Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1057 (2004) 107–113
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Fully automated online multi-dimensional protein profiling system for complex mixtures

Kiyonaga Fujii[a], Tomoyo Nakano[b], Hiroshi Hike[b], Fumihiko Usui[b], Yasuhiko Bando[b], Hiromasa Tojo[c], Toshihide Nishimura[a,*]

[a] *Clinical Proteome Center, Tokyo Medical University, 2-6-1, Nishi-shinjuku Shinjuku-ku, Tokyo 163-0217, Japan*
[b] *AMR Incorporated, 2-13-18, Nakane Meguro-ku, Tokyo 152-0031, Japan*
[c] *Department of Biochemistry and Molecular Biology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*

Received 21 July 2004; received in revised form 21 September 2004; accepted 24 September 2004

Abstract

For high throughput proteome analysis of highly complex protein mixtures, we have constructed a fully automated online system for multi-dimensional protein profiling, which utilizes a combination of two-dimensional liquid chromatography and tandem mass spectrometry (2D-LC–MS–MS), based on our well-established offline system described previously [K. Fujii, T. Nakano, T. Kawamura, F. Usui, Y. Bando, R. Wang, T. Nishimura, J. Proteome Res. 3 (2004) 712]. A two-valve switching system on a programmable auto sample injector is utilized for online two-dimensional chromatography with strong cation-exchange (SCX) and reversed-phase (RP) separations. The SCX separation is carried out during the equilibration of RP chromatography and the entire sequence of analysis was performed under fully automated conditions within 4 h, based on six SCX fractionations, and 40 min running time for the two-dimensional RP chromatography. In order to evaluate its performance in the detection and identification of proteins, digests of six standard proteins and yeast 20S proteasome have been analyzed and their results were compared to those obtained by the one-dimensional reversed-phase chromatography system (1D-LC–MS–MS). The 2D-LC–MS–MS system demonstrated that both the number of peptide fragments detected and the protein coverage had more than doubled. Furthermore, this multi-dimensional protein profiling system was also applied to the human 26S proteasome, which is one of the highly complex protein mixtures. Consequently, 723 peptide fragments were identified as 31 proteasome components, together with other coexisting proteins in the sample. The identification could be comprehensively performed with a 63% sequence coverage on an average, and additionally, with modifications at the *N*-terminus. These results indicated that the online 2D-LC–MS–MS system being described here is capable of analyzing highly complex protein mixtures in a high throughput manner, and that it would be applicable to dynamic proteomics.

Keywords: Proteomics; Multi-dimensional chromatography; Mass spectrometry; Protein profiling; Proteasome

1. Introduction

Protein samples of biological origin are highly complex in nature and require a sophisticated analytical methodology that is able to provide reliable results for identification of their components. In protein expression and functional analyses, proteomics is a powerful tool to provide a deep insight into biological mechanisms [1]. The biostatistical analysis on the large volume of data resulting from large-scale proteome profiling at every biological stage would become essential in finding significant functional proteins and a protein network associated with cellular mechanisms [2]. When a huge number of complex biological mixtures from different expression, stimulation, and apoptosis stages are subjected to quantitative analysis in a routine and reproducible manner in order to determine the proteins expressed, a protein significantly

* Corresponding author. Tel.: +81 3 5321 6623; fax: +81 3 5321 6624.
E-mail addresses: LINNE300@aol.com, nisimura@tokyo-med.ac.jp (T. Nishimura).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.09.078

associated with a biological function could be discovered. Thus, a simple, robust, and high throughput protein profiling methodology has to be established as a global platform for dynamic proteomics [3,4].

Several research groups have recently reported the development of multi-dimensional liquid chromatography/tandem mass spectrometry (LC–MS–MS) systems for static proteomics to catalog proteomes [3–11]. We have also constructed a two-dimensional (2D) LC–MS–MS system that combines strong cation-exchange (SCX) and reversed-phase (RP) micro-flowing liquid chromatography (μLC), followed by ion-trap mass spectrometry equipped with a nanoelectrospray ionization (NSI) interface as reported previously [12]. This 2D μLC–NSI-MS–MS is characterized technically by its feasibility and high resolution for protein profiling. The SCX separation has been optimized under offline conditions as the first dimensional chromatography for digested protein mixtures, and this protein profiling system was successfully applied to the identification of a mixture of six authentic proteins and a blood plasma sample.

