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Short communication

Characterization of interaction between protein and carbohydrate using CZE

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Abstract

The present study describes the application of the capillary zone electrophoresis (CZE) to investigate interactions between Concanavalin A (Con A) and the one of their major components (L-asparagin) with a variety of carbohydrates (D-glucose, D-fructose, D-mannose D-galactose, maltose and lactose). It was observed that the carbohydrates show different interactions with Con A and L-asparagin. Addition of carbohydrates to the electrolyte buffer and different concentrations of carbohydrates led to a change of the migration time and the ionic mobility of Con A and L-asparagin. A mathematical model for quantitative evaluation to calculate the aggregation constants (K) was used. Our investigations show that it is possible to characterize the interaction between carbohydrates and proteins or amino acid quantitatively using CZE.

Keywords: Protein; Carbohydrate; Capillary zone electrophoresis; Aggregation constants

1. Introduction

An important biological phenomenon is the specific recognition of proteins by sugar chains. The investigation of the molecular recognition properties of asparagin-linked (N-linked) saccharides and their interactions with protein have revealed that specially concanavalin A (Con A) many of these saccharides can bind, cross-link, and precipitate with the proteins [1,2]. Con A, the phytohemagglutinin from jack bean, first isolated by Summer [3], has been found to exhibit interesting biological properties. For example, the action of Con A in precipitating with various polysaccharides has many of the characteristics of an antigen–antibody system [4,5]. Capillary electrophoresis (CE) is a powerful tool for determining of such physicochemical properties, like aggregation constants [6], dissociation constants [7,8] and protein–ligand binding constants [9]. Furthermore, electrostatic interaction and hydro-

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gen bonding effects were observed between solutes and surfactants [10]. The hydrophobic interaction between solutes and micelles was evaluated by electrokinetic chromatography (EKC) [11,12]. Schwarz et al. have studied the interaction between drugs and bile salts using the micellar electrokinetic capillary electrophoresis (MEKC) [13,14]. Furthermore, the bond of protein–drugs and their interactions were determined using affinity capillary electrophoresis [15]. Plätzer et al. studied the determination of formation constants of cyclodextrin inclusion complexes using affinity capillary electrophresis [16]. Interactions between monosaccharides and protein were studied by different spectrophotometic methods. Honda et al. determined the association constant of monovalent mode protein–sugar interaction by capillary zone electrophoresis [17]. The present paper reports the use of capillary zone electrophoresis (CZE) as a technique to determine the association constants between carbohydrates and proteins. The effects of various concentrations of carbohydrates in the separation buffer on the migration time of Con A were used to obtain a quantitative measurement for the strength of interaction between carbohydrates and protein.

2. Theory

It was assumed that the protein (P) were formed with sugar (S) following complex equilibrium:

$$\mathrm{P} + m\mathrm{S} \rightarrow \mathrm{PS}_m \tag{1}$$

The complex forming equilibrium between protein and sugar was described as follows:

$$K = \frac{\mathrm{PS}_m}{\mathrm{P} \cdot \mathrm{S}^m} \tag{2}$$

where K is aggregation constant, P protein concentration, S ligand (sugar) concentration.

The effective mobility of the protein was related to the effective mobility of the free protein according to its degree of dissociation:

$$\mu_\mathrm{E} = \alpha \cdot \mu_\mathrm{P} + (1 - \alpha) \cdot \mu_{\mathrm{PS}_m} \tag{3}$$

where μ_E is effective mobility, α rate of dissociation.

The degree of dissociation of the complex was yielded from the concentration of the free protein (P) to the used total concentration (c_0):

$$\alpha = \frac{\mathrm{P}}{c_0} = \frac{\mathrm{P}}{\mathrm{P} + \mathrm{PS}_m} \tag{4}$$

Combining Eqs. (2) and (4) α is given as:

$$\alpha = \frac{1}{1 + K \cdot \mathrm{S}^m} \tag{5}$$

α depends here on K, S and m.

Calculated from Eqs. (3) and (5), the effective mobility is given by

$$\mu_\mathrm{E} = \frac{1}{1 + K \cdot \mathrm{S}^m} \cdot \mu_\mathrm{P} + \left(1 - \frac{1}{1 + K \cdot \mathrm{S}^m}\right) \cdot \mu_{\mathrm{PS}_m} \tag{6}$$

μ_P is the experimental value at $S = 0$ and $\mu_{\mathrm{PS}m}$ is the experimental value at the highest concentration of S.

