Visualization of metabolite change in skeletal muscle by contraction using imaging mass spectrometry

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Abstract Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) is a mass spectrometry-based imaging technique used to visualize the distribution of biomolecules without the need for extraction, purification, separation and labeling of the molecules. This technique can reveal the distribution of hundreds of ion signals in a single measurement, and also helps in understanding metabolite structure, overcoming the inadequate imaging probes for small metabolites. The versatility of MALDI-IMS has opened up new frontiers in several fields, especially in lipidomics. Lipids in skeletal muscle play a fundamental role in both normal muscle metabolism and disease states. Skeletal muscle lipid accumulation is associated with several chronic metabolic disorders, including obesity, insulin resistance, and type 2 diabetes. In this review, we show the applications of MALDI-IMS to mouse skeletal muscle for detecting changes in lipids induced by contraction.

Keywords: lipid, imaging mass spectrometry, contraction, skeletal muscle

Overview

Lipids contain many kinds of molecules, including fatty acyls, glycerolipids, glycerophospholipids, and sphingolipids. For comprehensive lipid analyses, we use a “lipidomics” approach, which provides further insights into complex metabolic networks of biological systems. Mainly, lipidomics approaches have been demonstrated by the use of liquid chromatography coupled with mass spectrometry (MS) to fully characterize lipid molecular species1-4). However, this technique includes extraction and purification steps, leading to the loss of lipid distribution over the biological tissue. Meanwhile, numerous techniques of chemical imaging enable the detection and localization of lipids. Staining with Nile Red, Oil Red O, osmium tetroxide, or BODIPY 493/503 is a common method to localize the total lipid fraction on frozen sections. On the other hand, few specific lipid antibodies5) are commercially available. These approaches target either the complete lipid fraction or only one specific family, not molecular species with varied composition, such as those of fatty acids.

Imaging mass spectrometry (IMS) is a relatively new imaging method based on MS. IMS is an analytical technique that detects the mass-to-charge ratio (m/z) of ionized molecules and visualizes the distribution of ions6-11). Several ionization methods, including secondary ion mass spectrometry (SIMS), desorption electrospray ionization, laser ablation electrospray ionization, and matrix-assisted laser desorption/ionization (MALDI)12), have been investigated as methods that can be combined with IMS. In particular, MALDI-IMS can detect a wide range of molecules and has the ability to characterize structure by tandem mass spectrometric analysis13). In this review, lipid imaging of mouse skeletal muscle by MALDI-IMS is shown to verify changes in lipids induced by contraction.

Application of IMS to skeletal muscle

Lipids in skeletal muscle play a fundamental role as an energy source in both normal muscle metabolism and disease states. Excess accumulation of lipids in skeletal muscle is associated with several chronic metabolic disorders, including obesity, insulin resistance, and type 2 diabetes14). There are some reports of IMS of skeletal muscles using time-of-flight (TOF)-SIMS. Magnusson et al demonstrated lipid accumulation in the skeletal muscle of leptin-deficient (ob/ob) mice by TOF-SIMS15), which revealed changes in the composition of monounsaturated fatty acids (FAs) and polyunsaturated FAs at high resolution. Tahellah et al also used TOF-SIMS in an attempt to visualize lipid distribution of Duchenne muscular dystrophy-affected muscles16). They showed that vita-
min E and phosphatidylinositols concentrated within the cells, whereas intact phosphocholines accumulated over the most damaged areas of dystrophic muscle, together with cholesterol and sphingomyelin (SM) species. TOF-SIMS is an excellent tool for high-spatial-resolution IMS (submicron order) of elements and small molecules at different organelle levels of the cell. However, due to in-source fragmentation and the lack of tandem mass spectrometric facility to identify the structure, SIMS lacks sensitivity for the mass range over \( m/z 800 \) and the ability to identify molecules. In the next section, the application of MALDI-IMS for the visualization of lipids in skeletal muscles is described. Although the spatial resolution is lower than that available with TOF-SIMS, MALDI-IMS has the advantages of being able to detect molecules over a wide range, including molecules higher than \( m/z 800 \), and identify them by the use of tandem mass spectrometric analyses directly on tissue sections.

**Metabolic change in lipids induced by contraction**

Lipids in skeletal muscle play a fundamental role in muscle metabolism. Small changes in total lipid amounts with muscle contraction have been detected by conventional biochemical methods, suggesting that dynamic change in lipid composition, rather than lipid amount, occurs during muscle contraction. However, it is poorly understood what types of molecular species are changed by muscle contractions due to the complexity and variety of lipid molecular species. In the present study, MALDI-IMS was used to analyze lipid metabolism. Fig. 1 shows the scheme of our measurements. We prepared tibias anterior muscles which underwent forced contraction by electrical stimulation of sciatic nerves in anesthetized mice. In this type of in situ contraction model, it is possible to provide one hind limb for the muscle contraction condition and the other hind limb for the basal condition (no contraction). After contraction, muscle tissues were immediately dissected and frozen in liquid nitrogen. Thin-sliced frozen sections of both basal and contraction muscles were thaw-mounted on glass slides and thoroughly sprayed with small organic compounds that acted as a matrix to assist the ionization of the metabolites. The laser spatial resolution was set at 25 \( \mu m \) and acquired the mass spectrum at each spot. Ion images were constructed at each \( m/z \) value.

