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A workflow for identification of isobaric isoforms of glycans using off-line MALDI-MS system

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Introduction

Analysis of glycosylation is one of the indispensable approaches for a development of antibody-drug and biomarker discovery, because a large number of proteins in eukaryotes are glycosylated and they play various roles in physiological function like a molecular recognizing. Therefore, a well-established workflow for characterization of glycosylation has been a one of the growing demands.

The author and co-workers has reported a system for identification of glycan structures using an observational MS^n spectral library obtained by MALDI-QIT-TOF MS[1), 2)]. In this study, we will demonstrate a practical workflow combined with the spectral library and off-line separation system for the glycan structure characterization.

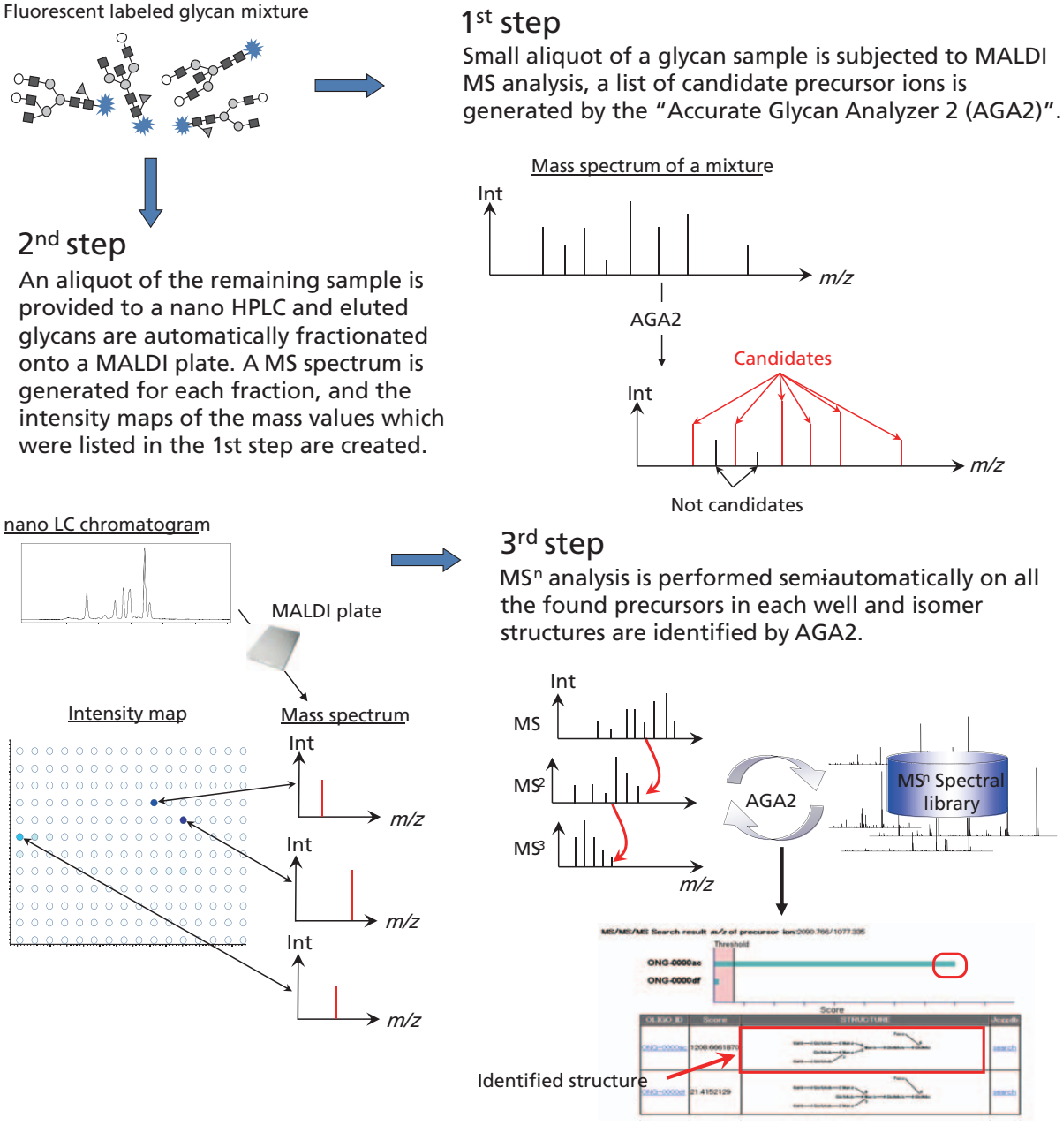

Fig. 1 NBS Biomarker Discovery System

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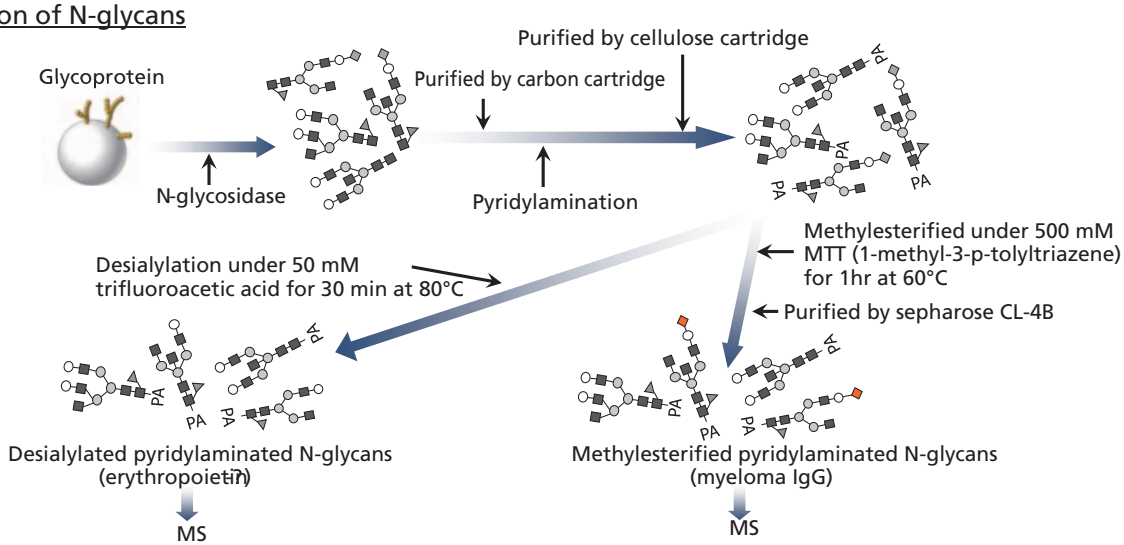
Analysis of N-glycans released from human myeloma IgG and human erythropoietin-α

To identify proteins associated with insulin resistance in adipocytes *in vitro*, differential proteome analysis using the NBS method was performed in 3T3-L1 adipocytes in which insulin resistance was induced by TNF-alpha or dexamethasone (Fig. 2a). The relative quantification and the identification of differentially expressed proteins were performed using LC-MALDI-TOF MS (Fig. 1b and 2a).

