Routine Analysis of Host Cell Proteins in Antibody Preparations using PASEF

ASMS 2018, MP 048

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Introduction

In the rapidly expanding arena of biotherapeutic analysis and bioprocess development, the analysis of host cell proteins (HCP) at the ppm level is critical. Immunospecific assays such as ELISA and non-targeted methods such as mass spectrometry are currently employed for this purpose. While ELISA is currently the gold standard for QC applications, the advantages of mass spectrometry are abundant and it has the potential to eventually become the favored approach for this analysis.

In this work we show how PASEF (parallel accumulation and serial fragmentation) scans, as implemented on the timsTOF PRO QTOF (Fig 1), can be applied to HCP analysis to achieve the goal of sensitive detection with enhanced speed and data quality.

Methods

The NISTmAb Reference Material 8671 and the Universal Proteomics Standard (UPS1, Sigma) were reduced using DTT in TFE solution and alkylated with iodacetamide prior to overnight digestion with trypsin (Promega). Peptides were separated on an Intensity Solo 2 1.8µm C18 100 x 2.1 mm column using an Elute UHPLC coupled to a timsTOF Pro ion mobility QTOF mass spectrometer (all Bruker Daltonics). A 150 minute gradient was used in a total runtime of 165 minutes. For nanospray, a nanoElute UHPLC was fitted with an IonOpticks 25 cm x 75 µm 1.6 µm C18 column using a 210 minute gradient. PASEF scans were recorded and searched against the mouse SwissProt database using Mascot.

Results: Standard flow UHPLC

The UPS1 standard was used to make a 5-step 1:3 dilution series in a constant background of NIST mAb over a concentration range from 0.3 to 934 ppm. Each dilution was measured in triplicate and the amount of NIST mAb loaded on column was 25 µg for each injection. PASEF enabled the detection of UPS1 proteins down to low single digit ppm concentrations in the presence of 25 µg NIST mAb. Figure 2 shows the linear response for the UPS1 protein beta-2-microglobulin in the concentration range from 132 ppm to 1.6 ppm.

The dilution series indicated that PASEF enabled detection of HCPs in the range of 1 to 100 ppm of the therapeutic protein, which usually requires special approaches such as 2D-LC or the use of libraries. In a model experiment, 30 µg NIST mAb was loaded onto the column to measure HCPs in the NIST mAb sample. This resulted in the detection of 8 proteins with 3 or more peptides and a further 17 proteins with 2 peptides, at 1 % FDR (Fig. 3). These results illustrate the use of PASEF to identify HCPs using a routine 1D-UPLC-MS configuration.

Results: nano flow UHPLC

A standard proteomics setup using nano UHPLC was also evaluated for its suitability for HCP identification. This setup has already been established as the new benchmark for bottom-up proteomics applications (1, 2). In comparison to other nano LC setups, robustness is improved by the CaptiveSpray ion source, which allows ions to be sprayed directly from the emitter for increased sensitivity. Figure 4 shows that > 200 HCPs were identified in 1.5 µg NIST mAb, including expected and previously unreported HCP proteins (Fig. 5). As shown in Figure 6 the depth of HCP coverage and sequence coverage was further extended to >280 HCP identifications by employing an alternate digestion method in which only the HCPs are digested allowing the mAb to be removed prior to analysis (3).

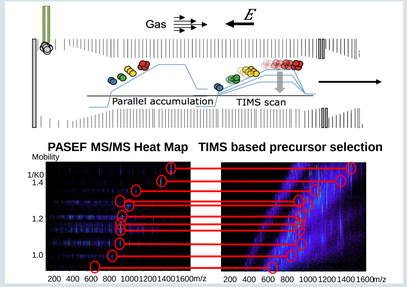

Fig. 1 Top: Accumulation and focusing of precursor ions in the TIMS cell. Bottom: PASEF scan allows 2 dimensional precursor isolation

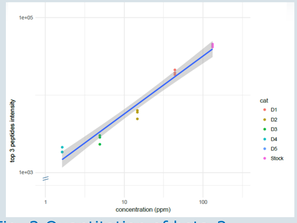

Fig. 2 Quantitation of beta-2-microglobulin Top 3 quantitation was performed using PEAKS Studio software (BSi)

