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Partial-filling affinity capillary electrophoresis of glycoprotein oligosaccharides derivatized with 8-aminopyrene-1,3,6-trisulfonic acid

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ABSTRACT

Partial-filling affinity capillary electrophoresis has been applied to the simultaneous analysis of interactions between glycoprotein oligosaccharides and certain plant lectins. A lectin solution and a mixture of glycoprotein-derived oligosaccharides labeled with 8-aminopyrene-1,3,6-trisulfonic acid were introduced to a neutrally coated capillary in this order, and separated by application of a negative voltage. Interaction of a lectin with each oligosaccharide in the mixture was observed as the specific retardation or dissipation of peaks, in addition to the size/charge separation of oligosaccharides by zone electrophoresis in the remainder (≈90%) of the capillary. The strength of the interaction with lectin was controlled by introducing an appropriate volume of lectin solution. Application of various specificities of lectins indicated characteristic migration profiles of the oligosaccharides. Moreover, sequential injection of four lectins (*Maachia amurensis* mitogen, *Sambucus sieboldiana* agglutinin, *Erythrina cristagalli* agglutinin, *Aleuria aurantia* lectin) induced complete dissipation of complex-type oligosaccharides and enabled specific determination of the presence of high-mannose oligosaccharides without the interference or alteration of the electropherogram in porcine thyroglobulin. This method was also applied to determine the binding constants of ovalbumin-derived oligosaccharides to wheat germ agglutinin.

1. Introduction

Capillary electrophoresis (CE) is a highly successful separation technique for the analysis of biomolecules. Capillaries with an i.d. of 50–100 μm have a high electrical resistance and a large surface area-to-volume ratio. Therefore, very high electrical fields (≈650 V/cm) can be applied. This results in short analysis times and high resolution of a mixture of closely related components in a sample.

Various separation techniques have been developed in the analysis of biochemical species. One versatile technique, affinity capillary electrophoresis (ACE), has been used to examine various interactions, including protein–drug, protein–carbohydrate, and antigen–antibody [1–5]. ACE is an analytical approach in which the change of migration patterns or times of interaction of molecules in an electrical field is recorded as the function of ligand concentration. It is used to identify specific binding and to determine binding constants. The availability and power of ACE results from four main advantages over other complementary techniques: (1) only minute quantities of sample are required; (2) the provided sample need not be pure because CE can be used to distinguish each analyte and those analytes of interest from the impurities; (3) automated CE instrumentation enhances the reliability of obtained data; and (4) molecular interactions can be characterized in free solution [6].

We previously applied this ACE technique for the profiling of oligosaccharides in glycoprotein specimens and calculation of their binding constants [7], screening of the specificity of tulip lectins [8], and characterization of milk oligosaccharides [9]. The ACE of lectins has certain advantages in the glycobiology era: (1) easy interpretation due to the combinatorial usage of lectins can provide detailed information of oligosaccharide structures; (2) high-speed analysis because the separation is completed within 30 min for a single run; and (3) high sensitivity by labeling with fluorescent tags enabling sensitive determination of the interaction. However, the method also has some disadvantages. The concentration, components and pH of the electrophoresis buffers have limited to sustain activity of the lectin used, and the usage of protein-containing buffers can complicate analyses due to adsorption of proteins to the capillary, vials and electrodes.

The use of a partial-filling method in ACE has been demonstrated to be effective for studying interactions [10]. In this technique, the capillary is filled with buffer. A "plug" of solution containing ligand

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is introduced to the inlet before injection of a sample containing the receptor. A high voltage is then applied. During electrophoresis, analytes flow through the zone of the "ligand plug" (where the receptor interacts with the ligand). This method seems very attractive in lectin ACE for oligosaccharide complexes because the affinity binding zone and the separation zone based on the charge/size ratio of analytes can be readily distinguished in a capillary. Also, the contribution of these two zones to the separation profile can be easily controlled by changing the injection time of the receptor solution. However, a higher concentration of lectin is required in the partial-filling technique because the length of the lectin plug is often very small compared with that of the capillary. This may be of particular concern for the adsorption of lectin to the capillary (not to the electrodes and vials) and physical adsorption of sample components to the lectin.

In the present study, we evaluated the partial-filling ACE technique as an alternative of the previous ACE method using a lectin-added electrophoresis buffer to ascertain if the: (1) specificity of lectins was fully maintained in partial-filling mode; (2) extent of the interaction between oligosaccharides and partially filled lectin could be controlled by changing the injection time of the lectin solution; (3) reproducibility of the introduction of lectin solution was sufficiently high for the calculation of binding constants. We also applied this technique for the one-step determination of high-mannose type oligosaccharides by sequential injection of a series of lectins.

