

EVIDENCE FOR LECTIN ACTIVITY ASSOCIATED WITH GLYCOPHORIN, THE MAJOR GLYCOPROTEIN OF HUMAN ERYTHROCYTE MEMBRANES

Implications for the structure of membranes

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1. Introduction

The human erythrocyte ghost membrane has been extensively investigated as a model system for the structure of biological membranes. Recent demonstrations that lectins are present as membrane components of plant cells [1–3] and examples of such lectins in preparations from animal cells [4–7] led us to consider the significance of the presence of lectins in membranes. In the present report, we demonstrate that lectin activity can be found in ghost membranes from human erythrocytes and that this activity is associated with the major glycoprotein of the membrane, known as glycophorin. We propose a possible role for lectins in membranes.

2. Methods

2.1. Preparation of glycophorin

Glycophorin was prepared according to the published techniques of Marchesi et al. by affinity chromatography on WGA-Sepharose [8,9]. Fresh blood (human type A⁺) was drawn into phosphate-buffered saline (PBS) pH 7.4, extensively washed

with PBS, and white cells and platelets removed before preparation of the ghosts [10]. Ghost membranes (40 ml packed cell vol.) were first extracted with 30 mM LIS, and the non-solubilized material incubated in 1% SDS, 250 mM NaCl and 15 mM sodium phosphate, pH 7.2, at 37°C for 15 min. After centrifugation the SDS concentration was lowered to 0.05% and the solution applied to a WGA-Sepharose column (bed vol. 3.0 ml) equilibrated in the same buffer. After washing the column through with buffer until the A_{280} of the eluant was < 0.05 the glycoprotein was eluted with 25 ml 100 mM *N*-acetyl-D-glucosamine. The column eluates were either dialysed against H₂O and freeze-dried or dialysed against PBS and concentrated by vacuum dialysis. Protein was determined using the method of Lowry [11] with BSA as a standard. SDS-equilibrated polyacrylamide gel electrophoresis was carried out according to the method of Laemmli [12].

2.2. Assays of agglutination

For assays of agglutination, fresh human blood was washed 5 times with PBS and the cells were used either untreated; after neuraminidase treatment: 50 units/ml, at room temperature, pH 7.4, for 30 min (Behringwerke, A. G. Marburg/Lahn FRG); or after trypsin-treatment: 1 mg/ml, at 37°C, pH 7.4, for

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45 min (Sigma Chemical Co.). After enzyme treatment the red blood cells were washed 6 times with PBS. Washed rabbit erythrocytes were also used for agglutination assays after treatment with trypsin (1 mg/ml, at 37°C, pH 7.4, for 45 min). A two-fold dilution series of the glycophorin preparation was made in PBS, pH 7.4, in microtitre plates (Cooke Engineering) and agglutination activity was assayed using a 1% suspension of erythrocytes. The effect of simple sugars to inhibit the agglutination against rabbit erythrocytes, were performed using a final concentration of 5×10^{-3} M. In addition, inhibition by various glycoproteins was checked using a two-fold dilution series of the substances in PBS. *N*-Butanol extraction of the glycophorin preparation was carried out at 4°C, the organic and aqueous phases were separated by centrifugation at $5000 \times g$ for 10 min, and lectin activity in the aqueous phase was assayed against human trypsin-treated erythrocytes. Anti-A antibodies (Hyland) were used to check the presence of blood group determinants in the glycophorin preparation.

3. Results and discussion

Glycophorin extracts prepared using affinity chromatography on WGA-Sepharose (fig.1) agglutinated erythrocytes. In an initial series of experiments, the activity was assayed against trypsin-treated rabbit erythrocytes. The minimal concentration required to agglutinate such erythrocytes was found to be 0.6 µg protein/50 µl; using a dilution of glycophorin that gave a titre of 128, 5×10^{-3} M D-mannose, L-fucose, D-xylose and *N*-acetyl-D-glucosamine were not inhibitory, whereas D-galactose reduced the titre from 128 to 64 and *N*-acetyl-D-galactosamine completely inhibited the agglutination.

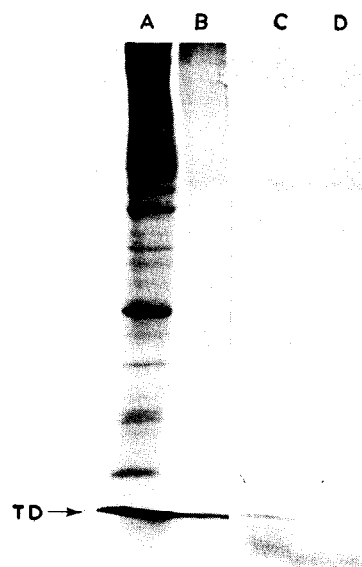


Fig.1. SDS-gel electrophoretograms of solubilized erythrocyte membranes (A and C) and substances eluted from WGA-Sepharose (B and D). Samples were solubilized by boiling for 3 min in 2% SDS, 5% mercaptoethanol, 10% glycerol and 50 mM Tris-HCl, pH 7.1. (TD) Tracking dye. The gels were stained either with Coomassie Blue for proteins (A and B) or with periodic acid Schiff reagent for carbohydrate (C and D).

