Structural Characterizations of Intact Monoclonal Antibodies by Native MS

Angela Criscuolo 1, 2; Tabiwang N. Arrey 2; Eugen Damoc 2; Thomas Moehring 2; Catharina Crone 2; Markus Kellmann 2
1Leipzig University, Leipzig, Germany; 2Thermo Fisher Scientific, Bremen, Germany

ABSTRACT

Proteolytic degradation of intact monoclonal antibodies (mAbs) has become a key step. In particular, the characterization of post-translational modifications (PTMs) is a fundamental regulatory requirement and an important aspect for the correct interpretation of biological activity. The high mass resolution and accuracy of the Orbitrap Exploris 480 mass spectrometer allowed confident identification of proteoforms which could not be resolved by other approaches. The combination of this approach with the high resolution, accurate-mass (HRAM) of the Orbitrap Exploris 480 mass spectrometer allowed confident identification of proteoforms which could not be resolved by other approaches. The combination of this approach with the high resolution, accurate-mass (HRAM) of the Orbitrap Exploris 480 mass spectrometer was used to study the proteoform diversity of intact mAbs.

INTRODUCTION

In the production of therapeutic mAbs, intact mass spectrometry analysis has become a key step. In particular, the characterization of PTMs is a fundamental regulatory requirement and an important aspect for the correct interpretation of biological activity. The high mass resolution and accuracy of the Orbitrap Exploris 480 mass spectrometer was used to study the proteoform diversity of intact mAbs.

MATERIALS AND METHODS

Sample Preparation

Tumor-induced 3D culture was labeled with 5 times a concentration range between 5.33 and 37 µg/mL. A series of buffer was labeled and analyzed as described below.

LC-MS Method

All experiments were performed using a Thermo Scientific™ Vanquish™ UHPLC system. For size exclusion chromatography, Trastuzumab was desalted online on a Thermo Scientific™ MAbPac™ 100A/100B 10/800 µm column. A combination of acetonitrile and water (0.03% formic acid) was used as the mobile phase for SEC. For charge variant chromatography, Trastuzumab was desalted online on a Thermo Scientific™ MAbPac™ 100A/100B 10/800 µm column. A combination of acetonitrile and water (0.03% formic acid) was used as the mobile phase for CV.

ESI–MS analyses were performed in positive ion mode on a Orbitrap Exploris 480 mass spectrometer using the Intact mAb Native  system template (Figure 2 and Table 1).

Table 1. LC (A) and MS (B) conditions used for analysis of Trastuzumab

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC (A)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UHPLC</td>
</tr>
<tr>
<td></td>
<td>Size exclusion chromatography</td>
</tr>
<tr>
<td></td>
<td>Charge variant chromatography</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (B)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Orbitrap Exploris 480 mass spectrometer</td>
</tr>
<tr>
<td></td>
<td>Intact mAb Native system template</td>
</tr>
</tbody>
</table>

RESULTS

SEC-MS

The base peak chromatograms from the two separation techniques are significantly different. For size exclusion chromatography, the elution times in a single peak, labeled as 20 minutes of the baseline, indicate the charge variant separation is in agreement with different contributions of the different charge variants. The total ion chromatograms for the amount of sample analyzed, as shown in Figures 5 and 6.

CV-MS

The charge variant chromatograms for the sample analyzed are significantly different. For charge variant chromatography the elution times in a single peak, labeled as 80 minutes of the baseline, indicate the charge variant separation is in agreement with different contributions of the different charge variants. The total ion chromatograms for the amount of sample analyzed, as shown in Figures 5 and 6.

CONCLUSIONS

The successful size exclusion and charge variant chromatography has shown the potential to reveal mAb heterogeneity. In the production of therapeutic mAbs, intact mass spectrometry analysis has become a key step. In particular, the characterization of PTMs is a fundamental regulatory requirement and an important aspect for the correct interpretation of biological activity. The high mass resolution and accuracy of the Orbitrap Exploris 480 mass spectrometer was used to study the proteoform diversity of intact mAbs.

REFERENCES


ACKNOWLEDGEMENTS

Financial support from the EU H2020 funded project MASSTRPLAN (Grant number 675132) is gratefully acknowledged. The authors thank Dr. Angela Criscuolo, Dr. Tabiwang N. Arrey, Dr. Eugen Damoc, and Dr. Thomas Moehring for scientific support and discussion.

TRADEMARKS/LICENSES

© 2019 Thermo Fisher Scientific Inc. All rights reserved. ReSpect is a trademark of Positive Sciences, Inc.

For Research Use Only. Not for use in diagnostic procedures.