ISOLATION OF GLYCOPROTEINS FROM PIG LYMPHOCYTE PLASMA MEMBRANE USING Lens culinaris PHYTOHEMAGGLUTININ

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Lymphocyte plasma membrane glycoproteins solubilized in 1% sodium deoxycholate were selectively purified by adsorption to LcH*, covalently attached to Sepharose 4B, and by subsequent elution with methyl-α-D-mannopyranoside. The yield of glycoprotein was more than twice that obtained using Concanavalin A-Sepharose. The purified glycoproteins inhibited the transformation of lymphocytes by LcH and PHA. The method is probably of general applicability to the purification of membrane glycoproteins.

Recently it was reported that lymphocyte plasma membrane glycoproteins can be purified by affinity chromatography on a column of Concanavalin A-Sepharose in 1% sodium deoxycholate (1). The procedure suffered, however, from two disadvantages that were apparently due to incomplete elution of material; firstly 80% only of the protein added to the column was recovered and, secondly, the yield of glycoproteins (5%) was low. The phytohemagglutinin isolated from Lens culinaris beans (2) binds the same sugar residues as Concanavalin A but with a 50-fold lower affinity (3); it also resembles Concanavalin A in stimulating lymphocytes to transform and undergo mitosis (4). In view of the decrease in strength of binding of

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* Abbreviations: LcH, Lens culinaris phytohemagglutinin; PHA, Phaseolus vulgaris phytohemagglutinin; SDS, sodium dodecyl sulphate.

carbohydrates by LcH it seemed likely that the above problems would be resolved by replacing Concanavalin A with LcH. The results indicate that LcH-Sepharose gave over a two-fold increase in the yield of glycoproteins. The purified material contained at least the majority of the membrane glycoproteins.

Materials and Methods

LcH was isolated from a commercial sample of lentils by a slight modification of the method described by Howard et al (2). The fraction precipitated between 30% and 80% saturation of ammonium sulphate was added to Sephadex G-75 and the adsorbed material was eluted with 0.1M-methyl-α-D-glucopyranoside [Sigma (London) Chemical Co. Ltd.] in 75mM-Na phosphate buffer-75mM-NaCl, pH 7.4. Polyacrylamide gel electrophoresis by the method of Davis (5) and amino acid analysis gave similar results to those reported previously (2). Equilibrium sedimentation (6) in 0.02 I-Na phosphate buffer-0.2M-NaCl, pH 7.1, at concentrations of 2 to 5 mg/ml gave apparent molecular weights of about 52000 using $\bar{v}$ of 0.745 (calc. from amino acid composition). Velocity sedimentation at 25°C of a 6.2 mg/ml soln. revealed one symmetrical peak with a $S_{20,w}$ of 3.8.

Sepharose 4B was activated with cyanogen bromide (20 mg/ml of settled beads) and coupling of LcH (1 mg/ml of settled beads) was performed in 0.1M-methyl-α-D-glucopyranoside-0.1M-$NaHCO_3$, pH 8.4, at 2°C for 18 hr. The slurry was then stirred for 1 hr in 5mM-ethanolamine HCl, pH 8.4, and washed successively with 0.1M-$NaHCO_3$, water, 1% sodium deoxycholate, 2% methyl-α-D-mannopyranoside in 1% sodium deoxycholate and finally with 1%

sodium deoxycholate. The absorbance at 280nm of the washings suggested that more than 90% of the LcH was bound. After prolonged standing at 2°C small amounts of LcH were eluted on washings. As a result, LcH-Sepharose was washed with 1% sodium deoxycholate immediately prior to addition of solubilized membrane.

Pig lymphocyte plasma membrane was prepared and solubilized in 1% sodium deoxycholate at 23°C as described by Allan & Crumpton (7). The fractionation of the solubilized membrane and the recovery of fractions were carried out as previously reported (1).

