IDENTIFICATION OF CONCANAVALIN A-BINDING PLASMA MEMBRANE ANTIGENS OF RAT LIVER

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1. Introduction

Lectins with specificities for different sugars have been extensively used as tools for characterization of surfaces of normal and neoplastic cells [1]. However, very little is known about the plasma membrane components carrying the carbohydrate moieties involved in the lectin-cell interaction.

The binding of different lectins to subcellular fractions of hepatocytes have earlier been studied by Henning and Uhlenbruck, using an agglutination technique [2]. Binding of liver carbohydrate structures to concanavalin A (Con A) has been investigated both with isolated membranes [3] and with detergent solubilized membranes [4]. Winquist et al. [4] identified the Con A-binding material from microsomes as membraneous oligosaccharide–lipid–protein complexes.

Con A-binding to rat liver plasma membranes competes with the binding of insulin [5] and inhibits the activity of 5′-nucleotidase [6], indicating that the lectin reacts with sugar moieties on or near the structures with these activities.

We attempted to resolve the Con A-binding plasma membrane fraction into individual antigens by using an immunodiffusion technique. For this purpose detergent extracts of isolated plasma membranes were subjected to affinity chromatography on a Con A-Sepharose column, followed by elution and analysis of the different fractions in rocket immunoelectrophoresis [7]. The various enzyme-active immunoprecipitates were identified by zymogram techniques [8].

Enzymes:
Nucleoside di- and triphosphatase;
NADH-neotetrazolium reductase (EC 1.6.99.2.);
L-leucyl-β-naphthylamidase (EC 3.4.11.1.);
Non-specific esterase (EC 3.1.1.-.).

2. Materials and methods

Liver plasma membranes were isolated from Sprague-Dawley rats according to Emmelot et al. [9]. The membranes were washed three times in 0.9% NaCl before use. Antisera against the plasma membranes were raised in rabbits as described earlier [8,10].

Membrane antigens were solubilized at 4°C for 2 hr with 1% (w/v) sodium deoxycholate (Merck, Darmstadt, W. Germany) in 0.05 M barbital buffer (pH 8.3) containing 0.1 M NaCl. Insoluble components were sedimented by centrifugation at 105 000 *g* for one hour. Protein concentrations were determined according to Lowry et al. [11] using bovine serum albumin (Armour Pharmaceutical Co., Ltd., Eastbourne, England) as the standard. The plasma membrane extracts were adjusted to 10 mg protein per ml.

Chromatography on Con A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) was performed on 0.6 × 9 cm columns equilibrated with 0.05 M barbital buffer (pH 8.3) containing 0.1 M NaCl and 0.25% (w/v) sodium deoxycholate. To decrease the detergent concentration in the sample before chromatography it was diluted (1:1) in 0.05 M barbital buffer containing 0.1 M NaCl. Two ml of the diluted plasma membrane extract was layered on the column. After washing with the buffer used for equilibration (20 ml) the Con A-bound components

were eluted with the same buffer, containing 0.3 M α-methyl-D-mannoside (20 ml). 1.5 ml fractions were collected and their absorbance at 280 nm was measured. The material which did not bind to Con A and the specifically eluted components were pooled separately. All samples were concentrated to 1 ml each in a Diaflo Ultrafilter UM 20E (Amicon, Lexington, Ma., USA) under N_2-pressure.

Fused rocket immunoelectrophoresis was performed according to Svendsen [7]. 1% (w/v) agarose (Behringwerke AG, Marburg-Lahn, W. Germany) in 0.05 M barbital buffer (pH 8.3) was used and 30 μl of either sample was applied to the holes. The electrophoresis was run at 4 V/cm for 20 hr. In order to confirm the efficiency of antigen-binding by the lectin an intermediate gel, containing con A-Sepharose (0.2 ml packed gel/ml of the 1% agarose gel), was inserted between the sample-gel and the antibody containing gel in some experiments as described by Bøg-Hansen [12]. If not otherwise indicated, 10% antiserum (v/v) was used in the antibody containing gels.

Dried electrophoretic plates were analysed by staining for the following enzyme activities: nucleoside di- and triphosphatase with ATP as substrate according to Wachstein and Meisel [13], NADH-neotetrazolium reductase as described by Raftell and Perlmann [14], L-leucyl-β-naphthylamidase according to Nachlas et al.

