

Title: a Powerful High-Resolution Mass Analyzer-Orbitrap

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Abstract: Organic mass spectrometry plays important roles in compound qualitative and quantitative analysis, proteomics, protein post-translational modification analysis, and metabolomics analysis. The mass analyzer is one of the most important components of the mass spectrometer. The sensitivity and resolution of a mass analyzer are important criteria for evaluating mass analyzers. Here, we review the research history, working principle, advantages and application of Orbitrap. We hope that through our review, readers can better understand the application value of Orbitrap and provide help to promote the application of mass spectrometry in biomedicine.

1. Introduction

The mass analyzer is one of the most important parts of a mass spectrometer. High-sensitivity and high-resolution mass analyzers have always been an important direction of mass spectrometer innovation. With the continuous development of proteomics, metabolomics and lipidomics, the analysis samples faced by mass spectrometry analysis will be more complicated and difficult to analyze. Therefore, higher-quality analytical instruments are required to solve these problems. In response to technical requirements, the first mass analyzer equipped with Orbitrap mass analyzer finally came out in 2015. This technological innovation has greatly promoted the development of proteomics, metabolomics and lipidomics, and has also greatly encouraged more researchers to develop new technologies based on mass spectrometry. [1].

In 2015, Thermo Fisher Scientific introduced for the first time a mass spectrometer equipped with an Orbitrap mass analyzer-LTQ Orbitrap mass spectrometer, which is also the world's first mass spectrometer equipped with an Orbitrap mass analyzer. This instrument has been widely used as soon as it was launched, not only because of its stable qualitative analysis capabilities, but also its good quantitative analysis capabilities. Thermo Fisher Scientific subsequently introduced a series of high-resolution mass spectrometers equipped with Orbitrap mass analyzers. Mass spectrometers based on Orbitrap mass analyzers play an important role in the analysis of complex biological samples. [2]. The variety of Orbitrap-based instruments developed reflects the scope of applications for which the Orbitrap Analyzer was used. These applications range from routine screening and quantitation analysis to complex analytical challenges ranging from proteomics to structural identification and characterization. [3].

2. The Advantages of Orbitrap

The mass spectrometer based on Orbitrap mass analyzer has greatly promoted the development of mass spectrometry in two aspects. On the one hand, it improves the speed of analysis, which is very important for obtaining more comprehensive information in complex samples. On the other hand, the Orbitrap mass analyzer improves the resolution and sensitivity of the analysis, which is vital for the analysis of complex samples. [4].

Over the last ten years, mass spectrometry has been revolutionized by access to ever-increasing instruments. The accuracy of Orbitrap mass analyzer can reach 2-5ppm. Recent report have shown that the accuracy of calibration with internal standards can be less than 1ppm, while the accuracy of calibration with external standards can be less than 3ppm. The sensitivity of the classic Orbitrap mass analyzer can reach 150,000, and the sensitivity of the new ultra-high field Orbitrap mass

analyzer can reach 600,000. Here we will review the principles and techniques of Orbitrap mass spectrometers and describe some important applications [5].

3. The Applications of Orbitrap

In recent years, proteomics has developed very rapidly. A mass spectrometer equipped with a high-resolution mass analyzer is a powerful tool for proteomics analysis. Orbitrap is widely used in the field of proteomics research due to its fast speed and high resolution [4]. In a recent article, researchers from the Max Planck Institute in Germany used quantitative proteomics to reveal new drug targets for the treatment of ovarian cancer. Most patients with high-grade serous ovarian cancer resistance to platinum-based chemotherapy and relapse, but 15% still have no disease for more than ten years. In order to discover the motivation for long-term survival, they quantitatively analyzed the proteome of platinum resistant high-grade serous ovarian cancer patients fixed from a trace of formalin, embedded in paraffin tumor. This indicates cancer/testicular antigen 45 (CT45) as a vital prognostic factor related to doubling disease-free survival in advanced high-grade serous ovarian cancer. Proteomics links CT45 to DNA damage pathways. Immunopeptidomics analysis reveal that CT45 derived HLA-I peptide can be activated in patient-derived cytotoxic T cells and promote tumor cell killing. This work suggest the power of proteomics to find targets for therapy and clarify its biological roles [6].

Researchers from the same research group used proteomics to analyze proteome changes in human serum. Their research shows that serum proteome analysis is expected to provide important information for disease diagnosis and target discovery. Proteins in the circulatory system reflect the individual's physiological. In daily clinical practice, protein Levels are usually determined using single protein Immunoassay. Quantitative analysis performed by mass spectrometry-based proteomics of blood, plasma and serum will be beneficial but is challenging due to high dynamic range protein abundance. In this article, they introduced a quick and powerful "plasma proteome analysis" channel. This single-run shotgun proteomics workflow does not require protein consumption and can be quantified analysis of hundreds of plasma proteomes. The elution was performed in a 20-minute gradient from 1 μ l of single finger prick. Apolipoprotein family, inflammation marker such as C-reactive protein, sex-related protein, and more than 40 FDA approved biomarkers are reproducible quantification. They showed that plasma proteome profiling provides an important information of a person health state [7].

In addition to quantitative proteomics analysis, protein post-translational modifications also play an important role in the physiological and pathological processes [8]. The mass spectrometer based on the high-resolution mass analyzer Orbitrap also played significant roles in the discovery of new protein modifications. A research team from the University of Chicago discovered for the first time that lactic acid can be modified to histones and affect the transcriptional regulation of specific genes. The Warburg effect, originally described as increased lactic acid production cancer is related to various cellular processes. This phenomenon is related to many diseases, including neoplasia, sepsis and autoimmune diseases. Lactic acid is converted from pyruvate in tumor cells and is widely regarded as an energy source and metabolic by-products. However, its non-metabolic function in physiology and the disease is still unclear. In this research article, they show the lactation of histones derived from lactate act as epigenetic modifications and can directly stimulate genes transcribe from chromatin. They identified 28 lactate modification sites on core histones. Hypoxia and bacterial challenges can induce the production of lactic acid from glycolysis. Using M1 macrophages as a model system, they showed that the lactation have different time dynamics from acetylation of histones. In late period of polarized M1 macrophages, histone lactylation is enhanced. In general, their results show that M1 attacked by bacteria has an endogenous "lactic acid clock". Therefore, histone lactylation reveals an opportunity to improve our understanding of the functions lactic acid and its role in a variety of pathophysiological conditions [9].

4. Discussion

Although there are many mass analyzers. Orbitrap as a high-resolution mass analyzer plays an important role in proteomics, metabolomics and lipidomics [1, 10]. This article reviews the development history of Orbitrap and the research progress in proteomics and protein post-translational modification in recent years. Orbitrap has a wider range of applications in biomedicine, such as drug metabolism analysis, metabolomics analysis and clinical laboratory analysis. The application of Orbitrap can solve many problems that the low-resolution quality analyzer could not solve before. But this does not mean that Orbitrap is the end of mass analyzers [2]. In the future, it is necessary to develop new mass analyzers with higher sensitivity and resolution to satisfy the analysis and identification of more complex systems. Mass spectrometry has a very strong advantage in qualitative and quantitative compounds. With the development of organic mass spectrometry, mass spectrometry is becoming more and more important in the research of biomedicine. However, the application of mass spectrometry in clinical analysis is still relatively small at present, so it will be an important direction to promote the application of mass spectrometry in clinical laboratory diagnosis in the future.

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