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INTERACTIONS OF INSOLUBILIZED LECTINS WITH MEMBRANE GLYCOPROTEINS IN PRESENCE OF DETERGENTS

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SUMMARY

The effects of several detergents commonly used to solubilize membrane glycoproteins have been investigated on the binding of hepatoma cell surface [^{3}H]-galactoglycoproteins to, and their elution from, concanavalin A or *Ricinus communis* lectins conjugated to Sepharose 4B. The optimum conditions (pH, ionic strength) in the presence of ionic [sodium deoxycholate (DOC) and sodium dodecyl sulphate (SDS)] and non-ionic detergents (Triton X-100) at a constant concentration were determined in order to ascertain which would yield the better efficiency. The effects of different detergent concentrations on binding and elution were then studied. The range of concentrations for each detergent to be used without modifying efficiency was determined. Triton X-100 and DOC (0.1–1%) did not change the efficiency on Ricinus lectin–Sepharose, whereas SDS, at a concentration greater than 0.05%, caused a dramatic decrease in efficiency. On concanavalin A–Sepharose, by contrast, the non-ionic detergent had no effect on the efficiency at all the concentrations tested (0.1–1%), while concentrations of more than 0.5% DOC and 0.1% SDS significantly decreased both binding and elution.

INTRODUCTION

Affinity chromatography using columns of insolubilized lectins is an effective procedure for the fractionation and isolation of glycoproteins and glycopeptides[1–3]. Since membrane glycoproteins are insoluble in neutral aqueous solutions, buffers containing ionic or non-ionic detergents must be used[4]. However, these detergents may either modify the native structure of the insolubilized lectins and/or change the interactions between the lectin and the membrane glycoproteins. Therefore, before using columns of insolubilized lectins to isolate glycoproteins, their fixation and elution should be studied under various experimental conditions (pH, ionic strength, detergent concentration) in order to determine the optimum procedure. Such studies have been done on concanavalin A and Ricinus lectin conjugated to Sepharose 4B using three ionic and non-ionic detergents and membrane glycoproteins from hepatoma cell surfaces. The optimum conditions were then applied to the fractionation of

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these glycoproteins in order to isolate the receptors of these lectins implicated in cell regulation[5] and in their toxic effect[6].

MATERIALS AND METHODS

Concanavalin A–Sepharose was obtained from Pharmacia, Uppsala, Sweden. *Ricinus communis* lectin (molecular weight: 120,000) was purified according to Nicolson and Blaustein[7] and was covalently conjugated to CNBr-activated Sepharose 4B (Pharmacia) as directed by the manufactures.

The detergents used were octylphenoxypolyethoxy ethanol (Triton X-100) from Sigma (St. Louis, MO, U.S.A.), sodium deoxycholate (DOC) from E. Merck (Darmstadt, G.F.R.) and sodium dodecyl sulphate (SDS) from Touzard and Matignon (Vitry-sur-Seine, France).

The labelling of the cell surface glycoproteins, using galactose oxidase and sodium [^{3}H] borohydride, has been described previously[8], as has the [^{3}H]galactoglycoprotein release from cells and their initial fractionation[9]. The specific radioactivity of [^{3}H]galactoglycoprotein was $0.18 \cdot 10^6$ dpm/mg of the protein.

Binding of [^{3}H]galactoglycoproteins to concanavalin A and Ricinus communis beads

The beads and the [^{3}H]galactoglycoproteins were equilibrated in 0.02 *M* Tris–HCl, pH 7 or 7.8, containing various concentrations of sodium chloride and the detergents. The binding studies were done in centrifuge tubes. The beads (500 μg of insolubilized concanavalin A or *Ricinus communis* lectin) were incubated routinely with [^{3}H]galactoglycoproteins (10,000 cpm) for 2 h at room temperature with gentle shaking. They were then centrifuged and washed five times with the buffer. To determine the radioactivity of each supernatant, an aliquot of each was added to 10 ml scintillation fluid (PCS, Amersham-Searle, Arlington Heights, IL, U.S.A.) and counted in a liquid scintillation spectrometer (Intertechnique SL 300). The efficiency of [^{3}H]galactoglycoprotein binding to the lectin–Sepharose was estimated by:

$$\frac{\text{cpm of } ^3\text{H added} - \text{cpm of } ^3\text{H unbound}}{\text{cpm of } ^3\text{H added}} \times 100\%$$

Elution of bound [^{3}H]galactoglycoprotein

Elution was carried out with the same buffer as that used for fixation, but containing 0.2 *M* α-methylglucoside (Sigma) for concanavalin A–Sepharose and 0.1 *M* lactose (E. Merck) for *Ricinus communis* lectin–Sepharose. The procedure was the same as that for fixation. The efficiency of [^{3}H]glycoprotein elution was estimated by:

$$\frac{\text{cpm of } ^3\text{H eluted}}{\text{cpm of } ^3\text{H added} - \text{cpm of } ^3\text{H unbound}} \times 100\%$$

The results were averages from four separate experiments and a new preparation of the glycoprotein fraction was used for each experiment.

