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Native mass analyses of membrane proteins with high resolution multi-turn TOF-MS system

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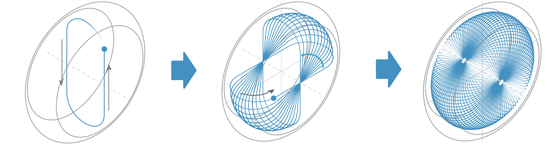
1. Introduction

Membrane proteins, such as G protein-coupled receptors, play crucial roles in various signal transduction processes within cells. As a result, they are major targets for drug development. In the field of structural and functional analysis of membrane proteins, native mass analysis is an important method, as it allows for the analysis of the proteins while maintaining their biological state[1)] . In native mass analysis, high-mass resolution in high m/z range is one of important specifications because target proteins are generally high mass molecules. We have been developing a novel high-resolution multi-turn (MT) TOF-MS system for the intact/native mass analysis of high-mass molecules, including membrane proteins and their complexes.

2. MT TOF-MS system

Our MT TOF-MS consists of rotationally symmetric sector electrodes, in which ions fly along a unique 3D open-loop orbit, thereby 50m flight path is achieved within a volume of 500 x 500 x 250 mm^3 (Figure 1).



1. Make 2D orbital flight pass. 2. Rotate around polar axis. 3. Adjust injection angle for 3D orbit.

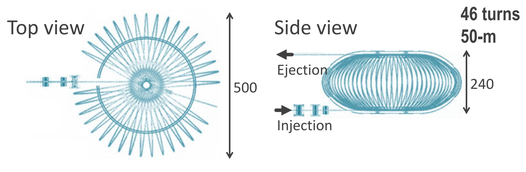


Figure 1. Ion trajectory of the multi-turn ion optical system

Figure 2 shows the overview of the MT TOF-MS system. The system consists of an electrospray ionization (ESI) source, a quadrupole mass filter, a collision cell, a linear ion trap, and the MT TOF.

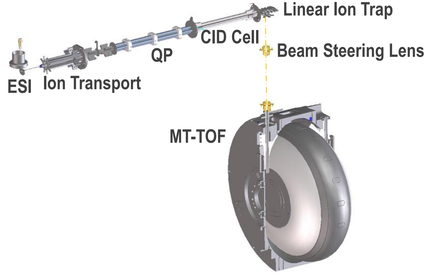


Figure 2. Overview of MT TOF-MS system

As a performance test result, a mass spectrum of Alcohol Dehydrogenase (ADH) is shown in Figure 3. Over 200k resolution was demonstrated in the analysis of ADH with a molecular weight of 37 kDa.

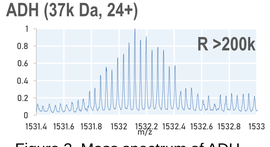


Figure 3. Mass spectrum of ADH

Furthermore, we measured ADH under native conditions with a nano ESI ion source. The mass spectrum of the ADH tetramer, which maintained non-covalent bonds, was observed (Figure 4).

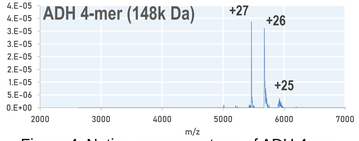


Figure 4. Native mass spectrum of ADH 4-mer

3. Native mass analysis of membrane proteins

3-1. Membrane protein analysis

In general, membrane protein samples are prepared in micellized forms with detergents. The samples are analyzed under mild conditions to avoid denaturation, whereas detergents should be completely removed by collisional dissociation (Figure 5).

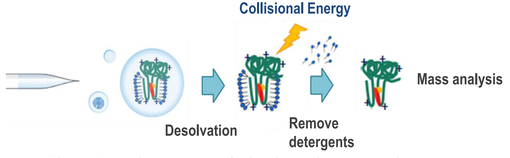


Figure 5. Native mass analysis of membrane proteins

3-2. Sample Preparations

Two membrane proteins, duodenal cytochrome *b* (Dcyt*b*) and bacterial intramembrane protease (*Aa*RseP), were exchanged with Micro Bio-Spin 6 column (Bio-Rad) into 200mM ammonium acetate (pH 7.4) and were prepared in micellized form with detergent. Batimastat (BAT) was used as a ligand to bind to *Aa*RseP.

3-3. MS conditions

All evaluations were performed with a nano-ESI source. The spray voltage was set to 1.2kV. The collision energies were adjusted within the range of 5 to 50 eV.

4. Results

4-1. Native mass analysis of Dcyt*b*

The analysis of Dcyt*b* was performed (Figure 6). The detergents were removed, and an isotope-separated mass spectrum was obtained. The measured mass is consistent with the mass of two hemes noncovalently bound to Dcyt*b*. The result demonstrate that membrane proteins could be analyzed under native conditions using the MT-TOF-MS system.

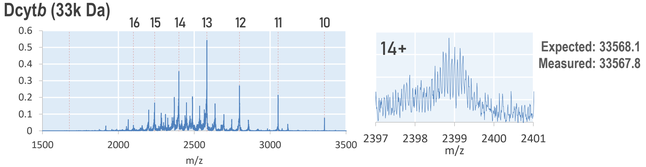


Figure 6. Native mass spectrum of Dcyt*b* with [G1]-ether-C12

4-2. Native mass analysis of *Aa*RseP

The analyses of *Aa*RseP bound only to Zn (Figure 7a) and bound to both Zn and BAT (Figure 7b) were performed. In the analysis of *Aa*RseP bound to Zn and BAT, in addition to the peak of *Aa*RseP bound to Zn only, a peak corresponding to *Aa*RseP bound to both Zn and BAT was also observed.

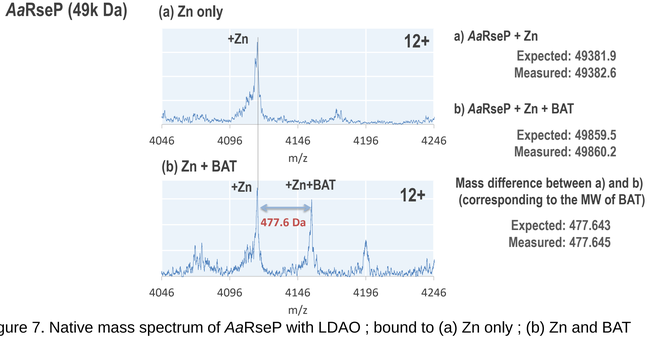


Figure 7. Native mass spectrum of *Aa*RseP with LDAO ; bound to (a) Zn only ; (b) Zn and BAT

5. Conclusions

- Native mass analyses of membrane proteins with MT TOF-MS were demonstrated.
- A high-resolution mass spectrum of native Dcyt*b* (33k Da) was obtained.
- A native mass spectrum of *Aa*RseP that maintained the ligand binding could be observed with high mass accuracy.

Reference

1) M. Tajiri et al., ACS Omega 2023, 8, 27, 24544–24551
2) Y. Tateishi et al., The 72nd ASMS Conference, ThP-401 (2024).