SHIMADZU

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Analyses of biopharmaceuticals using high-resolution multi-turn TOF-MS system

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1. Introduction

In recent years, the development of new biopharmaceuticals such as next-generation antibodies and viral vectors has been promoted [1]. In the field of quality control of these biopharmaceuticals, analyses of structural changes such as chemical modification have important roles. For this purpose, we have been developing a high-resolution multi-turn (MT) TOF-MS. In this presentation, we demonstrate the analyses of oxidized monoclonal antibodies (mAb) and the adeno-associated virus (AAV) capsid proteins.

2. MT TOF-MS system

Our MT TOF-MS consists of rotationally symmetric sector electrodes. Ions fly along a unique 3D open-loop orbit (Figure 1). It achieves nearly 50-m flight path within the compact size of Φ500 x 240 mm [2]. High resolution of over 150k has been demonstrated by simulation (Figure 2).

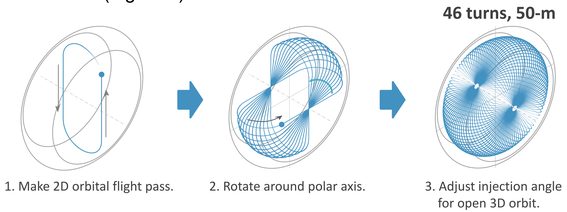

Figure 1 Ion trajectory of MT TOF-MS.

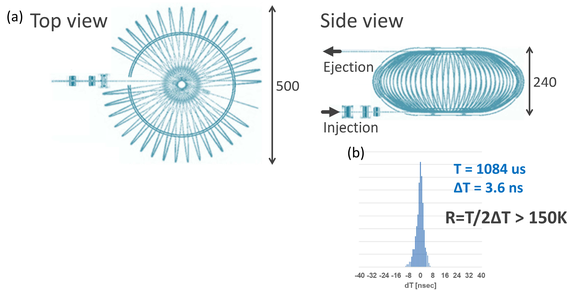

Figure 2 Simulated (a) ion trajectories and (b) TOF spectrum.

Figure 3 shows the overview of the MT TOF-MS system. The system consists of high-performance liquid chromatography (HPLC) system, UV detector, electrospray ionization (ESI) source, quadrupole (QP) mass filter, collision cell (CC), linear ion trap (LIT), and MT TOF-MS. Ions passed through QP and CC are temporary accumulated and compressed in LIT. Then these ions are extracted and injected toward the MT TOF-MS.

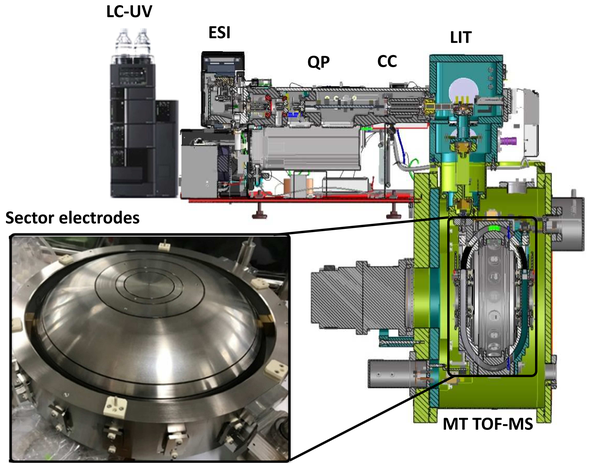

Figure 3 Overview of MT TOF-MS system.

A mass spectrum of myoglobin for a performance test is shown in Figure 4. We have achieved mass resolution of over 200k.

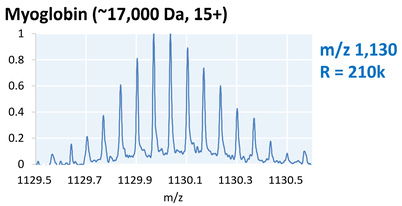

Figure 4 Mass spectrum of myoglobin.

