Proteomic analysis by MALDI-TOF mass spectrometry and its application to HL-60 cells

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Our research is concentrated on identification of newly synthesized and/or modified nuclear proteins during HL-60 cell differentiation. These proteins can be involved in cell differentiation signaling or can be responsible for reorganization of the nucleus at the maturation stage of HL-60 cells. The HL-60 human promyelocytic leukemia cell line was induced to differentiate by retinoic acid. To analyze changes in the steady-state amount of individual proteins, total nuclear proteins were fractionated by 2-DE and stained with Brilliant Blue R-250. Proteins of interest were cut out from the gel, in-gel digested with trypsin, supplied for MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometer) analysis, and mass information generated from the spectrum was submitted to a search performed with databases.

Key words: proteomic, MALDI-TOF MS, HL-60, nuclear proteins

INTRODUCTION

Much attention has been focused on the attempt to undertake global analysis of protein expression and in particular to analyze the changes in protein expression associated with, for example, disease states, knock-out of individual genes, drug treatments and changing extracellular conditions. By transducing extracellular signals from cell surface to the nucleus the incoming signals influence enzymatic activities, DNA binding, and transcription of other proteins, as well as the subcellular localization of these actions, and such activities cause G_0/G_1 cells to enter the commitment and differentiation stages of development [1–4]. In general, cell differentiation- or proliferation-specific gene expression requires *de novo* protein synthesis and/or post-translational modifications, in particular phosphorylation of proteins such as transcription factors. Consequently, there is a need to identify the proteins that are being modified, as well as to know when and where in the cell modifications occur.

In this paper we present a proteomic approach, which enables us to identify novel nuclear proteins that might be involved in the retinoic acid (RA) signaling events mediating the granulocytic differentiation and/or apoptosis of promyelocytic HL-60 cells. The proteomic approach empowers us to concentrate in the future on determination of protein localization, protein structure analysis, prediction of protein function, etc.

MATERIALS AND METHODS

Cell culture. Human promyelocytic leukemia HL-60 cells were cultured in RPMI 1640 medium (Gibco BRL, Life Technologies) supplemented with 10% of fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml of streptomycin (NordCell, Sweden) in a 5% CO_2-supplemented incubator at 37 °C. Granulocytic differentiation was induced with 1 μM retinoic acid (Sigma).

Isolation of nuclear proteins. Nuclear proteins were isolated as described by Kulytė et al. [5].

Gel electrophoresis and staining. The nuclear proteins were resolved by two-dimensional gel electrophoresis (IEF/SDS). Immobiline DryStrip Kit, pH range 3–10, and Exel Gel SDS, gradient 8–18% (Pharmacia Biotech, Uppsala, Sweden) were used for 2-DE. It was performed according to the manufacturer's instructions. For analysis of total nuclear proteins, 2-DE gels were stained with Brilliant Blue R-250 (Sigma) according to the manufacturers' instructions.

In-gel digestion and MALDI-TOF MS. Areas of interest were cut out from the gel and subjected to

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overnight in-gel tryptic digestion [6, 7]. Briefly, the gel slices were dehydrated with 50% acetonitrile and then dried completely using a centrifugal evaporator (DNA Mini, Eppendorf). The protein spot was rehydrated in 20 µl of 25 mM ammonium bicarbonate (pH 8.3) containing 20 µg/ml of modified trypsin (Promega), and the samples were incubated overnight at 37 °C. The tryptic peptides were subsequently extracted from the gel slices as follows. Any extraneous solution remaining after the digestion was removed and placed in a fresh tube. The gel slices were washed two times with 5% trifluoracetic acid in 50% acetonitrile, shaking occasionally. The digestion and extract solutions were then combined and evaporated to dryness. For MALDI-TOF analysis, the peptides were redissolved in 3 µl of 30%

