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Characterization of human transferrin glycoforms by capillary electrophoresis and electrospray ionization mass spectrometry

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Abstract

Carbohydrate Deficient Glycoprotein Syndrome (CDGS) is an inherited metabolic disease affecting all parts of the body. The biochemical diagnosis of this syndrome is based on the presence of a special marker in blood, Carbohydrate Deficient Transferrin (CDT), which is also a marker of chronic alcohol abuse. CDT is characterized by abnormal glycoforms of serum transferrin (Tf). In the present study, electrophoretic separation of human serum transferrin glycoforms was carried out using a bare fused-silica capillary and the glycoforms present in commercial Tf were baseline separated. The limit of detection (LOD) of human Tf was around the nmol concentration range. The LOD of the trisialo- and disialo-Tf, expressed as percentages of the tetrasialo-Tf peak area, were 0.5% for trisialo-Tf and 0.4% for disialo-Tf, and these values were appropriate for CDGS diagnosis. Moreover, Tf glycoforms were characterized using mass spectrometry (MS). The method was applied to the analysis of normal and pathological serum samples, after dilution. The results obtained suggest a way of making a rapid and simple CDGS diagnosis.

Keywords: Carbohydrate deficient glycoprotein syndrome; Glycoforms; Transferrin

1. Introduction

Glycoproteins contain carbohydrate chains covalently attached to their peptide structure by glycosidic linkages. There is a polymorphism associated with type, number and position of carbohydrate chains. This kind of diversity is known as microheterogeneity and the different species generated are called glycoforms [1]. Whole glycoforms found for a particular glycoprotein are related to the species and tissues involved. The glycoform range associated with a single glycoprotein may change during normal tissue development or as a result of pathological processes [2]. This is the case for carbohydrate deficient glycoprotein syndrome (CDGS). Patients with this syndrome present hypoglycosylation of different plasmatic glycoproteins, transferrin being one of these.

Transferrin (Tf) is the main iron-transporting glycoprotein in plasma [3]. It has a polypeptidic chain of 679 amino acids and two *N*-glycosylation sites at Asn^{413} and Asn^{611} (asparagine at positions 413 and 611, respectively) [2]. Over 6% of its molecular mass is accounted for by two sugar chains that may be mainly bi-antennary [4]. Each chain terminates with sialic acid residues (SA) which give a net negative charge to the protein.

The sialic acid content affects the electrophoretic behaviour of transferrin, making it possible to separate different sialoforms with the right technique [2]. Approximately 85% of Tf in normal serum is in the tetrasialo form, with two *N*-linked disialylated biantennary sugar chains, known as S_4. Other sialoforms such as pentasialo-, hexasialo- and trisialo-transferrin, known as S_5, S_6 and S_3, respectively, are present at low concentrations in healthy serum. The isoelectric point (p*I*) of transferrin glycoforms (Tf-glycoforms) is in the range of 5–6.

Carbohydrate-deficient transferrin (CDT) is typically considered to have less than three sialic acid residues. An increase in the di-, mono- and asialoforms (i.e. CDT), named

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S_2, S_1 and S_0, respectively, has been noted only with the above mentioned syndrome (CDGS) and chronic alcoholism [2,4]. For historical reasons, the diagnosis of CDGS is based upon the glycoform pattern observed for transferrin [2]. CDGS diagnosis is based on identification and quantitation of CDT, mainly of S_2 and S_0, in which one or both *N*-linked disialylated biantennary sugar chains are missing, respectively [2,4,5].

Until recently, the analytical determination of CDT was based on isoelectric focusing (IEF) combined with immunofixation, zone immunoelectrophoresis or Western blotting, as well as chromatographic techniques (i.e. anion-exchange chromatography or chromatofocusing) followed by immunoassays. These multistep techniques are clearly too complex and time consuming for routine application and are unreliable due to the complicated and manual procedures involved [6,7].

