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Capillary zone electrophoresis of α_1-acid glycoprotein fragments from trypsin and endoglycosidase digestions

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ABSTRACT

Capillary zone electrophoresis with fused-silica tubes having hydrophilic coating on the inner walls was evaluated in the separation of peptide and glycopeptide fragments from trypsin digestion of α_1-acid glycoprotein. Submapping of glycosylated and nonglycosylated tryptic fragments of the glycoprotein by capillary electrophoresis was facilitated by selective isolation of the glycopeptides on concanavalin A silica-based stationary phases prior to the electrophoretic run. In addition, the electrophoretic map and submaps of the whole tryptic digest and its concanavalin A fractions, respectively, allowed the elucidation of the microheterogeneity of the glycoprotein. Also, capillary zone electrophoresis proved suitable for the mapping of the oligosaccharide chains cleaved from the glycoproteins by endoglycosidase digestion. The oligosaccharides cleaved from human and bovine α_1-acid glycoprotein were analyzed after derivatization with 2-aminopyridine, which allowed their sensitive detection by on column UV absorption. The separation was best achieved when 0.1 *M* phosphate solution, pH 5.0, containing 50 m*M* tetrabutylammonium bromide was used as the running electrolyte. The effect of the organic salt on separation was attributed to ion-pair formation and/or hydrophobic interaction.

INTRODUCTION

High-performance capillary electrophoresis (HPCE) is rapidly developing and becoming an important tool for the separation of a wide variety of biological substances including peptides [1–3], proteins [4–6], nucleic acid fragments [7–9] and recently oligosaccharides [10]. The recent advances in instrumentation and the many sound features of HPCE, *e.g.*, various modes of separation, high resolving power, and small sample requirement, have made it possible for the technique to play this role.

This study is concerned with investigating the potential of capillary zone electrophoresis (CZE) in the separation and characterization of glycoprotein fragments, *i.e.*, peptides, glycopeptides and oligosaccharide chains. In this regard, we have selected human α_1-acid glycoprotein (AGP) as a model protein for the evaluation of CZE in analytical glycoprotein chemistry and analytical biotechnology. Besides being one of the best physicochemically characterized glycoproteins [11], human AGP is an attractive model because of its relatively high content of carbohydrates. Indeed, this single polypeptide chain of 181 amino acids has five glycosylation sites at asparagine residues in positions 15, 38, 54, 75 and 85 in the N-terminal of the molecule [12]. The N-linked oligosaccharide chains, which are of complex types at different degree of

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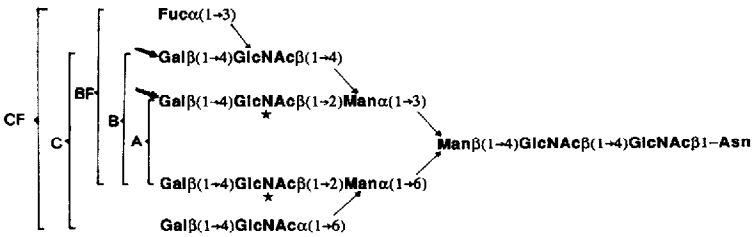

Fig. 1. Primary structures of the five carbohydrate classes as elucidated by Schmid *et al.* [12]. Classes A, B and C are the bi-, tri- and tetraantennary complex N-linked glycans, respectively, whereas BF and CF are the fucosylated B and C structures. Two additional glycans having the tetraantennary basic structure like class C were also reported [24]. One has two additional fucose each linked to the outer chain at the GlcNAc residues marked with a star, while the second one has an outer chain prolonged by Galβ(1-4) GlcNAc at either of the Gal residues marked with an arrow.

branching and sialylation (see Fig. 1), make up 45% of the total weight of the protein [11] (approx. mol.wt. = 41 000).