2. Materials and methods

2.1. Chemicals

8-Aminopyrene-1,3,6-trisulfonic acid (APTS), porcine thyroglobulin, human α_1-acid glycoprotein, human transferrin, and hen ovalbumin were obtained from Sigma–Aldrich Japan K.K. (Tokyo, Japan). *Aleuria aurantia* lectin (AAL), castor bean lectin (RCA_{120}), concanavalin A (Con A), *Datura stramonium* agglutinin (DSA), *Erythrina cristagalli* agglutinin (ECA), *Lens culinaris* agglutinin (LCA), *Maachia amurensis* mitogen (MAM), *Phaseolus vulgaris* agglutinin E_4 and L_4 (PHA-E_4 and PHA-L_4), pokeweed mitogen (PWM), *Sambucus sieboldiana* agglutinin (SSA), and wheat germ agglutinin (WGA) were obtained from Seikagaku Corporation (Tokyo, Japan). Peptide-N^4-(*N*-acetyl-β-D-glucosaminyl)asparagines amidase F (PNGase F, EC 3.5.1.52) was from Roche Applied Science (Tokyo, Japan). Sodium cyanoborohydride ($NaBH_3CN$) and iodoacetamide were obtained from Wako Pure Chemical Industries Limited (Osaka, Japan). Other reagents were of the highest commercial grade.

2.2. Preparation of APTS-labeled oligosaccharides

N-Linked oligosaccharides were prepared from 50 μg of lyophilized glycoprotein. A sample was dissolved in 50 μL of 50 mM phosphate buffer (pH 7.9) containing 0.1% sodium dodecyl sulfate and 2% 2-mercaptoethanol. The solution was heated at 100 °C for 5 min. After cooling, the solution was mixed with 5 μL of 7.5% NP-40 and 5 mU of recombinant PNGase F, and the reaction mixture incubated for 2 h at 37 °C. Deglycosylated proteins were precipitated by the addition of 180 μL of ice-cold ethanol and removed by centrifugation at 10,000 rpm for 5 min. The released oligosaccharides in the supernatant were dried in a centrifugal vacuum evaporator. They were labeled through reductive amination by the addition of 2 μL of 0.2 M APTS in 15% acetic acid and 2 μL of 1 M $NaBH_3CN$ in tetrahydrofuran. The labeling mixture was heated for 1 h at 80 °C. Usage of acetic acid and a higher reaction temperature causes partial loss of sialic acids from oligosaccharides. The solution was then diluted with water, and excess APTS was removed by chromatography using a Sephadex G-25 column (1 cm i.d. 30 cm) with 10 mM acetic acid as eluent. The first eluting fluorescent peak was observed at an excitation wavelength of 490 nm and an emission wavelength of 520 nm. The product was collected and evaporated to dryness. The residue was dissolved in 500 μL of water and stored in a refrigerator.

2.3. Partial-filling ACE of APTS-labeled oligosaccharides

In all CE separations, polydimethylsiloxane-coated capillaries (InertCap I®; GL Sciences Incorporated, Tokyo, Japan) of i.d. 50 μm with an effective length of 40 cm (50 cm in total) were used with 50 mM Tris–acetate buffer (pH 7.0) containing 0.8% hydroxypropylcellulose as running buffers. A P/ACE MDQ CE machine (Beckman Coulter Incorporated, Brea, CA, USA) equipped with a laser-induced fluorometric detection system was used. The capillary was thermostated at 25 °C. All lectins were dissolved in the running buffer at 1 mg/mL. A lectin solution was injected by application of a pressure of 2.07–6.9 kPa for 3–50 s; then a solution of APTS-oligosaccharides was injected for 5 s at 3.45 kPa. Separation was conducted by application of −15 kV. APTS oligosaccharides were detected by an argon laser-induced fluorometric detection with a band pass filter for fluorescein. After each run, a lectin solution in the capillary was removed by introducing buffer solution from the anodic side by pressure application (34.5 kPa, 2 min). The volume of lectin solution injected in a capillary was calculated using the method at the Internet website of the manufacturer according to the following equation [11].

$$V = \frac{\Delta P d^4 \pi t}{128 \eta L}$$

where V is the volume delivered across the capillary, ΔP denotes the pressure drop across the capillary (in Pascals), d signifies the internal diameter of the capillary (meters), t is the duration of pressure application (seconds), η represents the buffer viscosity (Pascal seconds), and L is the total capillary length (meters).