Capillary electrophoresis (CE) is a relative new tool for the analysis of biologically active compounds [8]. As this technique can improve the resolution of complex mixtures under non-denaturing conditions, based on the differences in charge-to-mass ratios, it has several advantages over conventional techniques. However, electrophoretic separation of proteins in uncoated fused-silica capillaries is more problematic, due mainly to protein interactions either with the inner capillary wall or with other proteins. This problem has been circumvented through the use of chemical additives that coat the wall in order to reduce protein–wall interactions [3,9]. It has also been shown that buffer composition plays a role in separation of the different species. This is the case for sodium borate buffer, which has been proposed to play a key role in separation by complexing with the diols of specific carbohydrate moieties on different glycoproteins [1,6,7,10,11]. CE has been used to separate the isoforms of different glycoproteins [1,8,10,12–17] Tf being one of them [6,7,18,19]. The sialic acid residues are deprotonated at basic pH and this generates different charge/mass ratios for each glycoform. This means that glycoforms can be separated by CE due to their different electrophoretic mobilities.

Mass spectrometry (MS) has also been widely used in biochemical analysis [20–28]. The development of ionization methods, such as electrospray (ES), has enabled it to be used for characterizing polar substances and biomolecules of molecular mass up to 100 000 Da. The ability of ES to produce multiply-charged ions, such as polyprotonated peptides and proteins, has generated a wide and interesting field of research. Thus, mass spectrometry with electrospray ionization is a useful tool in characterizing Tf-glycoforms.

In this work, an electrophoretic method was developed in order to separate glycoforms in a recombinant Tf. The method was applied to the analysis of normal and pathological serum samples, after dilution. Moreover, Tf glycoforms were characterized using mass spectrometry (MS). The results obtained permit us to propose a method for a rapid and simple CDGS diagnosis.

2. Experimental

2.1. Chemicals and reagents

Human transferrin (partially saturated, min. 98% pure) was purchased from Sigma (Madrid, Spain). Neuraminidase (from *Vibrio Cholerae*, 2.1 U/ml), sodium azide, L-glutamic acid and 1,4-butanediol of HPLC-grade were purchased from Fluka (Madrid, Spain). Acetonitrile, methanol, 2-propanol, ethyleneglycol, sodium tetraborate, boric acid, sodium hydrogencarbonate, hydrochloric acid, acetone, trifluoroacetic acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Heptafluorobutyric acid, ethanolamine, hexamethonium bromide and 1,4-diaminobutane (DAB) were purchased from Aldrich (Madrid, Spain). Tris, ethylenediamine and triethanolamine were obtained from J.T. Baker (Deventer, Holland). Iron chloride was purchased from Panreac (Barcelona, Spain). Water, with a resistivity of 18.2 MΩ cm, was obtained using a Milli-Q water purification system (Millipore, Molsheim, France).

2.2. Instrumental equipment

2.2.1. Capillary electrophoresis

All CE experiments were performed on a P/ACE System 5500 (Beckman Instruments, Palo Alto, CA, USA) equipped with an autosampler, automatic injector and a photodiode array detector. The distance between the detection window and the end of the capillary was 7 cm. Electropherograms were recorded using a computer program (P/ACE Station 1.0 with interface Golden System) supplied by Beckman. A 57 cm × 50 μm I.D. uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was inserted in a capillary cartridge thermostated to 25 °C (±0.1 °C). Samples were injected hydrodynamically at 0.5 p.s.i. (5–15 s). Experiments were conducted under normal polarity applying different separation voltages (10–30 kV). Detection of Tf-glycoforms was performed at 200 nm.

2.2.2. pH measurements

pH measurements were carried out with a Crison 2002 potentiometer (Crison Instruments, Barcelona, Spain), equipped with a Ross electrode 8102 (Orion Research, Boston, MA, USA).

2.2.3. Mass spectrometry

A VG Platform mass spectrometer (Fisons Instruments, Manchester, UK) equipped with a pneumatically assisted atmospheric pressure ionization (API) source was used. The instrument was periodically calibrated using a solution of bovine serum albumin (BSA). For the direct injection of transferrin solutions an injection loop of 5 μl with a flow-rate of 10 μl/min of mobile phase was used. The data acquisition was made in Multi Channel Analysis (MCA) mode. With this kind of acquisition, intensity data for each acquired scan

is added to the accumulated data of previous scans, and only one spectrum, obtained in full scan mode (m/z 1600–3000), is registered for every injection. Instrument and data analysis were carried out using MassLynx application software from Micromass (Manchester, UK). Data obtained were analyzed with the MaxEnt algorithm of MassLynx. The MaxEnt algorithm works iteratively to find the simplest molecular mass spectra from multiply charged electrospray spectra.

