AGGLUTINATION ACTIVITY ASSOCIATED WITH A GLYCOPROTEIN EXTRACT OF HUMAN PLATELET PLASMA MEMBRANES

Possible involvement in platelet aggregation

Dianna J. BOWLES+
Department of Biochemistry, Tennis Court Road, Cambridge, England

and

Avner ROTMAN
Department of Membrane Research, Weizmann Institute of Science, Rehovot, Israel

Received 13 April 1978

1. Introduction

Platelets are extremely active cells of the blood circulation system. Much attention has been focused on the components of the carbohydrate-rich glycocalyx surrounding these cells in an attempt to elucidate the role of the surface glycoproteins in the haemostatic function of platelets [1]. The recent demonstration that agglutination activity is associated with the major glycoprotein of erythocyte membranes and the likelihood that both a lectin and receptor are present as a self-neutralized pair within the membrane [2], led us to investigate the possibility of the presence of an analogous lectin and receptor in platelets. We demonstrate here that a glycoprotein extract isolated from human platelet plasma membranes exhibits haemagglutination and in addition causes agglutination of platelets.

2. Methods

Platelet membranes were prepared from fresh platelet rich plasma (obtained from the Blood Bank Tel Aviv-Jaffo) using the glycerol-lysis technique and subsequent sucrose density gradient centrifugation [3]. The membranes were solubilized by incubation at 37°C for 15 min in 1% SDS, 250 mM NaCl, and 15 mM sodium phosphate buffer, pH 7.2. After centrifugation at 50 000 × g for 15 min, the SDS concentration in the supernatant was lowered to 0.05% by dilution with 15 mM sodium phosphate buffer, pH 7.2, containing 250 mM NaCl and the solution was applied to an affinity column (5 ml) of wheat germ agglutinin (WGA) coupled to polyacryl hydrazide-agarose. WGA was purchased from Makor Chemicals Ltd, Jerusalem and was coupled using the technique in [4]. After extensive washing with buffer until the A_{280} of the eluant was < 0.05, the adsorbed material was eluted with 100 mM *N*-acetyl-D-glucosamine in the same buffer, dialysed against phosphate-buffered saline, concentrated by vacuum dialysis and haemagglutination activity was determined using native or trypsin-treated human erythrocytes as in [2]. The effect of the lectin on platelets was studied using either platelet-rich plasma or using a washed platelet suspension after the platelets had been separated from plasma by centrifugation through a discontinuous albumin gradient. The aggregation and effects of inhibitors was monitored visually using a light microscope.

+ Present address: EMBL, Postfach 10.2209, 69 Heidelberg, FRG

3. Results and discussion

The glycoprotein extract retained by WGA–agarose and eluted by *N*-acetylglucosamine agglutinated erythrocytes. Leakage from the lectin-column could not account for this activity since no agglutination activity could be washed from a newly-prepared column by 100 mM *N*-acetylglucosamine in SDS-containing elution buffer. A total solubilized plasma membrane fraction and the effluent through the WGA–agarose column did not exhibit agglutination activity. Since a total plasma membrane fraction contains both lectins and endogenous glycosylated receptors it is probable that activity cannot be expressed towards exogenous receptors in a test assay until the 2 self-neutralized components are separated from one another by purification. Recent studies [2] have indicated that the red cell is an analogous system, in which a total extract did not exhibit lectin activity but glycophorin when separated from the other membrane components (using 2 different purification procedures) did exhibit lectin activity towards trypsin-treated erythrocytes.

Results from the use of affinity chromatography to prepare platelet glycoproteins [5] suggested that glycoprotein I was the only component retained by WGA–Sepharose. However, preliminary data (unpublished) from our laboratory indicate that the situation is more complex with more than one glycosylated species present, and investigations are in progress to characterize which component in the glycoprotein extract is the lectin. Another report on lectin activity associated with platelet plasma membranes appeared recently, but the activity was assayed only as a membrane vesicle suspension and the ability to agglutinate platelets was not tested [6].

In all combinations tested, trypsin-treated erythrocytes of blood group specificity A^+ gave the highest titer with a glycoprotein extract prepared from either a mixed blood group population of platelets or from platelets of a single blood group type. It was found that 0.02 μg/100 μl was sufficient to just agglutinate trypsin-treated human A^+ erythrocytes, whereas approx. 2 orders of magnitude more (2–6 μg/100 μl) was necessary to cause agglutination of untreated erythrocytes. Table 1 shows the results of haemagglutination assays against erythrocytes of different blood groups. Haemagglutination could not be inhibited by any monosaccharide tested (these included: galactose, galactosamine–HCl, *N*-acetylgalactosamine) at final conc. 100 mM in PBS. Blood group substances were used as an initial probe into the specificity of the haemagglutination. Ovarian cyst blood group glycoprotein A was the most effective inhibitor of agglutination of all blood group types of trypsin-treated erythrocytes tested: 15.6 μg/ml blood group substance A was sufficient to inhibit agglutination caused by 225 μg/ml extract whereas concentrations of up to 125 μg/ml of blood group substances B and H were ineffective. Both the high titers exhibited against type A^+ erythrocytes and the efficiency of blood group substance A in inhibiting agglutination, would indicate that the carbohydrate specificity is related to residues of *N*-acetyl-D-galactosamine. The molecular nature of the receptor to the platelet lectin in the erythrocyte membrane is as yet unknown. It is of interest that the specificity of the lectin activity of glycophorin is very similar to that exhibited by the platelet lectin. This may reflect the common origin of the different cells of the circulatory system.