2.4. Calculation of binding constants

Migration time in the lectin plug (t_L) was calculated using the following equation.

$$t_L = (t - t_0)\frac{l_T - l_L}{l_T}$$

where t and t_0 are the migration time in the presence or absence of lectin, respectively, l_T is the effective length, and the length of the lectin plug l_L can be obtained as described in the section above. Binding constants of ovalbumin-derived oligosaccharides to WGA was calculated by *y*-reciprocal method [12] according to the following equation.

$$\frac{[L]}{\mu_p^{eff} - \mu_p^0} = \frac{1}{\mu_c^0 - \mu_p^0} \times [L] + \frac{1}{K_f(\mu_c^0 - \mu_p^0)} = f([L])$$

where $[L]$, K_f are the concentration of lectin in plug, and binding constant, respectively. μ_p^{eff}, μ_p^0, and μ_c^0 are effective electrophoretic mobility of APTS-oligosaccharide under the presence of lectin, mobility of free APTS-oligosaccharide and the mobility of APTS–WGA complex, respectively. From the linear relation in the plots of $[L]/(\mu_p^{eff} - \mu_p^0)$ versus $[L]$, we can obtain binding constants as slope/intercept.

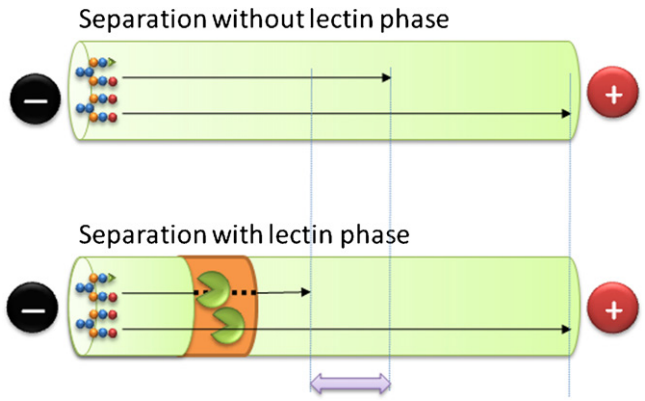

Fig. 1. Image of partial-filling affinity capillary electrophoresis of APTS-labeled oligosaccharides with lectins using a neutrally coated capillary. A specific delay indicated as an arrow (↔) indicates interaction with lectin partially filled in a capillary.

3. Results and discussion

3.1. Experimental design

Fig. 1 portrays the experimental design of partial-filling ACE. A lectin solution and then APTS-labeled oligosaccharides were introduced by hydrodynamic means to a neutrally coated capillary filled with electrophoresis buffer. Lectins with a high molecular mass had minimal electrophoretic velocity in a neutral background solution. In contrast, APTS derivatives, having three sulfonate groups, induced a high velocity in electric field. By applying a negative voltage to the capillary, APTS-labeled oligosaccharides quickly moved towards to the anode based on their size-to-charge ratio and passed through a zone of lectin solution. APTS oligosaccharides recognized by the lectin were slowed down in the lectin plug depending on affinity strength and complex mobility. The migration times were increased and often accompanied by peak broadening. In contrast, APTS oligosaccharides having no affinity for the lectin migrated with constant velocity over the capillary, and their elution profile did not change in the presence of lectin. Therefore, alteration of electrophoretic profiles in the presence of lectins directly indicated the affinity of specific oligosaccharides to the lectin.

In this experimental design, the removal of electroendoosmotic flow (EOF) and the suppression of adsorption of lectins was important. The static coating was very important for this purpose. A capillary neutrally coated with polydimethylsiloxane effectively removes EOF, and unfavorable adsorption of APTS oligosaccharides and lectins was suppressed by the addition of hydroxylpropylcellulose in the electrophoresis buffer. Moreover, to reduce analytical complications due to the adsorption of denatured lectins to the capillary, the lectin zone after each analytical run was removed from the inlet by the application of pressure to the capillary outlet. Under the optimized condition described above, the electrophoretic profiles did not change throughout this work.

3.2. Partial-filling lectin ACE of a mixture of high mannose and complex-type oligosaccharides derived from porcine thyroglobulin

In an initial series of experiments, we examined if partial-filling ACE could be used as an alternative to previous ACE using lectin-impregnated electrophoresis buffer [7]. We chose porcine thyroglobulin as an oligosaccharide pool to achieve this aim. This glycoprotein contains a series of high-mannose-type and complex-type oligosaccharides (Fig. 2(A)) [13–16]. We chose a series of lectins (MAM, DSA, PWM, PHA-L_4, SSA, LCA, ECA, WGA, Con A, AAL, and PHA-E_4) for this study. Before injection of APTS-labeled oligosaccharides, each lectin was dissolved in the running buffer at 1 mg/mL (10–30 μM), and introduced at 3.45 kPa for 30 s (which occupied 18 mm or 4.5% of the capillary to the detection window). Migrations of the oligosaccharides in the presence of lectins are shown in Fig. 2(B).

