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Grant Title: Rapid construction and application of O-glycan library for progressive glycomics

Abstract

I have recently developed a new method for the nonreductive release of O-glycans from glycoproteins, "eliminative oximation". The method uses aqueous conditions and is easy and rapid. The recovery rate of glycans is almost the same as that of gas-phase hydrazinolysis while the peeling is less than one-fifth. Unlike hydrazinolysis, the glycans containing NeuGc can be analyzed by this method. The obtained glycan-oximes can be fluorescently labeled by reductive amination as usual. Thus, the method will enable anyone to easily obtain fluorescent -labeled O-glycans from glycoproteins. The next challenge confronting the O-glycan analysis is the development of an easy method for structural elucidations. I will develop a multi-analytical database for the easy structural elucidation of O-glycans. In this study, we investigated how to construct an O-glycan library using a human glycosyltransferase library to develop such a database.



To efficiently construct the O-glycan library, we employed the four chemically synthesized glycosyl threonines (core-1 to core-4) as the starting materials, and diversified the glycosyl threonines by using human recombinant glycosyltransferases. Fluorescent labeling of the threonines enabled us to easily and sensitively monitor the glycosylations by TLC. The isolated products by HPTLC could be converted to the corresponding free O-glycans under the neutral condition. In addition, data on the substrate specificity of various glycosyltransferases have been accumulated in the process of carrying out many glycosylation reactions.

In the future, we plan to analyze the prepared glycan libraries by HPLC, CE, and MS, respectively, and accumulate the specific data of each glycan as a glycan analysis database. By combining this database with the eliminative oximation, it is expected that the era will come when anyone can easily perform O-glycan analysis, which has been difficult so far.

Eliminative Oximation (BBRC, 2019, 513(1):186)

