

Structural identification of novel skin bound ceramides using mass spectrometry

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ABSTRACT

The stratum corneum (SC) as the outermost layer of the skin has a barrier function and protects the organism against environmental influences and transepidermal water loss. It consists of non-viable keratin-filled cells, or corneocytes, embedded in a matrix of lipids (ceramides, free fatty acids, and cholesterol). Skin ceramides are sphingolipids consisting of sphingoid bases, which are linked to fatty acids by an amide bond. Typical fatty acid acyl chains are composed of non-hydroxy fatty acid (N), α -hydroxy fatty acid (A), ω -hydroxy fatty acid (O), and esterified ω -hydroxy fatty acid (EO). In this study, we identified 1-O-acylceramides as a novel epidermal bound ceramides using high-resolution mass spectrometry (HRMS) and chip-based direct infusion nanoelectrospray-ion trap mass spectrometry in mouse and guinea pig. These ceramides contained a long acyl chains linked to the 1-O-position of O-type ceramide. In addition, we have also established mass database for identifying 1-O-acylceramides in the stratum corneum (SC) of mouse and guinea pig. This information may be used to identify bound 1-O-acylceramides in the SC of animal skin.

METHODS

1. Materials

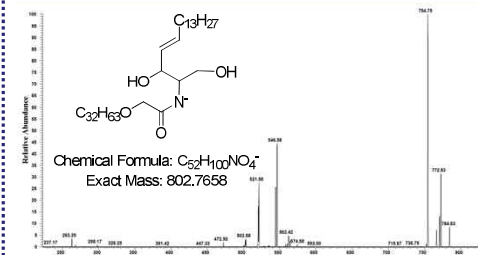
-Sample: mouse and guinea pig skin

2. Instrument

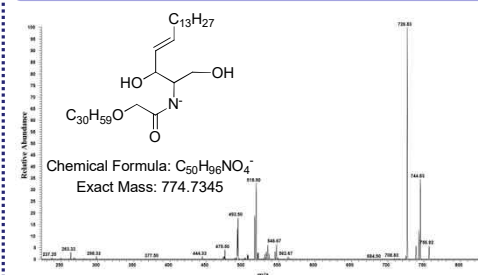


- ◆ LTQ XL mass spectrometer (Thermo Fischer Scientific, USA)
- ◆ TriVersa Nanomate (Advion Biosciences)
- ◆ Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fischer Scientific, USA)

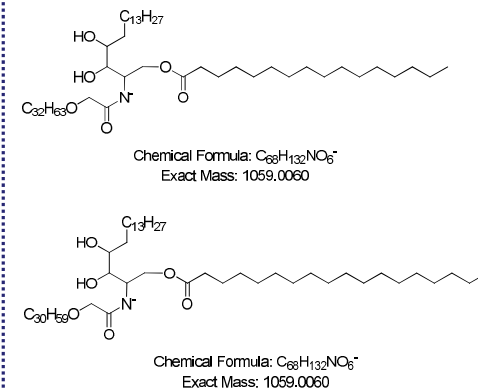
MS spectrum of m/z 1059 >802.8 using nanomate-LTQ mass spectrometry



MS spectrum of m/z 1059 >774.8 using nanomate-LTQ mass spectrometry

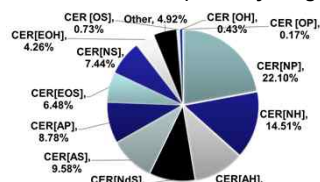


Structure of m/z 1059



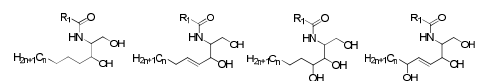
INTRODUCTION

◆ Ceramides are main lipid component existed in skin stratum corneum (>50% by weight).



◆ The structure of ceramides

DS (dihydrosphingosine) S (sphingosine) P (phytosphingosine) H (β -hydroxy-sphingosine)



R1 N (non-hydroxy fatty acid)



A (α -hydroxy fatty acid)



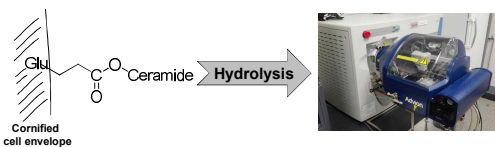
O (ω -hydroxy fatty acid)



EO (esterified ω -hydroxy fatty acid)



◆ Bound ceramide treatment

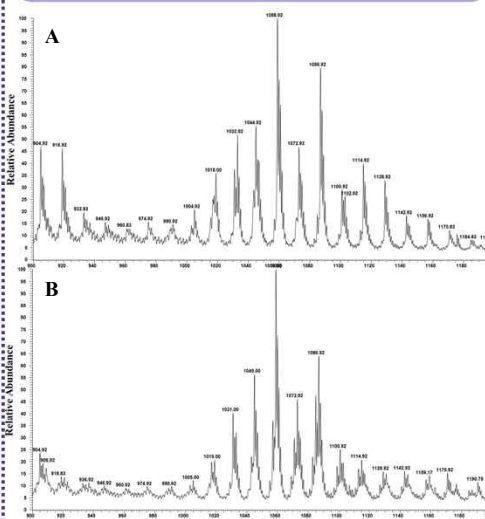


OBJECTIVE

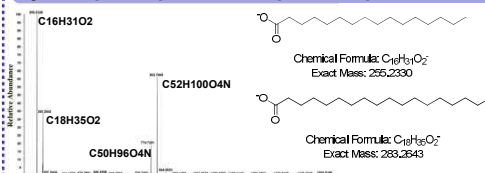
◆ To identify new skin bound ceramide using high-resolution mass spectrometry (HRMS) and nanomate-LTQ mass spectrometry

RESULTS

Typical mass spectrum of the ceramide extracts from the stratum corneum of guinea pig skin (a) and mouse skin (b) using chip-based direct infusion nanoelectrospray-ion trap mass spectrometry in negative ion mode



MS/MS spectrum of m/z 1059 using Q Exactive hybrid quadrupole-Orbitrap mass spectrometer



CONCLUSION

- ◆ We identified 1-O-acylceramides as a novel epidermal bound ceramides using high-resolution mass spectrometry (HRMS) and chip-based direct infusion nanoelectrospray-ion trap mass spectrometry in mouse and guinea pig skin.
- ◆ This information may be used to identify bound 1-O-acylceramides in the SC of animal skin.

REFERENCE

- ◆ t'Kindt R, Jorge L, Dumont E, Coututon P, David F, Sandra P, Sandra K. *Anal Chem* 84: 403-411 (2012)
- ◆ Wu Z, Shon JC, Lee D, Park KT, Park CS, Lee T, Lee HS, Liu KH. *Anal Bioanal Chem*, 408:2069-2082 (2016)
- ◆ Wu Z, Shon JC, Kim JY, Cho Y, Liu KH. *Arch Pharm Res*, 39:1426-1432 (2016)