Performance comparison of sublimated matrix and dried-droplet MALDI preparations for 2D-PAGE–based proteomics

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Introduction

- Optimized sample preparation prior to MS is crucial for successful and sensitive detection of peptides by MALDI-TOF. To address this, we have developed a novel technology for peptide sample preparation that uses chips with pre-deposited matrix spots of sub-micron sized CHCA crystals vacuum-sublimated onto an ultraphobic surface (Mass·Spec·Turbo Chips, QIAGEN; see below)
- Aqueous samples containing up to 50% ACN can be directly applied on top of the matrix spots, where they adsorb to the large surface area formed by the ultra-fine sublimated matrix crystals. Due to this sample concentration effect, a greater than 10x increase in sensitivity can be obtained
- The relative success of a sample preparation strategy can be measured by the increase in scoring for database search software such as MASCOT, reflecting increasing confidence of protein identification
- Here we present application data for a exemplary gel-based proteomic approach. Proteins of a human colon cancer carcinoma cell line were separated by 2D-PAGE and the protein ID results (No. of identified proteins, total and peptide Mascot score) of 50 randomly selected spots with differing pI, MW, and abundance were compared after sample preparation on steel targets or on the chip matrix.

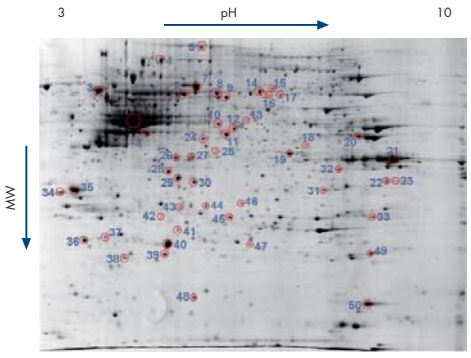
Mass·Spec·Turbo Chips: pre-deposited matrix spots containing sub-micron size CHCA crystals prepared by vacuum sublimation onto an ultraphobic surface.

Methods

- Cell culture: Cells of the premalignant human colonic PC/AA adenoma cell line (Williams et al., 1990) were grown in DMEM + Glutamax-I (4.5 g/l Glucose) at 37°C and 5% CO2.
- Sample preparation for 2D-PAGE: Cells were washed in PBS, lysed in standard 2D lysis buffer and centrifuged. After protein determination, an aliquot of 250 µg protein of the cleared lysate was separated on large format 2D-PAGE (pH 3–10 NL) under standard conditions using IPGphor and EttanDalt six electrophoresis equipment (GE Healthcare). Protein alkylation (DTT, iodoacetamide) was carried out between the first and second dimension. Protein spots were visualized by colloidal Coomassie (G-250) staining.
- Protein digestion: 50 randomly chosen spots were picked manually and prepared for digestion by successive washing of the gel plugs (water, 50% ACN, 100% ACN). Proteins were then digested with trypsin (15 ng/ml; Promega) overnight at RT and subsequently acidified by addition of TFA (final conc. 0.3%)
- Sample preparation for MALDI-TOF: 0.5 µl of the eluted peptides was mixed with the same volume of CHCA matrix (5 mg/ml in 66% ACN / 33% TFA [0.1%]) on a stainless steel plate (dried-droplet preparation) or 1 µl of the digest was applied directly on the sublimated chip matrix, dried down, and washed with finishing solution.
- Data acquisition and protein identification: Spectra (peptide mass fingerprints plus 6 result dependent MSMS spectra per spot) were acquired on a 4800 MALDI-TOF/TOF instrument (Applied Biosystems) under optimized conditions for both preparation types. Peak lists were extracted employing the "Peaks to Mascot" algorithm with the same parameters for both runs. Proteins were identified using the Mascot search engine to query the IPI_human database.