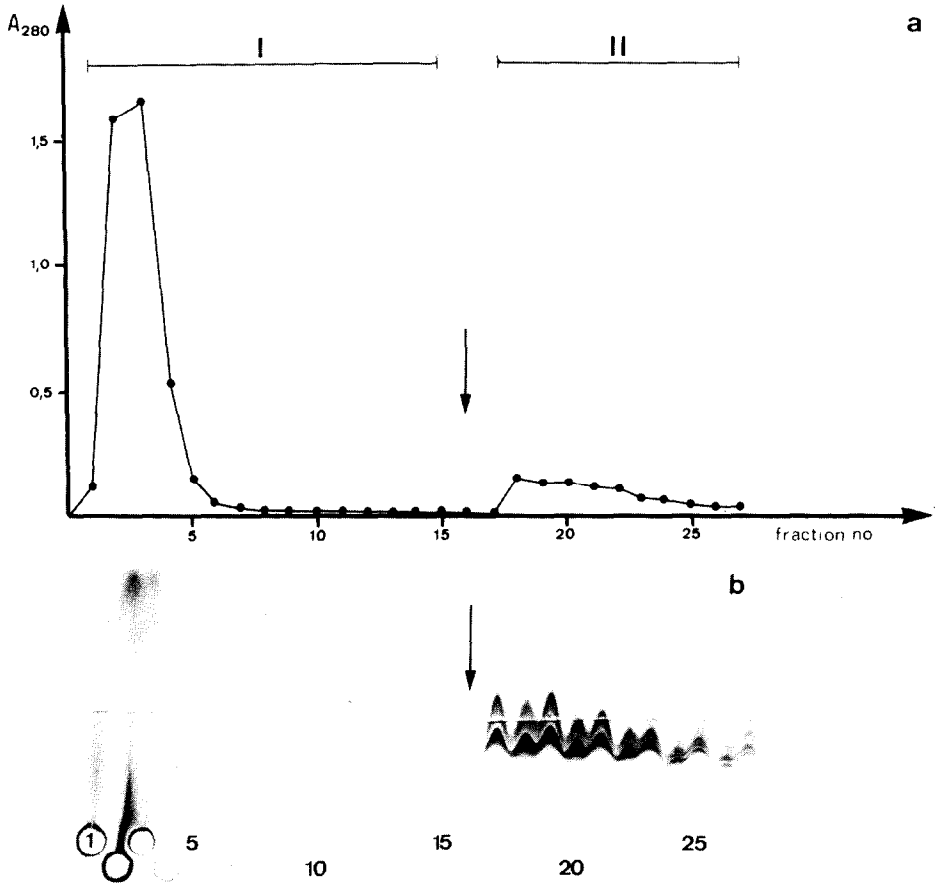

Fig.1. (a) A chromatogram showing the elution pattern when a plasma membrane extract was run through a concanavalin A-Sepharose column. I and II indicate the pools made from the eluted fractions. (b) Fused rocket immunoelectrophoresis with the fractions obtained in (a) and tested against anti-plasma membrane antiserum (5%). The electrophoretic plate was stained for ATPase activity. Arrows in (a) and (b) indicate where specific elution with 0.3 M α-methyl-D-mannoside was initiated.

[15] and non-specific esterase with α-naphthyl acetate as substrate as reported by Uriel [16]. All substrates were obtained from Sigma Chemical Co., St. Louis, Mo, USA.

3. Results

When a deoxycholate extract of plasma membranes was applied to a Con A-Sepharose column about 75% of the proteins could be washed through the column. The proteins bound to the column were specifically eluted by including α-methyl-D-mannoside in the buffer (fig.1). The specificity of the reaction of plasma membrane components with Con A was investigated by attempting to elute the column with a sugar not reacting with Con A. By including 0.3 M D-galactose in the buffer instead of α-methyl-D-mannoside, no protein was eluted from the column.

The Con A-bound and unbound material were tested in fused rocket immunoelectrophoresis against an anti-plasma membrane antiserum. By inserting a strip of agarose, containing Con A-Sepharose, between the antigen and the antibody containing gels, as mentioned above, a control for the reaction in the column was obtained.

The immunoelectrophoretic plates were dried and the precipitates were tested for different enzyme activities. Fig.2a shows a plate stained for nucleoside di- and triphosphatase activity with ATP as substrate. As can be seen, the majority of the ATPase-active antigens were specifically adsorbed to the column. Only two of the antigens with this activity were not bound to Con A. As the immunoelectrophoretic technique employed is semiquantitative and the volumes of the original extract and the fractions were comparable, it is obvious that not all the antigens were recovered. However, the recovery from the fractionation as such was in all experiments around 100% and the losses were mainly due to adsorption of membrane components to the test-tubes during concentration.