RESULTS

Effects of pH and ionic strength on [^{3}H]galactoglycoprotein binding at constant detergent concentration

The detergent concentrations used in these experiments were 0.25% for DOC and Triton X-100 and 0.05% for SDS.

The efficiency of [^{3}H]galactoglycoprotein binding to concanavalin A beads at pH 7 was similar irrespective of the detergent present and was unaffected by ionic strength. By contrast, at pH 7.8, the presence of saline (0.25 *M* NaCl) increased the efficiency to that at pH 7 and in the absence of NaCl. This was also the case with DOC and Triton X-100, but not with SDS (Fig. 1a,b). The optimum conditions for binding were obtained using either a Tris–HCl buffer at pH 7 (with no additional salt) or a Tris–HCl buffer at pH 7.8 (0.25 *M* NaCl) and DOC or Triton X-100. Only the Tris–HCl buffer pH 7 gave optimum conditions for binding when SDS was used (Fig. 1a, b). Under these conditions, 40% of the [^{3}H]galactoglycoprotein was bound to the concanavalin A beads.

The efficiency of [^{3}H]galactoglycoprotein binding to *Ricinus communis* beads

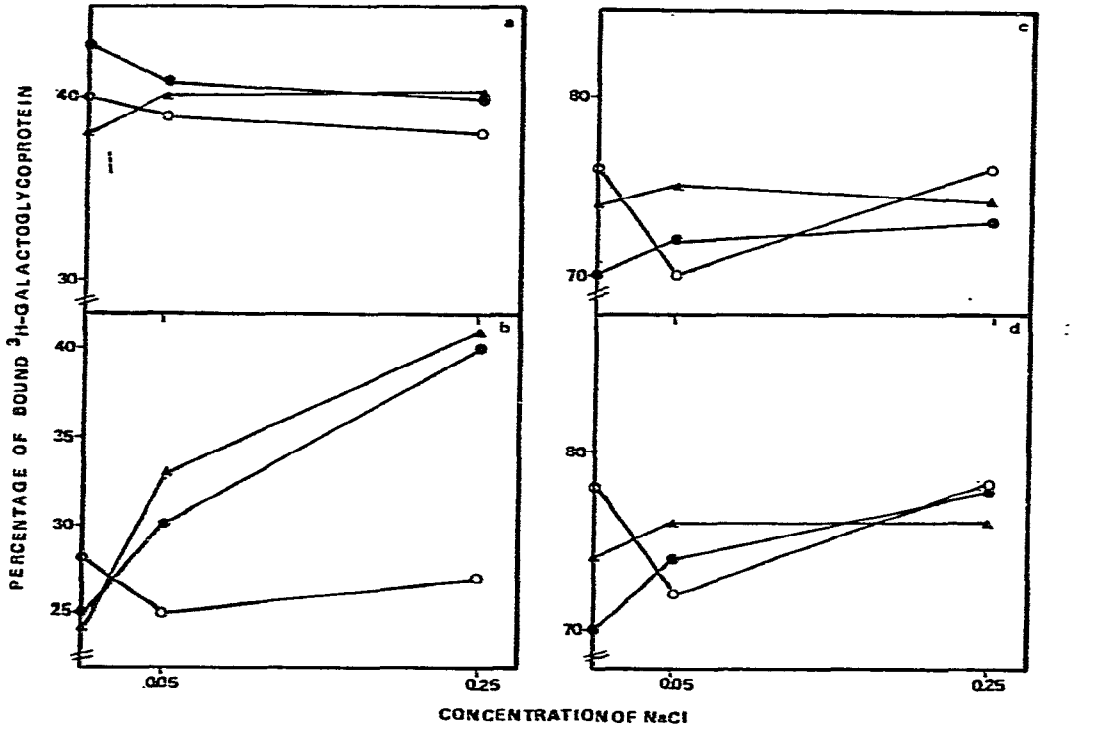

Fig. 1. Effects of pH and ionic strength on efficiency of [^{3}H]galactoglycoprotein binding to concanavalin A beads (left) and Ricinus lectin beads (right) in the presence of a constant concentration of detergent. The binding was performed using 0.02 *M* Tris–HCl buffer at pH 7 (a, c) or pH 7.8 (b, d), containing different concentrations of sodium chloride and DOC (0.25%) (●), Triton X-100 (0.25%) (▲), or SDS (0.05%) (○). Each point in this and other figures represents the average value obtained from four separate experiments.