Assuming a monovalent interaction between carbohydrats and protein ($m = 1$). Eq. (6) was a ranged into following linear forms:

$$\frac{1}{\mu_\mathrm{E} - \mu_\mathrm{P}} = \frac{1}{K(\mu_\mathrm{PS} - \mu_\mathrm{P})[\mathrm{S}]} + \frac{1}{\mu_\mathrm{PS} - \mu_\mathrm{P}} \tag{7}$$

The effective mobilities μ_x were determined at pH 10 using the following equation:

$$\mu_x = \frac{L_\mathrm{g} L_\mathrm{d}}{V}\left[\frac{1}{t_\mathrm{m}} - \frac{1}{t_0}\right] \tag{8}$$

where μ_x (x = E, P, PS) is the effective mobility, V the applied voltage, L_g the total capillary length, L_d the effective cappillary length (to the detector), t_m the migration time of the solute and t_0 the migration time of neutral marker (acetone).

3. Experimental

3.1. Apparatus

Capillary electrophoresis experiments were performed using a Hewlett Packard model G1600A (Waldbronn, Germany)^{3D} CE system with diode-array detector from 190 to 600 nm. CE ChemStation equipped with a HP Vectra 486/66U

workstation was used for instrument control, data acquisition, and data analysis. The system was controlled by windows software, which was modified to the HP system. The detection wavelength was 195 nm. The fused-silica capillaries obtained from Hewlett Packard (Waldbronn, Germany) with a total length (48.5 cm), length to detector (40 cm) and internal diameter (50 μm) were used for the determination and for the separation of protein and L-amino acid.

3.2. Chemicals

Concanavalin A and L-asparagin were obtained from ACROS ORGANICS (New Jersey, USA). D-glucose, D-fructose, D-mannose, D-galactose, maltose and lactose were obtained from Berlin-Chemie (Berlin, Germany). Acetone for chromatography, boric acid and sodium hydroxid were obtained from Merck (Darmstadt, Germany). The used borat buffer had a pH value of 10.

3.3. Sample preparation

Standard solutions of protein were prepared at 500 μg/ml in buffer containing different concentrations of sugar. The samples were injected immediately into the apparatus.

3.4. Buffer preparation

For capillary electrophoresis, 10 mM borate buffer solutions (pH 10) were prepared by dissolving 12.404 g boric acid and 100 ml sodium hydroxide in water filling up to a volume of 1000 ml (sol. 1) and 0.1 N sodium hydroxide (sol. 2). Both solutions were mixed and diluted with water 1:1 (v:v). The pH of the buffer was measured at 25 °C using a HI 9321 Microprozessor pH meter (HANNA instruments). The buffer solutions were filtered through a 0.2 μm syringe filter.

3.5. Analysis conditions

A new capillary was washed for 10 min with NaOH (1.0 M) at 40 °C, followed by washing for 10 min with water at the same temperature and for 5 min with water at 25 °C. Before each injection, the capillary was flushed with 0.1 M NaOH for 5 min and with the actual buffer solution for 5 min. The temperature was kept at 25 °C, a separation potential of 20 kV was used. Acetone was used as a marker substance for the determination of the electroosmotic mobility. The samples [buffer-acetone (99:1)] were injected at 50 mbar pressure for 5 s (hydrodynamic injection). Detailed experimental conditions are listed in the figures.

4. Results and discussion

The interaction of mono- and di-saccharide (D-glucose, -fructose, -mannose, -galactose, maltose and lactose) with Con A and L-asparagin at pH 10 were studied. The used saccharides exhibited a zero electrophoretic mobility at pH 10 and moved with the electroosmotic flow. Con A and L-asparagin had negatively electrophoretic mobilities at pH 10 and migrated in the direction of the cathode. The interaction between the anionic proteins and the neutral saccharides led to aggregation of saccharides to the protein compounds. Therefore, the electrophoretic mobility of the compounds changed from negative to zero. The relative standard deviation of the effective mobilities ($n = 5$) is between 1.5 and 2%. Acetone was used as a marker substance for the calculation of the electroosmotic mobility. The relative standard deviation of the EOF is between 0.5 and 2%. In the study we investigated the determination of the aggregation constants (K) by CZE as a new technique. For the determination of K by CZE some factors are important: (a) there is no interaction between saccharides or proteins with the capillary wall; (b) the influence of saccharide concentration on the electrophoretic mobility of the protein was discussed as a complex formation; (c) the formation constants and complex mobilities were of all calculated assuming an 1:1 interaction ($m = 1$). Table 1 summarizes the calculated aggregation constants estimated by application of the fitted function (Eq. (7)).