As will be shown in this report, CZE with its high resolving power and unique selectivity is well suited for the separation and characterization of peptide and glycopeptide fragments from the tryptic digest of human AGP, especially when combined with lectin affinity chromatography. For the selective isolation of glycopeptides prior to CZE analysis we have used concanavalin A silica-based stationary phases. This arrangement allowed CZE tryptic mapping of the whole digest as well as CZE submapping of glycosylated and non-glycosylated fragments. In addition, the usefulness of the technique in mapping the carbohydrate units of the protein liberated by endoglycosidase digestion is also demonstrated. The CZE methodology that generated the fingerprint for an endoglycosidase digest of human AGP, was also applied to the glycans of bovine AGP. In both cases the oligosaccharides were derivatized with 2-aminopyridine (2-AP), which provides the carbohydrates with a positive charge and a UV-absorbing (also fluorescent) tag [13].

EXPERIMENTAL

HPCE instrument

The instrument for capillary electrophoresis used in this study resemble that reported earlier [10]. It was constructed from a Glassman High Voltage (Whitehouse Station, NJ, U.S.A.) Model EH30P3 high-voltage power supply of positive polarity and a Linear (Reno, NV, U.S.A.) Model 200 UV–VIS variable-wavelength detector equipped with a cell for on-column capillary detection. In all experiments, sample injection was made by electromigration at a voltage and for a duration that varied from one mixture to another, and are indicated in the figure legends. The detection wavelength was set at 200 nm or 240 nm for sensing the polypeptides or derivatized oligosaccharides, respectively. The electropherograms were recorded with a Shimadzu computing integrator (Columbia, MD, U.S.A.) equipped with a floppy disk drive and a CRT monitor.

Capillary columns

Fused-silica capillary columns of 50-μm I.D. and 365-μm O.D. having polyimide-cladding were obtained from Polymicro Technology (Phoenix, AZ, U.S.A.). All capillaries (except where indicated) used in this study were modified in house with a hydrophilic coating on the inner walls. The inert coating, which consisted essentially of hydroxypolyether will be evaluated in CZE of proteins in an upcoming article [14]. It was prepared by allowing the capillary to react first with γ-glycidoxypropyltrimethoxysilane and then with a mixture of polyethylene glycol (mol.wt. 2000) and polyethylene glycol diglycidyl ether (mol.wt. 600) [14]. The running electrolyte was renewed after 5–6 runs and the capillary column was flushed successively with fresh buffer, water, methanol, water, and again running buffer before each injection in order to ensure reproducible separations.

High-performance liquid chromatography (HPLC) instrumentation and columns

The chromatograph was assembled from an LDC/Milton Roy (Riviera Beach, FL, U.S.A.) Model CM4000 solvent delivery pump with dual-beam variable-wavelength detector Model Spectro Monitor 3100. A Rheodyne (Cotati, CA, U.S.A.) Model 7125 sampling valve with a 100-μl sample loop was used for injection. Chromatograms were recorded with a Shimadzu (Columbia, MD, U.S.A.) Model C-R5A integrator.

A home made concanavalin A (Con A)–silica column (100 × 4.6 mm I.D.) was prepared by attaching Con A to Zorbax silica gel (DuPont, Willmington, DE, U.S.A.) using a procedure similar to that described by Larsson *et al.* [15]. Zorbax is a spherical silica with 300-Å and 7-μm pore and particle diameters, respectively. A bakerbond wide-pore octadecyl-silica column (250 × 4.6 mm I.D.) having mean particle and pore diameters of 5 μm and 300 Å, respectively, was a gift from J. T. Baker (Phillipsburg, NJ, U.S.A.).

Reagents and materials

Human and bovine AGPs, L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin, mannose (Man), galactose (Gal), N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), N-acetylneuraminic acid (NeuNAc), methyl-α-D-mannopyranoside, 2-AP, Brij 35 and Tris were obtained from Sigma (St. Louis, MO, U.S.A.). Peptide-N-glycosidase F (PNGase F) from *Flavobacterium meningosepticum* was obtained from Boehringer Mannheim (Indianapolis, IN, U.S.A.). Reagent-grade sodium phosphate monobasic and dibasic, boric acid, phosphoric acid, hydrochloric acid, acetic acid, sodium hydroxide and HPLC-grade methanol and acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.). Mercaptoethanol, sodium cyanoborohydride, polyethylene glycol and γ-glycidoxypropyltrimethoxysilane and tetrabutylammonium bromide were from Aldrich (Milwaukee, WI, U.S.A.). Distilled water was used to prepare the running electrolyte as well as the solutions used in column cleaning and pretreatment. All solutions were filtered with 0.2-μm UniPrep syringeless filters obtained from Genex (Gaithersburg, MD, U.S.A.) to avoid column plugging.

