IRMPD-based quantification of ER-catalyzed saturation and cyclopropanation reactions

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Introduction

The quantification of enzyme reactions with protein-linked substrates and products is highly challenging. Here we report a facile technique that allowed us to precisely quantify ratios of ACP-linked species in the ER-catalyzed saturation and cyclopropanation reactions by using the IRMPD-based method (**Figure 1**). This technique has wide application in natural product biosynthesis studies.

Reagents

Acyl-ACP samples and internal standards.

Equipment

ESI-FTICR-MS and reverse-phase HPLC.

Procedure

1. Prepare ESI-FTICR-MS samples by separating ACP samples from the reaction mixtures using the Source 15PRC reverse phase column (1).
2. Analyze freshly prepared samples by an actively shielded 7 Tesla quadrupole-FTICR mass spectrometer (APEX-Q, Bruker Daltonics, Billerica, MA). Infuse target analytes directly in electrospray solution (1:1 CH3CN:H2O with 0.1% HCOOH) into an electrospray ionization (ESI) source (Apollo II, Bruker Daltonics) operating in positive ion mode at a flow rate of 70 µL/h and a voltage of – 3.8 kV. Apply a counterflow of hot (240 degree) nitrogen gas to assist desolvation of ESI droplets. Externally accumulate multiply protonated ions generated by ESI in a hexapole and transfer them via high voltage ion optics to the ICR cell for analysis.
3. For IRMPD, accumulate precursor ions mass-selectively in the hexapole with a 5-10 m/z quadrupole isolation window, and transfer them to the ICR cell, and irradiate them for 100-300 ms by 10.6 µm photons at 10 W laser power (25 W CO_2 laser, Synrad, Mukilteo, WA).
4. Acquire data with XMASS software (version 6.1, Bruker Daltonics) in broadband mode from m/z = 200 to 2000 with 512k data points and summed over 10-30 scans. Analyze mass spectra using the MIDAS analysis software (2).
5. Measure peak abundances of PPant ejection products (PEPs) generated by IRMPD (3) to quantify the yields of the ER catalyzed saturation and cyclopropanation reactions

for time-course studies, or site mutagenesis assays. For ER saturation reaction, an addition of two hydrogens to the substrate is unlikely to affect ESI efficiency. Furthermore, products and substrates were observed with the same charge state distribution patterns, allowing a single charge state to be used for analysis (4). For Cur ER cyclopropanation, apply the ER saturation product as an internal standard to quantify the cyclopropanation product (with 2 Dalton mass difference). The ratio of these acyl-ACP species with 2 Dalton mass difference can be straightforwardly measured by analyzing their PEPs. With IRMPD, quantification results from a single charge state (+12) are identical to those based on an average of the entire charge state distribution. Thus, the +12 charge state can be utilized to simplify the IRMPD quantification analysis (**Figure 1**). The ratio of PEPs with 2 Dalton mass difference is calculated by measuring the abundance of the n and n+2 product ion peaks (the n+2 peak abundance is adjusted by subtracting the natural n+2 isotopic abundance for peak n). For saturation reactions, yields are calculated by measuring the ratios of substrates with respect to products whereas, for cyclopropanation reactions, yields are calculated by measuring the ratios of products with respect to the internal standard. Signal/noise ratios are typically ~10 but varied from 2-100 depending on the time point. All peak assignments are based on accurate mass. This IRMPD-based quantification has been validated by acyl-ACP standards (**Figure 2**).

Timing

Less than one hour.