The migration time of the protein is a quantitative parameter to characterize the strength of the

interactions between protein and carbohydrates. The mobility of a molecule in free solution is proportional to its electrical charge q, and inversely related to the hydrodynamic radius r, which depends on the molecular mass M. If the saccharide binds to a protein, the change in μ occurs to the mass saccharide–protein complex. K can be calculated by the quotient of intercept and slop in Eq. (7). The correlation coefficient of the $1/\mu_E - \mu_P$ versus 1/[S] plots were between 0.993 and 0.999. The estimation of the interaction strength is difficult when there are small differences between mobility of protein and mobility of carbohydrat–protein complex. This fact is given by use of D-galactose in our measurement conditions. Therefore, we can not calculate k of D-galactose in complex with Con A and L-asparagin. We have not observed here any a interactions of D-galactose with our proteins by increasing the concentration of D-mannose and D-galactose in run buffer. Fig. 1 shows typical electropherograms of protein using different concentrations of saccharides in running buffer. A strong change in the electrophoretic mobility was observed by increasing of D-glucose-, D-mannose-, D-fructose-, maltose-, and lactose-concentrations. A change in the mobility of saccharides of concentrations >0 and <20 mM was not observed. By increasing the concentration of these saccharides (≥ 20 mM) the protein peak (Fig. 1) shifted to the EOF.

It shows that with increasing the concentration of saccharides in buffer increases the degree of complexation too.

Table 1
Summarizes the calculated aggregation constants estimated by application of the fitted function (Eq. (7))

Compounds	Concanavalin A, K (l/mM)	Asparagin, K (l/mM)
D-Glucose	20.69	23.05
D-Fructose	27.39	3.755
D-Mannose	23.3	10.22
D-Galactose	0	0
Maltose	1786.6	83.24
Laktose	18.61	110.79

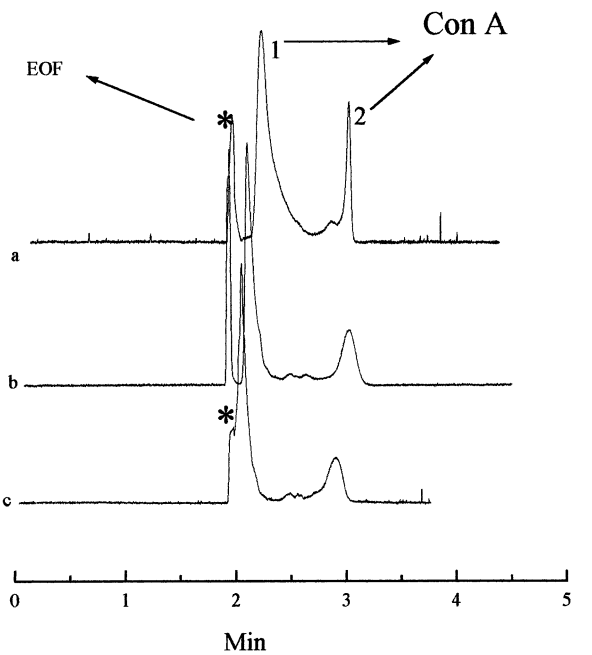

Fig. 1. Electropherogram of Con A at different concentrations of D-glucose in borat buffer, pH 10 (a) 0 mM D-glucose (b) 50 mM D-glucose (c) 90 mM D-glucose; uncoated fused-silica capillary with 48.5 cm effective length × 50 μm I.D.; 20 kV; temerature: 25 °C; pressure injection: 5 s at 50 mbar; detection: 195 nm.