Tryptic digest

The digestion of AGP with TPCK-treated trypsin was carried out in a 10 m*M*

Tris buffer containing 100 m*M* ammonium acetate and 0.1 m*M* calcium chloride, pH 8.3, at a trypsin substrate ratio of 1:100 and a temperature of 37°C [16]. Trypsin was added again after 2 h and the digestion was stopped after a total of 4 h by addition of 10% (v/v) phosphoric acid. Thereafter, the whole digest was desalted by passing it on a Bakerbond wide-pore octadecyl-silica column (250 × 4.6 mm I.D.) equilibrated with water at 0.05% (v/v) trifluoroacetic acid (TFA). The bound fragments were eluted stepwise with acetonitrile–water (80:20, v/v) at 0.05% TFA (v/v). In this desalting process, single amino acids in the tryptic digest are lost but no major peak showed in the dead volume of the column. The pooled fraction was evaporated to dryness using a SpeedVac Concentrator (from Savant, Farmingdale, NY, U.S.A.). The dried materials were dissolved in the appropriate buffer used in the subsequent experiments.

Part of the dried materials was used for the CZE tryptic mapping, another part was fractionated on Con A–silica column into groups of peptide and glycopeptide fragments which were employed to produce CZE submaps of glycosylated and non-glycosylated peptides, and a third part was incubated with PNGase F to generate the oligosaccharides.

Cleavage of oligosaccharides

The whole digest, which was desalted on the reversed-phase chromatographic (RPC) column as described above was then dissolved in 20 m*M* phosphate buffer containing 2 m*M* EDTA, 1% (v/v) mercaptoethanol, and 0.1% (w/v) Brij 35, pH 7.5. To this solution 3 units of peptide-N-glycosidase F were added, and the incubation was maintained at 37°C for 24 h [17]. Thereafter, the mixture was evaporated to dryness with a Savant SpeedVac Concentrator. The dried materials containing the cleaved oligosaccharides, the peptide fragments, and other reagents employed in the incubation step was used as is without a sample clean up prior to the derivatization of its oligosaccharide components.

Derivatization of mono- and oligosaccharides

Commercially available standard monosaccharides such as Man, Gal, GalNAc, GlcNAc and NeuNAc, which are the constituents of the carbohydrate chains of glycoproteins as well as the oligosaccharides cleaved from the glycoprotein were tagged with 2-aminopyridine (2-AP) at their reducing termini as described by Hase *et al.* [13]. The mixtures containing the pyridylamino (PA) derivatives of the various sugars were first evaporated to dryness using SpeedVac Concentrator. Subsequently, the dried materials were dissolved in water and then applied to capillary electrophoresis without any sample clean up from excess derivatizing agent and other components of the reaction mixture.

RESULTS AND DISCUSSION

CZE tryptic mapping and submapping

All CZE tryptic mapping and submapping of human AGP were performed on a capillary with hydrophilic coating on the inner walls, and using 0.1 *M* phosphate solution, pH 5.0, as the running electrolyte. The hydrophilic coating minimized solute-wall interactions and also permitted the electrophoresis of basic proteins in the pH range 2.0 to 7.0 with high separation efficiencies [14]. Furthermore, with the coated

