Journal of Chromatography, 240 (1982) 43–50
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,661

STUDIES ON LECTINS

LIII. AFFINITY ELECTROPHORESIS IN THE STUDY OF THE EFFECT OF DETERGENTS ON THE INTERACTION OF LECTINS WITH CARBOHYDRATES

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(Received December 17th, 1981)

SUMMARY

The effect of various types of detergents (Triton X-100, sodium dodecyl sulphate, cetyltrimethylammonium bromide) on the carbohydrate-binding activity of lectins was investigated by affinity electrophoresis on polyacrylamide gel. The non-ionic detergent Triton X-100 (0.5–2 %) did not cause dissociation of any of the lectins tested nor did it significantly affect the interaction of lectins with immobilized sugars. Application of the anionic detergent sodium dodecyl sulphate (0.1 %) resulted in the rapid dissociation of lectins into subunits. Subunits of none of the lectins studied interacted specifically with carbohydrates. If the dissociation of a lectin was incomplete, the carbohydrate-binding activity of undissociated lectin remained preserved. The cationic detergent cetyltrimethylammonium bromide (0.1 %) brought about a complete or partial dissociation into subunits. In the presence of cetyltrimethylammonium bromide neither subunits nor the undissociated molecules of most of the lectins studied interacted with sugars. Also, in the presence of this detergent, α-D-glucosyl and α-D-mannosyl ligands in polyacrylamide copolymers or mannan showed an enhancing effect in the dissociation into subunits of D-mannose-binding lectins (concanavalin A, seed lectins of *Pisum sativum*, *Lens esculenta* and *Lathyrus sativus*).

INTRODUCTION

Affinity chromatography using immobilized lectins is an effective procedure in the fractionation of glycoproteins and glycopeptides including cell surface glycoproteins[1]. However, membrane glycoproteins are not easily solubilized and separated in neutral aqueous solutions; therefore, chaotropic agents or detergents are commonly used for the solubilization of glycoproteins and as components of buffers used in affinity chromatography[2,3]. Chaotropic agents and detergents may influence the properties of the immobilized lectins in two ways: (i) modify and/or change the carbohydrate binding sites of lectins: (ii) bring about dissociation of the native lectin molecules into subunits which are held together by non-covalent forces.

0021-9673/82/0000–0000/$02.75

In spite of the increasing use of immobilized lectins in the isolation of membrane glycoproteins in detergent solutions, only Lotan *et al.*[3] have studied the effects of several commonly used detergents on the specific binding of asialo-[^{3}H]fetuin to immobilized lectins and on lectin-mediated agglutination of formalin-fixed erythrocytes. In this way they compared the effects of detergents on the ability of lectins, immobilized or free in a solution, to interact with carbohydrates. Dodeur and Jacquet[4] investigated the effect of detergents on the binding of hepatoma cell surface [^{3}H]galactoglycoproteins to concanavalin A or *Ricinus communis* lectin conjugated to Sepharose 4B.

In this study, affinity electrophoresis was used in the investigation of the effect of various types of detergents on the interaction of lectins with carbohydrates and on the stability and dissociation of lectin molecules.

EXPERIMENTAL

Water-soluble O-α-D-mannosyl and O-α-D-galactosyl polyacrylamide copolymers were prepared according to Hořejší *et al.*[5].

Lectins from the seeds of *Lens esculenta*[6], *Pisum sativum*[7], *Lathyrus sativus*[8] and *Canavalia ensiformis* (concanavalin A)[9] were isolated by affinity chromatography on Sephadex G-150. *Glycine soja* lectin was isolated from the seeds by affinity chromatography on α-D-galactosyl derivative of Separon (Spheron)[10]. Lectins from seeds of *Erythrina indica* and *Momordica charantia* were isolated by affinity chromatography on O-α-D-galactosyl polyacrylamide gel[11]. A mixture of lectins (toxin and agglutinin) from *Ricinus communis* seeds isolated by affinity chromatography on O-β-lactosyl polyacrylamide gel[12] was kindly provided by Dr. V. Hořejší.

Affinity electrophoresis was performed essentially as described previously[13]. The gel rods (0.5 × 8 cm) were prepared from a mixture containing 7% polyacrylamide, 0.2% N,N′-methylenebisacrylamide, an appropriate buffer system, water-soluble O-α-D-mannosyl or O-α-D-galactosyl polyacrylamide copolymer in a concentration giving the desired concentration of immobilized ligand (c_i) and an appropriate concentration of a detergent. Large-pore gels were omitted. Gels containing no specific glycosyl copolymer served as a control for checking the possible lectin dissociation. To prove the specific binding of lectins to immobilized sugar, either free D-mannose or D-galactose was added to the polymerization mixture to give a final concentration of 2%.