MAM is known to recognize complex-type oligosaccharides containing α2,3-linked NeuAc [17]. DSA is specific to polylactosamine and tetraantennary oligosaccharides [18]. PWM is specific to *N*-acetyllactosamine [19], and PHA-L_4 recognizes some tri- and tetraantennary oligosaccharides [20,21]. Electropherograms obtained in the presence of these lectins were approximately identical to the reference electropherogram shown at the bottom of Fig. 2(B). Thyroglobulin contains a series of biantennary complex oligosaccharides, indicating that it has no affinity for these lectins.

In contrast to the upper four traces of Fig. 2(B), ACE using other lectins as shown in the subsequent seven traces indicated affinity for thyroglobulin oligosaccharides, and showed specific migration profiles. SSA recognizes α2,6-linked NeuAc [22]. Partial-filling analysis using SSA did not alter the migration profiles of peak 4 and peaks 7–12, and indicated complete dissipation of peaks 1 to 3, 5, and 6. Disappearance of these peaks indicated the presence of α2,6-linked NeuAc. In contrast, other peaks showing identical migration times with reference data shown in the bottom trace of Fig. 2(B) did not contain α2,6-linked NeuAc. LCA has affinity for biantennary oligosaccharides with α1,6-linked Fuc in its core [23]. Severe broadening and slight retardation (*ca.* 10 s) of peaks 1 to 3, 5, and 6 implied that these peaks were assignable to the corresponding oligosaccharides. ECA shows specificity to terminal β-linked Gal residues [24]. Peak 2 and peak 5 were apparently retarded *ca.* 40 s by the addition of ECA, which implied oligosaccharides containing free Gal residues in their termini. WGA recognizes β-GlcNAc residues and also shows affinity for NeuAc-containing complex-type oligosaccharides [25]. Retardation and broadening of peaks 1 to 3, 5, and 6 were observed using WGA. Absence of terminal GlcNAc residues in thyroglobulin implied that these peaks were assignable to NeuAc-containing complex-type oligosaccharides. Con A shows strong affinity for high-mannose-type oligosaccharides and moderate affinity for biantennary complex-type oligosaccharides [26]. All oligosaccharides in thyroglobulin matched the specificity of Con A. Separation using Con A caused disappearance of most peaks. AAL is a Fuc-binding lectin; the binding strength to α1,6-linked Fuc is higher than those for α1,2-linked and α1,3-linked Fuc [27]. All complex oligosaccharides in this glycoprotein reportedly contain α1,6-linked Fuc residues, which implies that all complex-type oligosaccharides in thyroglobulin should be trapped by this lectin. The electropherogram indicated that peaks 1 to 3, 5, and 6 were recognized by AAL, and that their migration times were increased *ca.* 2 min. The peaks were assignable to complex-type oligosaccharides containing Fuc residues. PHA-E_4 recognizes biantennary oligosaccharides and triantennary oligosaccharides having two lactosamine branches at α1,3-linked Man; their affinity is reduced by the presence of terminal sialic acids [28]. From comparison of the reference electropherogram, the migration times of peaks 2, 5, and 6 were increased by ≈30 s, which indicated that these peaks were trapped by this lectin and were assignable to biantennary oligosaccharides with terminal Gal residues.

As shown above, lectins recognize specific oligosaccharides in oligosaccharide pools. The oligosaccharide peaks trapped by lectins indicate broadening of peaks and/or retardation of the migration time. By contrast, peaks of oligosaccharides with no affinity for lectins indicate an identical migration time and identical migration profile. The concentration of lectin solution in the partial-filling method was almost tenfold higher than that of our previous ACE method using lectin-impregnated electrophoresis buffer. In our previous work, we studied a conventional type of ACE of thyroglobulin oligosaccharides, and found that 10 μM of lectin induced severe tailing of peaks of excess reagent and unbound oligosac-

