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Affinity purification of IdeZ digest for Glycosylation profile of Immunoglobulins using a linear benchtop MALDI-TOFMS.

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Y. Yamazaki[1], S. Nakaya[1]
1 Shimadzu Corporation, Kyoto, Japan

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Overview

- A batch analysis of glycosylation of IgG using a bench-top linear MALDI-TOFMS was intended.
- A magnet beads linked with protein A was applied to affinity purification, following a digestion of IgG by IdeZ.
- Three different glycosylated Fc were clearly observed to classify them with a statistical software.

Introduction

Glycosylation on protein plays wide-range vital roles in biological processes from stabilization of protein conformation to expression of binding specificity. In this view, a characterization of the N-/O-linked glycan is quite significant, especially, in development of biopharmaceuticals. To date, whereas intensive efforts were conducted to characterize glycans precisely with high-end mass spectrometers, conventional instruments without time consuming preparation has been anticipated for batch analysis in screening or QA/QC. A newly developed bench-top MALDI-TOFMS is expected to be the conventional instrument in terms of sufficient specification, through-put, and cost effectiveness. We attempted to characterize glycosylation of IgG without a release of glycan using the bench-top MALDI-TOFMS. To do this, we examined a preparation using affinity beads and enzymatic cleavage.

Methods

- **IgGs and their preparations**

NIST mAb, IgG from mouse serum, and myeloma IgG were subject to MS analysis. IdeZ and Protein-A magnetic beads (Mag Sepharose) were purchased from Promega, and GE Healthcare respectively. The IgGs dissolved in tris-NaCl buffer were incubated with IdeZ at 37°C for two hours. Resultant solutions including Fc and F(ab')2 were applied to the beads equilibrated with the tris-buffer, then washed with the same buffer several times. Fc associated with Protein-A was eluted with acidic solution, then desalted with ZipTip C18.

- **MALDI-TOFMS**

All MS analysis in positive ion mode was performed with a bench-top linear MALDI-TOFMS (MALDI-8020, Shimadzu Corp., Japan). Sinapinic acid (SA) and Ferulic acid (FA) were applied to MS analysis as matrix at the same condition as follows; 20 mg/mL in 50% acetonitrile/milliQ water with 0.1% TFA.

- **Statistical data analysis**

eMSTAT Solution (Shimadzu Corp., Japan) was applied to perform classification/differentiation of glycosylated-Fc regions.

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MALDI-8020

Features:

- Compact design
- > 5,000 MS resolution
- High through-put with solid state laser and fast stage motion
- Rapid sample introduction
- UV laser-based source cleaning
- Quiet operation