References

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Acknowledgements

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Figures

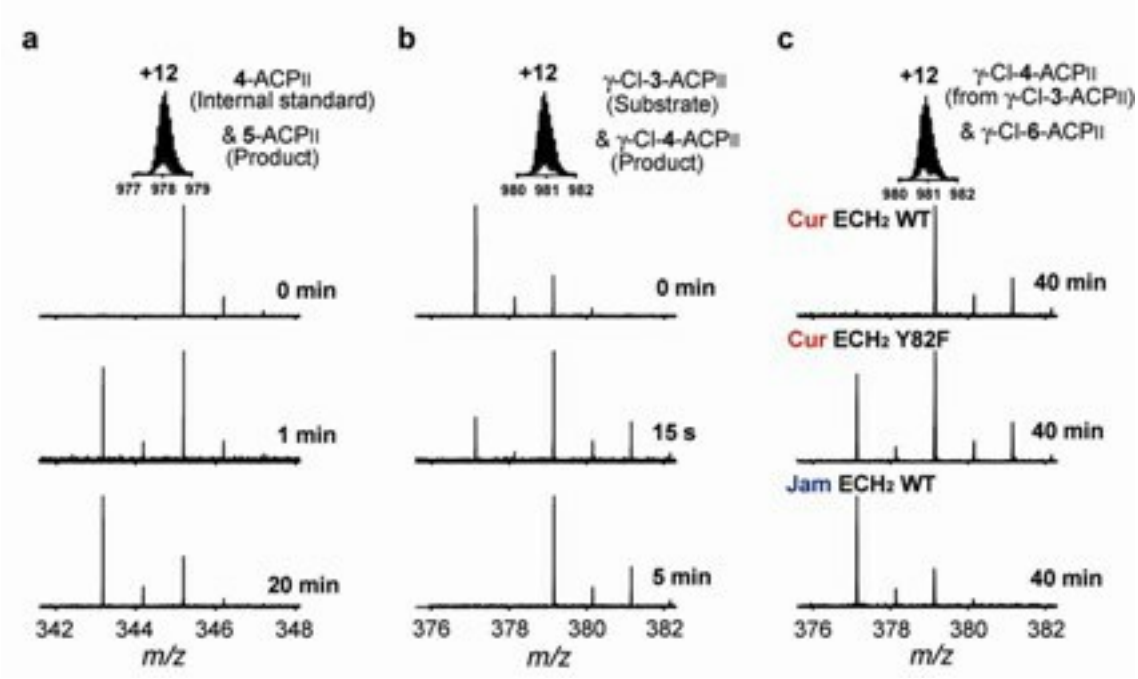

Figure 1

IRMPD-based quantification to measure the yields of a, Cur ER cyclopropanation, b, Jam ER saturation, and c, the ratio of alpha,beta and beta,gamma C=C products of ECH2s. a, To measure the yield of Cur ER cyclopropanation, 4-ACPII was added as internal standard for 5-ACPII. b, The yield of Jam ER saturation was measured by the ratio of gamma-Cl-3-ACPII substrate and -Cl-4-ACPII product. c, To measure the ratio of alpha,beta and beta,gamma C=C products, the alpha,beta C=C product was reduced by Jam ER by treating the mixtures of alpha,beta and beta,gamma C=C products with Jam ER for 30 min before IRMPD analysis. 3, 3-methylcrotonyl, 4, isovaleryl, 5, 2-methylcyclopropane-1-carboxyl, and 6, 3-methyl-3-butenoyl.

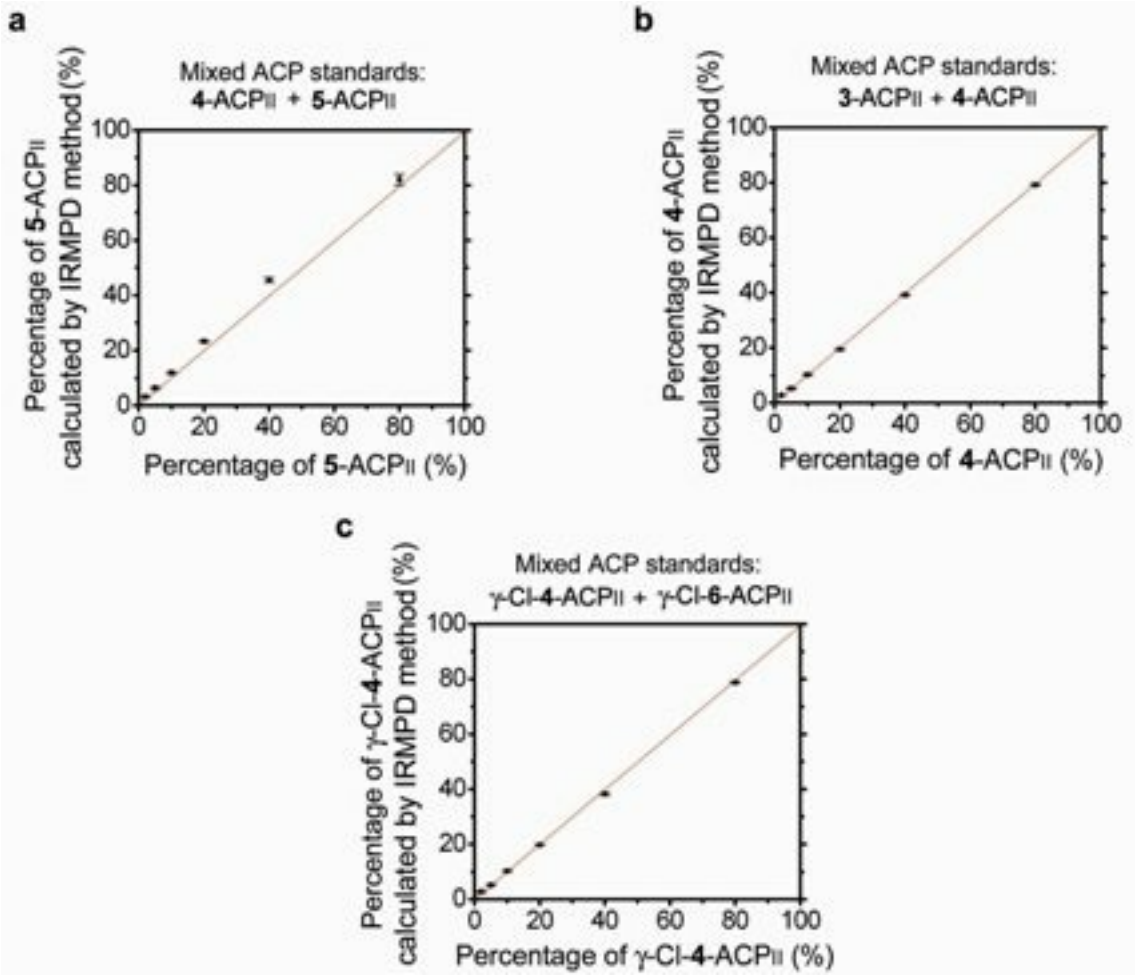

Figure 2

Validation of IRMPD-based quantification methods. 3-ACPII, 4-ACPII, and 5-ACPII were prepared by loading the corresponding CoA thioesters onto (apo) ACPII. gamma-Cl-4-ACPII and gamma-Cl-6-ACPII were enzymatically generated from 1-ACPII. The pairs of ACP standards were mixed with known ratios before the IRMPD analysis. Peak abundances of the PPant ejection products generated from the 12+ charge state of the pair of ACP standards were measured to calculate their ratio. The ratios of IRMPD products were compared with the known ratios of ACP standards. The assays were performed in triplicate, and standard deviation error bars are shown.

Metamorphic enzyme assembly in polyketide diversification

by Liangcai Gu, Bo Wang, Amol Kulkarni, +7
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