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Highly Sensitive Two-dimensional Profiling of N-linked Glycans by Hydrophilic Interaction Liquid Chromatography and Dual Stacking Capillary Gel Electrophoresis

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Summary

Glycosylation is one of most important post-translational modifications of proteins. Since biological samples often contain diverse glycans, highly sensitive, quantitative, and comprehensive profiling methods are required. Here, we newly developed a two dimensional (2D) separation system, which couples hydrophilic interaction liquid chromatography (HILIC) and capillary gel electrophoresis (CGE) via large-volume dual preconcentration by isotachophoresis and stacking (LDIS) [1]. Glycans labeled with 8-aminopyrene-1,3,6-trisulfonic acid were firstly separated into around 100 fractions by HILIC, which were then preconcentrated and separated by LDIS-CGE, and finally detected with laser-induced fluorescence. As a result, limit of detection was estimated to be 12 pM (60 amol, S/N = 3) and good linearity in the calibration curve (R^2 > 0.999) was realized in the 2D analysis of maltoheptaose.

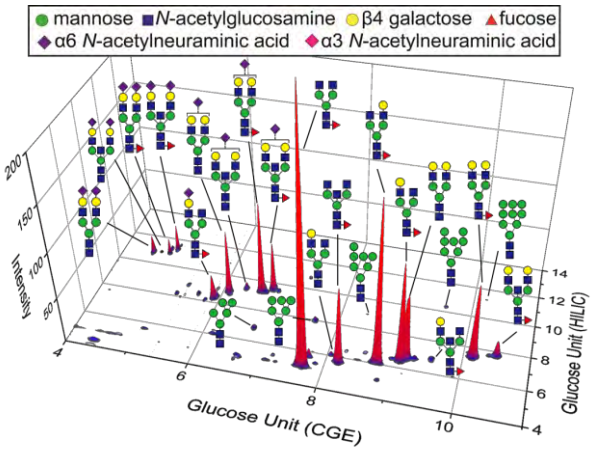

Figure 1. HILIC/CGE 2D profile of IgG N-glycans.

Finally, 2D profiling of N-linked glycans obtained from standard glycoproteins and cell lysates were demonstrated. As shown in Figure 1, high resolution 2D profile of IgG N-glycans was successfully obtained by the data alignment using triple internal standards [2]. N-glycans were well distributed on the HILIC/CGE 2D plane based on the glycan size, number of sialic acids, linkage type, and so on. Specific minor glycans were also identified from HeLa cell lysates.

References

[1] T. Kawai, N. Ota, A. Imasato, Y. Shirasaki, K. Otsuka, Y. Tanaka, Profiling of N-linked glycans from 100 cells by capillary electrophoresis with large-volume dual preconcentration by isotachophoresis and stacking, J. Chromatogr. A 1565 (2018) 138–144.

[2] G. Jarvas, M. Szigeti, J. Chapman, A. Guttman, Triple-Internal Standard Based Glycan Structural Assignment Method for Capillary Electrophoresis Analysis of Carbohydrates, Anal. Chem. 88 (2016) 11364–11367.