Protein	#Peptides	Seq. Cov. [%]	Scores	MW [kDa]	pI
Fructose-bisphosphate aldolase A	20	64,3	902.6	39,3	8,3
Fructose-bisphosphate aldolase C	13	47,1	679.4	39,4	6,7
Glucose-6-phosphate isomerase	10	24,6	322.5	62,7	8,1
Protein disulfide-isomerase A6	5	13,4	222.3	48,1	5,0
Granulins	4	12,1	103.6	63,4	6,4
Beta-2-microglobulin	3	22,7	116.8	13,8	8,6
Low affinity immunoglobulin gamma Fc region receptor II	3	10,0	75.0	36,7	6,2
Adenylate kinase 2, mitochondrial	3	17,2	65.8	26,5	7,0

Fig. 3 HCPs identified in NIST mAb with >3 peptides using analytical LC

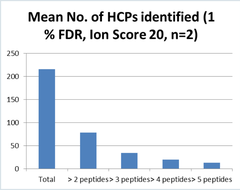

Fig. 4 HCPs detected in NIST mAb using nano LC

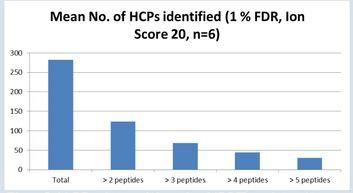

Fig. 6 HCPs identified in NIST mAb after digestion with alternate method (3) using nano LC

Protein	#Peptides	S.C. [%]
Fructose-bisphosphate aldolase A (i)	32	76.9
Fructose-bisphosphate aldolase C	19	64.7
* Glucose-6-phosphate isomerase	19	50.4
Granulins	14	42.8
Papilin	11	12.7
Hepatocyte growth factor-like protein	9	16.2
Polypeptide N-acetylgalactosaminyltransferase 6	8	20.6
Peptidyl-prolyl cis-trans isomerase FKBP2	7	48.6
Protein disulfide-isomerase A6	6	12.7
Syntaxin-12	5	27.0
Adenylate kinase 2, mitochondrial	5	33.1
Heterogeneous nuclear ribonucleoproteins A2/B1	5	15.9
NSFL1 cofactor p47	5	20.0
* Titin	5	0.2
Clathrin interactor 1	4	6.7
Adenylyl cyclase-associated protein 1	4	16.2
Beta-2-microglobulin	4	16.0
Eukaryotic translation initiation factor 4B	4	9.5
Ubiquitin-conjugating enzyme E2 variant 2	4	37.9
* Protein NipSnap homolog 3B	4	25.1
Heterogeneous nuclear ribonucleoprotein A1 (ii)	3	12.8
Methionine--tRNA ligase, cytoplasmic	3	6.0
Semaphorin-4B	3	3.4
* Fumarate hydratase, mitochondrial	3	12.0
Stress-induced-phosphoprotein 1	3	6.1
Heterogeneous nuclear ribonucleoprotein A/B	3	16.1
Properdin	3	9.5
Protein ABHD11	3	21.5
* Nucleoprotein TPR	3	2.1
Protein enabled homolog	3	4.4
* Ubiquitin-60S ribosomal protein L40	3	29.7
* 40S ribosomal protein S12 (iii)	3	21.2
* Dapper homolog 3	3	1.8
Polypeptide N-acetylgalactosaminyltransferase 2	3	5.1
* Bcl-2-associated transcription factor 1	3	3.8
* Pancreatic lipase-related protein 2	3	13.1

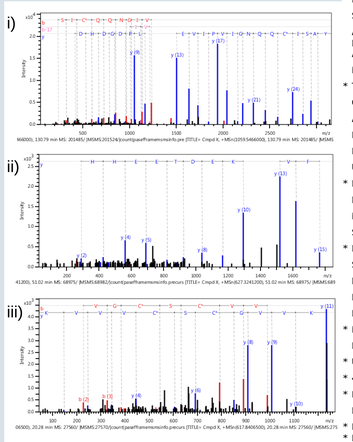

Fig. 5 Abridged list of HCPs detected in NIST mAb using nano LC. Example MSMS spectra shown on left for HCPs marked i-iii. *Previously unreported HCPs

References

(1) Meier et al.; J Proteome Res. 2015 Dec 4;14(12):5378-87

(2) Lubeck et al.; Bruker Application Note 131

(3) Huang et al.; Anal. Chem. 2017, 89, 5436-5444

Conclusions

- PASEF scans improve the sensitivity of routine peptide mapping enabling the detection of HCPs at required sub 100 ppm levels
- PASEF coupled to nanoLC facilitates detection of previously unreported trace level HCPs to help interpret ELISA results
- The quality of MS/MS sequence spectra provided by the timsTOF Pro with PASEF allows high confidence in protein ID even with only 1-2 peptides
- The depth of HCP identification provided by PASEF technology allows fingerprinting of biomanufacturing processes and the ability to easily identify and monitor the affects of changes in these procedures

Technology