Affinity electrophoresis in the presence of Triton X-100

To study the effect of Triton X-100 the acidic buffer system described by Reisfeld *et al.*[14] was used. Triton X-100 was added to the polymerization mixture and sample solution in such an amount as to obtain a final concentration in the gel of 0.5–2%.

Affinity electrophoresis in the presence of sodium dodecyl sulphate (SDS)

The effect of SDS on the interaction of lectins with saccharides was studied in two systems: (i) in an alkaline buffer system[15] and (ii) in a buffer system of pH 7.2 according to Weber and Osborn[16]. SDS was added to the polymerization mixture, the electrode buffer and sample solution in amounts yielding final concentrations of 0.05–0.7%.

Affinity electrophoresis in the presence of cetyltrimethylammonium bromide (CTAB)

For the investigation of the effect of CTAB on the interaction of lectins with saccharides, the buffer system and conditions of electrophoresis according to Eley *et al.*[17] were used. The final concentration of CTAB in the polymerization mixture and in the electrode buffer was 0.1–0.2%.

Freshly prepared protein samples (50 μg) in 20% glycerol solution containing an appropriate amount of detergent (20 μl) were applied to the top of the gels. Alkaline discontinuous electrophoresis[15] was run for 1.5 h at a current density of 4 mA per tube, acidic discontinuous electrophoresis[14] for 2 h at 7 mA per tube, electrophoresis according to Weber and Osborn[16] for 2–2.5 h at 7 mA per tube and electrophoresis in the presence of CTAB[17] for 3–4 h at 8 mA per tube.

The gels were stained with Amido Black 10B, except for the gels obtained after electrophoresis in the presence of CTAB, which were stained, after fixation with hot 10% trichloroacetic acid, with Coomassie Blue R-250 according to Eley *et al.*[17].

The migration distances of the protein zones were measured with an accuracy of ±0.5 mm after staining. Dissociation constants were estimated graphically from the dependence of $1/d_0 - d$ on $1/c_i$ (for definitions see ref. 18).

RESULTS

For the investigation of the effect of detergents on the interaction of lectine with saccharides, three types of detergents were chosen: non-ionic (Triton X-100), anionic (SDS) and cationic (CTAB). With SDS, the interaction was studied at pH 7.2 and in an alkaline buffer system[15].

The interaction of following lectins with immobilized saccharides (given in parentheses) was tested: concanavalin A (D-Man), lectins from the seeds of *Pisum sativum* (D-Man), *Lens esculenta* (D-Man), *Lathyrus sativus* (D-Man), *Ricinus communis* (D-Gal), *Erythrina indica* (D-Gal), *Glycine soja* (D-Gal) and *Momordica charantia* (D-Gal).

Non-ionic detergent: Triton X-100

The presence of Triton X-100 at concentrations of 0.5–2% did not influence the electrophoretic mobility of the lectins studied. None of the lectins dissociated under these conditions.

The results of affinity electrophoresis showed that at concentrations of 0.5–2% the non-ionic detergent affected the interaction of the lectins with immobilized saccharides only very slightly. With concanavalin A, a slight decrease in the binding activity of this lectin to immobilized D-mannosyl residues was observed; the determined value of the dissociation constant of the lectin–immobilized α-D-mannosyl residues complex was higher ($7.9 \cdot 10^{-5}$ *M*) than that determined in the absence of detergent ($5.3 \cdot 10^{-5}$ *M*). On the other hand, the interaction of soybean lectin and both lectins from *Ricinus communis* seeds (toxin and agglutinin) with immobilized α-D-galactosyl residues was enhanced in the presence of Triton X-100; the dissociation constants of complexes of these lectins with the specific immobilized saccharide (soybean lectin $2.9 \cdot 10^{-4}$ *M*, agglutinin $4.1 \cdot 10^{-4}$ *M*, toxin $1.0 \cdot 10^{-3}$ *M*) were slightly lower than those determined in the absence of the detergent ($5.0 \cdot 10^{-4}$ *M*, $6.0 \cdot 10^{-4}$ *M* and $1.8 \cdot 10^{-3}$ *M*, respectively). Interaction of the lectins from seeds of *Pisum*

sativum, *Lens seculenta* and *Lathyrus sativus* with immobilized α-D-mannosyl residues was not influenced by the presence of the non-ionic detergent.