2.3. Electrolyte solutions

2.3.1. Capillary electrophoresis

A pH range of 7–9.5 was covered using two types of running buffer solutions: 25 m*M* sodium tetraborate, adjusting until the desired pH with 100 m*M* boric acid; Tris–glutamate buffer (50 m*M* Tris and 50 m*M* monosodium glutamate), adjusting the pH with concentrated sodium hydroxide. For CE experiments, Tf was dissolved in water (1 μg/μl) and saturated with iron, adding 18 μl of 10 m*M* $FeCl_3$ and 25 μl of 500 m*M* $NaHCO_3$, in order to avoid heterogeneity in the charge of the individual Tf glycoforms due to incomplete iron saturation. The sample also contained acetone at 5% (v/v) as electroosmotic flow (EOF) marker [29,30]. Samples and running electrolytes were passed through a 0.45-μm nylon filter (MSI).

2.3.2. Mass spectrometry

For mass spectrometry experiments, Tf was dissolved in water (0.4–4 μg/μl). The solvent used for these experiments was a mixture of water/acetonitrile (50:50 v/v) containing 0.25% (v/v) trifluoroacetic acid (TFAA) or 0.1% (v/v) heptafluorobutyric acid (HFBA). Samples and running electrolytes were passed through a 0.45-μm nylon filter (MSI).

2.4. Capillary treatment

All capillary rinses were performed at high pressure (20 p.s.i.). New capillaries were activated by flushing them for 20 min with 1 *M* NaOH followed by water for 20 min and finally conditioned with running buffer for 60 min. Between days or after a change of buffer, the capillary was conditioned by rinsing successively for 20 min with 0.1 *M* NaOH, 20 min with water and 60 min with running buffer. Finally, the system was conditioned by applying a constant voltage for about 10 min, until a steady baseline was obtained. It was empirically demonstrated that this final step accelerates capillary equilibration. Between runs, the capillary was successively rinsed with 30 s of water and 1 min of running buffer. It was stored overnight filled with working buffer electrolyte.

2.5. Experimental procedure

2.5.1. Capillary electrophoresis

Different capillary electrophoretic separations were carried out in order to optimize the Tf-glycoforms separation.

Table 1
Additives tested in the Transferrin CE analysis

Additive	Concentration
Hydroxylamines	
Ethanolamine	0.1% (w/v)
Triethanolamine	50 m*M*
Alkyldiamines	
Ethylenediamine	50 m*M*
1,4-Diaminobutane (DAB)	1 m*M*
α,β-bis-quaternary ammonium	
Hexamethonium bromide	0.75 m*M*
Organic solvents	
Acetonitrile	30% (v/v)
Methanol	30% (v/v)
2-Propanol	15% (v/v)
1,4-Butanediol	15% (v/v)
Ethyleneglycol	15% (v/v)

The glycoforms were identified by the elution order and named as S_0, S_1, S_2, S_3 and S_4 according to the number of contained sialic acid. A pH range of 7–9.5 was covered testing two types of buffer solutions: borate and Tris–glutamate and different additives to buffer solution were tested. These modifiers are shown in Table 1. Different capillary dimensions (inner diameters of 50 and 75 μm, and lengths of 57, 77 and 107 cm) were also tested.

2.5.2. Mass spectrometry

For mass spectrometry experiments, the solvent used consisted of a mixture of water/acetonitrile (50:50 v/v) and the ES response was optimized by adding different acids, such as trifluoroacetic acid (TFAA) and heptafluorobutyric acid (HFBA), at different concentrations (0.05–0.25%). In order to optimize the ES-MS source and analyzer parameters, an aqueous Tf solution (0.4 μg/μl) was introduced directly into the ES source at a flow-rate of 10 μl/min. The optimal values for sample cone voltage and source temperature were: 75 V and 120 °C, respectively, when 0.25% TFAA was used as additive; 110 V and 60 °C when 0.1% HFBA was used as additive.

