1. Introduction

The analysis of intact antibodies expressed in E. coli is challenging at the 1-5 kDa level, mainly due to difficulties in obtaining high-quality samples. The application of Orbitrap mass spectrometry has been enhanced by the use of complementary techniques such as LC-MS/MS. The ETD technique has been shown to be effective in the fragmentation of intact proteins, allowing for the identification of sequence-specific tryptic peptides. This method is particularly useful in the analysis of intact antibodies, where the intact structure is crucial for understanding the functionality of the protein.

2. Methods

ETD-enabled Orbitrap mass spectrometry (Thermo Scientific) was employed for protein top-down analysis. ETD was compared with nanoLC-FTICR MS peptide fragmentation, which is a time-consuming method due to the complexity of the spectra. The ETD experiments were performed on a Q Exactive mass spectrometer, and the ETD spectra were acquired at a resolution of 70,000 FWHM. The sample preparation involved the digestion of intact proteins using trypsin or chymotrypsin.

3. ETD of human Humira IgG

The ETD of human Humira IgG was performed using ETD and nanoLC-FTICR MS. The ETD spectra showed a clear fragmentation pattern, with the identification of specific tryptic peptides. The nanoLC-FTICR MS spectra were less informative, with a lower number of identified peptides.

4. ETD of murine NPY

The ETD of murine NPY was also performed using ETD and nanoLC-FTICR MS. The ETD spectra showed a clear fragmentation pattern, with the identification of specific tryptic peptides. The nanoLC-FTICR MS spectra were less informative, with a lower number of identified peptides.

5. Method optimization

The optimization of the ETD method involved the modification of the ETD parameters, such as the collision energy and the ion transmission time. The optimization was performed using a series of test samples, and the best conditions were identified.

6. ETD of murine IgG2b

The ETD of murine IgG2b was performed using ETD and nanoLC-FTICR MS. The ETD spectra showed a clear fragmentation pattern, with the identification of specific tryptic peptides. The nanoLC-FTICR MS spectra were less informative, with a lower number of identified peptides.

7. Structure-fragmentation relationship: the Fc

The structure-fragmentation relationship of the Fc domain was analyzed using ETD and nanoLC-FTICR MS. The ETD spectra showed a clear fragmentation pattern, with the identification of specific tryptic peptides. The nanoLC-FTICR MS spectra were less informative, with a lower number of identified peptides.

8. Structure-fragmentation relationship: ECD PIA

The fragmentation of the ECD PIA was performed using ETD and nanoLC-FTICR MS. The ETD spectra showed a clear fragmentation pattern, with the identification of specific tryptic peptides. The nanoLC-FTICR MS spectra were less informative, with a lower number of identified peptides.

9. Conclusions

ETD fragmentation of intact IgGs was shown to be a valuable tool for the analysis of intact antibodies. The ETD spectra showed a clear fragmentation pattern, with the identification of specific tryptic peptides. The nanoLC-FTICR MS spectra were less informative, with a lower number of identified peptides. Further improvement in SNR is required for the identification of ITMs and increased sequence coverage.

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11. References