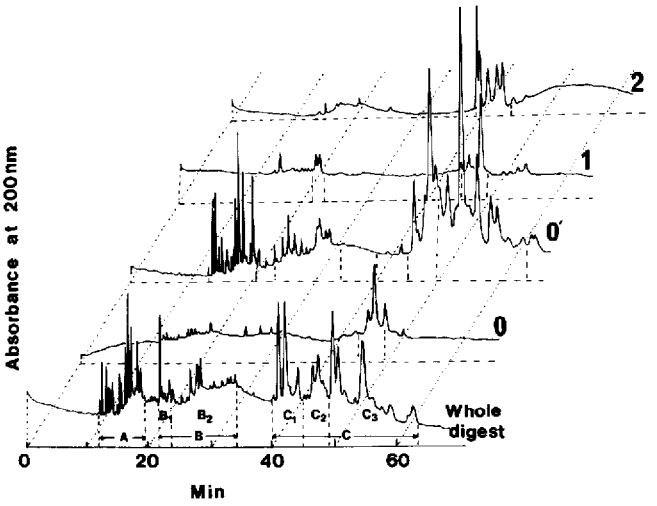

Fig. 2. CZE tryptic mapping and submapping of human AGP. Capillary, fused-silica tube with hydrophilic coating on the inner walls, 45 cm (to the detection point), 80 cm total length × 50 μm I.D.; electrolyte, 0.1 *M* phosphate solution, pH 5.0; running voltage, 22.5 kV; current was *ca.* 55 μA; injection by electromigration for 4 s at 22.5 kV. Fractions: 0 = Con A non-reactive (excluded from the column); 0′ = Con A non-reactive (unretained by the column); 1 = Con A slightly reactive (eluted with buffer); 2 = Con A strongly reactive (eluted with the haptenic sugar).

capillaries, the electroosmotic flow decreased by a factor of *ca.* 3.5 on the average when compared with the uncoated capillary.

Fig. 2 illustrates the CZE mapping and submapping of peptide fragments from the tryptic digest of human AGP. The electropherogram of the whole digest has an elution pattern characteristic of a mixture whose peptide fragments are widely different in size and net charge. Three areas, with different peak capacity, denoted by A, B and C can be distinguished on the electropherogram of the whole digest. They are separated by two quasi peakless areas (see whole digest, Fig. 2). Obviously, small and positively charged peptide fragments are likely to be present in area A, slightly neutral peptides may be located in area B, and large and negatively charged tryptic fragments may be seggregated in area C. The values of the nominal "overall mobility", $\bar{\mu}_{\text{overall}}$, for areas A, B and C are $2.94 \cdot 10^{-4}$, $1.7 \cdot 10^{-4}$ and $0.88 \cdot 10^{-4}$ cm^2/V · s, respectively. The nominal overall mobility refers to the mobility at the center of each area. The overall mobility, which is the sum of electrophoretic mobility and electroosmotic mobility, was calculated by the equation, $\bar{\mu}_{\text{overall}} = Ll/Vt$, where L, l, V and t are the total length of the capillary, the distance from the injection point to the detection point, the applied voltage and the time for the solute to migrate the length l, respectively.

As seen in Fig. 2, the map for the whole tryptic digest of human AGP demonstrates the high resolving power of CZE and reveals the microheterogeneities of the glycoprotein as manifested by the excessive number of peaks for a protein of 181 amino acid residues with 20 trypsin cleavage sites (8 lysine and 12 arginine residues) [11,18]. In fact, by neglecting all sources of heterogeneity in the protein, the tryptic digest should result only into 12 peptide and 5 glycopeptide fragments, and three single amino acids (two lysine and one arginine). However, more than any other serum

glycoproteins, AGP is a highly heterogeneous protein [11,18,19]. One of the unique aspects of the primary structure of polypeptide chain of pooled human AGP is its peculiar structural polymorphism [11]. Substitutions were found at 21 of the 181 amino acids in the single polypeptide chain, which is responsible in part for multiple peptide and glycopeptide fragments in the tryptic digest. More details concerning the primary structure of the protein can be found in refs. 11 and 18. Another source of multiple fragments in the tryptic digest is the microheterogeneities of the oligosaccharide chains attached [11,19,20]. Indeed, the variation in the terminal sialic acid causes charge heterogeneity in the glycopeptide fragments cleaved at the same location by trypsin, the differences in the extent of glycosylation among a population of the protein molecules lead to fragments having the same peptide backbone but with or without carbohydrate chains, and the variation in the nature of the oligosaccharide chains at each location yields several glycopeptides that have the same peptide backbone, but different in their oligosaccharide structures.