Agglutination activity was also assayed using human erythrocytes. The results are shown in table 1. The effect of various glycoproteins on the activity was examined and the results are shown in table 2. Glycophorin only agglutinated human erythrocytes after trypsin treatment. The major effect of trypsin treatment is to cleave glycopeptides from the external carbohydrate-rich N-terminal of the glycophorin

Table 1
Agglutination activity of glycophorin with human erythrocytes

Type of blood cell	Titre shown by glycophorin (11.25 µg/50 µl)
Untreated A ⁺ , B ⁺ , O ⁺	0
Neuraminidase-treated A ⁺ , B ⁺ , O ⁺	0
Trypsin-treated A ⁺	32
Trypsin-treated B ⁺	4
Trypsin-treated O ⁺	2

Table 2
Effect of glycoproteins on agglutination of trypsin-treated human A⁺ erythrocytes by glycophorin

Glycoprotein	Minimal concentration ^a ($\mu\text{g/ml}$)
Fetuin	> 500
Asialofetuin	> 500
Asialo-ovine submaxillary glycoprotein	16
Ovarian cyst blood group substance A	34
Ovarian cyst blood group substance B	> 250
Ovarian cyst blood group substance H	> 250

^a Concentration required to completely inhibit agglutination by 1.9 $\mu\text{g}/50\text{ ml}$ glycophorin

polypeptide. In such circumstances, the carbohydrate moieties of other glycosylated species in the membrane would be more exposed, and presumably it is towards these that the lectin activity of the glycophorin extract is directed. The high titre against type A erythrocytes and the effectiveness of asialomucin and ovarian blood group substance A in inhibiting agglutination, confirm the specificity of the lectin activity for residues of *N*-acetyl-D-galactosamine.

The agglutination activity exhibited by glycophorin extracts could be due to factors other than a direct protein-carbohydrate interaction between glycophorin and carbohydrate residues available on the erythrocyte surface (e.g., refs [13–15]). The lectin activity cannot be explained by ligand leakage from the affinity Sepharose column, since:

- (i) The agglutination of erythrocytes by the ligand (WGA: Sigma) was not inhibited by *N*-acetyl-D-galactosamine.
- (ii) The lectin activity of the glycophorin preparation was not inhibited by *N*-acetyl-D-glucosamine (a specific inhibitor of WGA).

Indeed, the possibility that activity results from the preparative method can be ruled out since glycophorin prepared by an alternative method, the LIS-solubilization procedure [15], also agglutinated erythrocytes, but at a much lower specific activity (unpublished results).

Agglutination cannot be caused by some component of the erythrocyte surface recognising A blood group determinants in the glycoprotein extract since, using anti-A antibodies, the glycophorin preparation

was shown to possess no A blood group determinants although from fig.1, and thin-layer chromatography in a propanol-H₂O 7:3 system (unpublished data), glycolipids are present in the extract. However, it is unlikely that glycolipids are responsible for the agglutination of erythrocytes since:

- (i) removal of substantial amounts of glycolipids by *N*-butanol extraction did not reduce the agglutination activity of the aqueous phase.
- (ii) Agglutination activity was completely destroyed by boiling for 10 min.

Agglutination activity towards erythrocytes requires a minimum of two binding sites per functional molecule of lectin. If it is assumed that the agglutination activity of glycophorin resides in the protein moiety of the molecule, comparatively few amino acid residues (approx. 70) may be on the external face of the bilayer and available to provide a binding pocket for receptors [17]. It is possible that each molecule may in fact possess only one sugar-binding site and agglutination activity is possible because glycophorin molecules aggregate. It has been shown that dimerization of glycophorin can occur in vitro [18], but there is no evidence, as yet, for its occurrence in situ. It is interesting to consider what might act as the receptor of the lectin in vivo. Recent studies by us have shown that in protoplasts prepared from soybean callus, a lectin and its receptor are present in the plasma membrane. [3]. Both components are available for binding to externally supplied lectin or sugar, respectively, up until the time when the surface

membrane of the protoplast is masked by cell wall deposition. Lectins and their receptors are also present in plasma membranes of aggregating amoebae of two slime mould species [19–21]. We suggest that there may be an interaction between an endogenous lectin and its receptor(s) in the plane of the membrane in both plant and animal cells. There are two main possibilities for lectin receptors in the membrane: glycoproteins and glycolipids. As yet, no information on the protein or lipid nature of the receptor for endogenous membrane lectins has been reported. Liposomes prepared from protein-free lipids of human and rabbit erythrocyte ghosts can be agglutinated by soybean agglutinin [22], indicating that the carbohydrate moieties of the glycolipids contain galactose and/or *N*-acetyl-D-galactosamine. Therefore an endogenous receptor for glycophorin could be glycolipid. Alternatively, another glycosylated component of erythrocyte membranes is the glycoprotein(s) known as Band 3; although only 6–8% glycosylated the component in the specific receptor for several seed lectins [23,24] and thus could act as endogenous receptor for glycophorin.

The implications of endogenous lectin–receptor interaction in the plane of the membrane are numerous. Proteins and lipids exhibit lateral movement, either by a passive process described as lipid flow [25], or by an active mechanism involving attachment of proteins/glycoproteins to an underlying cytoskeletal system [26,27]. We would like to put forward the hypothesis that an important factor in the control of lateral mobility of membrane components is lectin–receptor interaction. Many observations have suggested that the movement of components relative to one another may be decisive in the determination of the metabolic state of the cell. We suggest a model in which the inherent reversibility, characteristic of lectin binding, provides the cell with a sensitive mechanism for rearranging functional complexes within a membrane. One could foresee a ‘closed’ system, whereby lectin and receptor interact in the plane of the membrane, and an ‘open’ system where the individual components of the pair are freed from interaction (e.g., by exogenous competitor, metabolic signal or modification by enzyme action etc.) and are thereby allowed both increased movement in the membrane and the potential to interact with components of neighbouring cells.

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