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Note

Studies on lectins

XLV. Effect of ionic strength on the interaction of lectins with carbohydrates as determined by affinity electrophoresis

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In previous papers, affinity electrophoresis on polyacrylamide gels containing O-glycosyl polyacrylamide copolymers was used for the determination of dissociation constants of lectin–sugar complexes[1], in the investigation of the interaction of modified lectins with saccharides[2] and in following the pH dependence of lectin interactions with sugars[3]. In the study of the pH dependence of the dissociation constants of lectin–sugar complexes several continuous and discontinuous buffer systems were used. These buffer systems differed in their composition and consequently also in ionic strength. An interesting question arose as to whether the different ionic strengths of the buffer systems used affected the binding parameters of lectins.

This paper describes the results of a study of the effect of the ionic strength of buffers on the interaction of lectins with saccharides.

EXPERIMENTAL

Water-soluble O-α-D-mannosyl and O-α-D-galactosyl polyacrylamide copolymers were prepared as described by Hořejší *et al.*[4].

Lectin from the seeds of *Lens esculenta* and concanavalin A were isolated using affinity chromatography on Sephadex[5,6]. Lectin from the seeds of *Glycine soja* was isolated by affinity chromatography on an O-D-galactosyl derivative of Separon (Spheron)[7].

Affinity electrophoresis was performed essentially as described previously[1]. The gel rods (0.5 × 8 cm) were prepared from a mixture containing 7% polyacrylamide, 0.2% N,N′-methylenebisacrylamide, an appropriate buffer system and the water-soluble O-α-D-mannosyl or O-α-D-galactosyl polyacrylamide copolymer in a concentration giving the desired concentration of immobilized sugar (c_i). Large pore gels were omitted.

Two continuous buffer systems were used: 0.04 *M* β-alanine–acetic acid buffer (pH 4.0) and 0.05 *M* glycine–sodium hydroxide buffer (pH 9.5)[3]. To study the effect of ionic strength on the interaction of lectins with immobilized saccharide, sodium chloride was added to the buffer systems in such amounts that its total concentrations in the gel and in the electrode solution were 20, 40 and 80 m*M*.

A mixture of 50 μg of protein sample in 20 μl of 20% glycerol solution was applied to the top of the gel. Electrophoresis in alkaline buffer (pH 9.5) was run at 4 mA per tube for 1–3 h and in acidic buffer (pH 4.0) at 7 mA per tube for 1–3 h. With increasing concentration of sodium chloride it was necessary to increase the time of electrophoresis.

Migration distances of the protein zones were measured with an accuracy of $\pm$ 0.5 mm after staining with amido black. Dissociation constants were estimated graphically from the dependence of $1/(d_0 - d)$ on $1/c_i$ (ref. 8) (see Figs. 1 and 4 for definition of symbols).

RESULTS AND DISCUSSION

For the study of the effect of ionic strength on the interaction of lectins with immobilized saccharides, two continuous buffer systems (pH 4.0 and 9.5) were chosen. An increase in ionic strength was achieved by addition of sodium chloride to the buffer. The ionic strengths used were chosen to cover the whole range of ionic strength of the different buffer systems used in the study of the dependence of the interaction of lectin with saccharides on pH[3] ($I = 0.011$–0.090 M).

The effect of ionic strength was tested on examples of lectins differring in their sugar specificity (α-D-mannosyl and D-galactosyl binding lectins) and in the dissociation constants of the lectin–saccharide complexes (lentil lectin, concanavalin A).

The effect of ionic strength on the interaction of the lentil lectin with immobilized α-D-mannosyl residues was studied in an alkaline buffer system ($I = 0.0085$–0.017 M) and in an acidic buffer system ($I = 0.013$–0.093 M). The dependences of $1/d_0 - d$ on $1/c_i$, from which K_i was estimated, for the lentil lectin in systems containing different amounts of sodium chloride are shown in Figs. 1 and 2. Dissociation constants of lectin-immobilized saccharide complexes (K_i) were in the range $1.49 \cdot 10^{-3}$ constants of lectin-immobilized saccharide complexes (K_i) were in the range $1.49 \cdot 10^{-3}$–$1.55 \cdot 10^{-3}$ M for the acidic buffer system and $1.03 \cdot 10^{-3}$–$1.06 \cdot 10^{-3}$ M for the alkaline buffer system.