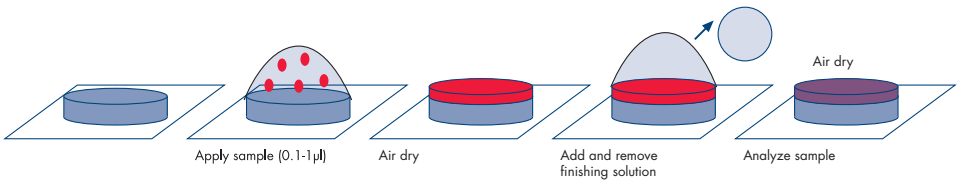
Reference: Williams AC, Harper SJ, Paraskeva C. (1990) Neoplastic transformation of a human colonic epithelial cell line: in vitro evidence for the adenoma to carcinoma sequence. Cancer Res. **150**. 4724.

2D-PAGE gel and protein identifications



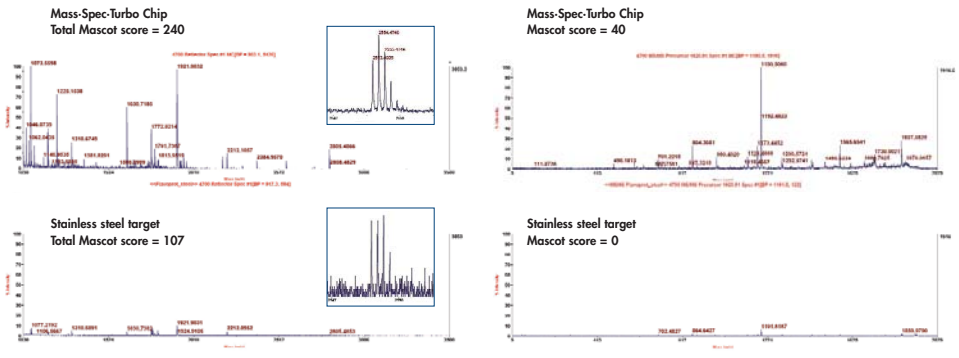
High-resolution 2D-PAGE separation of 250 µg protein from colon cancer cell lysate.

Spot no.	Protein name	Spot no.	Protein name
1	ACTB Actin	26	acidic ribosomal prot. RPLP0
2	KRT19 Keratin	27	Isoform 1 of Pyridoxal kinase
3	Tubulin beta-2C	28	PPA1 Inorganic pyrophosphatase
4	HSPA9	29	Annexin A3
5	Gelsolin, isoform 2	30	annexin IV
6	KRT8 Keratin	31	Electron transfer flavoprotein subunit alpha
7	PDIA3 55 kDa protein	32	Voltage-dependent anion-selective channel protein
8	Mitochondrial aldehyde dehydrogenase 2	33	Electron transfer flavoprotein subunit beta
9	Heterogeneous nuclear ribonucleoprotein H	34	(Keratin, type I) contaminant
10	Aminoacylase-1	35	tropomyosin 3 isoform 5
11	Macrophage capping protein	36	TPT1 Tumor protein, translationally-controlled 1
12	SERPINB1	37	Catechol O-methyltransferase
13	Adenosylhomocysteinase	38	ATP5H Isoform 1 of ATP synthase D chain
14	CCT2 T-complex protein 1	39	Peroxiredoxin-2
15	Retinal dehydrogenase 1	40	GSTP1 Glutathione S-transferase P
16	RUVBL1	41	Proteasome subunit beta type 4
17	-	42	Heat-shock protein beta-1
18	Sialic acid synthase	43	Platelet-activating factor acetylhydrolase IB
19	Annexin A1	44	Proteasome subunit beta type 7
20	Fructose-bisphosphate aldolase A	45	ECHS1 Enoyl-CoA hydratase
21	GAPDH	46	Glutathione transferase omega-1
22	Voltage-dependent anion-selective channel protein 1	47	PARK7 Protein DJ-1
23	Hydroxyacyl-coenzyme A dehydrogenase	48	-
24	Serpin B5	49	PRDX1 Peroxiredoxin-1
25	(Keratin, type I) contaminant	50	CFL1 Cofilin-1



