Steping into the dark matter of metabolomics: Quantification of protein-bound fatty acid synthesis intermediates in *Escherichia coli*.

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1. Introduction

Bacterial fatty acid biosynthesis (FAS) pathway is an important anabolic pathway, extensively studied as an antibiotic target and a source of biofuels. During the elongation process the acyl chain remains covalently attached to specialised acyl carrier protein (ACP) via post-translationally attached phosphopantetheine moiety. This unique feature blurs traditional division between proteomics and metabolomics, as pathway intermediates exist as variants of a single post translational modification.

2. Approach

We present a hybrid proteomics-metabolomics LCMS method capable of simultaneous detection of over 30 intermediates of fatty acid biosynthesis pathway (acyl-ACP) from Escherichia coli[1]. We apply this method to characterise state of the pathway under different growth conditions and study its response to nutritional upshifts and antibiotic treatment, demonstrating highly dynamic character of the pathway

Acyl-ACP is extracted from samples E. coli cultures by chloroform/methanol precipitation, proteolytically digested using protease GluC in presence of a detergent. Resulting peptide mixture is analysed by reversed phase LC coupled with QQQ MS operating in dynamic MRM mode. MRM transitions were developed in silico and peak identities verified by triggered MRM, isotopic labeling and biological controls. Isotopically-labeled internal standards prepared as extracts of E. coli grown on 15N-labeled minimal media were used to correct for analytical variation during the measurements.

3. Results

Highly acidic character of E. coli ACP results in low number of tryptic cleavage sites and phospho-pantetheinylated peptide incompatible with direct detection. Use of protease GluC specific for glutamic acid residues results in 11 amino acid long modified peptide that is viable for detection with MRM. We demonstrate that successful digestion and detection of very hydrophobic peptides binding long-chain acyl chains requires use of a detergent during digestion.

Dynamic character of thioester bonds linking acyl chain to phosphopantetheine group requires fast quenching during sampling to prevent ex-vivo alteration of acyl-ACPs. We show that sampling into TCA solution (2% final) prevents alteration of acyl-ACP pools observed when samples are harvested by centrifugation.

We apply the method to demonstrate that treatment of bacterial culture with antibiotic cerulenin results in significant changes in acyl-ACP species already 5s after treatment, showing highly dynamic character of the pathway.

We demonstrate that during steady-state growth using different carbon sources total amount of acyl carrier protein do not change significantly with the growth rate, however the growth rate correlation is visible for majority of acyl-ACP intermediates in the pathway. Interestingly, final products of the pathway, used as substrates for phospholipid synthesis do not correlate with the growth rate suggesting that phospholipid synthesis flux is not regulated by substrate levels. Following nutritional upshift, acyl-ACP species undergo significant changes within one minute after upshift, showing increased levels of intermediates especially in unsaturated branch of the pathway.

4. Discussion

Specific chemical character of FAS intermediates makes them unavailable for analysis using generic metabolomics or proteomics approaches. We demonstrate that successful quantitation can be achieved using specialised method. This result paves the way to investigate acylation state of FAS intermediates in other species, including human FAS, being important but unexplored cancer biomarker.

Results indicate LC-MS may be used to study different carrier proteins which opens the way for studying human fatty acid synthases in a similar way. other protein-bound metabolites, for example acylation state of lipoate-carrier protein (E2 protein of pyruvate dehydrogenase complex or protein H of glycine cleavage system) that would enable studying these tetrahydrofolate-dependent systems from unique, complementary perspective.

References

1. M.J. Noga, et al. “Mass-Spectrometry-Based Quantification of Protein-Bound Fatty Acid Synthesis Intermediates From Escherichia Coli..” *Journal of proteome research* 15(10):3617–23. 2016