The effect of ionic strength on the interaction of concanavalin A with immobilized α-D-mannosyl residues was investigated in an acidic buffer system ($I = 0.013$–0.093 M). As with the lentil lectin, the interaction of concanavalin A with immobilized saccharide was not affected by increasing ionic strength in the region used (Fig. 3). The dissociation constants (K_i) of complexes of concanavalin A with immobilized α-D-mannosyl residues were in the range $8.8 \cdot 10^{-5}$–$9.01 \cdot 10^{-5}$ M.

In contrast to the above-mentioned lectins, a weak dependence of the interaction of soybean lectin with immobilized α-D-galactosyl residues on ionic strength ($I = 0.013$–0.093 M) in an acidic buffer system was observed, as can be seen in Fig. 4 and Table I.

The results show that the ionic strength in the range tested did not significantly affect the interaction of the examined lectins with saccharides; hence the determined pH dependence of this interaction as described previously[3] is not influenced by variations in the ionic strength of the buffer systems used.

The use of a higher ionic strength than that used here has limitations due to the unfavourable electrophoretic mobility of lectins. With increasing ionic strength of the buffer, the electrophoretic mobility of lectins is low, even after prolonged

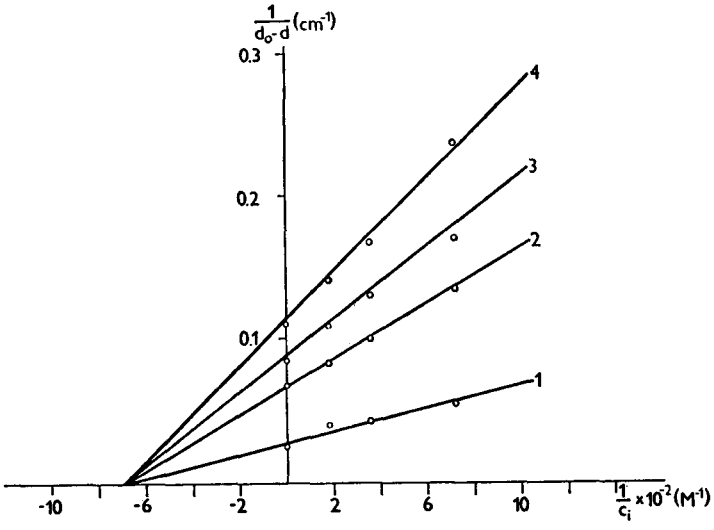

Fig. 1. Determination of dissociation constants of the lentil lectin–immobilized α-D-mannosyl residues complex in an acidic buffer system. 1, Affinity electrophoresis carried out without addition of NaCl; 2, 3 and 4, affinity electrophoresis carried out in the buffer system containing total concentrations of NaCl of 20, 40 and 80 mM, respectively. d_0 = mobility of the lectin on α-D-galactosyl copolymer-containing gel; d = mobility of the lectin at given concentration of immobilized α-D-mannosyl residues; c_i = concentration of immobilized α-D-mannosyl residues.

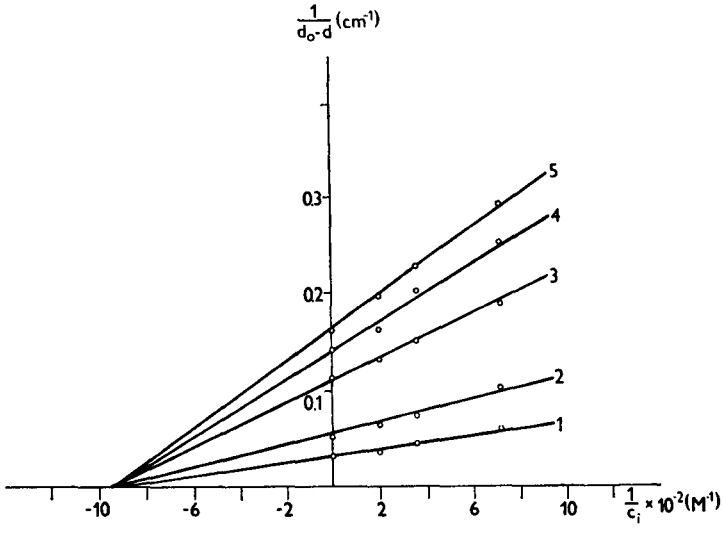

Fig. 2. Determination of dissociation constants of the lentil lectin–immobilized α-D-mannosyl residues complex in an alkaline buffer system. 1, Affinity electrophoresis carried out in the buffer system diluted with deionized water in the ratio 1:1; 2, affinity electrophoresis carried out without addition of NaCl; 3, 4 and 5, affinity electrophoresis carried out in the buffer system containing total concentrations of NaCl of 20, 40 and 80 mM, respectively. Symbols as in Fig. 1.