To further elucidate the microheterogeneity of the glycoprotein and to generate CZE submaps for the tryptic digest, *i.e.*, maps of groups of peptide or glycopeptide fragments, we have combined high performance lectin affinity chromatography and capillary electrophoresis. Fig. 3 shows affinity chromatography group separation of the peptide fragments from human AGP performed on silica-bound Con A column. The pooled fractions labelled 0 and 0′ contain the Con A non-reactive species, whereas those labelled 1 and 2 have the Con A slightly and strongly reactive fragments, respectively. Whereas the components of fraction 1 were slightly retained by Con A and eluted from the column with the equilibrating buffer (binding buffer), the components of fraction 2 interacted strongly with Con A and eluted from the column with the haptenic sugar, methyl-α-D-mannopyranoside.

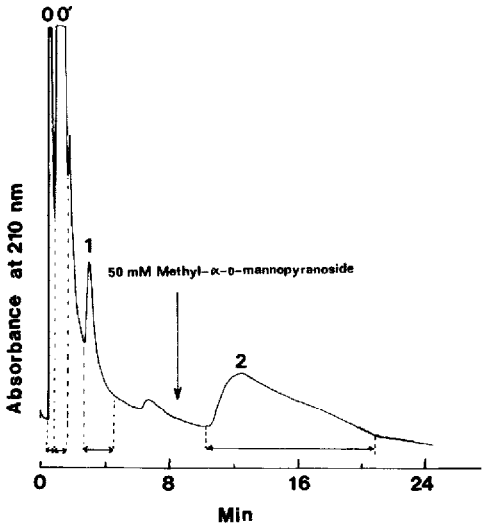

Fig. 3. High-performance lectin affinity chromatography of human AGP tryptic digest. Column, silica-bound Con A, 100 × 4.6 mm I.D.; flow-rate, 1 ml/min; temperature, 25°C. Binding buffer, 20 m*M* phosphate, pH 6.5, containing 0.1 *M* NaCl; debinding buffer, 50 m*M* methyl-α-D-mannopyranoside in the binding buffer.

The different Con A fractions were first desugared and/or desalted on an RPC column (see Experimental) and then analyzed by CZE. The results are depicted in Fig. 2, and are refered to as submaps denoted by 0, 0′, 1 and 2. The tryptic fragments of fraction 0 were excluded from the Con A column, may be due to electrostatic repulsion between the peptides of the fraction and the Con A surface of same charge at pH 6.5 (p*I* value of Con A is *ca.* 4.5–5.5 [21]). Indeed, the CZE submap 0 exhibited peaks eluting late from the capillary, and corresponding to area C_2 on the whole map, which is mainly populated by negatively charged peptide fragments as reflected from the value of its nominal overall mobility ($\bar{\mu}_{overall} = 1.0 \cdot 10^{-4}$ cm^2/V · s). Fraction 0′, the peptide fragments of which did not interact with the Con A and eluted in the column void volume, yielded the CZE submap 0′ containing most peaks that are in the map of the whole digest except those in areas B_1 and C_1. The two submaps 0 and 0′ are mostly populated by non-glycosylated fragments, since their components are repulsed from or not retained by the Con A column.

Con A fraction 1 produced a CZE submap containing glycopeptide fragments with tri- and tetraantennary glycans that are known to interact weakly with Con A [22]. The submap 1 has some of the fragments that are missing in submap 0′ (area B_1), but has fragments from area C_2 and minor peaks from area C_3 on the whole map. Fraction 2 produced a CZE submap with peaks corresponding to area C_1 on the whole map that are missing from submaps 0, 0′ and 1. They are believed to be the glycopeptides with biantennary glycans that strongly bind to a Con A affinity column [22,23]. An area on the whole map worth mentioning is area C_2 whose components are found in submaps 0, 0′ and 1. The components of this area on submaps 0, 0′ and 1 are likely to be different from each others but overlap in the whole digest due to insufficient selectivity.

The approach tested and developed here is capable of elucidating the micro-heterogeneity of the protein, and is convenient for CZE submapping of tryptic fragments of interest, *e.g.*, glycopeptides. This methodology is expected to work also with other glycoproteins, and the CZE submapping of all the glycosylated tryptic fragments with different type of glycans may require the use of more than one lectin column in the prefractionation step.