2.6. Neuraminidase treatment

As the most interesting sialoforms in CDGS studies present fewer sialic acid residues (S_2 and S_0) than the main glycoform (S_4) present in healthy serum, Tf was incubated with the neuraminidase enzyme in order to split off sialic acid residues and to obtain the different sialoforms with lower sialic acid content.

2.6.1. Capillary electrophoresis

For capillary electrophoresis experiments, a 10-μl volume of enzyme solution (2.1 U/mg in aqueous solution, pH∼5.5) was incubated with 1 ml of Tf solution (1 μg/μl) at room temperature. The reaction was followed at different times by direct injections of aliquots, until the peak attributed to asialo-Tf appeared. The sialoforms of Tf generated by

neuraminidase were labelled S'_3, S'_2, S'_1 and S'_0, respectively, according to the number of sialic acid residues they contained, in order to distinguish them from the S_3, S_2, S_1 and S_0 glycoforms, which have lost the complete carbohydrate chain. The S'_i sialoforms differ in $\sim n \times 292$ Da, n being the number of sialic acids split off by the neuraminidase.

2.6.2. Mass spectrometry

For mass spectrometry experiments, 1 ml of Tf (4 μg/μl) was incubated with 40 μl of enzyme. The reaction was followed by direct injection of different aliquots to MS taken at several times. A 15-μl sample aliquot was acidified with 5 μl of the eluent described in Section 2.5.2 prior to injection.

2.7. Analysis of serum samples

Normal serum samples from healthy individuals and pathological serum samples from patients with the CDG Syndrome were supplied by the Institut de Bioquímica Clínica of the University of Barcelona. Serum samples were saturated with iron by incubation with 10 m*M* $FeCl_3$ and 500 m*M* $NaHCO_3$ for 30 min, and diluted 1/10–1/3 in water before injection. Normal Tf contents of human serum ranges from 2 to 3 μg/μl.

3. Results and discussion

3.1. Capillary electrophoretic separation of Tf-glycoforms

In the present study, Tf isoforms were separated according to the different mobility of the Tf-sialoforms, which differ in the number of sialic acid residues and, consequently, in the number of negative charges. Under basic CZE separation conditions, the high electroosmotic flow generated pushes the negatively-charged Tf isoforms to the detector (cathodic end). Several factors, such as type and pH of running buffer, type and concentration of buffer additives, length and inner diameter of the capillary, and other instrumental parameters (e.g. applied voltage, injection time) have been optimized in order to achieve a good resolution of the different sialoforms.

The parameters with most influence, among those indicated above, were the pH of running buffer and the type and concentration of buffer additives. Despite obtaining similar results with both assayed buffers, borate and Tris–glutamate (Fig. 1), we chose the borate buffer because of the role of borate anions in glycoform separations [6,7,10,11,31] and the fact that borate's high UV transparency allows higher sensitivity detection [6]. Buffer pH ranged from 7 to 9.5 and a loss of resolution was observed working at extreme pH values (Fig. 1). Therefore, an optimal value of 8.3—giving the best resolution—was chosen.

In order to reduce protein adsorption onto the fused-silica capillary walls, different additives were tested (Table 1). Hexamethonium bromide and 1,4-diaminobutane (DAB)