Results

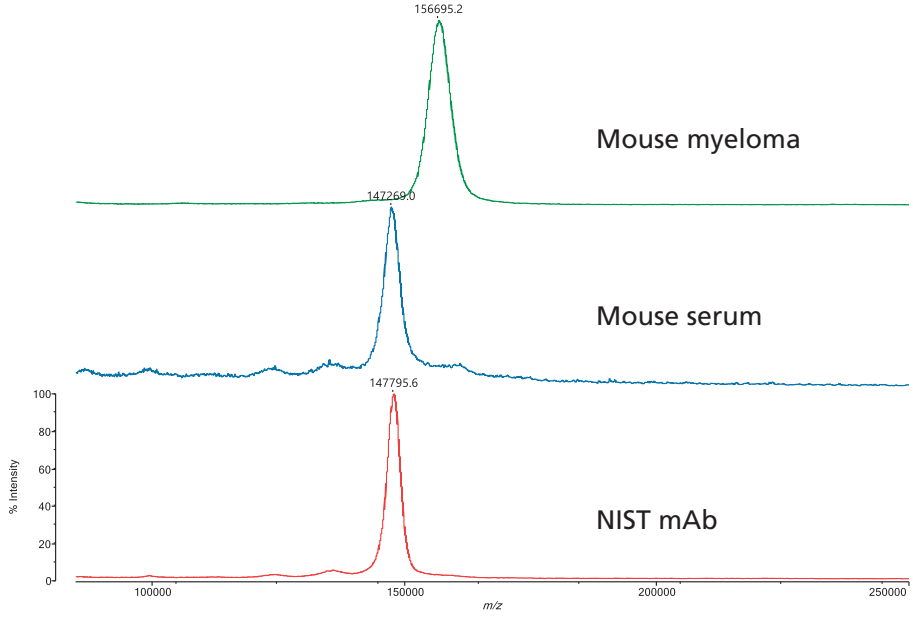

Fig.1 MS of whole IgGs

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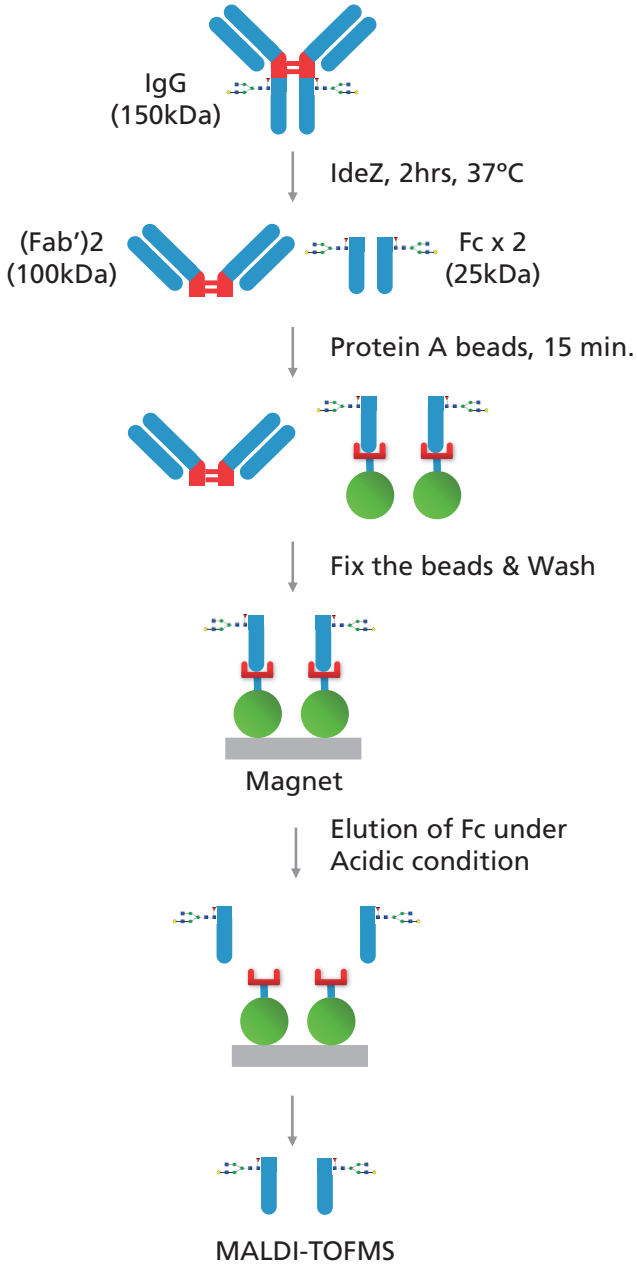

Fig.2 Work-flow of Fc affinity separation/detection

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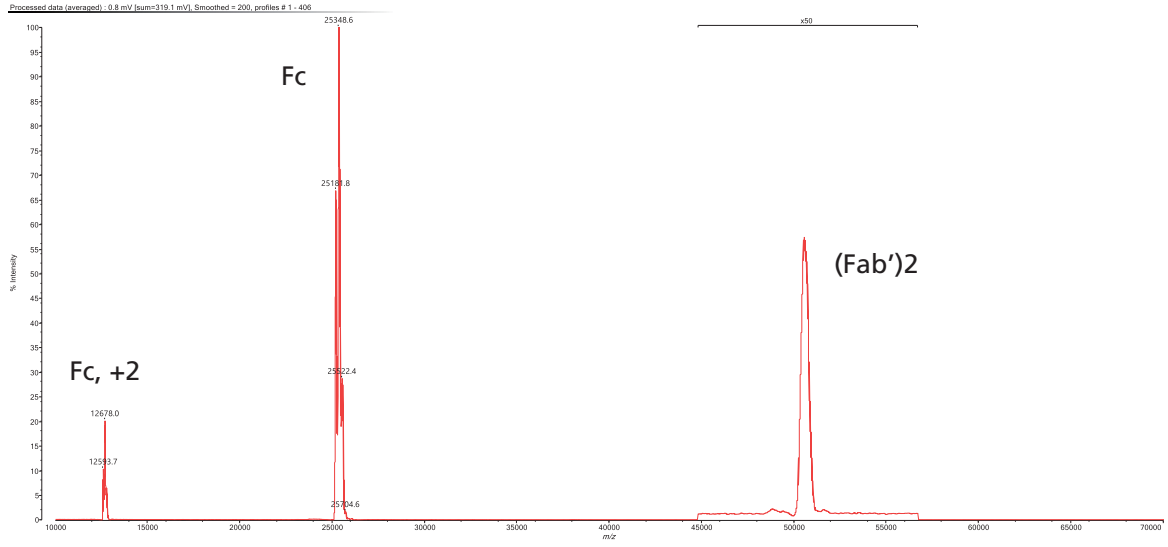