was similar to the above at both pH 7 and pH 7.8 and unaffected by saline concentration whichever the detergent used. 70–75% of the [^{3}H]galactoglycoproteins were bound (Fig. 1c, d).

Effects of pH and ionic strength on the elution of bound [^{3}H]galactoglycoprotein

The elution of [^{3}H]galactoglycoproteins bound to concanavalin A beads was increased at pH 7.8 in the absence of NaCl and in the presence of either DOC or Triton X-100 (Fig. 2a, b). Using SDS, optimum elution was obtained with a buffer at pH 7 and in the absence of NaCl, or at pH 7.8 with 0.25 *M* NaCl (Fig. 2a,b). 50% of the bound [^{3}H]galactoglycoprotein was eluted using DOC and Triton X-100 and 60% with SDS (Fig. 2a, b). With each detergent, the elution of the [^{3}H]galactoglycoproteins bound to *Ricinus communis* beads was increased at pH 7.8 and with no NaCl. The optimum efficiency of elution was unaffected by the saline concentration in the buffer. 70% of the [^{3}H]galactoglycoproteins were eluted (Fig. 2c, d).

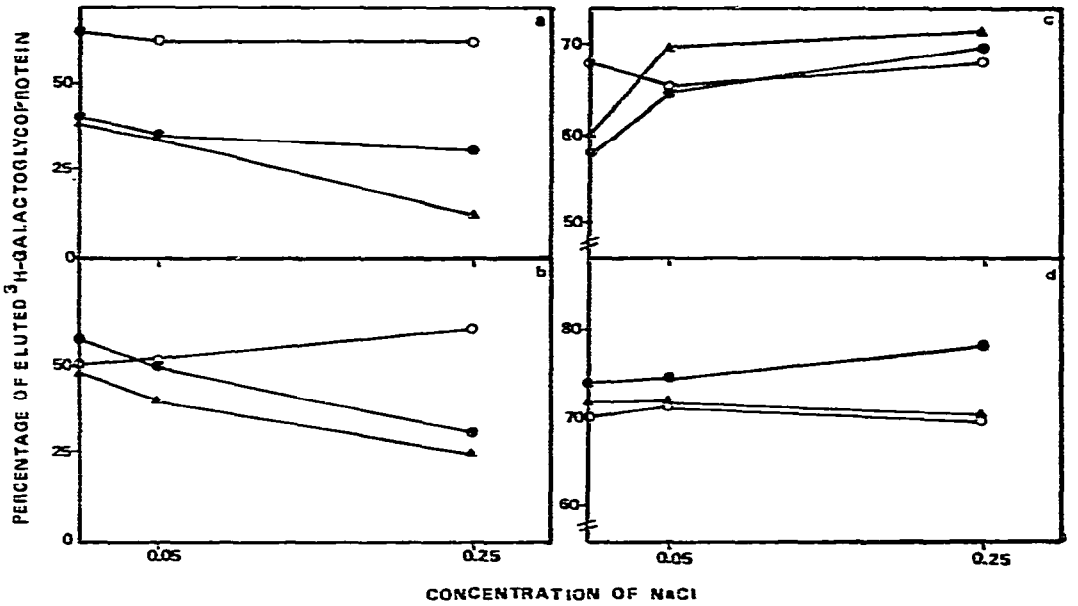

Fig. 2. Effects of pH and ionic strength on [^{3}H]galactoglycoprotein elution efficiencies from concanavalin A beads (left) and Ricinus beads (right) in the presence of a constant concentration of detergent. Elution was performed using 0.02 *M* Tris–HCl buffer at pH 7 (a, c) or pH 7.8 (b, d), containing either 0.2 *M* α-methylglucoside (for concanavalin A) or 0.1 *M* lactose (for Ricinus lectin) and different concentrations of sodium chloride and detergent (see Fig. 1).