3. Analyses of biopharmaceuticals

Analyses of oxidized mAb subunits and the AAV viral proteins (VPs) were conducted.

3-1. Sample Preparations

Oxidized mAbs

For oxidation, 70% tBHP was added to NISTmAb (NIST8671, Sigma-Aldrich) and incubated at room temperature. Then, the whole amount of samples was applied to MicroSpin G-25 columns and the buffer was exchanged to PBS (pH7.4). FabRICATOR Enzyme and IgGZERO Enzyme (Genovis AB) were added and digested at 37°C for 60 min. PBS and 1M TCEP hydrochloride solution was added and incubated at 37°C for 60 min to reduce them to subunits. 6% TFA was added to stop the reaction.

AAV-VPs

10% acetic acid was added to AAV6-empty and incubated at room temperature for 15 min to fragment into VP1, VP2 and VP3 [3].

3-2. Analytical Conditions

LC-UV-MT TOF-MS system has been constructed, with which quantitative analysis by LC-UV and high-resolution mass spectrometry by MT TOF-MS were achieved.

HPLC conditions (Shimadzu HPLC)

	Oxidized mAbs	AAV-VPs
Column	Restek C4 5um, 150 x 2.1	Waters ACQUITY BEH C4 1.7um, 150 x 2.1
Mobile Phase A	0.1%FA in H_2O	0.1%DFA in H2O
Mobile Phase B	0.1%FA in ACN	0.1%DFA in ACN
Flow Rate	200 uL/min	200 uL/min
Injection volume	20 uL	50 uL
UV	-	280 nm

MS conditions (MT TOF-MS)

	Oxidized mAbs	AAV-VPs
Ionization	ESI	ESI
Neb gas	3 L/min	1.5 L/min
Heat gas	10 L/min	5 L/min
Dry gas	10 L/min	10 L/min
I.F. voltage	+4 kV	+3.5 kV
DL temp.	250 °C	200 °C
I.F. temp.	300 °C	200 °C
H.B. temp.	400 °C	300 °C

3-3. Results

Analysis of oxidized mAb subunits

Changes of oxidation products as a function of oxidation time were confirmed with over 100k resolution (Figure 5).

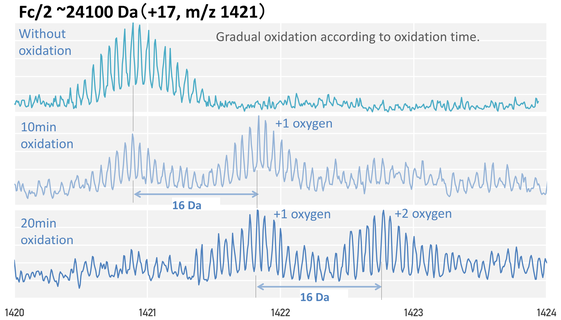

Figure 5 Changes in oxidant formation of mAb subunits.

Analysis of AAV-VPs

LC chromatogram of VPs and mass spectrum of VP3 were obtained. Phosphorylated VP3 was observed in mass spectrum (Figure 6).

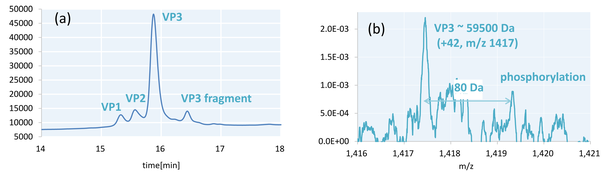

Figure 6 (a) TIC of AAV6 and (b) Mass spectrum of VP3 and its phosphorylation product.

4. Conclusions

- We demonstrated the high-resolution mass analyses of oxidized mAbs and the AAV capsid proteins with MT TOF-MS.

References

[1] M. Amano et al., Anal. Chem., 86, 7536-7543 (2014)
[2] Y. Tateishi et al., The 70th ASMS Conference, MP-019 (2022)
[3] H. Oyama et al., HUMAN GENE THERAPY, vol 32, number 21-22, 1403–1416 (2021)

The authors declare no competing financial interest.