Fig.3 MS of NIST mAb treated with IdeZ

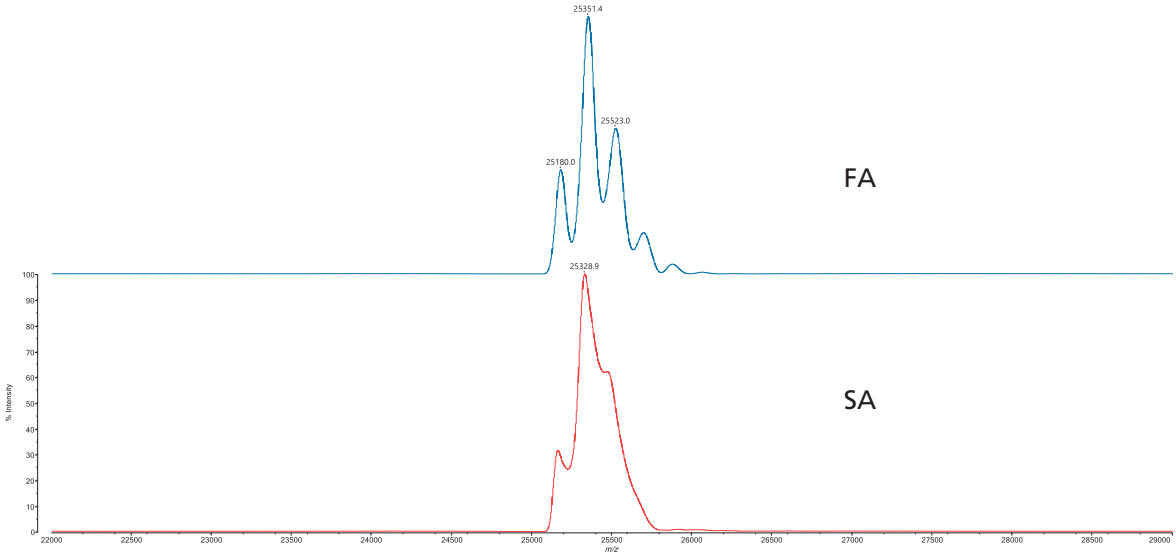

Fig.4 A comparison of Fc measured with SA and FA under the identical MS condition

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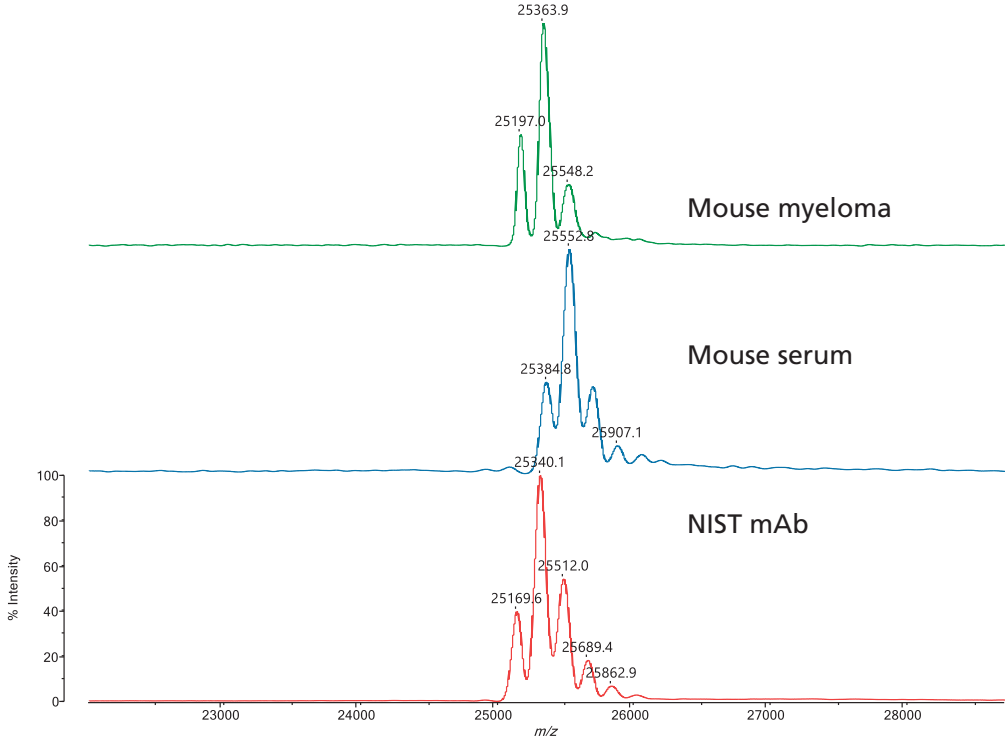