Specificity of the interaction between [^{3}H]galactoglycoproteins and the lectin beads

The [^{3}H]galactoglycoproteins were incubated with the beads using the optimum buffer conditions determined above and, in addition, either 0.2 *M* α-methylglucoside (concanavalin A beads) or 0.1 *M* lactose (Ricinus beads) was added to the buffer. The binding of [^{3}H]galactoglycoprotein to the concanavalin A or Ricinus beads was either 70% or 90% inhibited by the saccharide inhibitor of lectin.

Effects of different detergent concentrations on the binding and elution steps

For these experiments, the optimum conditions determined were used. The [^{3}H]galactoglycoprotein fractions were soluble at all of the detergent concentrations studied, *i.e.*, in the presence of detergent concentrations greater than 0.02% of SDS or 0.1% of Triton X-100 or DOC.

On concanavalin A beads, the efficiency of binding and elution was unaffected by the concentration of Triton X-100. By contrast, concentrations greater than 0.1% SDS or 0.5% DOC caused a marked decrease in the binding and elution efficiencies (Fig. 3).

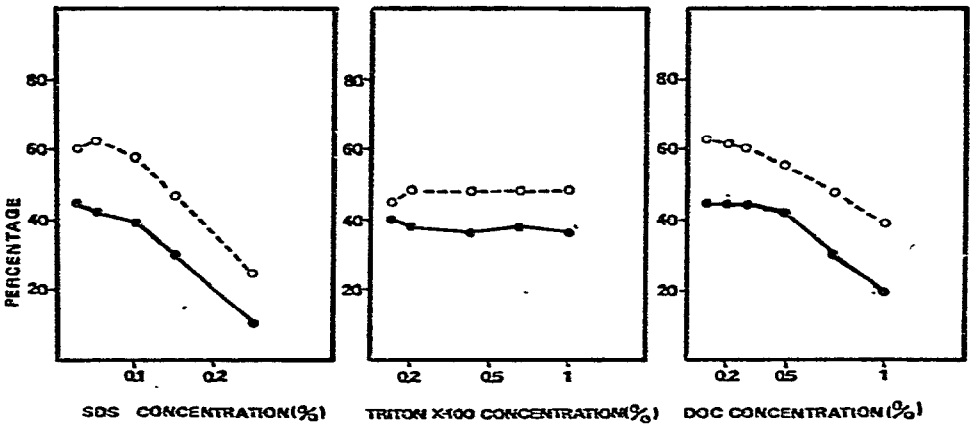

Fig. 3. Effect of different detergent concentrations on the binding and elution steps for [^{3}H]-galactoglycoproteins and concanavalin A beads. In the presence of SDS, fixation was performed using 0.02 *M* Tris–HCl pH 7 and elution using the same buffer containing 0.2 *M* α-methylglucoside. In the presence of Triton X-100 and DOC, fixation was performed using 0.02 *M* Tris–HCl pH 7 and elution using 0.02 *M* Tris–HCl pH 7.8 containing 0.2 *M* α-methylglucoside. The percentages of bound (●) and eluted (○) [^{3}H]galactoglycoprotein are shown.

On *Ricinus communis* beads, the efficiencies of binding and elution were unaffected by the concentration of Triton X-100 or DOC, but in the presence of SDS these efficiencies were dramatically decreased at concentrations greater than 0.05% (Fig. 4).

DISCUSSION

We have determined the optimum conditions for lectin affinity chromatography in the presence of different detergents commonly used to solubilize membrane constituents. In such procedures, the detergent and particularly its concentration has to be chosen so as to maintain the solubility of the membrane constituents and the eluted products, and to allow the specific interactions between the glycoproteins and the conjugated lectin to take place without modifying their native structure.