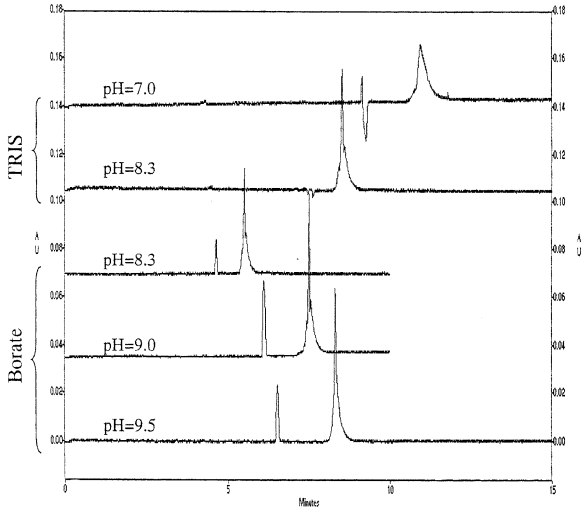

Fig. 1. Electropherograms at 15 kV of 1 μg/μl Tf solution (injection time: 5 s), using Tris–glutamate and borate buffers, at different pH values.

gave the best results (Fig. 2). We chose DAB because the baseline obtained was cleaner and simpler than that obtained with hexamethonium bromide. Previously, other workers have also found DAB to be the most suitable additive in the separation of transferrin glycoforms in uncoated fused silica capillaries [6,7]. The positively charged amino groups of DAB interact with the negatively charged free silanol groups on the capillary wall, and thereby reducing protein adsorption, decreasing EOF and, therefore, increasing the migration time of the sample. These factors allow an enhanced resolution of the different glycoforms. After testing different DAB concentrations, from 0.5 to 4 m*M*, in the separation borate buffer, the optimal concentration of DAB was taken to be 1 m*M*. The use of higher DAB concentrations failed to improve resolution and led to an

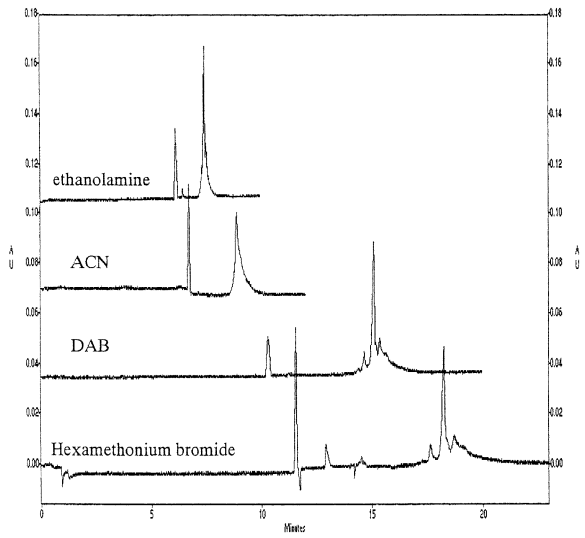

Fig. 2. Electropherograms at 15 kV of 1 μg/μl Tf solution (injection time: 5 s) using different mobile phase additives.

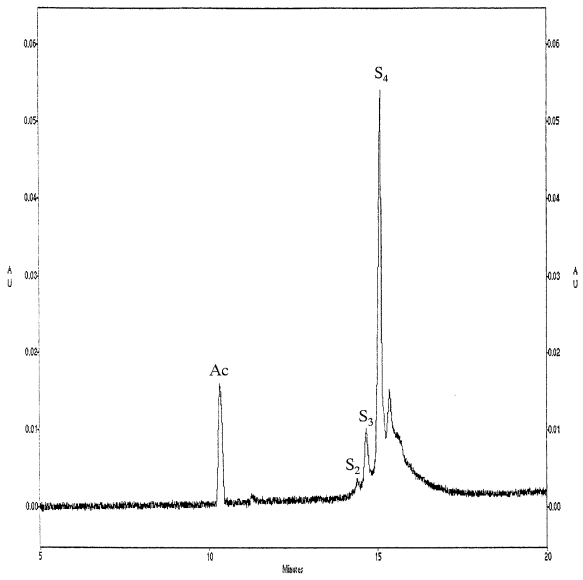

Fig. 3. Electropherogram of 1 μg/μl Tf solution obtained with the following optimized conditions: 15 s injection time, 15 kV separation voltage, 100 m*M* borate containing 1 m*M* DAB at pH 8.3 as separation electrolyte, 25 °C temperature, detection at 200 nm, normal polarity.

increase in analysis time. Using these optimal conditions, a good resolution of Tf sialoforms was achieved in less than 18 min with a bare capillary of 50 μm I.D. and 57 cm length, as can be seen in Fig. 3.

