γ-thrombin. This is evidence that the effect of thrombomodulin is specific for α-thrombin, which has a recognition center for high molecular weight compounds in its structure.

Thus interaction between thrombin and thrombomodulin disturbs the basic functions of thrombin as an activator of the clotting system, its ability to induce conversion of fibrinogen into fibrin and to stimulate platelet aggregation.

Meanwhile thrombomodulin is a coenzyme for activation of protein C — the most effective of the known endogenous anticoagulants — by thrombin. In the presence of thrombomodulin, K_m for the reaction of protein C activation by thrombin was reduced 20-fold [11].

The question arises: is this change in the specificity of thrombin on interaction with thrombomodulin connected with a change in the catalytic properties of the active center proper of this enzyme? To answer this question the effect of thrombomodulin on thrombin activity relative to low molecular weight substrates (BAME and H-D-phenylalanyl-L-pipecolyl-L-arginine paranitroanilide) was investigated. The experiments showed that thrombomodulin had virtually no effect on the kinetic characteristics of interaction of thrombin with these substrates. It can be tentatively suggested that during formation of a thrombin–thrombomodulin complex the active center of the enzyme is unchanged, and the altered specificity of thrombin is the result of a change in accessibility of additional recognition sites for high molecular weight compounds on the thrombin molecule. Further research is necessary to confirm this hypothesis.

The suggestion that the recognition site for high molecular weight compounds plays a definite role in the mechanism of action of thrombin as a regulator of hemostasis was thus confirmed.

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