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PAWEŁ NOWAK[a], TADEUSZ KRAJEWSKI[a], TADEUSZ PIETRUCHA[b] and CZESŁAW CIERNIEWSKI[b]

INTERACTION OF HETEROLOGOUS FIBRINOGENS WITH HUMAN PLATELET RECEPTORS

[a] *Department of Biochemistry, Institute of Biochemistry, University of Łódź, Banacha 12/16; 90-237 Łódź, and*
[b] *Department of Biophysics, Institute of Physiology and Biochemistry, Medical School of Łódź, Lindleya 3; 90-237 Łódź, Poland*

The interaction of ADP-stimulated human platelets with human ^{125}I-fibrinogen as well as with pig and bovine fibrinogens was analysed. It was found that the fibrinogens studied were bound to the same platelet receptors but the affinity of animal preparations was about half the value observed for human fibrinogen (in a homologous system).

Fibrinogen is an essential cofactor in the aggregation of human platelets [1, 2]. When they are stimulated by ADP, specific fibrinogen receptors (complexes of glycoproteins IIb-IIIa) are expressed on the platelet surface and then a dimeric molecule of fibrinogen binds to these receptors causing platelet aggregation [3, 4, 5]. It has been demonstrated that both γ and α chains of human fibrinogen interact directly with ADP-activated platelets [6]. The γ-chain domain is composed of a sequence of 12 carboxyterminal residues, $\gamma_{400-411}$, and α chain contains two *loci* at residues 95-97 and 572-574 showing activity towards human platelet receptors [7, 8, 9].

Our previous observations indicated that ADP-stimulated human platelets may be able to aggregate in the presence of heterologous (pig and bovine) fibrinogens as well but the extent and rate of this process were lower than in the presence of human fibrinogen [10].

In this paper we report the results of kinetic analyses of binding of ^{125}I-labelled human, pig and bovine fibrinogens to human platelet receptors.

MATERIALS AND METHODS

Platelets were isolated from fresh human blood by differential centrifugation [11]. Blood was collected into ACD (acid-citrate-dextrose) anticoagulant solution (5:1) containing benzamidine hydrochloride and

trasylol, and centrifuged at 22°C and 200 × *g* for 20 min. Platelet-rich plasma was collected and recentrifuged for 20 min at 1000 × *g*. The platelet pellet was washed according to Mustard *et al.* [12] and then suspended in Tyrode's-2% albumin buffer (pH 7.4).

Fibrinogen was isolated from human, pig and bovine plasma according to Doolittle *et al.* [13]. Fibrinogen preparations were dissolved in 0.14 M NaCl buffered with 0.01 M sodium phosphate, pH 7.4, and passed through a Lys-Sepharose column to remove plasminogen. Isolated mammalian fibrinogens were 95-97% coagulable with bovine α-thrombin. Reduced fibrinogen preparations consisted of intact Aα, Bβ and γ chains as estimated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

Fibrinogen was labelled with ^{125}I using a modified chloramine T method [14]. Specific radioactivity of the ^{125}I-labelled fibrinogen ranged from 50 000 c.p.m./μg to 200 000 c.p.m./μg. The clottability of labelled fibrinogens was between 92-95%. Protein concentration was determined by the microbiuret method [15] or spectrophotometrically [16].

For experiments on binding of ^{125}I-labelled fibrinogen to platelets, 200 μl platelets (5×10^8/ml) suspended in Tyrode's-2% albumin buffer, pH 7.4, were incubated for 10 min at 37°C with ^{125}I-labelled fibrinogen (0-1.47 μM) and stimulated by 10 μM ADP in the presence of 1 mM $CaCl_2$. Then 200 μl of the platelet suspension was layered on the top of 250 μl of 15% sucrose solution and centrifuged in a conical bottom tube for 2.5 min at 2500 × *g*. The number of fibrinogen molecules bound to the platelets was determined by counting the radioactivity in the tip of the centrifuge tube. Nonspecific ^{125}I-fibrinogen binding was measured in samples incubated without ADP.

RESULTS

Binding of ^{125}I-labelled human, pig and bovine fibrinogens to ADP-stimulated human platelets is shown in Fig. 1. All the fibrinogen preparations tested, bound to platelets in a specific and saturable manner.

The values of specific binding (shown in Fig. 1) were analysed according to Scatchard [11]. Under the optimal conditions for binding of fibrinogen to human platelets, the number of fibrinogen molecules bound per platelet was similar in each case, but the affinity of animal fibrinogens to human platelet receptors was about half that of homologous fibrinogen (Fig. 2, Table 1).