CZE mapping of oligosaccharide chains from AGP

Human AGP oligosaccharides were cleaved from the glycopeptide fragments of the tryptic digest using PNGase F, an endoglycosidase that cleaves all types of N-linked oligosaccharide chains between the asparagine and the carbohydrate units [17]. The CZE mapping of the PA derivatives of these oligosaccharides is portrayed in Fig. 4a. It was performed on a coated capillary using 0.1 *M* phosphate solution, pH 5.0, containing 50 m*M* tetrabutylammonium bromide as the running electrolyte. As can be seen in Fig. 4a, the electropherogram shows six well defined peaks and a few minor peaks, eluting after the excess 2-AP and some spikes. The glycans of AGP (see Fig. 1) are known for their microheterogeneities caused mainly by variation in their terminal sialic acid. This may explain the presence of several peaks in the CZE map. The spikes indicated the presence of undissolved matters in the sample, since they were more numerous when applying the mixture to CZE without centrifugation.

Based on the above results, CZE can play an important role in the field of glycan separation and characterization. In addition, it holds promise for rapidly monitoring

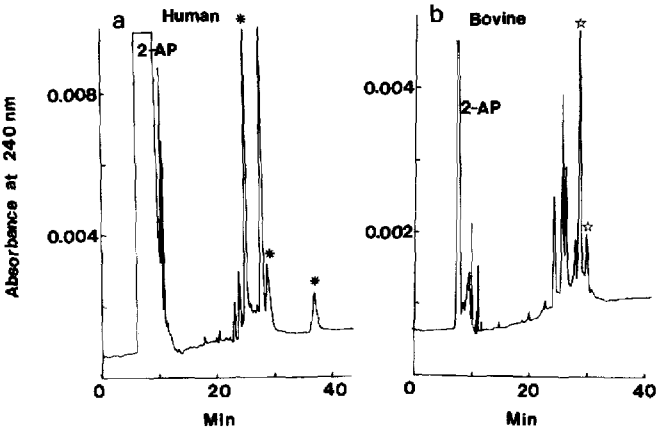

Fig. 4. CZE mapping of pyridylamino derivatives of human (a) and bovine (b) AGP oligosaccharides. Capillary, fused-silica tube with hydrophilic coating on the inner walls, 45 cm (to the detection point), 80 cm total length × 50 μm I.D.; electrolyte, 0.1 *M* phosphate solution, pH 5.0, containing 50 m*M* tetrabutylammonium bromide; running voltage, 18 kV; current, 80 μA; injection by electromigration for 2 s at 18 kV. For stars and asterisks, see text.

the extent of deglycosylation. Indeed, in another set of experiments, the oligosaccharides were cleaved directly from the glycoprotein using the same amount of protein starting materials as in the preceding experiment and keeping other conditions identical, *i.e.*, pH, temperature, number of PNGase F units and the time of digestion. The extent of deglycosylation from the whole protein decreased when compared to that from the glycopeptide fragments. This was ascertained from the disappearance of three major peaks from the CZE map (indicated by asterisks in Fig. 4a). This can be explained by the better accessibility of the endoglycosidase to the cleaving site in the glycopeptides than in the intact glycoprotein.

To evaluate the potential of CZE in elucidating the difference in glycan structures, the oligosaccharides from bovine AGP were cleaved from the glycopeptides and derivatized with 2-AP as in the case of human glycoprotein. Subsequently, the derivatized bovine AGP oligosaccharides were analyzed by CZE using the same electrophoretic conditions as in Fig. 4a, and their corresponding CZE map is depicted in Fig. 4b. The map shows an elution pattern different from that of the oligosaccharides derived from the human glycoprotein. Both human and bovine AGPs have been found to have the same sialic acid, galactose and mannose content [25]. The major differences are such that 50% of the sialic acid in bovine AGP are N-glycolylneuraminic acid and the fucose content is very low [25]. These differences are accentuated by CZE mapping of both types of glycans; compare Fig. 4a and b.