Fig.5 A comparison of three Fc separated with the work-flow

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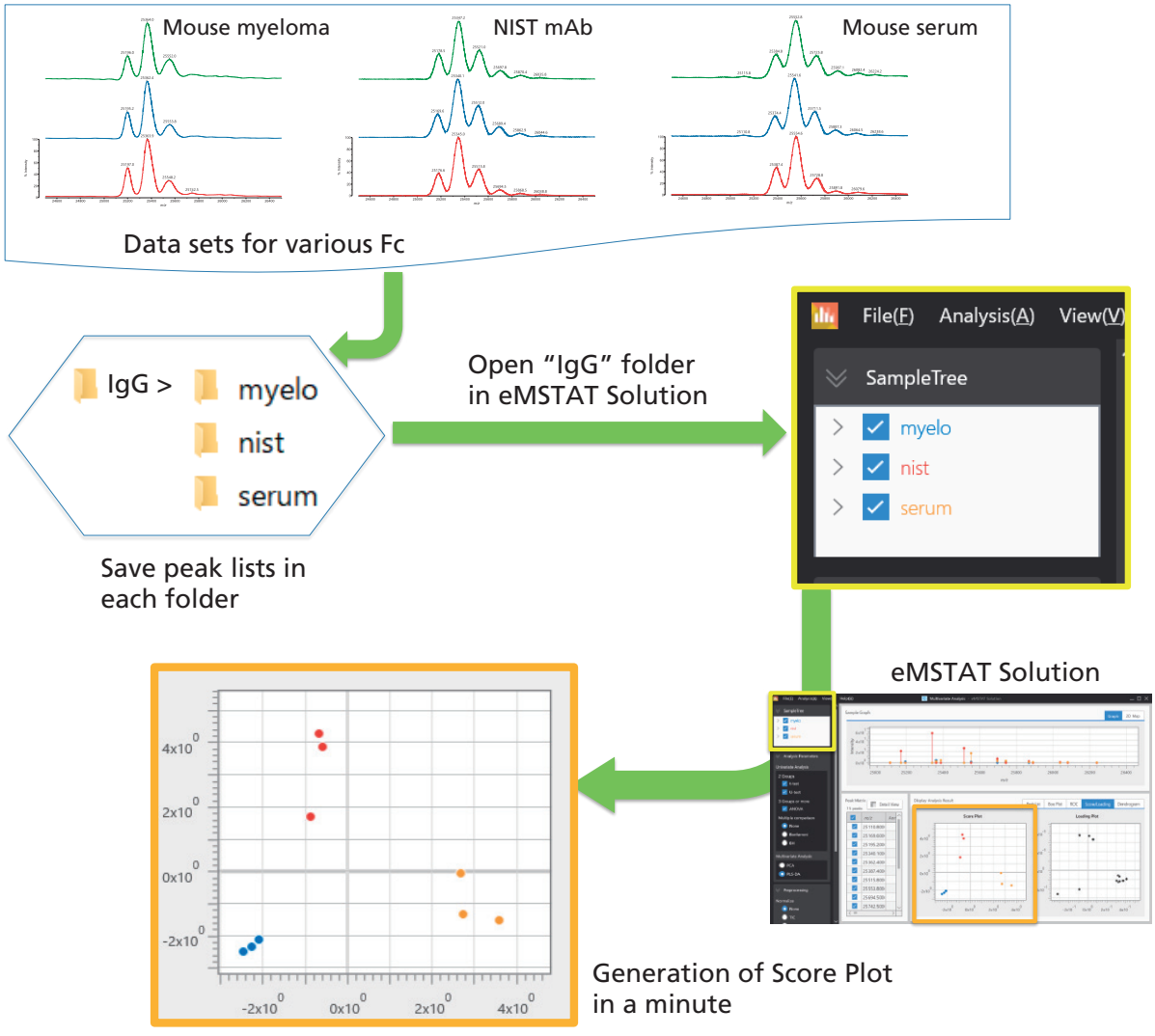

Fig.6 Classification of three Fc using eMSTAT Solution

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Conclusions

- Purification of enzymatically fragmented IgGs by affinity-beads enables batch analysis for the glycosylation using a bench-top MALDI-TOFMS.
- MS resolution of the bench-top MALDI-TOFMS is sufficient to recognize three Fc that differ in glycosylation mainly.
- A statistical analysis by eMSTAT Solution enables a classification of three glycosylated Fc smoothly and quickly, which could be applicable to QA/QC.

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