If the concentration of saccharides in buffer is higher than 90 mM there is no further decrease in the mobility of proteins, because it reaches the

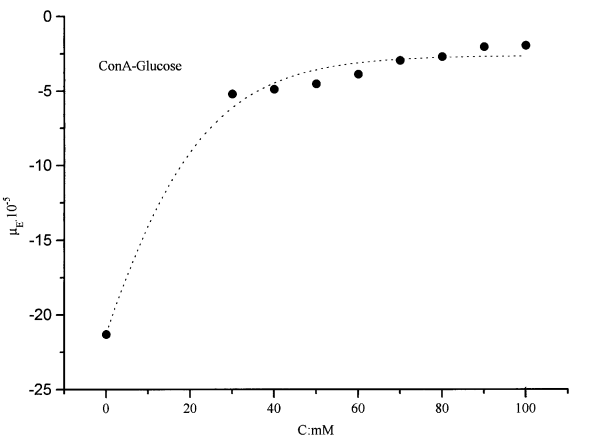

Fig. 2. Influence of different concentration of D-glucose on the ionenmobility of the Con A.

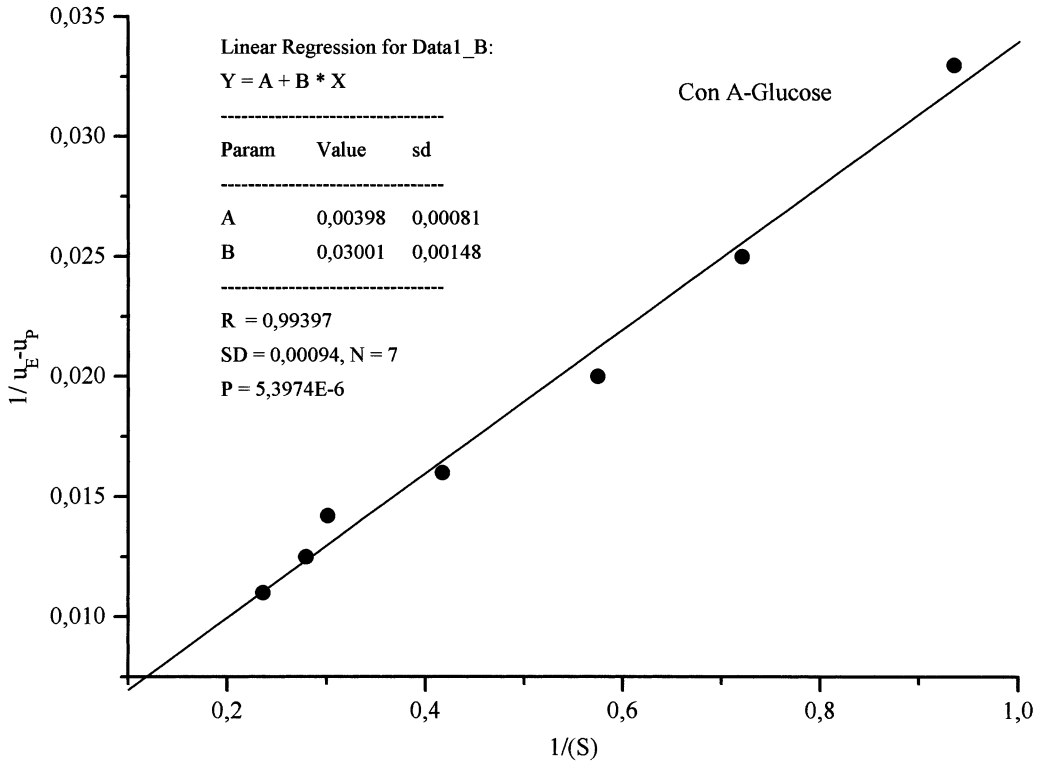

Fig. 3. Relationship between $1/\mu_E - \mu_P$ and $1/(S)$.

saturation equilibrium. This fact is shown in Figs. 2 and 3.

K gives information of the strength of interaction on one hand and of the affinity of saccharides to the proteins on the other hand. The value of *K* is higher, where the interaction of saccharides with protein is strong. It can readily be seen that substitution or modification of any one of the hydroxyl groups at c-3, c-4, or c-6 results in a complete loss of activity, indicating a specific interaction with the lectine used.

The strength of the interaction of the carbohydrates with Con A was found to be as follows:

Maltose > D-fructose > D-mannose > D-glucose > Laktose

and with asparagin:

Laktose > Maltose > D-glucose > D-mannose > D-fructose

5. Conclusion

The present study shows it is possible to characterize interactions between Concanavalin A and carbohydrates quantitatively using CZE as a new technique. A physicochemical model was used to calculate the aggregation constants and to interpret the interaction between proteins and carbohydrate. CZE is advantageous as a rapid and simple screening method which provides quantitative results of the interactions of carbohydrates with protein with a minimum of substance consumption and time spent.

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