DIRECT AUTORADIOGRAPHIC VISUALISATION IN SDS-GELS OF LECTIN-BINDING COMPONENTS OF THE HUMAN ERYTHROCYTE MEMBRANE

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1. Introduction

The glycoprotein composition of plasma membranes and changes therein accompanying a variety of cellular events [1] are of interest in many areas of molecular biology. Lectins [2] are useful reagents for investigating and isolating glycoproteins and we describe here the use of a new technique for both identification and quantification of specific lectin binding sites in plasma membranes. This technique has enabled us to show that the diversity of lectin binding components of the human erythrocyte membrane is greater than was previously suggested [3–5].

Human erythrocyte membranes were solubilised in sodium dodecyl sulphate (SDS) and the components resolved by polyacrylamide gel electrophoresis. After staining with Coomassie blue, the gels were fixed in glutaraldehyde, treated with sodium borohydride to block excess aldehyde groups and incubated with [^{131}I]-lectin conjugates. The glycopeptides acting as specific lectin 'receptors' were then visualised by autoradiography.

2. Materials and methods

Lens culinaris phytohaemagglutinin (LCPHA) and *Axinella* sponge agglutinin (AX) were gifts of Dr D. Snary; *Phaseolus vulgaris* agglutinin (PHA) purified mitogenic grade was purchased from Wellcome Laboratories, Beckenham, England and bovine pancreatic ribonuclease, *Clostridium perfringens* neuraminidase and Concanavalin A (Con A) from Sigma Chemical Co. Wheatgerm agglutinin (WGA) was prepared by the method of Allen et al. [6]. Human erythrocyte membranes were a gift of Dr Jenny Fordham.

2.1 *Separation of membrane components*

Erythrocyte membranes (5–10 mg protein ml^{-1}) were incubated for 30 min at 37°C in 0.1 M sodium phosphate pH 6.5 and 1 mM phenylmethylsulphonyl fluoride with or without 0.05 I.U. ml^{-1} neuraminidase. Membranes were immediately solubilized with SDS and 2-mercaptoethanol at final concentrations of 2% and boiled for 2 min. Membrane and mouse serum proteins were resolved in 8% w/v polyacrylamide gel slabs [7]. Gels were stained with either Coomassie blue or Schiff's reagent [8] and photographed. The gels were fixed in 0.1 M phosphate pH 7.0 (buffer A) containing 0.05% w/v glutaraldehyde for 6 h changing twice and then reduced in buffer A containing 10 μg ml^{-1} $BaBH_4$ for 24 h changing twice.

2.2. *Preparation of [^{131}I]-lectins and labelling of gels*

[^{131}I]-lectin conjugates were prepared using carrier-free Na[^{131}I] by the chloramine T procedure [9]; the spec. act. were approx. 1 mCi·mg^{-1} protein. Conjugates (1 mg·ml^{-1}) contained ribonuclease (5 mg·ml^{-1}) either in buffer A or 0.1 M Tris pH 7.0 with 1 mM $CaCl_2$ and 1 mM $MnCl_2$ (buffer B) for Con A. Ribonuclease was included as it entered the gels rapidly and minimized non-specific binding of the conjugates. Gels were supported on glass plates in a humidified chamber and 0.5 ml of conjugate solution applied to the surface of each gel with a brush. After 24 h gels were washed

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with buffer A (buffer B for Con A) for 3 days, changing frequently. The gels on glass plates were sealed with cellulose film and autoradiographs prepared by exposing Kodirex X-ray film for 1–24 h. Densitometry of autoradiographs was performed using a Joyce-Loebl instrument.

3. Results

The numbered components of Fairbanks et al. [3] were identified here by two-dimensional electrophoresis, the conditions for the first dimension being those of Fairbanks et al.

In fig.1, autoradiographs of gels developed with ^{131}I-conjugates of PHA, LCPHA, Con A, AX and WGA are compared with protein and glycoprotein staining patterns of identical gels.

The major sialoglycoprotein, PAS 1 [3], was labelled strongly by WGA, LCPHA and AX and weakly by PHA and Con A. This region was stained only poorly with Coomassie blue.

PAS 2 was labelled by WGA, LCPHA and AX, but PAS 3 only by WGA and AX Protein III bound Con A most strongly, LCPHA to a lesser extent and WGA, AX and PHA only weakly.

A diffuse region of lower mobility than proteins I and II was revealed by WGA and AX. The latter also labelled a Coomassie blue-positive band (arrow).

