Capillary electrophoresis - mass spectrometry for the determination of carbohydrate-deficient transferrin: challenges and issues

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Introduction

Transferrin (TI) is the most important iron transporting glycoprotein, mainly expressed in hepatocytes. TI contains two metal binding sites and two apparently N-linked glycosylation chains, usually occupied with oligosaccharides and negatively charged side chains. The two N-linked chains differ in the degree of branching, showing bi-, tri-, or tetra-antennary structures.

TI encompasses isoforms with zero to eight acidic residues. The major glycans (75-80 %) of TI contains four acidic residues (tetrasialo-TI) but other variations in size, sugar content, and net charge occur. Acidic residues can be identified in normal human serum.

In 1978, Sibley et al. reported the presence of TI glycoforms with ≥2.5 % in serum from alcohol abusers, while decreased amounts or absence after alcohol abstinence. Anato-TI (PT) and Disialo-TI (PT) were referred to as carbohydrate-deficient transferrin (CDT). CDT has been widely used as the most reliable indirect marker of chronic alcohol abuse.

Applications

Capillary electrophoresis (CE) has been used extensively for the determination of CDT in chronic alcoholics over the years. CE is a powerful technique that allows for high-resolution separation of proteins and carbohydrates, making it suitable for detecting TI glycoforms.

Method development

Capillary electrophoresis (CE)

Numerous static coatings compatible with MS were investigated in terms of polymer concentrations, coating procedure, capillary lumen, and glycoforms. The coating was considered stable with migration times RSDa ≤ 2 %. Efficiency and protein mobilities were measured to monitor reversible adsorption, while EOF mobility was measured to estimate irreversible adsorption. Glycophores™ resolution was assessed to estimate the performance of the procedure.

Capillary electrophoresis is a sensitive and selective technique for the separation and detection of glycoforms. However, the absorption of proteins onto the capillary surface can occur. This reversible or irreversible interaction can affect peak efficiency, migration time, and protein recovery. A suitable coating can be used to prevent protein adsorption.

A commercial procedure based on a capillary coating strategy to prevent protein adsorption has been implemented for the routine analysis of TI glycoforms. The CEspher™ CDT kit (Anatech, Sussex, Belgium) contains a TRIS borate separation buffer at pH 8.5 with propylene glycol, a buffer modifier with polyacrylamide, and an iron solution (PAC) for TIE saturation before analysis in CE-UV.

Results obtained with this CE-UV procedure may suffer from a lack of sensitivity. CE can be hyphenated to mass spectrometry (MS) to enhance the sensitivity of the method and provide additional selectivity.

Mass spectrometry

The CE was hyphenated to a time-of-flight mass spectrometer (TOF/MS) via an electrospray ionization (ESI) interface. ESI-TOFMS parameters were investigated via TI injection through the capillary, including reducing gas flow rate, drying gas flow rates, and temperature, and ESI and desolvation voltages. The composition of the sheath gas was investigated in terms of ethanol (anisopropyl, acetonitrile, and methanol) and acid (formic and acetic acid) nature and proportions.

Soft or neutral接口

In CE-ESI-TOFMS experiments brought out the challenges faced for intact protein analysis with a consequent loss of glycoforms resolution inherent to the configuration, as well as poor TI ionization efficiency. The use of other interface geometries may be considered to avoid the sensitivity and resolution issues experienced in this study.

Conclusions

CE-USV has been widely used over the past years for the determination of CDT in case of chronic alcohol use evaluation with a commercially available procedure. However, this method presents some limitations in terms of analytical selectivity and sensitivity which could be overcome with MS detection. The current method is not MS-compatible due to the presence of non-volatile BGE and capillary coating. This study consisted in developing new conditions to evaluate the TI glycoforms’ separation by CE-ESI-TOF-MS.

Numerous coating compositions and procedures were investigated. Different coatings were tested, i.e., cationic, neutral, and anionic coatings. BGE composition was also optimized for each coating, covering a broad range of pH values. An anionic PB-DS coating (10%) and polyacrylamide (0.1 %) provided the best results in terms of efficiency and resolution. Glycoforms resolution, efficiency, and protein mobility were all monitored, and MS-TOFMS parameters were investigated to enable the ionization and detection of TI. Tetrasialo-TI, the more abundant and major isoform, was detected over the range 2000-3000 m/z with a resolution of 5000 (ESI+/w) or 500 (ESI-). Less abundant glycoforms were poorly or not detected with these conditions due to poor ionization efficiencies.

The loading quantity was increased up to 2.7 % of the capillary volume (41 nl injected), no peak was detected, clearly demonstrating a significant sensitivity explained by a poor ionization.

CE-ESI-TOFMS experiments brought out the challenges faced for intact protein analysis with a consequent loss of glycoforms resolution inherent to the configuration, as well as poor TI ionization efficiency. The use of other interface geometries may be considered to avoid the sensitivity and resolution issues experienced in this study.