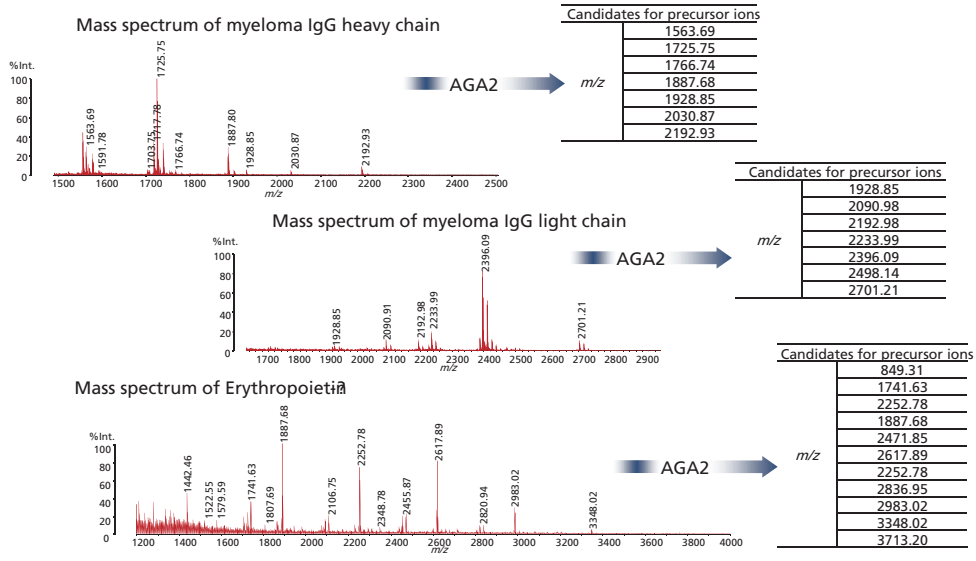
I) Preparation of N-glycans



Human myeloma IgG (Carbiochem) was separated by 1D SDS-PAGE and treated with N-glycosidase (Takara Bio Inc.) in gel. On the other hand, Human erythropoietin-a (Carbiochem) was treated with N-glycosidase in solution.

Released N-glycans were pyridylaminated. Additionally, sialic acids of N-glycans from IgG were methylesterified, and N-glycans from erythropoietin were desialylated.

II) MS analysis of each sample and detection of candidate N-glycans by AGA2



7, 7, and 11 signals were generated from MS analysis of the methylesterified N-glycans of IgG heavy chain, light chain, and desialylated N-glycans of erythropoietin by AGA2, respectively.

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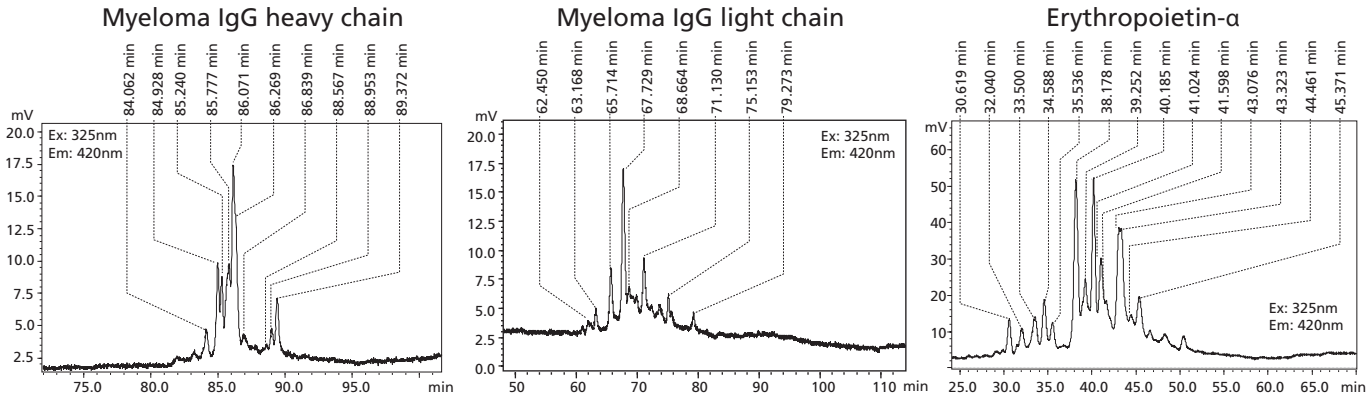
III) Separation of isomers using nano HPLC



N-glycans were separated by the carbon column (Hypercarb KAPPA Capillary column, Length: 100 mm, ID 180um; Thermo scientific) under the linear gradient of 90% CH3CN solution containing 0.1% formic acid. The total flow rate of the nano HPLC was set at 1 uL/min. Each fraction was spotted onto a MALDI plate with the MALDI matrix solution (2.5 mg/mL DHBA in 80% EtOH, containing 5 mM NaCl). Separated N-glycans were detected by the Laser induced fluorescent detector (ZETALIF 2000 He/Cd Laser; Picometrics).