In a recent report from our laboratory [10], we have demonstrated the capability of CZE in the separation of the PA derivatives of maltooligosaccharides. This study has shown the effectiveness of tetrabutylammonium bromide in affecting full separation of the oligomers. The organic salt was also useful in the separation of glycans. The comparison of Fig. 5a and b demonstrates the high selectivity and high resolution attained upon adding tetrabutylammonium bromide to the running

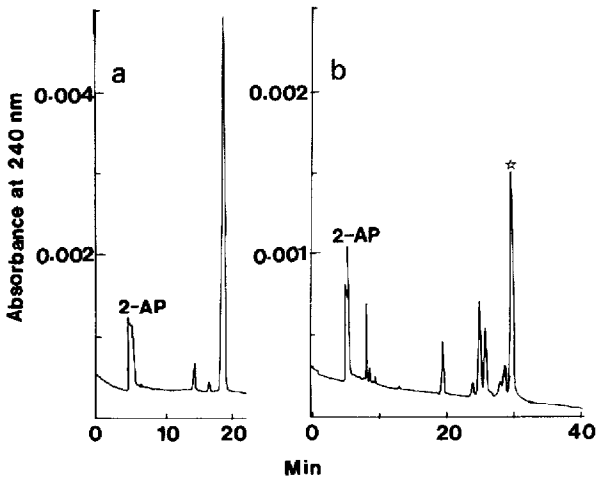

Fig. 5. CZE mapping of pyridylamino derivatives of bovine AGP oligosaccharides without (a) or with (b) 50 m*M* tetrabutylammonium bromide in the running electrolyte. Capillary, fused-silica tube with hydrophilic coating on the inner walls, 35 cm (to the detection point), 70 cm total length × 50 μm I.D.; electrolyte, 0.1 *M* phosphate solution, pH 5.0; running voltage, 15 kV; currents were *ca.* 55 μA in (a) and 90 μA in (b); injection by electromigration for 8 s at 15 kV. For star, see text.

electrolyte. Indeed, in the absence of the organic salt (Fig. 5a), the glycans were separated as two minor peaks and one large peak, whereas upon adding tetrabutylammonium bromide to the running electrolyte several additional peaks appeared in the map (Fig. 5b). The improvement in selectivity upon adding the organic salt to the running electrolyte can be explained by ion pair formation between the quarternary ammonium salt and the sialic acid of the carbohydrate chains and/or by hydrophobic interaction between the alkyl chains of the organic cation and the PA-oligosaccharides. On the other hand, the addition of tetrabutylammonium bromide increased the ionic strength of the running electrolyte, and as expected the residence time of the separated glycans increased. This is due to the reduction in electroosmotic flow [6], as a consequence of a decrease in both the thickness of the double layer and the ζ potential of the capillary wall [26]. The decrease in the rate of the flow is more reflected on the late eluting zones than 2-AP. The migration time of 2-AP increased slightly by a factor of 1.08.

The comparison of electropherograms in Figs. 4b and 5b reveals the influence of the effective capillary length, *i.e.*, the distance from the injection end to the detection point, on the separation of glycans at approximately the same field strength. The difference between these two electropherograms is such that the former was performed on a capillary of 80 cm total length and 45 cm effective length whereas the latter was obtained with a capillary of 70 cm total length and 35 cm separation distance. Although a better selectivity was obtained in Fig. 5b, especially for the fast migrating zones, the peaks of the electropherogram in Fig. 4b are sharper and concomitantly complete resolution was obtained for the last two peaks (indicated by stars in Figs. 4b and 5b).

As pointed out in the Experimental section, the derivatization of the oligo-

saccharides was carried out in the presence of the protein or the peptide fragments without an isolation step of the cleaved oligosaccharides. Also the mixture containing the derivatized oligosaccharides, excess derivatizing agent and other components of the reaction mixture (see Experimental) was applied directly to CZE. Since the remaining protein, peptides and other components of the reaction mixture do not form an interference at the wavelength of detection (240 nm) and the excess 2-AP elute first in the electrophoretic run, solid phase extraction or other isolation procedures were not needed prior to CZE analysis. This represents an advantage for the method established here in terms of reduced time and labor. In addition, since no extraction step is involved, the approach used here eliminates sample loss.