Anionic detergent: sodium dodecyl sulphate (SDS)

In contrast to Triton X-100, SDS, even at a low concentration (0.1 %), very easily caused dissociation of several of the lectins into subunits, as was revealed by polyacrylamide gel electrophoresis at pH 7.2 and in the alkaline buffer system.

Affinity electrophoresis on polyacrylamide gel containing immobilized specific sugars has shown that subunits of none of the lectins interacted specifically with sugars. An interaction with saccharides in the presence of SDS was observed only when the lectin was not dissociated into subunits. The concentration of SDS at which a lectin molecule dissociated into subunits was different for different lectins and was slightly dependent on the pH and composition of the buffers (ionic strength) used for the electrophoresis.

Concanavalin A was very easily dissociated into subunits even with 0.1 % SDS under the conditions of electrophoresis described by Weber and Osborn[16] (pH 7.2). When polyacrylamide gel electrophoresis was carried out in an alkaline buffer system according to Davis[15], with 0.1 % SDS concanavalin A was not completely dissociated and undissociated lectin molecules interacted with immobilized α-D-mannosyl residues. At SDS concentrations higher than 0.1 %, the concanavalin A molecule was completely dissociated and the subunits did not interact with the sugar ligand.

Lectins from *Pisum sativum*, *Lens esculenta* and *Lathyrus sativus* seeds were characterized by similar electrophoretic behaviours in the presence of SDS. Under the conditions of alkaline electrophoresis[15], these lectins were not completely dissociated into subunits at SDS concentrations of 0.1–0.3 %. A portion of the lectin molecules undissociated at these SDS concentrations interacted with immobilized α-D-mannosyl residues. Under the conditions of electrophoresis at pH 7.2 (ref. 16), the amount of undissociated lectins was greatly decreased and only at 0.1 % SDS was an interaction with immobilized saccharides observable (mostly with *Pisum sativum* lectin); even at this low SDS concentration most of the lectin was dissociated and neither of the two types of subunits interacted with the immobilized specific saccharide.

The interactions of both lectins from *Ricinus communis* seeds, which are not dissociated in the absence of mercaptoethanol, with immobilized α-D-galactosyl residues were very sensitive to SDS. Under conditions of alkaline electrophoresis, only at a 0.1 % SDS concentration were interactions of both lectins with immobilized saccharides observed. At pH 7.2, at the same SDS concentration, no interaction could be detected.

The least sensitive to the action of SDS were D-galactose-binding lectins from seeds of *Glycine soja*, *Erythrina indica* and *Momordica charantia*. Very similar results were obtained both in the alkaline buffer system[15] and in the system according to Weber and Osborn[16]. With 0.1–0.5 % SDS lectins from soybean and *Erythrina indica* seeds were not completely dissociated; part of the lectins did not dissociate into subunits and was specifically bound to the immobilized D-galactose residues (see Fig. 1). The lectin from *Momordica charantia* seeds, which does not dissociate in SDS medium in the absence of mercaptoethanol, interacted with α-D-galactosyl residues even at a 0.7 % SDS concentration. In the alkaline buffer system containing SDS, isolectins present in the preparation of *Momordica charantia* lectin were detected even on con-