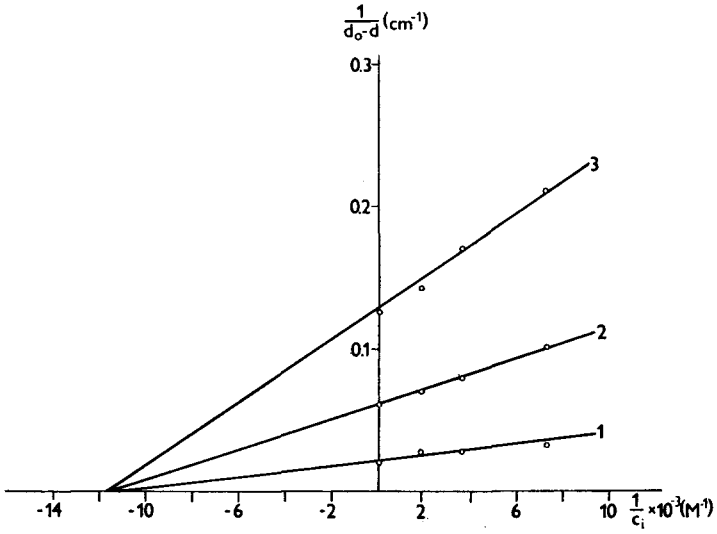

Fig. 3. Determination of dissociation constants of the concanavalin A–immobilized α-D-mannosyl residues complex. 1, Affinity electrophoresis carried out without addition of NaCl; 2 and 3, affinity electrophoresis carried out in buffer system containing total concentrations of NaCl of 20 and 80 m*M*, respectively. Symbols as in Fig. 1.

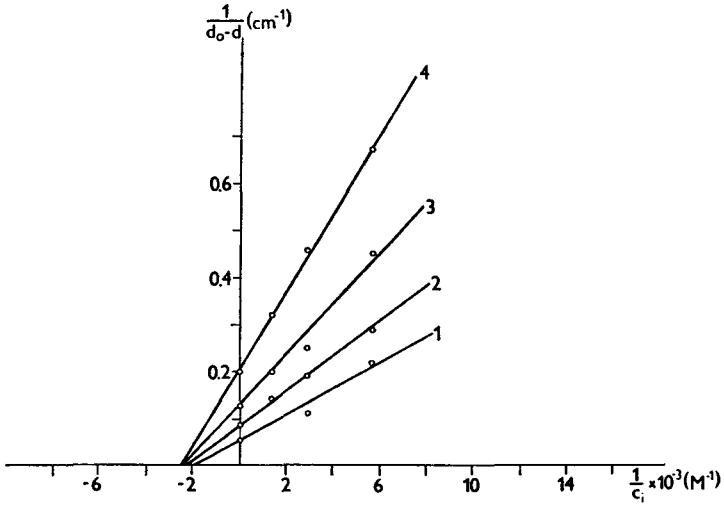

Fig. 4. Determination of dissociation constants of the soybean lectin–immobilized α-D-galactosyl residues complex. 1, Affinity electrophoresis carried out without addition of NaCl; 2, 3 and 4, affinity electrophoresis carried out in the system containing total concentrations of NaCl of 20, 40 and 80 m*M*, respectively. d_0 = mobility of the lectin on α-D-mannosyl copolymer-containing gel; d = mobility of the lectin at given concentration of immobilized α-D-galactosyl residues; c_i = concentration of immobilized α-D-galactosyl residues.

electrophoresis, and the determination of K_i is therefore less accurate. Under such conditions it is possible to show only qualitatively whether or not the lectins interact with immobilized saccharides.

TABLE I

DISSOCIATION CONSTANTS OF THE COMPLEX OF SOYBEAN LECTIN WITH IM MOBILIZED α-D-GALACTOSYL RESIDUES

Concentration of NaCl in buffer (mM)	K_i *(M)*
0	$4.8 \cdot 10^{-4}$
20	$4.5 \cdot 10^{-4}$
40	$4.2 \cdot 10^{-4}$
80	$4.1 \cdot 10^{-4}$

Although the dissociation constants of lectin–immobilized saccharide complexes (K_i) are probably less exactly defined than those of lectin-free sugar complexes (K), as discussed in previous papers in this series, K_i values are very useful for comparative purposes, as has been shown on the examples of the pH dependence of dissociation constants[3] and the dependence of dissociation constants of lectin–sugar complexes on the length of the spacer and anomeric configuration of a sugar derivative[9].

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