IV) Characterization of N-glycans by MS^n analysis combined with AGA2

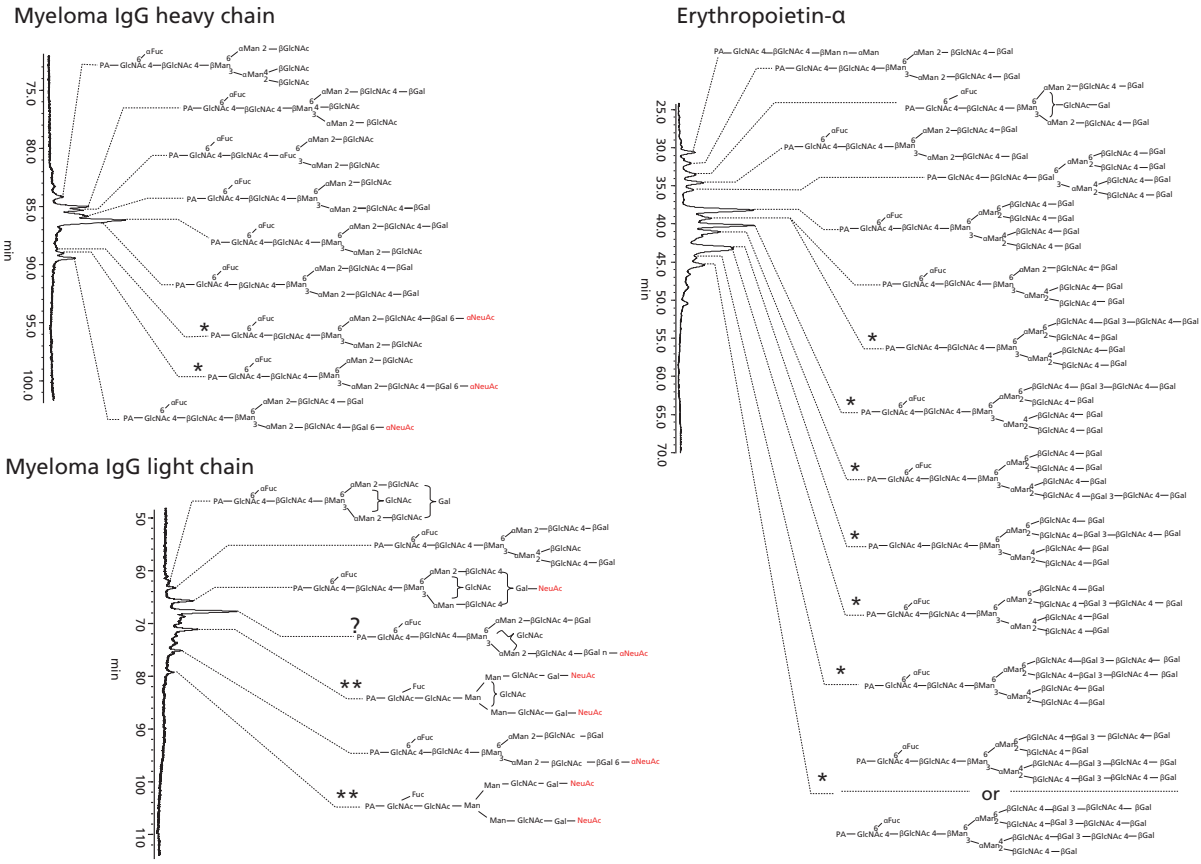


Fig. 3 Identification and functional analysis of novel adipokine

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Conclusions

- We identified various glycan isomers using our workflow.
- Our workflow could be applicable to various areas of research where screening of glycans is required.

References

1) A. Kameyama et.al. Anal. Chem., 77, 4719-4725 (2005)
2) A. Kameyama et.al. J. Proteome. Res., 5, 808-814 (2006)

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