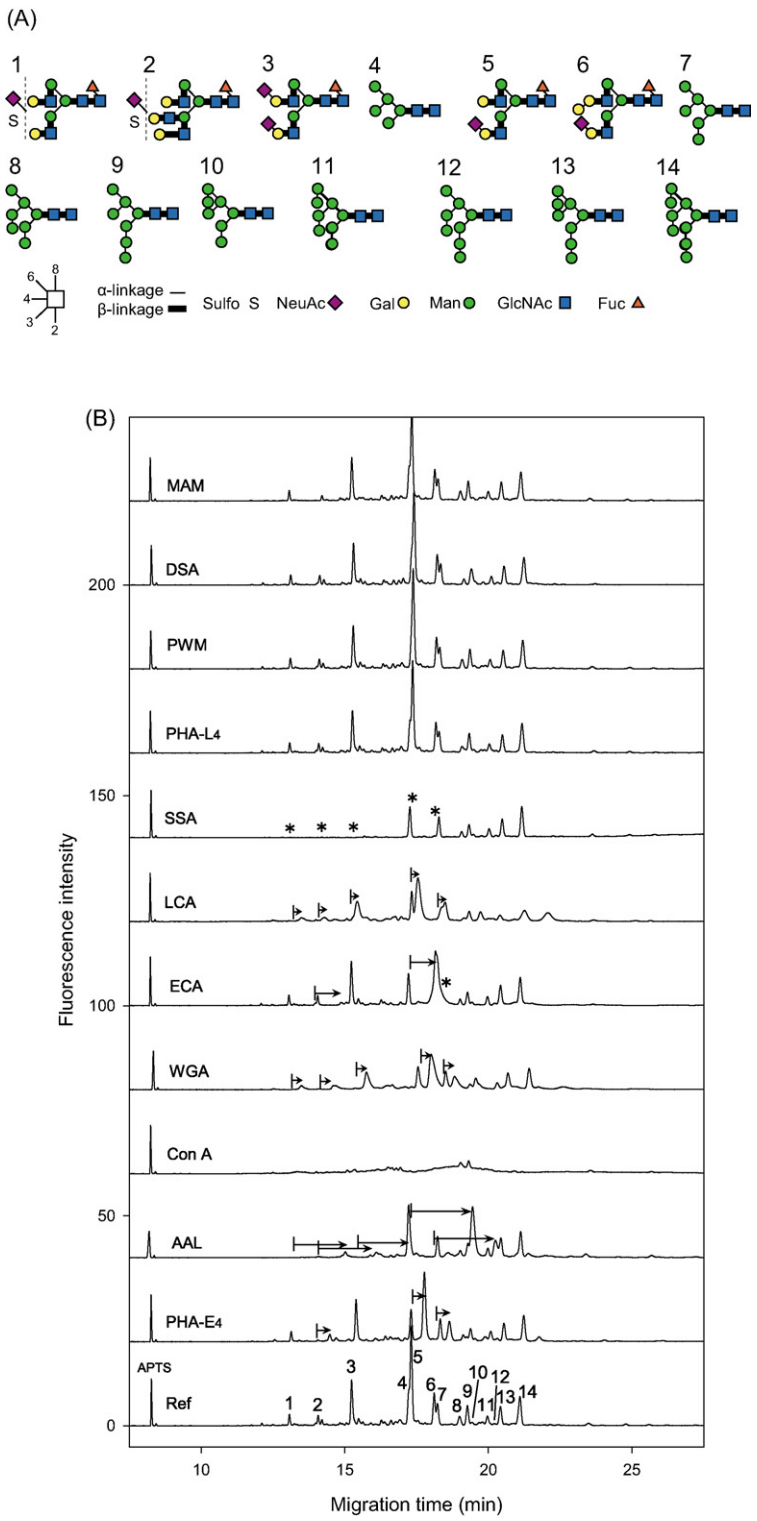

Fig. 2. Oligosaccharide structures previously reported and tentative assignment of peaks (A) and partial-filling affinity capillary electrophoresis of APTS-labeled oligosaccharides derived from porcine thyroglobulin with various lectins (B). Lectin solution (1 mg/mL, 0.25 mg/mL for Con A) was injected at 3.45 kPa for 30 s; then APTS oligosaccharides (0.1 mg/mL as glycoprotein) were injected at 3.45 kPa for 30 s. Conditions: running buffer, 50 mM Tris–acetate buffer, pH 7.0 containing 0.8% hydroxypropylcellulose; capillary, polydimethylsiloxane-coated, 50 μm i.d., 40 cm/50 cm; applied voltage, −15 kV; detection, 488 nm (excitation)/520 nm (emission).

charides [8]. In contrast, partial-filling techniques using a higher concentration of lectins indicated good separation, and did not indicate non-specific adsorption. This may be due to the separation of the affinity zone and separation zone in the partial-filling method.

3.3. Partial-filling ACE by changing the injection volume of lectins

As a second step, we confirmed the applicability of partial-filling ACE for various oligosaccharides derived from other glycoproteins