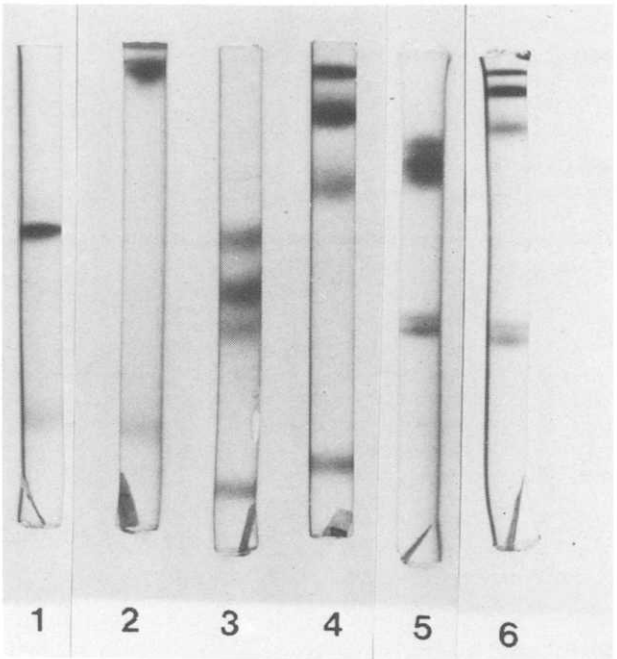

Fig. 1. Affinity electrophoresis of lectins from seeds of *Glycine soja* and *Erythrina indica* in the presence of 0.3 % SDS. 1, 2 = *Glycine soja* lectin; 3–6 = *Erythrina indica* lectin. 1, 2, 3, 4: Electrophoresis carried out in the alkaline buffer system[15]; 5, 6: electrophoresis carried out in the system according to Weber and Osborn[16]. 1, 3, 5: Control gels containing no glycosyl copolymer ($c_i = 0$); 2, 4, 6: affinity gels containing immobilized α-D-galactosyl residues ($c_i = 3.5 \cdot 10^{-3}$ *M*).

trol gels, whereas in the absence of the detergent these multiple forms could be separated only on affinity gels[11].

Cationic detergent: cetyltrimethylammonium bromide (CTAB)

As with SDS, the cationic detergent CTAB even at a 0.1 % concentration caused the dissociation of several lectins, subunits of which were held by non-covalent bonds. In some lectins the dissociation was incomplete.

In contrast to SDS medium, none of the lectins, which were not fully dissociated in the presence of CTAB, interacted specifically with immobilized saccharides. The mobilities of zones corresponding to undissociated lectins and the lectin subunits were the same both on affinity and the control gels in the presence or absence of the free specific sugar. The only exception was the lectin from *Erythrina indica* seeds: in the presence of 0.1 % CTAB, the undissociated lectin interacted with immobilized α-D-galactosyl residues; the interaction was inhibited by the addition of free D-galactose to the affinity gel.

The soybean lectin was partially dissociated in the presence of 0.1–0.2 % CTAB, but neither undissociated lectin nor its subunits interacted with immobilized specific sugar. *Ricinus communis* lectins did not interact with immobilized α-D-galactosyl residues even at a 0.1 % concentration of CTAB.

As with D-galactose-binding lectins, lectins binding α-D-mannosyl residues

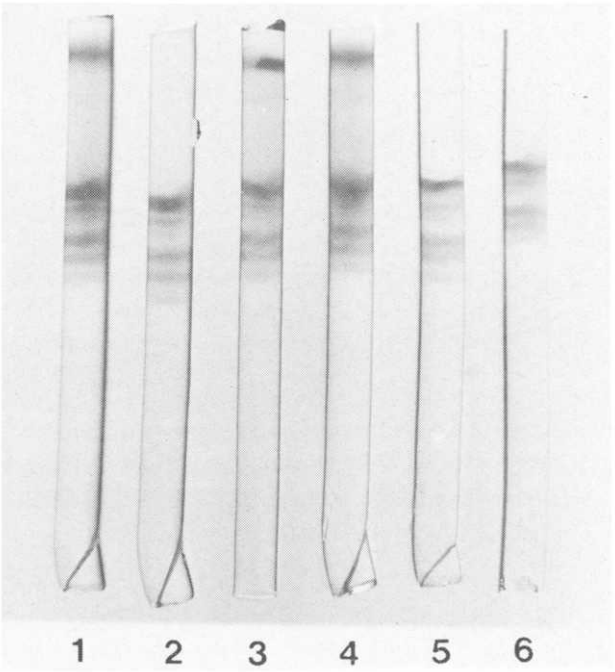

Fig. 2. Affinity electrophoresis of concanavalin A in the presence of 0.1 % CTAB. 1 = Control gel without any glycosyl copolymer ($c_i = 0$); 2 = affinity gel containing immobilized α-D-mannosyl residues ($c_i = 2.5 \cdot 10^{-3}$ *M*); 3 = affinity gel containing immobilized α-D-mannosyl residues ($c_i = 2.5 \cdot 10^{-3}$ *M*) and free D-mannose ($c = 1.1 \cdot 10^{-1}$ *M*); 4 = gel containing immobilized α-D-galactosyl residues; 5 = control gel; in contrast to other gels, the sample solution applied to the gel contained α-D-mannosyl polyacrylamide copolymer in the same concentration as in affinity gels; 6 = affinity gel containing mannan (2%).