Two-step MALDI sample preparation process

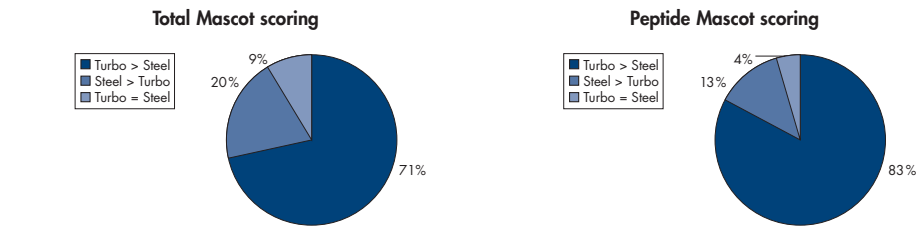
MALDI MS analysis of 2D-PAGE gel samples



MS spectra of spot # 31 **MS/MS spectra of the m/z = 1920 precursor**

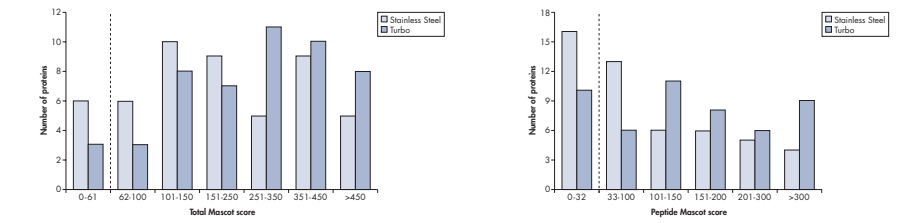
Direct comparison of A MS spectra and B MS/MS spectra of precursor ion m/z = 1920 of spot no. 31, identified as Electron transfer flavoprotein, subunit alpha. Inset: Close-up of m/z = 2553 peak (123–146 VAAKLEVAPISDIIAIKSPDTFVR) after sample application on Mass·Spec·Turbo Chip (top) or stainless steel (bottom). Note that signal intensity on stainless steel is too weak for peak annotation under given parameters.

Statistical analysis of protein identification performance



Comparison of individual protein identification performance between stainless steel and chip-based sample preparation (total Mascot score).

Comparison of individual protein identification performance between stainless steel and chip-based sample preparation (peptide Mascot score).

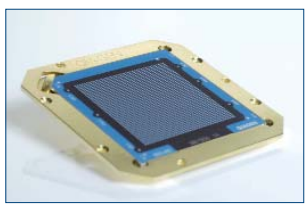


Total Mascot score distribution for the 50 selected proteins obtained using Mass·Spec·Turbo Chip and stainless steel target. Broken line indicates significance limit for the IPI_human database (Mascot protein score = 61).

Peptide Mascot score distribution for the 50 selected proteins obtained using Mass·Spec·Focus Chip and stainless steel target. Broken line indicates significance limit for the IPI_human database (Mascot peptide score = 32).

Mascot score is a measure of the significance of protein identification and increases if — for a constant mass accuracy, sensitivity and sequence coverage — the number of characteristic fragment peaks can be enhanced simultaneously.

Summary



- In an exemplary study, protein identification performance of Mass·Spec·Turbo Chips was compared to that of classical dried-droplet preparations on stainless steel plates by randomly selecting 2D-gel spots. Of 50 selected spots, 47 proteins were identified using the chip-based approach, whereas 45 were identified on stainless steel targets.
- Comparison of spectra demonstrated higher sensitivity using the pre-spotted MALDI chips vs. standard dried-droplet preparations for the analysis of peptides in MALDI-TOF MS and MS/MS, especially in the higher mass range.
- Statistical analysis of the Mascot results demonstrated that in more than 2/3 of the cases, Mascot database searches provided significantly higher total and peptide (MS/MS) scores (and therefore higher confidence levels) for samples prepared on Mass·Spec·Turbo Chips.
- These properties together with the support of high-throughput automation make Mass·Spec·Turbo Chips well-suited for use in larger proteomics projects.

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