Absolute Quantification of Metabolic Enzymes via Targeted Proteomics using a new Kit of SpikeTides™ Peptides

F. Bindel, G. Mastrobuoni, A. Eisenberger, S. Kempa

Integrative Metabolomics and Proteomics Laboratory, Berlin Institute of Medical Systems Biology, Max Delbrueck Center, Robert-Roessle-Str. 25, 13125 Berlin

A panel of metabolic enzymes involved in the central carbon metabolism that are often deregulated in cancer was reliably quantified with the help of a set of stable isotope labeled (SIL) and absolutely quantified SpikeTides™ peptides. To establish the process, using proteomics data and bioinformatics predictions, we established a set of reliably detectable and quantifiable peptides. The absolute quantities of metabolic enzymes are important input parameters for computational models of biological processes and crucial for a metabolic characterization of tumour tissues.

Introduction

The central carbon metabolism (CCM) is the major pathway for the energy generation and biomass production for a cell. It is highly regulated on different levels and is prone to changes during tumorigenesis. The most important metabolic phenotype of cancer is aerobic glycolysis – also known as the Warburg effect. The Warburg effect is characterized by a higher glycolytic flux, leading to higher lactate secretion even with ample supply of oxygen. A diverse set of processes can result from aerobic glycolysis[1], which can be accompanied with changes of expressed proteins and/or variation in their abundance. The determination of absolute abundances of involved metabolic enzymes is an inportant prerequisite for cancer phenotyping based on these enzymes. Additionally, this knowledge helps to further refine mathematical models estimating metabolic fluxes, as the metabolic flux can be estimated through Michaelis-Menten rate law[2].

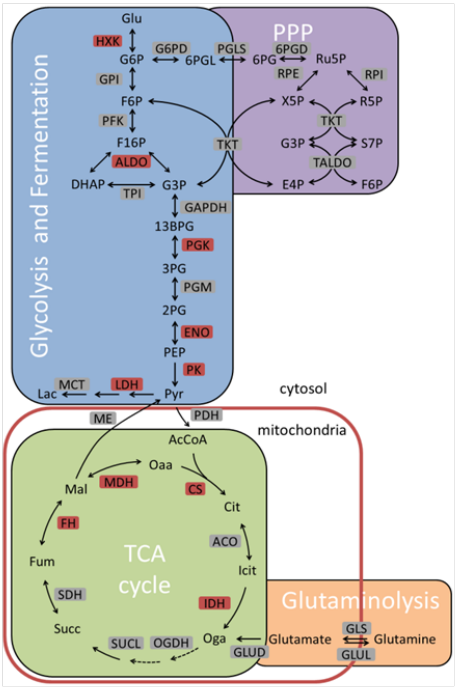

Figure 1: Schematic representation of the central carbon metabolism and the covered metabolic enzymes marked in red.

Results

A panel of metabolic enzymes highly relevant in cancer phenotyping was selected (see Figure 1 and Table 1).

No.	Protein Name	# Peptides / Protein
1	Isocitrate dehydrogenase [NAD] subunit beta	3
2	Citrate synthase, mitochondrial	2
3	Isocitrate dehydrogenase [NADP] cytoplasmic	2
4	L-lactate dehydrogenase A chain	3
5	Phosphoglycerate kinase 1	2
6	Fructose-bisphosphate aldolase A	2
7	Glyceraldehyde-3-phosphate dehydrogenase	2
8	Fructose-bisphosphate aldolase B	2
9	Alpha-enolase	2
10	Fumarate hydratase, mitochondrial	2
11	Pyruvate dehydrogenase E1 component subunit α	2
12	Gamma-enolase	2
13	Fructose-bisphosphate aldolase C	2
14	Beta-enolase	2
15	Pyruvate kinase PKM	3
16	Phosphoglycerate mutase 1,2,4	1
17	Hexokinase-1	1
18	Hexokinase-1	2
19	Malate dehydrogenase, cytoplasmic	4
20	Isocitrate dehydrogenase [NAD] subunit alpha	2
21	Isocitrate dehydrogenase [NAD] subunit gamma	2
22	Hexokinase-2	2
23	Probable D-lactate dehydrogenase, mitochondrial	2
24	Isocitrate dehydrogenase [NADP], mitochondrial	2

Table 1: Metabolic Enzymes quantified in the study.

The identification of suitable proteotypic peptides was done with great care as this is a crucial step in targeted proteomics.[3] As a first step, different in-depth proteome datasets (either generated in-house or publically available[4]) were used. After filtering for tryptic sequences that are unique for CCM-proteins, peptides carrying potential PTMs (e.g. phosphorylation, cysteines, methionines, etc.) were discarded where possible. Furthermore, peptide intensity and detectability was taken into account when more candidate sequences were available.