Few studies have been carried out on either the effects of different types of detergent or the binding and elution steps of these methods. The fixation of solubilized erythrocyte membrane constituents has been studied using conjugated

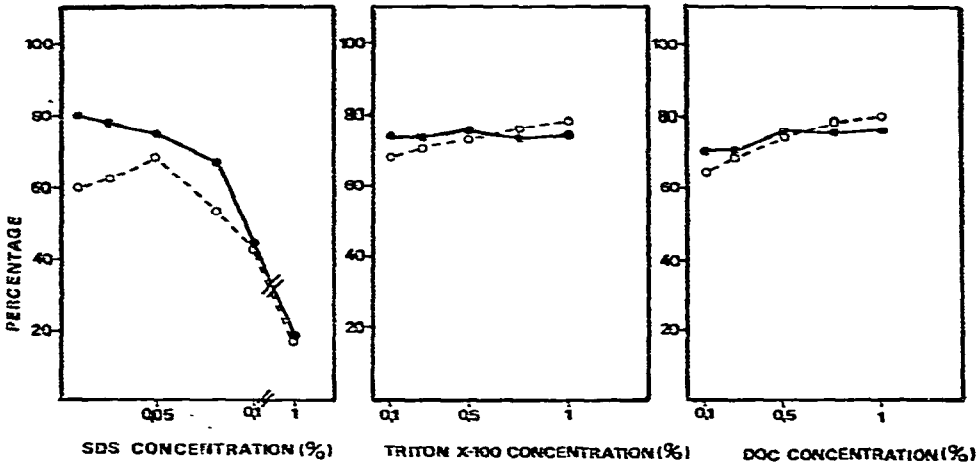

Fig. 4. Effects of different detergent concentrations on the binding and elution steps for [^{3}H]galactoglycoprotein and Ricinus lectin beads. For each detergent, fixation was performed using 0.02 *M* Tris–HCl pH 7.8 and elution using the same buffer containing 0.1 *M* lactose. Other details as in Fig. 3.

wheat germ agglutinin in order to purify the glycophorin[10], and another study has been reported[11] using the hydrophobic seroglycoprotein, fetuin, which had previously been purified. Since both solubilization and affinity chromatography may be modified by the presence of other membrane hydrophobic constituents such as lipids, membrane glycoproteins could prove to be a more reliable model.

Our data indicate that at a constant detergent concentration the buffer pH is of primary importance. However, Lotan *et al.*[11], using conjugated concanavalin A, found that the maximum efficiencies of binding and elution were affected by ionic strength. In our experience, the choice of buffer pH depends on both the conjugated lectin and the detergent. The maximum recovery of bound product may require a change of pH between fixation and elution. Thus, in the presence of DOC or Triton X-100, the fixation on conjugated concanavalin A increased at pH 7 while the elution was facilitated at pH 7.8.

If the solubilization of the membrane constituents and eluted products requires high concentrations of detergent, a non-ionic detergent (*e.g.*, Triton X-100) is more appropriate than an ionic detergent. The effects on the insolubilized lectin have been shown to be negligible at all the concentrations studied. Similar results have been reported by Lotan *et al.*[11]. The most likely explanation is that the non-covalent bound of the protein structure are not broken by Triton X-100[4].

Ionic detergents such as SDS or DOC may be used without affecting the insolubilized lectins or the affinity, but the range of concentrations applicable is limited and depends on the lectins involved. For example, sodium deoxycholate did not change the efficiency on insolubilized Ricinus beads, but a concentration greater than 0.5% caused a marked decrease of fixation and elution on insolubilized concanavalin A. Our results have shown that both ionic and non-ionic detergents can be used. Identical results have been obtained using affinity chromatography under the optimum conditions determined above. During filtration, insolubility could

be a complication owing to the high concentration of protein in the filtered product. This results in excessive fixation and a low elution efficiency, but can be avoided if the product is used in solution at very low concentration.

Good specificity of [^{3}H]galactoglycoprotein binding was obtained in our experiments as shown by the inhibition of the binding to the lectin beads in the presence of a saccharide inhibitor. The differences in [^{3}H]galactoglycoprotein binding efficiencies between insolubilized concanavalin A and the Ricinus lectin could be explained in terms of the direct accessibility of unmasked galactosyl residues to the insolubilized Ricinus lectin. This would also agree with the number of receptor sites available for each lectin as determined previously[5,6].

While simple sugar elution resulted in the release of almost all the glycoprotein that was initially bound to the Ricinus lectin, only 50% of the material bound to concanavalin A was found to be released under optimum conditions. This result is in agreement with the findings of Nachbar *et al.*[12] and would suggest that some hydrophobic interaction takes place between the insolubilized lectin and the glycoproteins. Our results also indicate that the same receptor molecules bind different lectins. If each lectin receptor bound only one distinct class of glycoprotein, the sum of the percentages of [^{3}H]glycoprotein bound to concanavalin A and Ricinus lectin would not exceed 100. The fact that addition of the percentages yields a sum greater than 100 suggests that these two lectins share the same receptors.

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