In order to validate the proposed method, the quality parameters were calculated [20]. Analytical precision of the proposed method was studied by calculating run-to-run and day-to-day reproducibility of migration times and peak areas of disialo-, trisialo- and tetrasialo-Tf, using a standard Tf solution of 1 μg/μl. The results are shown in Table 2. Precision of migration times and peak areas were satisfactory. Tf glycoforms with a higher number of sialic acid residues than tetrasialo-Tf were not considered in the present study, because of their minor relevance in CDGS.

With the proposed CE method, the limit of detection (LOD, signal-to-noise ratio of 3) and the limit of quantification (LOQ, signal-to-noise ratio of 10) of human Tf were about 4 and 25 ng/μl, respectively. The LOD and LOQ of the trisialo- and disialo-Tf, expressed as percentages of the tetrasialo-Tf peak area of healthy serum, were 0.5 and 2.1% for S_3, respectively, and 0.4 and 1.2% for S_2, respectively. That means that the total Tf concentrations required to detect S3 and S2 sialoforms are 35 and 200 ng/μl, respectively. For quantification purposes, the total Tf concentrations required are 125 ng/μl for S_3 and 550 ng/μl for S_2. According to the percentage concentrations reported by Martensson et al. [32] of about 2 and 6% for S_2 and S_3, respectively in healthy serum, the sensitivity achieved with the present method is perfectly adequate for the determination of disialo- and trisialo-Tf in serum samples.

Table 2
Repeatability and reproducibility of migration times (min) and absolute peak area of S_2, S_3 and S_4 with the proposed CE method

	Intra-day ($n=5$)		Day-to-day ($n=12$)	
	Mean	RSD (%)	Mean	RSD (%)
Migration time (min)				
Disialo-Tf	15.5	1.0	15.4	1.6
Trisialo-Tf	15.8	1.1	15.7	1.7
Tetrasialo-Tf	16.4	1.1	16.2	1.7
Absolute peak area[a]				
Disialo-Tf	6621	4.8	5952	5.2
Trisialo-Tf	98 637	4.3	91 915	5.5
Tetrasialo-Tf	694 156	5.3	654 769	6.3

[a] Arbitrary units.

Table 3
Calibration equations and linear ranges of the different Tf-glycoforms present in commercial transferrin

Glycoform	Equation	Regression coefficient, *R*	Number of considered points	Linear range (ng/μl)
S_4	$y=793x-1984$	0.999	12	5–1000
S_3	$y=112x-55$	0.999	6	35–1000
S_2	$y=13x-897$	0.996	5	200–1000

In the calibration equations, x represents the commercial Tf concentration expressed in ng/μl and y represents the peak area.

Quantitation was performed by external calibration, plotting peak area versus concentration of commercial Tf injected into the electrophoretic system. Calibration equations and linear ranges of the different sialoforms are shown in Table 3 (correlation coefficients >0.999).

The commercial Tf was treated with neuraminidase enzyme for increasing times. Neuraminidase only split off the sialic acid residues from Tf, not the carbohydrate chain [4,6,19]. However, the generated sialoforms, known as S_i', are frequently used as models in separation studies of CDGS Tf-glycoforms because they present the same electrophoretic behaviour based on the different negative charge. The electropherograms obtained by injection of different reaction aliquots show a good resolution of the Tf sialoforms generated (Fig. 4). Therefore, the proposed method should also be applicable to the separation of CDGS glycoforms.