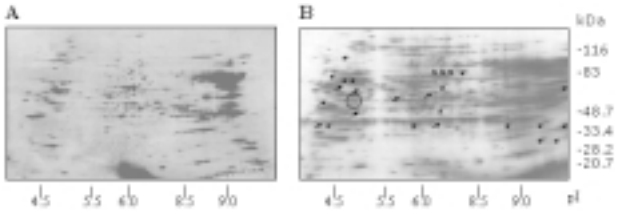
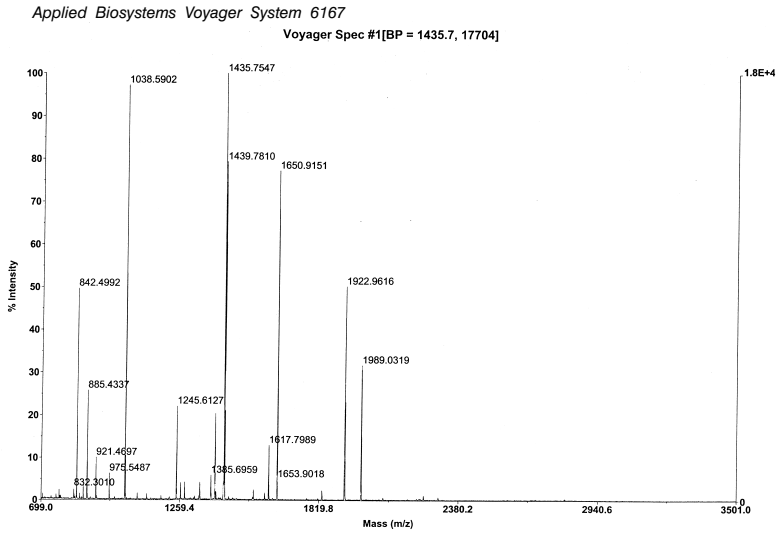


Fig. 1. Two-dimensional electrophoretic patterns of total nuclear proteins of proliferating and differentiating HL-60 cells. Total nuclear proteins were isolated from proliferating cells (A) and from HL-60 cells induced to differentiation with retinoic acid (B). Proteins were fractionated by 2-DE and then stained with Brilliant Blue R-250. Arrows in (B) show proteins that are absent in proliferating cell nucleus. These proteins were supplied for MALDI-TOF MS. In this study we have presented the proteomic analysis only of protein marked by circle

A



B

PeptIdent

Peptide mass fingerprinting

Name given to unknown protein:	51-020221
Species searched:	HOMO SAPIENS (HUMAN)
Database searched:	SWISS-PROT
pI: 4.85	**range:** 3.85 - 5.85
Mw: 52000	**range:** 41600 - 62400
Peptide masses for unknown protein:	760.5046 772.5851 825.2887 842.6515 845.2465 855.2023 861.2221 877.1879 885.6059 921.6473 975.7098 1038.7916 1245.8376 1262.8678 1341.3298 1385.973 1401.9646 1406.9226 1436.0349 1440.0749 1458.0624 1602.1387 1618.13 1651.2459 1922.339 1988.4326 1990.4275
Tolerance:	±200 ppm
Minimum number of peptides required to match:	2
Maximum number of matching proteins to print:	20

Using **monoisotopic** masses of the occurring amino acid residues and interpreting your peptide masses as **[M+H]$^+$**.
Enzyme: **Trypsin**, allowing for **up to 1 missed cleavages (#MC).**
Cysteine treated with **Iodoacetamide** to form carbamidomethyl-cysteine (Cys_CAM).
Scan done on 01-Mar-2002.
SWISS-PROT Release 40.11 of 21-Feb-2002: 105322 entries

Score: 0.37, 10 matching peptides: P06576 (ATPB_HUMAN) pI: 5.00, Mw: 51769.25
CHAIN 1: ATP SYNTHASE BETA CHAIN. - Homo sapiens (Human).

GlycoMod | Find Mod | FindPept | PeptideMass | BioGraph

user mass	matching mass	Δmass (ppm)	#MC	modification	position	peptide
975.7098	975.5622	-151.45	0		202-212	IGLFGGAGVGK
1038.7916	1038.5942	-190.23	0		134-143	IPVGPETLGR
1262.8678	1262.6409	-179.83	0		110-121	TIAMDGTEGLVR
1385.973	1385.7093	-190.41	0		144-155	IMNVIGEPIDER
1406.9226	1406.6811	-171.79	0		226-239	AHGGYSVFAGVGER
1436.0349	1435.754	-195.76	0		311-324	FTQAGSEVSALLGR
1440.0749	1439.7893	-198.47	0		282-294	VALTGLTVAEYFR
1458.0624	1457.8396	-152.92	0		213-225	TVLIMELINNVAK
1651.2459	1650.9173	-199.13	0		95-109	LVLEVAQHLGESTVR
1922.339	1921.9654	-194.45	0		295-310	DQEGQDVLLFIDNIFR

ΔpI: 0.15, ΔMw: 230.8 Da (0.4%)
27.0% of sequence covered:

