University of Pannonia
Doctoral School of Chemistry and Environmental Sciences

Development of a novel sugar separation and determination method for capillary electrophoresis

Ph.D. Thesis

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Introduction and objectives

The qualitative and quantitative analysis of complex sugars (glycans) bound to proteins is playing an increasingly important role in the diagnosis of certain diseases and in monitoring the quality stability of protein-based drugs. These sugar structures play key roles in many physiological processes, such as immune processes, signal transduction, protein stability and solubility. In some diseases, including cancer and autoimmune diseases, the quantitative ratio of sugar structures bound to certain proteins is altered and can be used as a biomarker. In most cases, some separation method, such as high-performance liquid chromatography or capillary electrophoresis, are used to analyze glycan structures separately. The latter is an increasingly common method worldwide, a relatively new technique compared to liquid chromatography, orthogonal to it, both with advantages and disadvantages. The development of glycan analysis by capillary electrophoresis still has a lot of potential, and research into the role of glycans in disease is still in its infancy. The sample preparation for capillary gel electrophoresis-based glycan analysis includes a step in which free unreacted fluorescent dye is removed. This is a rather resource-intensive task, so eliminating it would greatly facilitate the process of glycan analysis by capillary electrophoresis.

In my work, I designed a capillary electrophoresis-based glycan separation system that utilizes the electroosmotic flow (EOF) phenomenon to achieve on-line purification. This is possible by moving the EOF in the separation capillary towards the outlet end, with the relative movement of the free dye and the dyed sugars in opposite directions. While the mobility of the free dye in absolute terms is greater than the speed of the EOF (so that the hydrodynamically injected dye leaves the capillary at its inlet end), it is less for the labeled sugars, so the EOF moves them towards the detector while separating due to their unequal mobility relative to each other. In such a separation system, the purification step is not necessary.

In addition, I planned to test the efficiency of the magnetic microbead based sample purification method with the developed novel separation system and to develop a computational algorithm for the separation method that allows structure determination, which is able to determine the structure of the separated molecules with sufficiently high accuracy and reproducibility, regardless of the capillary length.

Experimental work

In my research, I have developed a novel capillary electrophoresis method for the separation of fluorescently labelled glycans, where the sample preparation step to remove free dye can be omitted. For this purpose, I developed a buffer system that uses EOF for the separation. The buffer concentration, pH, and other parameters were adjusted so that the EOF velocity flowing towards the outlet end of the capillary falls between the absolute (otherwise negative sign) effective mobility values of the label glycans and the free dye, so that the free dye leaves the system at the inlet of the capillary at the start of the separation, while the label glycans can be detected due to their positive apparent mobility. The buffer composition developed was 253 mM Tris-base, 150 mM caproic acid, pH=8.1. In addition, I implemented a preconcentration method based on large volume sample stacking (LVSS) with the developed system, which resulted in an order of magnitude increase in intensity.

Then I used the novel separation method to investigate the effectiveness of an industrial paramagnetic microbead sample purification procedure. I found that for different samples (maltodextrin ladder, human immunoglobulin G, ribonuclease b and fetuin glycan samples), the purification step resulted in significant changes in the amount of some glycan structures, which can cause critical errors in the evaluation of the glycan profile, as the information obtained from the quantitative ratios of the structures is distorted.

Furthermore, for the EOF-driven capillary electrophoresis method, I have designed a structure determination method based on Glucose Unit (GU), which is based on the maltodextrin ladder. The method uses two internal standards, two structures (maltotriose (DP3) and maltodecapentaose (DP15)), which are also part of the maltodextrin ladder. The robustness of the method was evaluated by calculating GU of 8 randomly selected structures from human immunoglobulin G glycan samples measured 6 times a day for 6 days, with a %RSD > 0.1%. The reproducibility of the method was performed on 3 capillary of different lengths with the same sample, using triplicate measurements, for which the mean %RSD > 0.1% standard deviation was also obtained for the 8 glycan structures, which is very good.

New scientific results: Theses

1. I have developed a capillary electrophoresis method for the high-resolution separation of APTS-labelled glycans by online electrokinetic purification, suitable for the analysis of samples from which the fluorescent dye has not been removed in large excess from the sample matrix. I have optimized the electrolyte composition and pH of the separation background and the separation parameters, and demonstrated comparable resolution to the capillary gel electrophoresis method currently used in industry, which requires a purification step, using 253 mM Tris(hydroxymethyl)aminomethane - 150 mM caproic acid, pH=8.1 buffer system for samples not cleaned from excess dye, and I have also achieved a special large volume sample stacking with focusing to achieve an increase in signal intensity of up to one order of magnitude.

2. Using the developed capillary electrophoresis method, I have demonstrated that the sample purification process with magnetic beads containing carboxyl groups, as used in industry, can change the quantitative ratio of certain glycan structures, thus distorting the qualitative information. I examined the ratio of sample components in purified and unpurified fetuin, human immunoglobulin G, ribonuclease B, and maltodextrin ladder, as well as in three eluents of the purification step, and demonstrated peak ratio changes.

3. Using a novel approach, a numerical method for calculating Glucose Units has been developed for the capillary electrophoresis technique, which relates the effective migration time of unknown glycan structures to the degree of polymerization of the maltodextrin ladder, allowing the identification of analytes with very high accuracy and repeatability, independent of capillary length and sample conductivity. I have demonstrated the repeatability of the algorithm by testing six human immunoglobulin G N-glycan profiles per day for six days and by 3-3 measurements on capillary lengths of 40 cm, 50 cm and 60 cm total lengths to demonstrate the good reproducibility of the calculation method regardless of capillary column length.

Publications related to theses

Patent

Farsang, R., Jarvas, G., & Guttman, A. Method for purification free capillary electrophoresis of labeled carbohydrates; WO2025078849

Farsang, R., Jarvas, G., & Guttman, A. Glucose unit determination based on a numeric virtual eof marker search method; WO2025141293A1

Scientific Articles

Farsang, R., Hogyor, K., Jarvas, G., & Guttman, A. (2023). Capillary zone electrophoresis of 8-aminopyrene-1, 3, 6-trisulfonic acid labeled carbohydrates with online electrokinetic sample cleanup. Analytical Chemistry, 95(45), 16459-16464.

Farsang, R., Jarvas, G., & Guttman, A. (2024). Purification free N-glycan analysis by capillary zone electrophoresis: Hunt for the lost glycans. Journal of Pharmaceutical and Biomedical Analysis, 238, 115812.

Farsang, R., Farkas, A., Jarvas, G., & Guttman, A. (2024). Glucose unit computation in capillary zone electrophoresis of carbohydrates using a numerical approximation based search for a virtual EOF marker. A tutorial. TrAC Trends in Analytical Chemistry, 176, 117762.

Other scientific publications

Patent

Kovács, N., **Farsang, R.**, Szigeti, M., Jarvas, G., & Jankovics, H.; Tagged exoglycosidase enzymes and immobilized glycan sequencing approach; US20240417708A1

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Kovacs, N., **Farsang, R.**, Szigeti, M., Vonderviszt, F., & Jankovics, H. (2022). Enhanced recombinant protein production of soluble, highly active and immobilizable PNGase F. Molecular Biotechnology, 64(8), 914-918.

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Torok, R., Auer, F., **Farsang, R.**, Jona, E., Jarvas, G., & Guttman, A. (2022). The effect of sample glucose content on PNGase F-mediated N-glycan release analyzed by capillary electrophoresis. Molecules, 27(23), 8192.