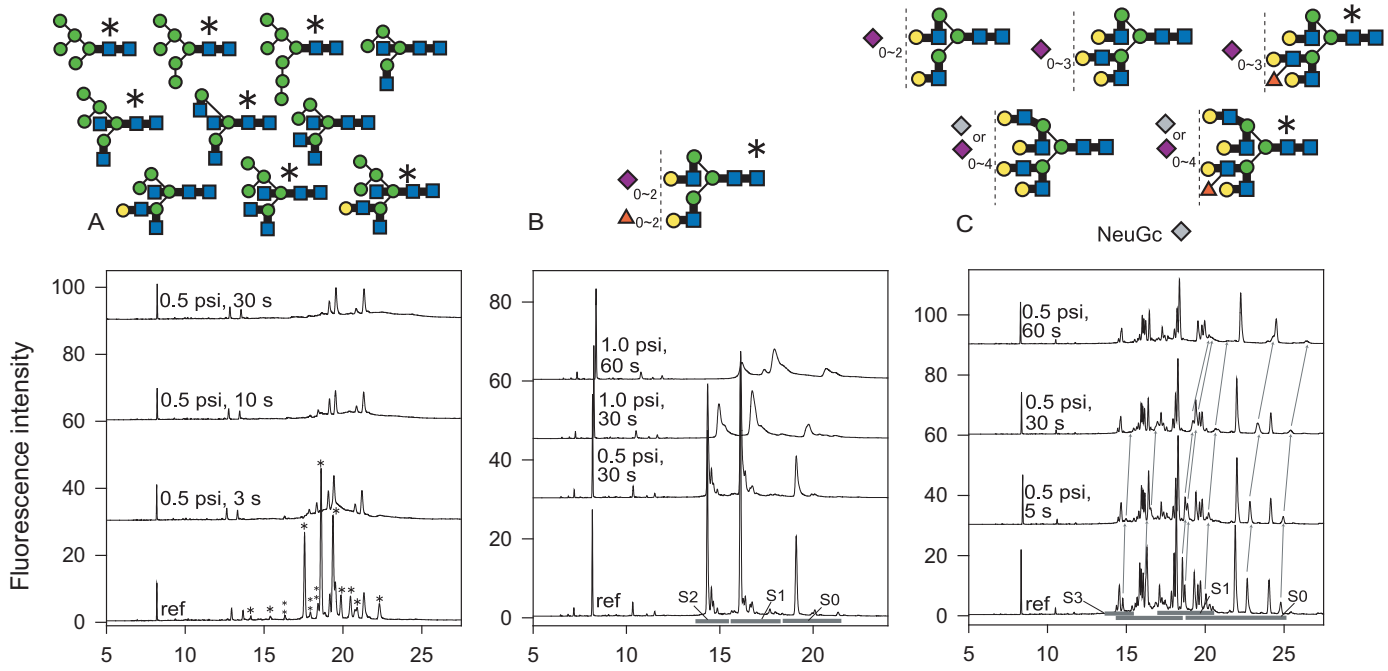

Fig. 3. Representative electropherograms of APTS-labeled oligosaccharides derived from ovalbumin with Con A (A), human transferrin with RCA_{120} (B), and α_1-acid glycoprotein with AAL (C). Lectin injection conditions are shown in each electropherogram. Gray bars with S*x* indicate estimated elution area of trisialo-, disialo-, monosialo- and asialooligosaccharides. Other symbols and the other conditions were identical to those shown in Fig. 2. Starred oligosaccharides are expected to show affinity to the lectin.

(ovalbumin, transferrin, and α_1-acid glycoprotein) by changing the injection time of lectins. Some of the results are depicted in Fig. 3.

Ovalbumin contains a series of high mannose-type oligosaccharides ($Man_{5-7}GlcNAc_2$), a complex-type oligosaccharide ($Man_3GlcNAc_5$), and some hybrid-type oligosaccharides with bisecting GlcNAc ($Man_{4-5}GlcNAc_{5-6}Gal_{0-1}$) [29,30]. Fig. 3(A) shows selected electropherograms obtained by changing the injection time of Con A. Peaks with a single asterisk disappeared after injection of 0.25 mg/mL of Con A at 3.45 kPa for 3 s (although retardation was not observed). In contrast, some peaks with two asterisks slightly broadened, and injection at 3.45 kPa and 30 s was needed for complete dissipation. Four other unmarked peaks that appeared at 19.2, 19.5, 20.8, and 21.3 were not altered by injection of Con A. According to the binding specificity of Con A, hybrid-type oligosaccharides with non-branched oligomannosyl sequences may not have affinity for Con A. Peak dissipation without retardation indicated that the recognition of oligosaccharides in the plug of Con A was fast but that dissociation of the oligosaccharide–Con A complex was kinetically very slow. This phenomenon is not specific for the partial-filling method; our previous work also indicated the dissipation profiles in affinity electrophoresis using electrophoretic buffer containing Con A [7].

Fig. 3(B) shows the change in migration of transferrin oligosaccharides by changing the injection times of RCA_{120}. This glycoprotein contains mainly biantennary complex-type oligosaccharides with α2,6-linked NeuAc (some of which also contain α1,6-linked Fuc in core chitobiose and/or α1-3-linked Fuc in terminal lactosamine residues). We applied drastic conditions for APTS labeling, which caused partial loss of sialic acids. As shown in the lower part of Fig. 3(B), tall peaks corresponding to disialo-, monosialo- and asialooligosaccharides appeared at 14.3, 16.1, and 19.1 min, respectively. Small peaks following major peaks therefore corresponded to fucosylated oligosaccharides. Injection of RCA_{120} lectin induced severe tailing and broadening of all transferrin peaks. Moreover, the long period of injection of this lectin induced slight retardation of migration.