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     1         11        21        31        41        51
     |         |         |         |         |         |
   1                                          aqt spspkagaat    60
  61 grivavigav vdvqfdeglp pilnalevqg retrLVLEVA QHLGESTVRT IAMDGTEGLV   120
 121 Rgqkvldsga pikIPVGPET LGRIMNVIGE PIDERgpikt kqfapihaea pefmemsveq   180
 181 eilvtgikvv dllapyakgg kIGLFGGAGV GKTVLIMELI NNVAKAHGGY SVFAGVGERt   240
 241 regndlyhem iesgvinlkd atskvalvyg qmneppgara rVALTGLTVA EYFRDQEGQD   300
 301 VLLFIDNIFR FTQAGSEVSA LLGRipsavg yqptlatdmg tmqeritttk kgsitsvqai   360
 361 yvpaddltdp apattfahld attvlsraia elgiypavdp ldstsrimdp nivgsehydv   420
 421 argvqkilqd ykslqdiiai lgmdelseed kltvsrarki qrflsqpfqv aevftghmqk   480
 481 lvpiketikg fqqilageyd hlpeqafymv gpieeavaka dklaeehss
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Fig. 2. Proteomic analysis by using MALDI-TOF MS of proteins from differentiating HL-60 cells. Peptide mass information generated from the composite spectrum (A) was submitted to a search performed with the Protana, EXPASY and EMBL database, using the MS-Fit, PeptIdent and PeptidSearch algorithms. B – results from EXPASY database

acetonitrile and 0.01% trifluoracetic acid and were then prepared with a matrix (α-cyano-4-hydroxycinnamic acid) on the target plate. The analysis was performed on a Voyager MALDI-TOF MS (Perspective Biosystems Inc., Town State) and externally calibrated using synthetic peptides with known masses. The spectra were obtained in the positive ionization mode at 25 kV. The mass information generated from the composite spectrum was submitted to a search performed with the Protana, EXPASY or EMBL database.

RESULTS AND DISCUSSION

In this study, we present the proteomic approach for identification of nuclear proteins of HL-60 cells. Separation of nuclear proteins of proliferating and differentiated HL-60 cells by 2DE was performed by using a wide pH range IPG strip (pH 3–10). These 2D maps are shown in Fig. 1. Optimized sample preparation and separation by successfully adapted ExcelGel Gradient 8–16% improved the sensitivity and resolution of 2D protein maps obtained from proliferating and differentiated HL-60 cell nuclei. Nevertheless, in case the sample concentration is not enough for mass spectrometry, an excellent resolution with higher sample loadings could be obtained by using narrow pH range IPG strips (pH 4–7). Using this method, we were able to identify a protein in a minor spot. Novel nuclear proteins of differentiated HL-60 cells were cut out and prepared for MALDI-TOF MS analysis as described in Materials and Methods. All newly synthesized nuclear proteins in differentiated HL-60 cells were supplied for proteomic analysis (Fig. 1, panel B marked by arrows). However, in this study we present the analysis of one protein which is marked by circle in Fig. 1, panel B. A high-quality mass spectrum was obtained (Fig. 2, panel A). Three software packages, MS-Fit (with combined molecular weight, pI and species searches select 7626 entries), PeptIdent (Swiss-Prot, release 40.7, 103373 entries) and PeptideSearch (EMBL) were used to identify the protein spot. In Fig. 2, panel B we present PeptIdent results. This method is fast, needs no modification, and only small amounts of protein are needed (typically a few pmol). For a detailed discussion of MS applied to proteome analysis, several recent outstanding reviews are available [8–10].

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HL-60 LĄSTELIŲ PROTEOMINĖ ANALIZĖ NAUDOJANT MALDI-TOF MS

S a n t r a u k a

Šiame darbe pateikiama HL-60 ląstelių, indukuotų retinoine rūgštimi, branduolio baltymų proteominė analizė. Individualių baltymų analizei išskirti HL-60 ląstelių branduolio baltymai buvo frakcionuoti dvikryptėje sistemoje. Naujai sintetinti baltymai buvo „iškirpti“ iš gelio, tripsinizuoti ir analizuojami MALDI-TOF MS sistemoje. Peptidų masės buvo naudojamos baltymų analizei su Protana, EXPASY ir EMBL duomenų bazėmis, pasitelkus MS-Fit, PeptIdent ir PeptidSearch algoritmus.