Neuraminidase treatment abolished all well-defined labelling by WGA. Labelling of PAS 1 by PHA, LCPHA and AX showed that removal of sialic acid residues resulted in an increase in mobility of the leading edge of this component. The labelling of PAS 2 by LCPHA and of PAS 2 and 3 by AX was more diffuse, also with AX two new bands between PAS 2 and 3 were just discernible. Con A labelling of protein III was not affected by neuraminidase treatment.

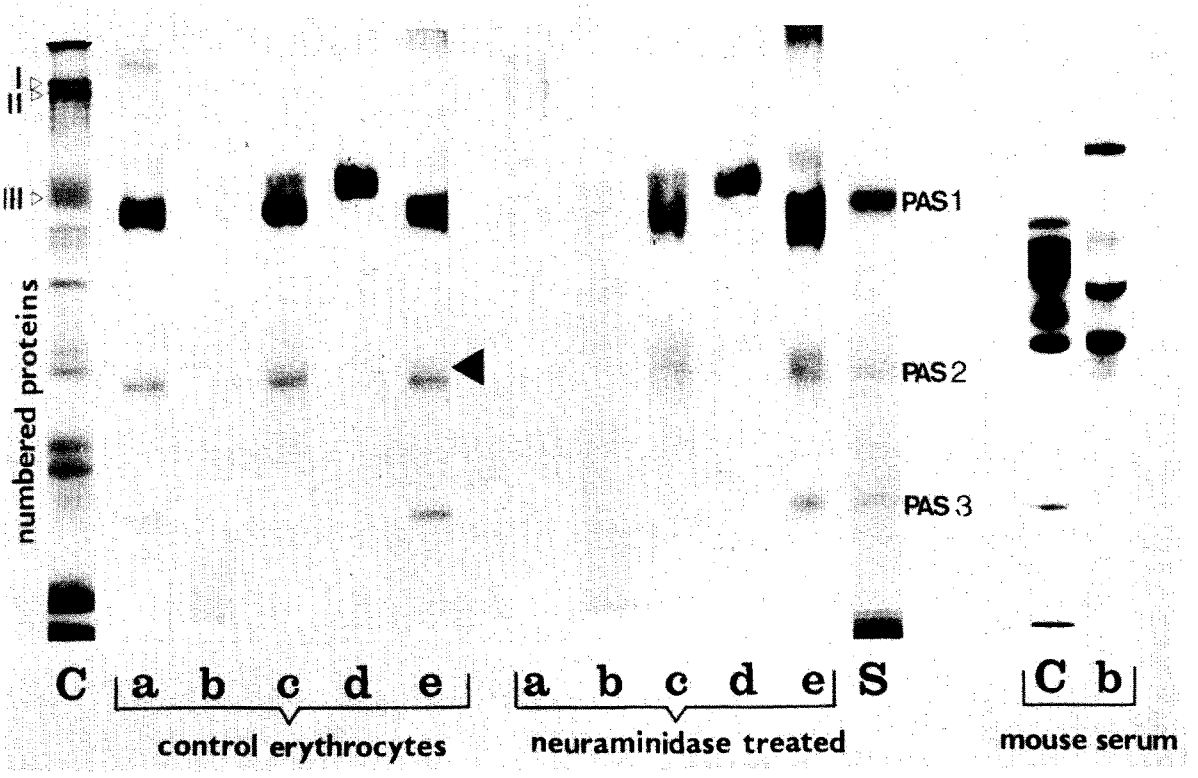
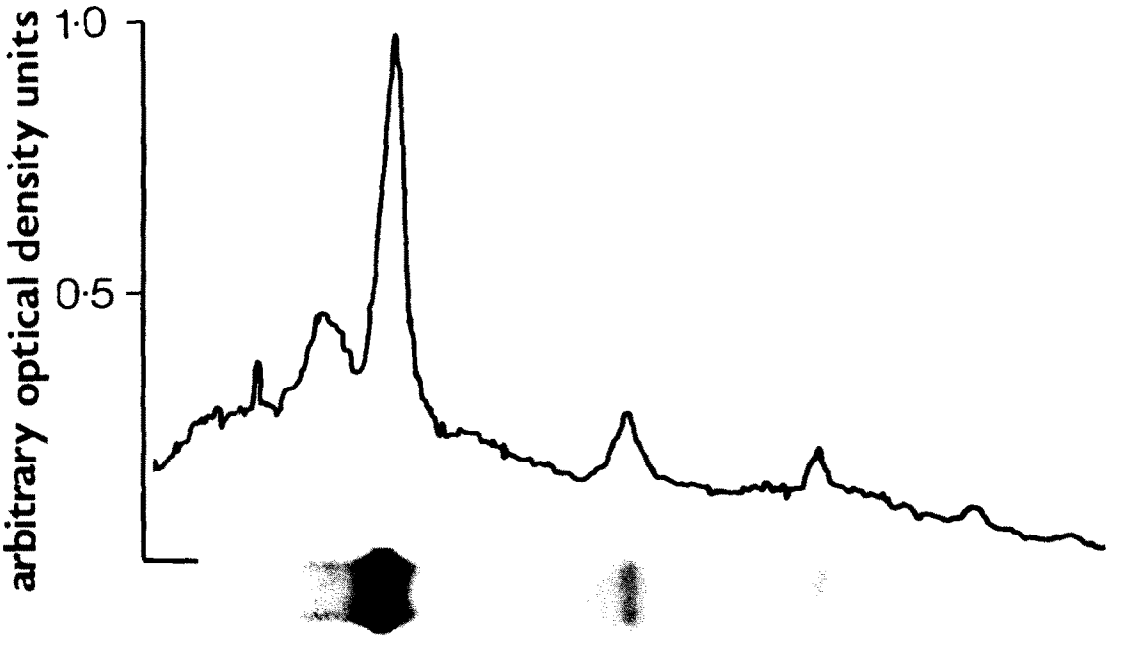