Normal and pathological serum samples were saturated with iron, diluted and injected into the CE system using the proposed method. Fig. 5 shows comparisons between injection of unextracted sera from a control subject and from a subject with CDG Syndrome. The Tf isoforms can be identified on the basis of the correspondence of migration time with that of standard Tf (Fig. 3). The CE elution profile of normal serum shows high concentrations of tetrasialotransferrin and low amounts of disialo-, trisialo-, and pentasialotransferrin. In the CE elution profile of CDGS serum (Fig. 5), transferrin glycoforms with di- and asialylated carbohydrate chains are increased. This profile corresponds to CDGS type I serum, which typically shows dramatic reductions in S_4

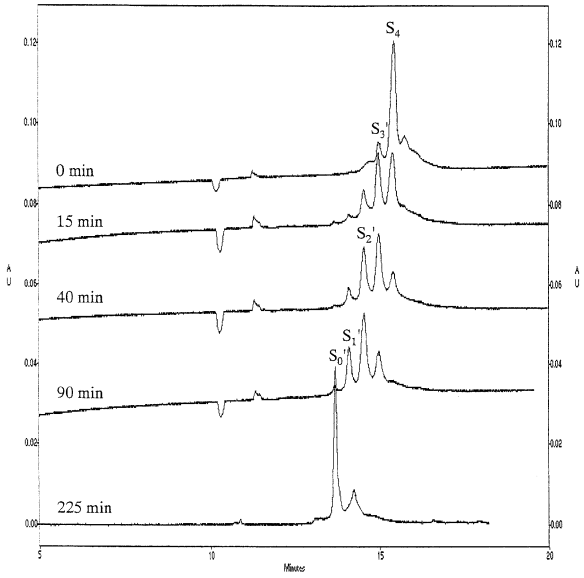

Fig. 4. Electropherograms obtained by injection of different aliquots of solution obtained after digestion with neuraminidase at different reaction times. Experimental conditions are as in Fig. 3.

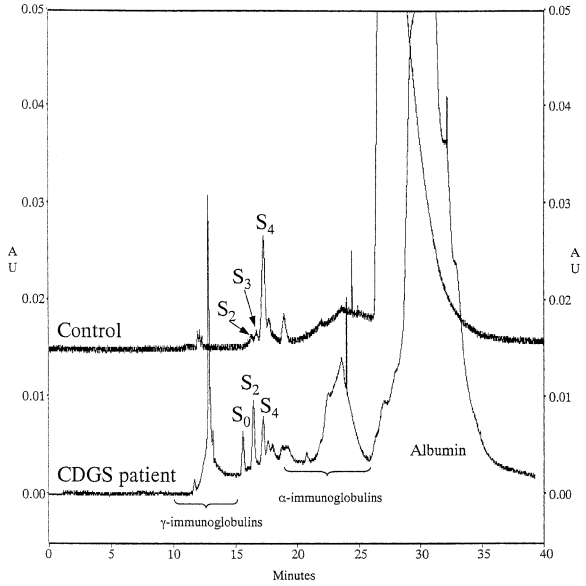

Fig. 5. Electropherograms obtained by injection of diluted serum samples from healthy individuals and from CDGS patients. Albumin and immunoglobulins are also labeled. Experimental conditions are as in Fig. 3.

and dramatic increases in S_0 and S_2 sialylated forms [19,33]. In fact, the increased appearance of less sialylated transferrins, especially of disialotransferrin, is the specific marker for CDG Syndrome [34]. These results suggest that the proposed CE method can be used to resolve Tf glycoforms in serum, suggesting a rapid diagnostic test for the Carbohydrate Deficient Glycoprotein Syndromes group of diseases.

3.2. Characterization of Tf-glycoforms by ES-MS

Fig. 6 shows the electrospray mass spectra of a standard solution of Tf (4 μg/μl) obtained at the optimal mass spectrometer conditions. Applying the MaxEnt algorithm to the spectral data, gave a molecular masses for the S_4 glycoform of 79 529 and 79 525 with the solvent composed of water/acetonitrile (50:50 v/v) with 0.10% HFBA and 0.25% TFAA, respectively. These molecular mass results are in agreement with the theoretical value for the Tf-glycoform S_4, the main sialoform in serum, with a molecular mass of 79 570 Da [3–5].