Results and Discussion

Fig. 1 shows the separation of pig lymphocyte plasma membrane into two fractions by using LcH-Sepharose. Approx. 95% of the added

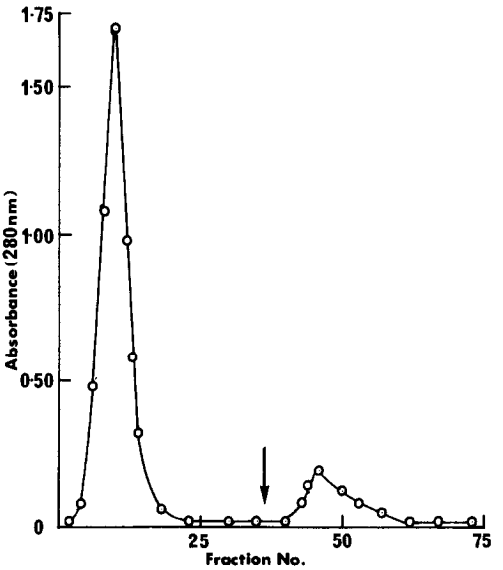

Fig. 1. Fractionation of lymphocyte plasma membrane using LcH-Sepharose. Membrane solubilized in 1% sodium deoxycholate (2 ml containing 8 mg of protein) was eluted from a column (8 x 1 cm) of LcH-Sepharose with 1% deoxycholate until the absorbance at 280nm of the eluate returned to the base-line. The column was then washed (arrow) with 2% (w/v) methyl-α-D-mannopyranoside in 1% deoxycholate.

protein was recovered as determined from the absorbance at 280nm and by the method of Lowry et al (8). The unretarded and eluted fractions were recovered by precipitation with 0.1 vol. of 2% acetic acid and by washing with 95% ethanol. The glycolipids of the membrane were discarded during this procedure together with the deoxycholic acid and the sugar used for elution. As a result the recovered carbohydrate represents glycoprotein. The relative amounts of protein, carbohydrate (neutral sugar) and 5'-nucleotidase associated with the two fractions are shown in Table 1. The eluted fraction contained 83% of the neutral sugar but only 13% of the protein. It was concluded that the major portion of the membrane glycoproteins were present in this fraction. This conclusion is supported by a comparison of the carbohydrate patterns of the membrane and the fractions after polyacrylamide gel electrophoresis in SDS (Fig.2A-C). These results emphasise that the unretarded fraction is markedly depleted and the eluted fraction

Table 1. Distribution of protein, neutral sugar and 5'-nucleotidase activity between the unretarded and eluted fractions.

Fraction	Protein	Neutral sugar	5'-Nucleotidase
Unretarded	87	17	15
Eluted	13	83	85

The results are expressed as per cent of the total material recovered and represent the average of 3 experiments. Protein was determined by the method of Lowry et al (8), neutral sugar by the anthrone method using glucose as standard (9) and 5'-nucleotidase according to Michell & Hawthorne (10).

is enriched in carbohydrate relative to the membrane. The yield of glycoproteins (12% of the protein added to the column) was more than twice that obtained using Concanavalin A-Sepharose (1).

The distribution of 5'-nucleotidase paralleled that of carbohydrate (Table 1) and suggested that this enzyme is a glycoprotein. The exact significance of the carbohydrate associated with the unretarded fraction is not clear. It probably does not,

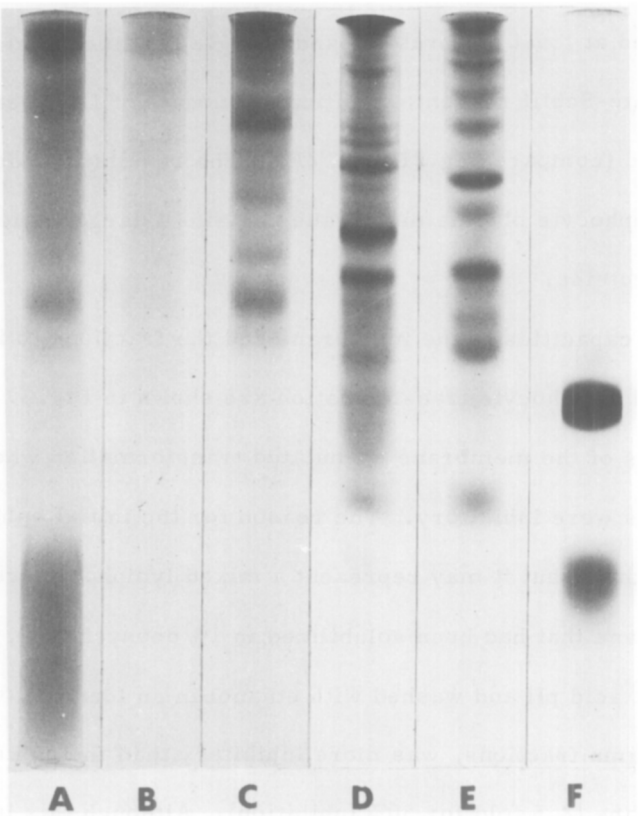

Fig.2. Carbohydrate and protein patterns after polyacrylamide disc gel electrophoresis in SDS. Electrophoresis was carried out as described previously (7). A and D, Pig lymphocyte plasma membrane (400 and 200μg of protein respectively); B, unretarded fraction (200μg of protein); C and E, eluted fraction (200 and 100μg of protein respectively); F, LcH (50μg). Gels A-C were stained for carbohydrate with periodate-Schiff reagent (11), and gels D-F for protein with 'Naphthalene Black 10B'.