The data obtained permit to conclude that the interaction of pig and bovine fibrinogens with activated human platelets is mediated by the same platelet receptors that recognized specific domains in human fibrinogen. Lower affinity of the examined animal fibrinogens to human platelet receptors as compared with homologous fibrinogen seems to indicate that only some of these domains are present in pig and bovine fibrinogens.

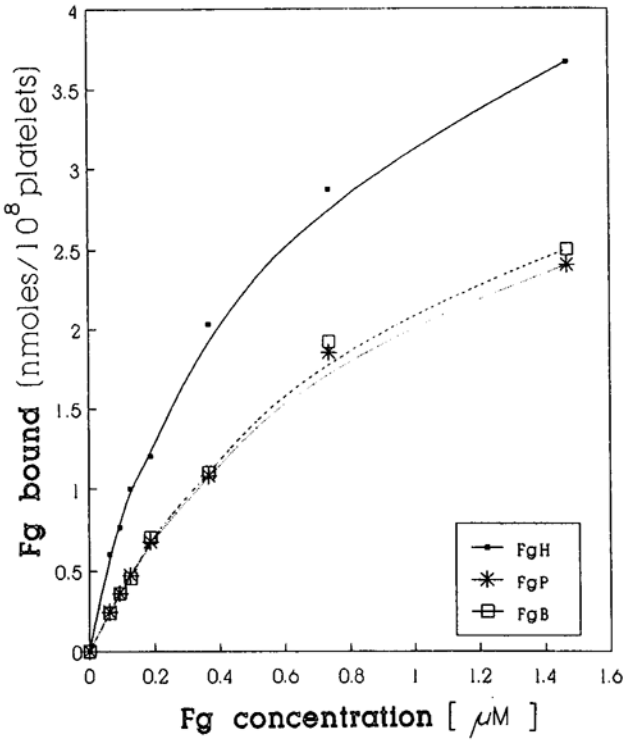

Fig. 1. Binding of ^{125}I-labelled human (FgH), pig (FgP) and bovine (FgB) fibrinogens to ADP-stimulated human platelets, as a function of ligand concentration. The platelets (1×10^8/ml) were incubated with 1 mM $CaCl_2$, 10 μM ADP and fibrinogen (0-1.47 μM) for 10 min at 37°C. A typical experiment is presented

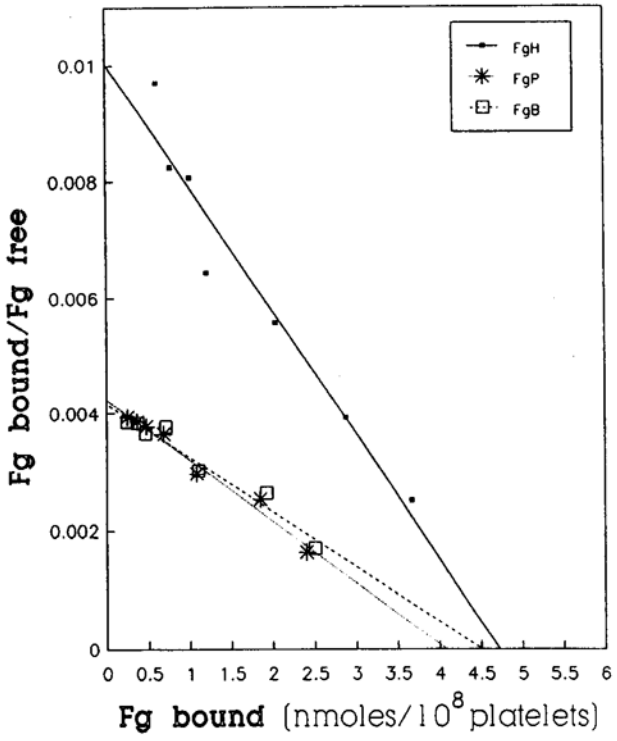

Fig. 2. Scatchard analysis of specific binding of human, pig and bovine fibrinogens. A typical experiment is presented

Table 1

Affinity of ^{125}I-labelled human, pig and bovine fibrinogens to ADP-stimulated human platelets

The results are average values of five experiments (±SD)

Fibrinogen bound	Dissociation constant (μM)	Number of fibrinogen receptors per single platelet
Human	0.47±0.03	28 000±4000
Pig	1.07±0.04	25 000±3000
Bovine	0.95±0.04	27 000±3000

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