In order to characterize the sialoforms generated by neuraminidase treatment, aliquots obtained at different reaction times were directly injected into the electrospray ionization mass spectrometry system. The cluster of peaks obtained in the spectra was different at every injection, showing that transformation of Tf was taking place, new Tf-sialoforms

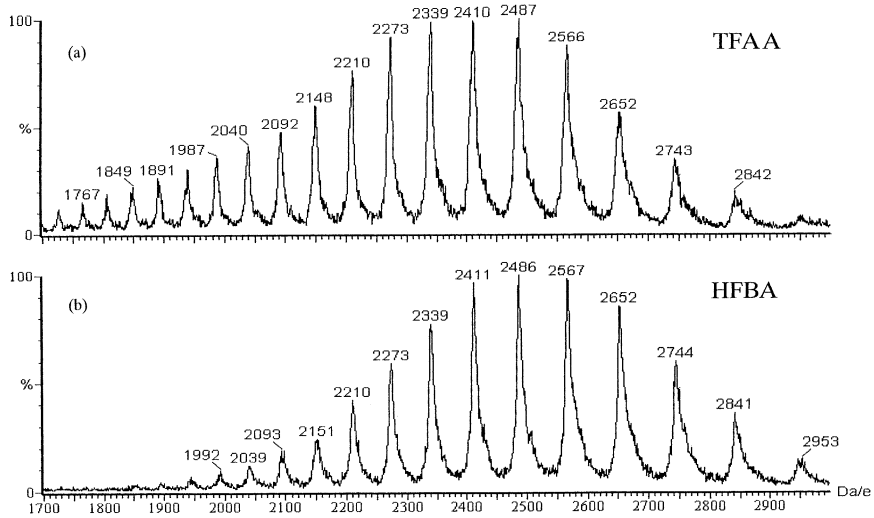

Fig. 6. Electrospray mass spectra of Tf (4 μg/μl) obtained with a mobile phase of acetonitrile–water (50:50, v/v) containing: (a) 0.25% TFAA and (b) 0.10% HFBA.

Table 4
Molecular masses obtained by MaxEnt algorithm for the different sialoforms of Tf generated by addition of neuraminidase using both solvents considered: acetonitrile–water (50:50, v/v) with 0.25% TFAA (solvent A) and acetonitrile–water (50:50, v/v) with 0.10% HFBA (solvent B)

Glycoform	Molecular masses (Da)	
	Solvent A	Solvent B
S_4	79 525 ± 13	79 529 ± 13
S'_3	79 232 ± 13	79 237 ± 23
S'_2	78 964 ± 15	78 945 ± 10
S'_1	78 659 ± 18	78 661 ± 14
S'_0	78 367 ± 10	79 376 ± 11

The standard deviation is also indicated.

being generated. The molecular masses of the different sialoforms generated were obtained by applying the MaxEnt algorithm and are summarized in Table 4. The results obtained are consistent with the molecular masses expected after consecutive loss of different sialic acid residues. Both solvents considered prove to be useful for characterizing the different Tf sialoforms generated, as there are no significant differences between the results obtained with either of them (Table 4).

These results are simply a first step in our ultimate goal: the development of a CE–MS method for the analysis of Tf sialoforms. For this purpose, the modification of the CE conditions to increase the compatibility with the MS detection and the improvement of the MS sensitivity in order to obtain suitable spectra at lower concentrations will be necessary.

Even at this early stage, the results obtained here demonstrate the potential of mass spectrometry with electrospray as an ionization source for the characterization of glycoforms with small mass differences (∼292 Da) and, consequently, the proposed method should also be applicable to glycoforms present in CDGS patients' serum samples, which differ in about ∼2200 Da, because of the loss of a biantennary sugar chain.

4. Conclusions

In this study, a CE method has been proposed for separating human serum commercial Tf-glycoforms, a good resolution being achieved for the most relevant sialoforms of CDGS, those containing fewer than four sialic acid residues. The separation is carried out in less than 18 min without the need for complex sample preparation. The precision, sensitivity and linearity of this method enable a satisfactory quantitation of samples. The proposed method was applied to control and CDGS sera samples and different profiles were obtained which can be used for diagnosis purposes. Moreover, characterization of all Tf-glycoforms, using a neuraminidase treatment, was achieved through ES-MS. The CE method developed enables a quick and simple CDGS diagnosis to be made.

Acknowledgements

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