Human α_1-acid glycoprotein contains bi-, tri-, and tetraantennary complex-type oligosaccharides. Some triantennary and tetraantennary oligosaccharides have α1,3-linked Fuc at a lactosamine branch; their non-reducing ends are terminated with NeuAc and NeuGc [31]. Therefore, the oligosaccharide mixture has very complicated electropherograms. For partial-filling analysis using a Fuc-specific lectin, AAL is depicted in Fig. 3(C). The Fuc-containing peaks are distributed irregularly throughout the electropherogram; some peaks with asterisks are gradually broadened and retarded along with the increase of injection time of AAL.

An advantage of partial-filling ACE can be seen by changing the effective plug length of the lectin solution without varying the concentration of the components. This feature enhances the applicability of this method.

3.4. Multiple-injection analyses

Combinatorial usage of lectins having various specificities might be useful for the profiling of specific oligosaccharides in a complex mixture [32]. However, reports on the use of a lectin mixture for structural profiling of oligosaccharides are lacking because some lectins such as ECA, LCA, PHA, PWM and SSA are glycoproteins. Therefore mixing of lectins including them often causes aggregation [33]. Therefore, lectin mixing is unfavorable. In our method, a combinatorial assay using a series of lectins can be realized by injecting each lectin solution sequentially into the capillary before the analysis. We chose porcine thyroglobulin as a model oligosaccharide pool and tried specific detection of high-mannose-type oligosaccharides from a pool including a series of complex-type oligosaccharides. To trap all complex-type oligosaccharides, we chose four lectins: α2,3-NeuAc-specific MAM, α2,6-NeuAc-specific SSA, β-Gal-specific ECA, and α-Fuc-specific AAL. These four lectins were injected at 3.45 kPa for 30 s in this order. Thyroglobulin oligosaccharides were then injected and separated. The separation profile is shown in the upper panel of Fig. 4 (which shows only selected peaks). Peaks observed at 12–17 min, large peaks appear-

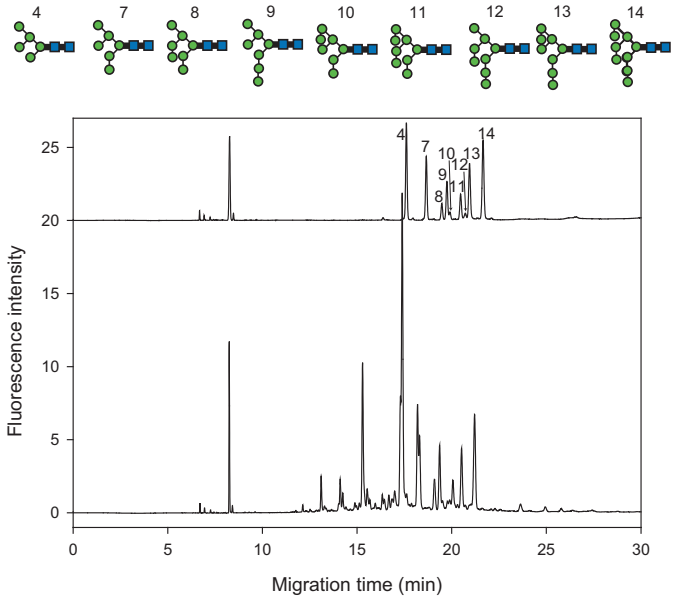

Fig. 4. Partial-filling affinity capillary electrophoresis using a series of lectins to identify high-mannose-type oligosaccharides in an oligosaccharide pool derived from porcine thyroglobulin. A 1 mg/mL solution of MAM, SSA, ECA, and AAL was introduced at 3.45 kPa for 30 s in this order; then APTS oligosaccharides from porcine thyroglobulin were injected for 5 s. The lower trace shows the reference data obtained without lectins. The other conditions were identical to those shown in Fig. 2.

ing at 17.5 min and 18.1 min, and other minor peaks appearing in reference electropherograms had disappeared completely. As shown in Fig. 2, most of the disappeared peaks were trapped by SSA. However, several minor peaks appearing at ≈15 min, 19 min, and 23–27 min, also disappeared from this profile. The same profile was also obtained by changing the injection order of the lectin solution. Moreover, the upper trace was very similar to the separation of APTS-labeled high-mannose-type oligosaccharides [34]. We speculated peaks at 17.3 min for $Man_5GlcNAc_2$, 18.2 min for $Man_6GlcNAc_2$, 19.3 min and 19.4 min for $Man_7GlcNAc_2$, 20.0 min and 20.5 min for $Man_8GlcNAc_2$, and 21.2 min for $Man_9GlcNAc_2$. This technique can be used to specifically detect oligosaccharides in a complex mixture if a suitable set of lectins is chosen.

