Application of Imaging TOF-SIMS in Cell and Tissue Research

AKADEMISK AVHANDLING

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av

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Avhandlingen baseras på följande delarbeten:

I Börner, K., Nygren, H., Hagenhoff, B., Malmberg, P., Tallarek, E. & Mansson, J. E.
Distribution of cholesterol and galactosylceramide in rat cerebellar white matter
Biochimica et Biophysica Acta 1761(3): 335-44 (2006)

II Richter, K., Nygren, H., Malmberg, P. & Hagenhoff, B.
Localization of fatty acids with selective chain length by imaging time-of-flight secondary ion mass spectrometry
Microscopy Research and Technique 70(7): 640-7 (2007)

III Nygren, H., Börner, K., Malmberg, P., Tallarek, E. & Hagenhoff, B.
Imaging TOF-SIMS of rat kidney prepared by high-pressure freezing
Microscopy Research and Technique 68(6): 329-34 (2005)

IV Pernber, Z., Richter, K., Mansson, J. E. & Nygren, H.
Sulfatide with different fatty acids has unique distributions in cerebellum as imaged by time-of-flight secondary ion mass spectrometry (TOF-SIMS)
Biochimica et Biophysica Acta 1771(2): 202-9 (2007)

GÖTEBORGS UNIVERSITET

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Abstract

This thesis is aimed at extending the use of TOF-SIMS (time of flight secondary ion mass spectrometry) as an analytical tool in cell and tissue research. TOF-SIMS is a relatively new method that allows analysis of the chemical composition of sample surfaces. Originally, it was used for imaging the distribution of elements on surfaces in materials science. Technical improvements have made analysis of fragile biological samples possible through localization of relevant secondary ions, e.g. lipid fragments and inorganic ion patterns. TOF-SIMS has several advantages over alternative imaging methods e.g. its sensitivity to all elements, detection of all isotopes, and it allows composite imaging of the surface distribution of detected elements and molecules.

The present work comprises new applications of cryomethods for preparation of TOF-SIMS tissue samples in an effort to obtain analytical results that reflect the vital situation as closely as possible. Lipid species (galactosylceramides, sulfatides or fatty acids) and ions (Na/K) were identified and localized in specimens from cerebellum, kidney and intestine with instruments equipped with a bismuth cluster primary ion source.

Two sample preparation sequences were employed: (i) plunge freezing in liquid nitrogen and cryosectioning; (ii) high-pressure freezing followed by freeze-fracturing and freeze-drying. The two preparation methods resulted in similar distributions of lipids and fatty acids but only sample preparation with high-pressure freezing allowed demonstration of separate distributions of sodium and potassium in rat cerebellum and kidney.

TOF-SIMS analysis revealed specific distributions of lipids cholesterol, phosphocholine, sulfatides, two galactosylceramide species and ions sodium and potassium in rat cerebellum. It could be shown that the white matter is separated into a "cytoplasm-rich" compartment containing phosphocholine, Na^+, K^+ and N-lignoceroylgalactosylceramide and a cholesterol-rich compartment containing cholesterol and N-stearoyl-galactosylceramide. Sulfatides with short chain fatty acids displayed a uniform distribution in the white matter and a patchy distribution for sulfatide with C24 fatty acids.

Fatty acid signals showed high intensities for stearic acid in Purkinje cell bodies of the rat cerebellum; the fatty acids palmitic and oleic acid displayed most intense signals located to the molecular and granular layer. Fatty acid imaging in mouse intestine revealed highest palmitic acid signals in the secretory crypt cells and the intestinal lumen and highest oleic acid signals in intestinal villi.

New data on the cellular and subcellular distribution of lipid molecular species in tissues have been presented that are not possible to achieve with other microscopical techniques. It is concluded that TOF-SIMS at the present state is a powerful tool in imaging of ions and organic compounds at a MW up to ≈ 1,000 in biological tissues. TOF-SIMS has a potential to provide very significant information for the understanding of physiological functions and pathological processes in biomedical research. Further technical development will extend the range of applications.

Key words: TOF-SIMS, imaging mass spectrometry, cryopreparation, ions, lipids, galactosylceramide, sulfatide, fatty acids

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