The method used has two modes for detecting ionized molecules, positive and negative ion modes that can detect cation-added ions and de-protonated ions, respectively. Fig. 2a represents ion images detected in the positive-ion mode. In this mode, phospholipids and neutral glycerolipids could be detected. Since the molecules are ionized as various types of adduct ions, \([M+H]^+\), \([M+Na]^+\), and \([M+K]^+\), it is difficult to identify the molecules by comparing their molecular mass alone. Therefore, tandem mass spectrometric analysis was attempted to identify all of the molecules. Interestingly, peaks under \( m/z 700 \) were drastically reduced by muscle contraction, and the peak at \( m/z 661 \) was identified as diacyl-glycerol (DAG) \((18:1/18:0)\), suggesting that muscle contraction decreased DAGs in skeletal muscles. The major lipids in the muscle tissues are phosphatidylcholines (PCs), and various kinds of PCs could be detected. Some polyunsaturated FA-containing PC ions were present at a significantly higher level in contracted muscles compared to resting muscles, for example, those of the ion at \( m/z 844 \), assigned as PC \((\text{diacyl-18:2/20:4})\), and the ion at \( m/z 848 \), assigned as PC \((\text{diacyl-38:4})\). However, the distributions of these two types of ion in the muscle tissue were completely different: while the accumulation of ion signals of \( m/z 844 \) was

![Fig. 1 Scheme of MALDI-IMS](image-url)
ubiquitous throughout muscle myofibers, signals of m/z 848 were distributed specifically inside each myofiber. It was speculated that this relocation was derived from the remodeling process of FAs during contraction.

The molecular ion at m/z 741 was assigned as SM (d18:1/16:0). The SM distribution and amount were not changed by muscle contraction. Triacylglycerol (TAG) is also a major lipid type in muscles, and it is easy to detect with this method. A representative ion at m/z 889 was assigned as TAG (16:1/16:1/20:4), and the amounts were lowered by muscle contraction. This is the first report to show the change in lipids in distribution and composition during contraction. Muscle contraction (or physical exercise) is a potent stimulus that enhances insulin sensitivity, though the mechanism is still unknown\(^2\). By the use of MALDI-IMS, these molecules will be followed to confirm lipid-induced insulin sensitivity or a glucose-uptake mechanism in skeletal muscles.

Energy charge is an index of metabolic activity, and low signals of the index indicate an up-regulation of metabolism\(^2\). To evaluate change in the energy charge in skeletal muscles by electrical pulse-evoked contraction, adenosine phosphate-related ions were examined. Energy charge was calculated from pixels of ATP, ADP, and AMP and displayed as images (Fig. 2b). Generally, the normal energy charge is around 0.8, and a lower energy charge represents an up-regulation of metabolism. The histogram showed average energy charges of 0.849 in basal muscle and 0.785 in contracted muscle. These data indicate that ATP was generated at a high degree in contraction samples compared with basal samples. Thus, this is considered a good tool to investigate local metabolic activity with contraction in skeletal muscle. The results here show that changes in the lipid amount, lipid composition, and energy metabolic activity can be evaluated in each local spot of cells and tissues at the same time using MALDI-IMS and tandem mass spectrometric analyses.

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**Fig. 2** MALDI-IMS of metabolites

(a) The ion images of each m/z value are shown in the positive-ion mode. (b) In the negative-ion mode, ATP, ADP, and AMP distribution was visualized in the tissue. The energy charge was calculated and visualized with SIMenergy software (Shimadzu Co., Japan). The histogram was calculated by examining each pixel. Scale bar is 400 μm. Modified with permission from\(^1\).
Conclusion

This study showed dynamic changes in the lipid composition of murine skeletal muscle following contraction using the MALDI-IMS technique. Although conventional methods did not show significant changes in lipid amounts from basal to contraction conditions, it was proved that the lipid composition, i.e., the relative amounts of PC, DAG and TAG, was actually changed by contraction. Moreover, some molecules showed characteristic distributions after contraction that could be related to contraction-induced metabolic dynamics. MALDI-IMS is becoming an essential tool for molecular imaging of biological samples. Many great advances have been made in MALDI-IMS technology, allowing it to visualize lipids in various types of biological samples, though there is still room for improvement in sample preparation, ionization, and instrumentation. Moreover, to make the relatively new MALDI-IMS strategy a routine tool for biomarker discovery, it needs to be validated on a larger sample scale.

References