The three antigens with L-leucyl-β-naphthylamidase activity previously detected in plasma membranes [8] were all bound to Con A and were specifically eluted from the column with α-methyl-D-mannoside

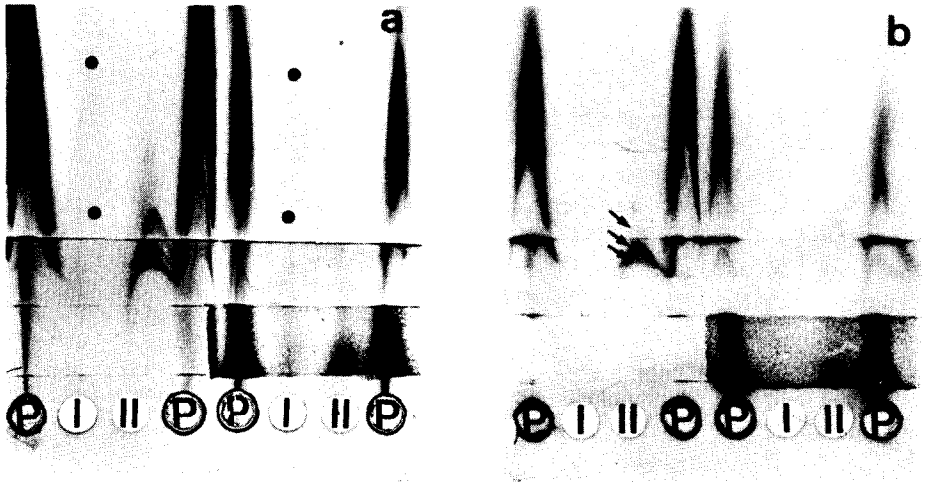

Fig.2. Fused rocket immunoelectrophoresis of whole plasma membrane extract (P) and pools I and II of the fractions obtained by chromatography on Con A-Sepharose as shown in fig.1. The samples were percipitated by anti-plasma membrane antiserum (10%) included in the top gel. The left part of each plate shows a test of pools I and II compared with whole plasma membrane extract. (a) A plate stained for ATPase activity. Two weakley stained ATPase-active antigens (indicated by black dots) were found in pool I. Most of the ATPase-active antigens were recovered in the specifically eluted pool II. As a control, a gel containing Con A-Sepharose was inserted between the sample and the antibody-containing gel in the right part of the plates. This confirmed the differences between the pools I and II. (b) A plate run in parallel, but stained for L-leucyl-β-naphthyl-amidase activity. The arrows indicate the specifically stained precipitates of pool II.

(fig.2b arrows). No such activity was found in the precipitates of the material washed through the column (Fraction I). Also the single antigen with non-specific esterase activity present in plasma membranes [8,10] was found in the specifically eluted material. In contrast, the catalase-active antigen [10] appeared in the material which was not bound to Con A. The NADH-neotetrazolium reductase activity was apparently inhibited during the chromatographic procedure as no activity could be recovered in any of the precipitates after chromatography.

In additional experiments the material specifically adsorbed to the Con A-Sepharose column was eluted with a continuous gradient of α-methyl-D-mannoside (0–0.3 M). However, no clear-cut differences in the elution patterns of the different antigens were obtained with various sugar concentrations.

In conventional immunoelectrophoresis all the enzyme-active antigens we have detected in plasma membrane extracts migrate towards the anode. However, some antigens lacking known enzyme activities migrate cathodically [10]. These latter antigens were not bound to Con A.

4. Discussion

The results show that most of the enzyme-active plasma membrane antigens earlier described by us [8,10] bind to Con A-Sepharose. In line with the results of Winquist et al. [4] on rat liver microsomes, the Con A-bound material comprises about 25% of the total detergent-soluble membrane proteins. These authors concluded that the material bound to Con A contained oligosaccharide–lipid–protein complexes. These complexes most probably correspond to the phospholipid-containing multienzyme complexes earlier identified by us in both microsomes and plasma membranes [8,17]. The multienzyme complexes found in plasma membranes were shown to contain nucleoside di- and triphosphatase activity together with NADH-neotetrazolium reductase activity [8]. Two antigens additionally exhibited L-leucyl-β-naphthylamidase activity. In this study most ATPase and all L-leucyl-β-naphthylamidase activities were bound to Con A. The reason for the loss of NADH-neotetrazolium reductase activity upon chromatography remains to be established. As the substructure of the multienzyme complexes is as yet unknown, the molecules carrying the carbohydrate moiety binding to the lectin could be either of glycoprotein or glycolipid nature.

Unpublished results indicate that two of the L-leucyl-β-naphthylamidase-active antigens present in multienzyme complexes of the plasma membranes also are present in a soluble form in the lysosomal content. These soluble antigens bind to Con A although they are not associated with phospholipids or other enzyme activities, indicating a glycoprotein nature of these enzyme molecules.

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