(concanavalin A, lectins from seeds of *Pisum sativum*, *Lens esculenta* and *Lathyrus sativus*) were dissociated of the major part into subunits even at a 0.1 % CTAB concentration. However, on affinity gels these lectins showed interesting behaviour.

In the presence of 0.1 % CTAB on affinity gels containing immobilized α-D-mannosyl residues, zones corresponding to undissociated lectins could not be detected, whereas on control gels without D-mannosyl or D-glucosyl copolymers or gels containing α-D-galactosyl copolymer under the same conditions incompletely dissociated lectins were found. In the presence of a free specific sugar (2 % D-mannose) in the affinity gel, the electrophoretic behaviour of all of these lectins did not differ from that on a control gel. This phenomenon was observed for all of the α-D-mannose binding lectins studied in the presence of 0.1–0.2 % CTAB. Subunits of none of the studied lectins interacted specifically with immobilized α-D-mannosyl residues (see Fig. 2).

The same effect was observed, even when α-D-mannosyl copolymer was added only to the sample solutions prior to application to the gel. No zones corresponding to undissociated lectins were observed on affinity gels. A similar effect, *viz.*, an enhancement of dissociation of lectins in the presence of 0.1 % CTAB, was also brought about in affinity gels containing the α-D-glucosyl copolymer or mannan. On the other hand, addition of the α-D-galactosyl copolymer to the sample solution did not result in complete dissociation of the studied lectins.

DISCUSSION

In the experiments described above we used affinity electrophoresis to establish the effect of detergents directly on lectin molecules. This method enabled us also to study the possible interactions of lectin subunits with specific saccharides.

Dodeur and Jacquet[4] found that the interaction of lectins with saccharides depends on the ionic strength and pH of the medium in which affinity chromatography is carried out. We have also observed the dependence on composition of buffers and pH with SDS when the experiments were carried out in a buffer system of pH 7.2 (ref. 16) and in an alkaline buffer system[15].

In agreement with results of Lotan *et al.*[3] and Dodeur and Jacquet[4], our results have shown that the non-ionic detergent Triton X-100, even at a 2% concentration, had no effect on the interaction of any of the lectins with saccharides; with some lectins the interaction in the presence of Triton X-100 was sligthly higher than in its absence. Subunits of none of the studied lectins, even in trace amounts, were detected.

As reported previously[3,4], the anionic detergent SDS strongly affected the interaction of lectins with carbohydrates in affinity chromatographic experiments. Our results on affinity electrophoresis have shown that the anionic detergent SDS brought about rapid dissociation of most of the lectins to subunits. However, if under the given conditions the dissociation was incomplete, the undissociated part of some lectins interacted specifically with the sugar ligands even at relatively high SDS concentrations (0.5%). This phenomenon was observed especially with D-galactose binding lectins. Contrary to Lotan *et al.*[3], who described a very easy dissociation of soybean lectin in the presence of SDS, we have observed that this lectin belongs to the group of lectins that are the most insensitive to the action of this anionic detergent. Even though the presence of SDS does not inhibit the interaction of undissociated lectins with immobilized sugar ligands in affinity gels, no saccharide-binding activity of subunits of any of the studied lectins could be detected.

In the presence of the cationic detergent CTAB, we observed no specific interactions with sugars of most of the lectins even at low detergent concentrations (0.1%); the only exception was the lectin from *Erythrina indica* seeds. Our results are not in agreement with those of the affinity chromatographic experiments described by Lotan *et al.*[3]; the interaction of the glycoprotein with immobilized lectins in some instances was not affected, in some instances was decreased (depending on the deteregent concentration), but with 0.1% CTAB the interaction was still detectable. The results of their hemagglutination tests also showed that CTAB affected lectins less than SDS. These differences in the results could probably be explained by different experimental conditions under which examination of the detergent effect was carried out.

The behaviour of D-mannose-binding lectins in the presence of CTAB was very interesting; the presence of a high-molecular-weight substance specifically interacting with these lectins, either synthetic (D-glucosyl or D-mannosyl polyacrylamide copolymers) or a natural polysaccharide (mannan) caused an easier dissociation of D-mannose-binding lectins. The effect could be reversed by addition of a free specific sugar to the affinity gel. With D-galactose-binding lectins, this phenomenon was not observed.

In addition to the previously reported studies on the effect of ionic strength[19] and pH[20] on binding activities of lectins, this paper gives another example of the

applicability of affinity electrophoresis to the investigation of the effect of different factors on the interaction of proteins with specific ligands.

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