Fig.1. SDS-gels of erythrocyte membranes, neuraminidase treated membranes and mouse serum. Gels were stained with Coomassie blue (C) for Schiff's (S). Autoradiographs were made with [^{131}I]-lectin conjugates of (a) WGA, (b) PHA, (c) LCPHA, (d) Con A and (e) AX. Bands were numbered according to Fairbanks et al. [3].



sample volume (μl)	PAS 1	PAS 2	PAS 3
75	48	5·1	3·0
25	18	2·0	1·0
8	6·9	-	-
2	2·7	-	-

Fig.2. Densitometric tracing of an autoradiograph of [^{131}I]-WGA labelled gel of erythrocyte membranes and table showing relative peak areas of PAS 1, 2 and 3 as a function of sample volume loaded.

The PHA used was a mitogenic, low erythroagglutinating grade and this probably accounts for the weak labelling of the membrane components. This same preparation of conjugate gave sharp labelling of a number of components in mouse serum (fig.1).

Preincubation of gels with 0.1 M D-galactose prevented labelling of erythrocyte membrane components by AX. However, 0.1 M D-galactose did not inhibit binding of WGA. Thus, the results suggest that labelling of glycoproteins by ^{131}I-conjugated lectins following SDS-gel electrophoresis appears to be a function of the carbohydrate specificities of the lectins.

Densitometry of autoradiographs from gels with different sample loadings has shown that the labelling is quantitative and that the relative proportions of WGA bound to PAS 1, 2 and 3 respectively are 18:2:1 (fig.2).

4. Discussion

The data presented demonstrate that glycopeptides which bind lectins can be identified in polyacrylamide gels by treatment with the appropriate radio-labelled lectin following electrophoresis. Our results indicate that SDS and fixation do not prevent lectin binding to glycoproteins under these conditions. The inhibition of binding by monomeric sugars show that lectin

binding is sugar specific [10] and this is confirmed by the labelling patterns of erythrocyte components which are consistent with previous studies. Thus, Findlay [4] has shown that Con A binds only to protein III but that LCPHA which has similar monomeric sugar specificity, binds also to the major sialoglycoprotein (PAS 1). Our results also confirm those of Adair and Kornfeld [5] showing that the principal 'receptor' for WGA is the major sialoglycoprotein. However, these workers did not isolate the other minor WGA-binding components detected on our gels.

The numerous AX-binding components indicate a large spectrum of D-galactose-containing glycoproteins in the erythrocyte membrane. This has previously been suggested from labelling studies using galactose oxidase and [^{35}S] methioninesulphone hydrazide [11].

As well as *N*-acetyl glucosamine, WGA is known to bind to sialic acid [12] which may account for neuraminidase treatment abolishing WGA binding. The increased mobility of the major sialoglycoprotein (PAS 1) observed after neuraminidase treatment is clearly shown by labelling with AX and LCPHA as has previously been noted by Fairbanks et al. [3].

This accord with previous work on erythrocyte glycoproteins is strong evidence for the validity of the present technique which is now being employed to characterise membrane and other glycoproteins and, in particular, to investigate changes in membrane glycoproteins occurring during activation of lymphoid cells.

Acknowledgement

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