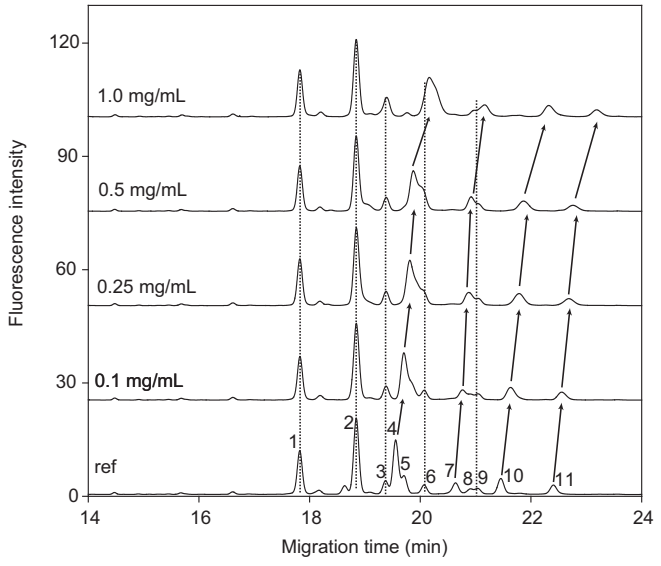

Fig. 5. Partial-filling affinity capillary electrophoresis of APTS-labeled oligosaccharides derived from ovalbumin using WGA (0, 0.1, 0.25, 0.5 and 1.0 mg/mL) for the calculation of binding constants of selected peaks. Tentative assignments of peaks are shown in Table 1. The other conditions were identical to those shown in Fig. 2.

Table 1
Tentative peak assignment and their binding constant to WGA.

Peak no.	Structure	Ka (M^{-1})
1		$<10^2$
2		$<10^2$
3		$<10^2$
4		91,000
5		ND
6		$<10^2$
7		82,000
8		ND
9		$<10^2$
10	Unknown	88,000
11		70,000

ND: not determined.

3.5. Calculation of binding constants

Calculation of binding constants is an important step in the evaluation of protein–oligosaccharide interactions, and understanding the biological significance of oligosaccharides attached to glycoproteins. ACE is important because this method is available for simultaneous analyses of the resolution of components and affinity for ligands. In the present study, we extended the use of partial-filling ACE to estimate the binding constants between WGA and APTS-labeled oligosaccharides derived from ovalbumin. These experiments were conducted by injecting an identical volume of a series of different concentrations of WGA solution. The migration times of some peaks were retarded with the increase of WGA concentration as shown in Fig. 5. Peaks 1, 2, 3, 6, and 9 did not change, but the migration times of peaks 4, 5, 7, 8, 10, and 11 increased gradually with WGA concentration. The difference in the mobilities of these peaks and concentration of WGA indicated a linear relationship in the *y*-reciprocal plots. Binding constants were obtained from the linear plot in the slope/intercept form, and the results presented in Table 1. The binding constants obtained were distributed

between 7.0×10^4 and 9.1×10^4 M^{-1}. The results obtained using the present method indicated good correlation with our previous work using lectin-immobilized silica LC [35]. Moreover, we believe that the binding constants obtained in free solution are more reliable and accurate.

4. Conclusion

Partial-filling lectin ACE can be used effectively to study the interaction between glycoprotein oligosaccharides with some plant lectins. Usage of a polydimethylsiloxane-coated capillary and hydroxypropylcellulose-impregnated electrophoretic buffer prohibits the generation of EOF and adsorption of lectins to the inner wall of the capillary. Moreover, APTS labeling enables sensitive determination of affinity profiles using minute amounts of oligosaccharides. We have shown these features of partial-filling lectin ACE by applying a series of lectins to examine affinity with a mixture of high-mannose and complex-type oligosaccharides derived from thyroglobulin, and by changing the injection conditions of lectins to selected glycoprotein oligosaccharides. Changes in electrophoretic profiles were observed as mobility shift, peak broadening, and peak disappearance. Sequential injection of a suitable set of lectins may enables detection and quantitation of specific oligosaccharides. This feature is applicable to quality control in the production of various glycoprotein pharmaceuticals, and an application will be shown near future. The method was also applied to obtain binding constants between WGA and ovalbumin oligosaccharides. Therefore, this is a convenient candidate method to detect specific oligosaccharides in glycoproteins.

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