After transition-specific collision energy optimization, a mixture of 51 stable-isotope labelled SpikeTides™ peptides (1-4 peptides per protein, see Table 1 for protein list) was used in a serial dilution for the determination of the linear dynamic quantification range of each peptide (see Figure 2 for a typical response curve). Additionally, background compatibility of each peptide was checked by spiking different amounts of the peptide mix into a constant complex background.

For the absolute quantification of metabolic enzymes the AQUA strategy[5] was used. Absolutely quantified SIL peptides were obtained after tryptic digestion of respective SpikeTides™_TQL peptides.[6] The chosen peptides were developed to absolutely quantify the corresponding proteins in cell cultures and human tissues.

SpikeTides™ Sets – heavy - quantified

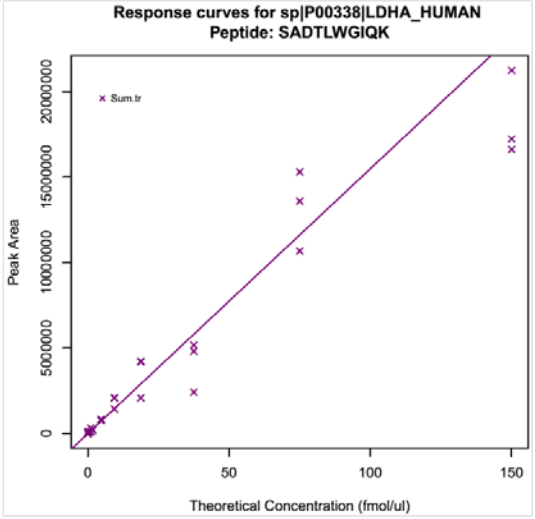

Figure 2: Snapshot of the QuaSAR report for the peptide SADTLWGIQK belonging to the protein LDHA.

Discussion & Conclusions

For absolute quantification of proteins by targeted proteomics, the linear dynamic quantification range is crucial for the detection of absolute values. The herein used SpikeTides™ Set Metabolic Enzymes – heavy – quantified offers the possibility to absolutely quantify metabolic enzymes. After proving the suitability of the linear dynamic range, the peptides were successfully used for the determination of absolute abundance of selected proteins in selected cell lines. This metabolic enzymes kit allows the absolute quantification of several CCM enzymes in diverse cancer cells. It can be easily extended to cover more proteins of interest and is easily implemented in an in-solution digestion workflow.

Materials & Methods

The peptides from the SpikeTides™ Set Metabolic Enzymes – heavy – quantified (JPT) were dissolved as described in the JPT instruction manual. A MasterMix (MM) containing 50 nM of each peptide was digested with Trypsin at room temparature for 16 h in order to remove the quantification tag and used for the SRM assay generation. The program Skyline[7] (Version ranging from 1.5 to 2.5) was used for SRM method generation and collision energy optimization[8]. Corresponding transitions were calculated with the following settings: precursor charge states were set to 2 and 3. Corresponding fragment ion charge states were set to 1 and 2. Additionally, only y-ions and products ions ranging from "m/z < precursor" to "last ion" were monitored. Peptides were detected on a nanoLC (nanoLC Ultra 1D+, Eksigent) coupled to a triple quadrupole (TSQ Vantage, ThermoScientific). The TSQ Vantage was equipped with a nanospray ion source (Nanospray Flex Proxeon Ion Source, Thermo). A spray voltage of 1.8-2.1 kV was used for the electro spray ionization. MS spectra were acquired with a mass range of 300-1500 m/z with a resolution 0.7 amu. 1.5 mTorr of Argon was used for fragmentation. Skyline and QuaSAR[9] (Version 1.2) were used for data analysis. The R^2 value of each peptide was calculated with QuaSAR. An optimized MM was spiked in prior to the digestion of a MDA-MB-231 cell line sample and measured in biological triplicates.

References

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The Author

Fabian Bindel
fabian.bindel@mdc-berlin.de
Integrative Metabolomics and Proteomics Laboratory, Max Delbrueck Center
Berlin, Germany

Fabian Bindel studied "Technical Biology" at the University of Stuttgart and did his PhD at the Lab of Dr. Kempa of Integrative Proteomics and Metabolomics located at the Berlin Institute of Medical Systems Biology. There he set up a targeted proteomics workflow in order to absolutely quantify different metabolic enzymes. He aims to continue SRM-driven research.

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