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Authors
Kind, Tobias
Okazaki, Yozo
Saito, Kazuki
et al.

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LipidBlast Templates As Flexible Tools for Creating New in-Silico Tandem Mass Spectral Libraries

Tobias Kind,*† Yozo Okazaki,‡ Kazuki Saito,‡§ and Oliver Fiehn*†

†West Coast Metabolomics Center, University of California Davis, Davis, California 95616, United States
‡RIKEN Center for Sustainable Resource Science, Metabolomics Research Group, Yokohama 230-0045, Japan
§Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260-8675, Japan

ABSTRACT: Tandem mass spectral libraries (MS/MS) are usually built by acquiring experimentally measured mass spectra from chemical reference compounds. We here show the versatility of in-silico or computer generated tandem mass spectra that are directly obtained from compound structures. We use the freely available LipidBlast development software to generate 15 000 MS/MS spectra of the glucuronosyl-diacylglycerol (GlcADG) lipid class, recently discovered for the first time in plants. The generation of such an in-silico MS/MS library for positive and negative ionization mode took 5 h development time, including the validation of the obtained mass spectra. Such libraries allow for high-throughput annotations of previously unknown glycolipids. The publicly available LipidBlast templates are universally applicable for the development of MS/MS libraries for novel lipid classes.

The development of new LipidBlast MS/MS spectra requires a development sheet (Microsoft Excel) and a Visual Basic for Applications software (Microsoft Excel VBA) that contains programmatic code to export the MS/MS spectra. Extensive method details can be found in the original LipidBlast publication.2 We concisely describe the general approach here. The creation of new in-silico MS/MS libraries requires sample MS/MS spectra for development and a series of MS/MS spectra for validation. Spectra were taken from original experimental measurements and the publication itself.1 These tandem mass spectra were used for developing the library. The spectra were acquired on a hybrid ion trap/time-of-flight mass spectrometer with electrospray ionization (ESI-IT-TOF-MS). Additional supplemental raw MS/MS spectra from the same instrument were used for validation. All spectra are freely available for download from our Web site. Instead of a completely independent development, we simply copied an

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existing member from the LipidBlast development sheet (MSMS-prediction-distribute-v49.xls) and chose a lipid class (sulfoquinovosyldiacylglycerol, SQDG) similar to the novel GlcADG lipid class as a starter template. In-silico structures were generated by replacing the SQDG core structure with GlcADG in the SMILES (Simplified Molecular Input Line Entry System) structure codes using text-based find-and-replace in Microsoft Excel. The resulting SMILES codes were used to calculate a series of required molecular properties using the ChemAxon cxcalc command line tools. The properties included were accurate mass, octanol–water partition coefficient (log P), and the InChIKey. The values in the copied SQDG Microsoft Excel template sheet were subsequently adjusted for the novel GlcADG adduct ions as well as observed GlcADG fragmentations. The associated VBA code was modified to allow the export of the GlcADG lipids. Tandem mass spectrometry MSP files were converted by the LIB2NIST program, and the resulting libraries were copied as a subdirectory into the NIST MS Search program. The libraries were then used to further validate MS/MS spectra using the NIST MS Search program and for batch-wise comparison using the NIST MSPepSearch program.

### RESULTS AND DISCUSSION

We created a total of 15 000 novel in-silico MS/MS spectra for the glucuronosyldiacylglycerol lipid class using the LipidBlast development templates. A total of 5000 tandem mass spectra were modeled for positive [M + NH₄]⁺ ionization mode and 10 000 MS/MS spectra for negative ionization mode [M − H]⁻. The negative ionization mode numbers are twice the size because they cover spectra for low-CID (collision-induced dissociation) and high-CID voltage mode. Lipids with acyl carbon chain lengths C₂ to C₂₆ and degrees of unsaturation with double bond counts of 0–6 are included. The peak fragments and their individual abundances were modeled according to a reference spectrum obtained from an ion trap/time-of-flight mass spectrometer (see Figure 1). This approach is feasible because lipids follow very consistent fragmentation rules. For MS/MS library search we used accurate mass precursor search with subsequent product ion matching. The details of the matching procedure are outlined in the original publication.
LipidBlast paper. Short, the precursor filter removes many false candidates that fall outside a given mass window. The subsequent product ion matching algorithm uses traditional similarity scoring of remaining candidates. Reverse search scores can be used in case of impurities or nonexplained peaks.10