The results of the above study demonstrate the effectiveness of the technique in the area of glycoconjugate research. In addition, since CZE uses nanoliter quantities for several runs, the small sample size most often encountered in the domain of glycans is not a limiting factor and the technique can be explored further in the separation and analysis of carbohydrate chains of glycoproteins provided that authentic standards are available.

CZE analysis of monosaccharide constituents of glycoproteins

The above electrophoretic systems were also employed in the separation of the constituents of carbohydrate chains of glycoproteins using coated capillaries. The standards Gal, Man, GlcNAc, GalNAc and NeuNAc were first derivatized with 2-AP. Following, the PA-sugars were electrophoresed on a coated capillary, using 0.1 *M* phosphate solution, pH 5.0, with or without tetrabutylammonium bromide. In the

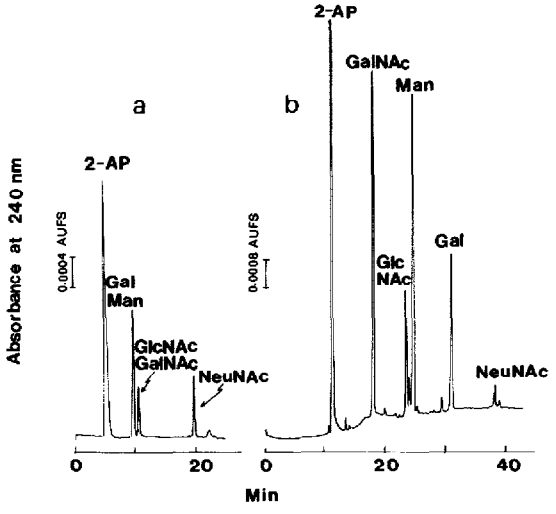

Fig. 6. CZE of pyridylamino derivatives of standard monosaccharides. (a) Capillary, fused-silica tube with hydrophilic coating on the inner wall, 35 cm (to the detection point), 70 cm total length × 50 μm I.D.; electrolyte, 0.1 *M* sodium phosphate solution, pH 5.0, containing 50 m*M* tetrabutylammonium bromide; running voltage, 15 kV; current was *ca.* 90 μA; injection by electromigration for 8 s at 15 kV. (b) Capillary, uncoated fused-silica tube, 50 cm (to the detection point), 80 cm total length × 50 μm I.D.; electrolyte, 0.2 *M* sodium borate, pH 10.5; running voltage, 18 kV; current was 75 μA; injection by electromigration for 1 s at 10 kV.

presence of 50 m*M* tetrabutylammonium bromide in the running electrolyte (see Fig. 6a), the PA derivatives of the monosaccharides were only separated into groups whereby, Man and Gal separated from GalNAc and GlcNAc and the PA derivative of NeuNAc being a zwitterion at pH 5.0 migrated at slower paste. In the absence of tetrabutylammonium bromide in the running electrolyte the acetylated (GlcNAc and GalNAc) and nonacetylated (Man and Gal) monosaccharides coeluted. The migration modulus, η, which is the ratio of the migration times in the presence to that in the absence of tetrabutylammonium bromide were 1.13 for Gal and Man, 1.16 for GlcNAc and GalNAc and 1.04 for NeuNAc. Although, only group separation can be obtained when tetrabutylammonium bromide is added to the running electrolyte, the method established here may prove useful in the analysis of sugar residues of glycans cleaved by sequential exoglycosidases.

The full resolution of the PA derivatives of the standard monosaccharides was brought about by using uncoated capillary and borate-sugar complexes at high pH (see Fig. 6b), an electrophoretic system exploited earlier by Honda *et al.* [27] for a series of monosaccharides and by Wallingford and Ewing [28] for catecholamines. The anionic borate-sugar complexes created the charge for electrophoretic separation, as shown in Fig. 6b. It is well known that compounds containing *cis*-oriented hydroxyl groups form stronger complexes with borate than those having *trans*-oriented diols [29]. Mannose has *trans*-diols at C-3/C-4 position, and therefore migrated faster than galactose which has *cis*-diols at the same location. This trend does not follow for GalNAc and GlcNAc, may be due to the presence of N-acetyl group which may affect the binding of borate. NeuNAc is a negatively charged species at high pH and the binding of borate to this molecule slows even further its migration.

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