Table 1
Haemagglutination activity of the glycoprotein extract

Blood group type	Titer exhibited by 21 μg/100 μl glycoprotein extract.
A^+ native	8
B^+ native	8
O^+ native	4
A^+ trypsin-treated	1024
B^+ trypsin-treated	128
O^+ trypsin-treated	128

Haemagglutination activity was determined by a serial dilution technique using microtiter plates and a 1% suspension of erythrocytes. Trypsin treatment was a described previously [2]

Preliminary results indicate that the glycoprotein extract can cause agglutination of platelets which can be specifically inhibited by preincubation of extracts with blood group substance A (fig.1). At this stage, it is not known whether this agglutination of platelets is an ADP-mediated response or whether it is due to a simple bridging of the cells by a multivalent lectin present in the extract. However, it is clear that since clumping is induced by the extract there is a native receptor to the endogenous lectin available on the

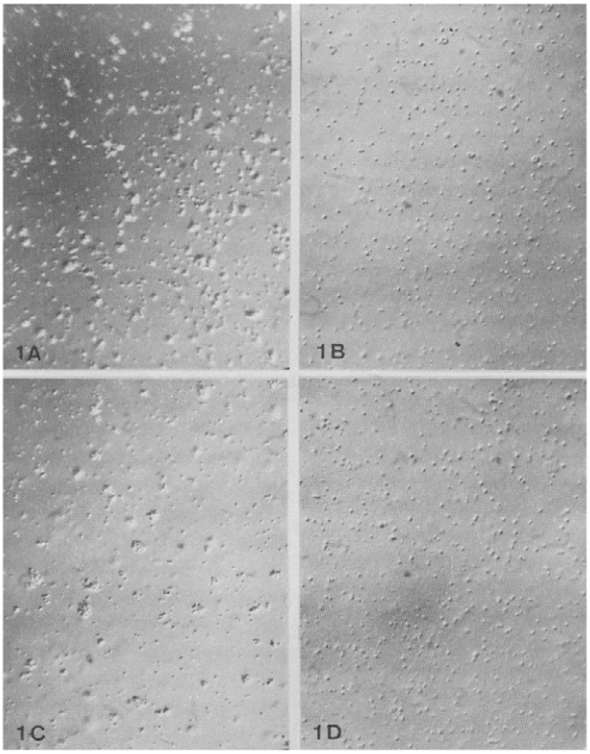

Fig.1. Platelet suspensions viewed using a Zeiss microscope and Nomaski optics. (A) Platelet-rich plasma (blood group A) was incubated with 64 μg/ml blood group A platelet lectin extract. (B) as in (A), but the lectin was preincubated with 500 μg/ml blood group substance A. (C) and (D) are the same incubation conditions as in (A) and (B) but using a washed platelet suspension rather than platelet-rich plasma. (magnification 50 X.)

outer surface of plasma membranes of intact platelets. This raises the exciting possibility that platelet–platelet aggregation during the physiological process of blood clot formation, may be mediated through endogenous lectin–receptor interaction. A basic requirement of this hypothesis is that in freely-circulating platelet the plasma membrane lectin must be present but inactive and upon subsequent triggering of the cell become activated and able to bind to adjacent cells. This requirement could be satisfied by 2 main models. Firstly, the lectin could be buried in the membrane and on activation and shape change become more exposed and able to bind to receptors continuously available at the cell surface. Alternatively, both the lectin and receptor may be present at the surface of the cell but self-neutralized in a freely-circulating platelet, and on triggering of cell aggregation the pair-complex dissociates and intercellular interaction outside of the plane of the surface membrane occurs. This implies that induction of cell aggregation may be controlled by the same parameters as those that cause dissociation of an endogenous lectin–receptor pair complex.

Acknowledgements

D.J.B. acknowledges financial support from an EMBO short term fellowship to the Weizmann Institute, and an SRC fellowship at the Department of Biochemistry, University of Cambridge. A. R. is a Charles E. Smith fellow at the Weizman Institute. D.J.B. would like to thank Euan and Carol for their help and advice in taking blood samples and the preparation of washed platelets. The blood group substances were a kind gift from W. Watkins for CRC Harrow. Many thanks also are due to Nathan Sharon and Carlos Gitler for their enthusiasm and stimulating discussions.

References

[1] Nurden, A. T. and Caen, J. P. (1975) Nature 255, 720–722.
[2] Bowles, D. J. and Hanke, D. E. (1977) FEBS Lett. 82, 34–38.
[3] Barber, A. J. and Hamieson, G. A. (1970) J. Biol. Chem. 245, 6357–6365.
[4] Miron, T., Carter, W. G. and Wilchek, M. (1976) J. Solid Phase Biochem. 1, 225–234.
[5] Clemetson, J., Pfueller, S. L., Luscher, E. F. and Jenkins, C. S. P. (1977) Biochim. Biophys. Acta 464, 493–508.
[6] Gartner, T. K., Williams, D. C. and Phillips, D. R. (1977) Biochem. Biophys. Res. Commun. 79, 592–599.