We validated the negative ionization mode in-silico MS/MS spectra with four experimentally obtained tandem mass spectra from the same class (see Figure 3). These experimental spectra matched the in-silico generated spectra, due to low-resolved ion peaks and overlapping product ions. In the case of overlapping or not completely resolved peaks by liquid chromatography as shown for GlcADG 36:4, lower hit scores with more ambiguous compound annotations are obtained. In order to increase hit scores we additionally modeled spectra for low-CID and high-CID voltage mode. In low-CID voltage the precursor ion has a higher peak intensity because it is not completely fragmented. In high-CID voltage mode the precursor ion disappears due to complete fragmentation and the fatty acyl intensities highly increase. The CID voltage specific modeling allows for analysis of experimental spectra from a wider range of instruments such as triple quadrupole or Fourier transform (FT) mass spectrometers (see Figure 3). A final validation and application step was performed on tandem mass spectra obtained from authentic reference standards synthesized by Cao and Williams.11 The paper also discussed specific product ion ratios for [M − H−sn2 + H2O]− and for [M − H−sn1 + H2O]− that can lead to the correct positional assignment of sn1 and sn2 fatty acyls. Such total synthesis approaches and detailed CID investigations with different ionization voltages will be extremely valuable for assignments of regioisomers in future versions of LipidBlast. Currently LipidBlast libraries cannot annotate stereochemistry, regiospecificity, and position of double bonds correctly. Also a number of bacterial fatty acids such as cyclic-, prenyl-, and epoxy fatty acids are not yet included. However, the lipid class as well as the total carbon and degree of unsaturation of each of the fatty acyl chains can be correctly annotated. The positive ionization mode [M + NH4]+ spectra were developed in a similar way and validated with two independent MS/MS spectra. The positive ion mode spectra are specific for hybrid ion trap/time-of-flight mass spectrometers. No voltage optimization has been performed due to the lack of additional reference spectra.

Our publicly available GlcADG glycolipid library has direct transatlantic aspects that go beyond plant lipid research.12,13 Glucuronidated glycolipids occur across several domains of life or phylogenetic branches. Glucuronidyl (glucuronosyl) lipids containing tuberculostearic acid (C18-methyl) were found and analyzed in mycobacterial lipid extracts11,14,15 from *Mycobacterium smegmatis* (see left panel of Figure 3) and other bacteria, *C18-methyl* group (tuberculostearic acid) is not directly assigned but rather annotated as C19 fatty acid. Glycolipids with similar structures were also observed in Gram-negative bacteria, *Pseudomonas diminuta*,16,17 *Hyphomonas jannaschiana*,18 *Agrobacterium tumefaciens*,19 and Gram-positive bacteria such as *Corynebacterium glutamicum*.20 GlcADG related glycolipids were also found in the fungus *Aspergillus fumigatus*.21 Diacylglycerol-alpha-D-glucuronide algal lipids have been found in *Pavlova lutheri* algae.23,24 The GlcADG lyso-forms (one acyl chain) as well as ether analogues (plasmenyl, plasmanyl) have been described in the literature for use as lipid haptons25 and synthesized for membrane property estimations,26 but no evidence has been found that they exist in nature yet.

Most of the publications did not report MS/MS spectra in the past. Subsequently such spectra could not be accumulated in large electronic mass spectral databases such as Wiley MSforID,27 ReSpect,28 MassBank,29 NIST,30 or Metlin.31 We close that gap with our publicly available in-silico MS/MS library, enabling future research groups to perform high-throughput analysis of complex glycolipid mixtures by simply extending and using LipidBlast.

The fast development of in-silico MS/MS spectra using the LipidBlast Excel templates shows the versatility and broad application domains of our LipidBlast software. The developed libraries and new templates are freely provided for commercial and noncommercial reuse with a Creative Commons-By Attribution (CC-BY) license and can be found under http://feihnlab.ucdavis.edu/projects/LipidBlast.

**AUTHOR INFORMATION**

**Corresponding Authors**

*E-mail: tkind@ucdavis.edu.*

*E-mail: ofiehn@ucdavis.edu.*

**Notes**

The authors declare no competing financial interest.

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