however, represent a unique class of glycoproteins but rather membrane that was not dissociated in 1% deoxycholate. This explanation is supported by the residual 5'-nucleotidase activity of the unretarded fraction and the capacity of this fraction to inhibit lymphocyte transformation (Fig. 3), and by the observation that about 10% of the solubilized membrane protein was excluded by Sepharose 6B (see Fig. 6; ref. no. 7).

Polyacrylamide gel electrophoresis in SDS of the eluted fraction revealed at least ten protein bands (Fig. 2E) that also stained with periodate-Schiff reagent; significant amounts of LcH were not detected (compare Fig. 2E with 2F). The results indicate that the lymphocyte plasma membrane contains a large variety of glycoproteins.

The capacities of the membrane and the fractions to inhibit LcH-induced lymphocyte transformation are shown in Fig. 3. Small amounts of the membrane stimulated transformation whereas larger amounts were inhibitory. The reason for the initial enhancement is not known but it may represent a mixed lymphocyte reaction. Membrane that had been solubilized in 1% deoxycholate, precipitated at acid pH and washed with ethanol in an identical way with the column fractions, was more inhibitory than the untreated membrane (2.5 fold for 50% inhibition). Although this increase in activity was probably due to the exposure of 'cryptic' sites in the membrane that bind LcH, it may be related to the loss of the membrane's capacity to stimulate transformation (Fig. 3). The inhibitory activity of the unretarded fraction indicated that it contained small amounts of glycoproteins that bind LcH.

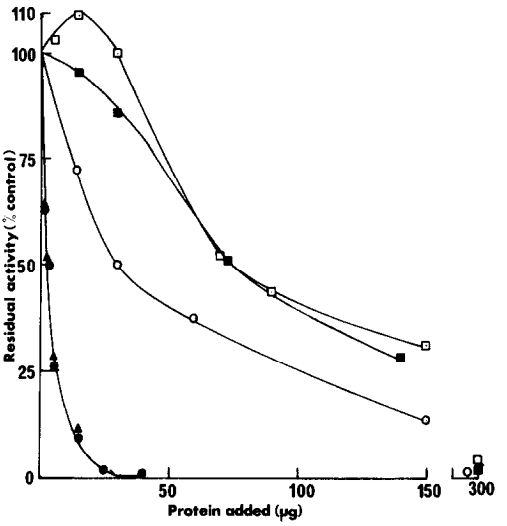

Fig. 3. Inhibition of LcH-induced lymphocyte transformation by pig lymphocyte plasma membrane (□), membrane recovered after solubilization in deoxycholate (○), the unretarded fraction (■) and the eluted fraction (●). The capacity of the eluted fraction to inhibit lymphocyte transformation by PHA is also shown (▲). Inhibition of transformation was measured as described previously (1) except that 5μg of LcH or 1μg of PHA was used.

In contrast, the eluted fraction was 25-fold more effective in causing 50% inhibition than the unadsorbed material and 10-fold more effective than the treated membrane. The relationship between the large variety of glycoproteins in the eluted fraction and the transformation of lymphocytes by LcH is uncertain. It is, however, feasible that one glycoprotein only mediates transformation. Fractionation of the lymphocyte glycoproteins by using phytohemagglutinins with different specificities should help to resolve this problem. As the eluted fraction also inhibited lymphocyte transformation by PHA (Fig. 3), at least one glycoproteins must be bound by both PHA and LcH. Although this does not mean that the glycoproteins which mediate LcH- and

PHA-transformation are the same, it makes it more likely especially since PHA does not bind all the glycoproteins that interact with LcH (unpublished observations).

Conclusions

LcH-Sepharose is superior to Concanavalin A-Sepharose for the isolation of lymphocyte plasma membrane glycoproteins. In view of the fairly broad specificity of LcH for carbohydrates and the similar carbohydrate compositions of glycoproteins, the method is probably of general applicability to the isolation of membrane glycoproteins.

Acknowledgements

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