On the other hand, such an offline multi-dimensional separation methodology has the inevitable drawback that manual operation is unable to frequently ensure the reliability and reproducibility of results during a series of experiments on analytical quality. A loss of digests of low abundant proteins in samples tends to be caused due to adsorption on receptacles such as vials and tubes used during fractionation by manual separation. To overcome the drawbacks of the offline analysis described above, we have established a fully automated online system for multi-dimensional separation so that several biological samples can be analyzed under a uniform quality standard in a high throughput manner, as a global platform for dynamic proteomics. In this paper, we describe our comprehensive online 2D μLC–NSI-MS–MS system equipped with a two-valve switching auto sample injection system.

2. Experimental

2.1. Materials

HPLC-grade acetonitrile, formic acid, and trifluoroacetic acid (TFA) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The water used was Milli-Q grade (Millipore, Bedford, MA). Six bovine proteins (β-lactoglobulin, glutamic dehydrogenase, bovine serum albumin, apotransferrin, lactoperoxidase, and catalase), ammonium formate, ammonium bicarbonate (ABC), and iodoacetamide (IAM) were purchased from Sigma (St. Louis, MO). Human 26S proteasome (PW9310) and yeast 20S proteasome (PW8775) were obtained from Affiniti Research Products Ltd. (Devon, UK). Tris[2-carboxyethyl]phosphine (TCEP) was purchased from Pierce (Rockford, IL). Sequencing grade-modified trypsin was a product of Promega (Madison, WI).

2.2. Sample preparation of BSA digests and digests of six standard proteins

One nanomole of bovine serum albumin (BSA) was diluted with 230 μL of 100 mM ABC (aq.). Following this, 12.5 μL of 10 mM TCEP was added for reduction and the mixed solution was maintained at 37 °C for 45 min. Subsequently, 12.5 μL of 50 mM IAM was added, and the mixed solution was maintained at 24 °C for 1 h in darkness, for alkylation. The resulting sample was digested by a trypsine:protein ratio of 1:50 (w/w), and the resultant 250 μL solution was incubated at 37 °C for 15 h in darkness. All these reactions were carried out in methylpentene polymer (TPX) tubes (Hitech Inc., Tokyo, Japan). The solutions were interval-mixed (10 s) at 850 rpm using an Eppendorf thermomixer R (Brinkmann, Westbury, NY), which uses a 1.5 mL microtube. The in-solution digestion of the six standard proteins (β-lactoglobulin, glutamic dehydrogenase, BSA, apotransferrin, lactoperoxidase, and catalase) was carried out in the same manner as described previously [12].

2.3. Sample preparation of the digested 26S and 20S proteasomes

Each of the purchased 50 μg of 26S and 20S proteasome samples was diluted to 190 μL with 50 mM ABC (aq.) containing 10% acetonitrile, in TPX microtubes. For reduction, 2.5 μL of 10 mM TCEP was added, and the mixed solution was maintained at 37 °C for 45 min. Subsequently, 2.5 μL of 50 mM IAM was added, and the mixed solution was maintained at 24 °C for 1 h in darkness for alkylation. For digestion, 2 μg of trypsin was added with 5 μL of 50 mM ABC, and a total of 200 μL of the resulting solution was incubated at 37 °C for 18 h in darkness. All these reactions were carried out using the Eppendorf thermomixer R and mixing at 850 rpm for periods of 10 s at intervals of 10 s. Fifty microliters of the digested samples was diluted with 125 μL of 2% acetonitrile (aq.) containing 0.005% TFA (aq.), after adjusting the pH level to about 3 with 50 μL of 1% TFA (aq.). Subsequently, 25 μL of the samples were injected into the system mentioned below.

2.4. 2D-SCX/RP μLC–NSI-MS–MS system

The 2D μLC–NSI-MS–MS system was composed of a MAGIC 2002 dual solvent delivery system (Michrom BioResources Inc., Auburn, CA) for HPLC, a HTS PAL auto sampler with two 10-port injector valves (CTC Analytics, Zwingen, Switzerland), and a Finnigan LCQ Deca XP ion trap mass spectrometer (ThermoElectron KK, San Jose, CA) equipped with a nanoelectrospray ion (NSI) source (AMR Inc., Tokyo, Japan). FortisTip (OmniSeparo-TJ, Hyogo, Japan), which is a recently developed electrospray emitter coated with a fluorinated polymer, was used as the NSI needle [13]. Sample injection and SCX separation were automatically carried out using the HTS PAL auto

sample injection system. SCX separation was performed on a SCX microtrap cartridge (12 μm, 300 Å, 8 mm × 1.0 mm i.d., Michrom) by stepwise elution at an injection speed of 30 μL/min on the first injector valve. The solvent system containing 2% acetonitrile was composed of 0.005% TFA (aq.) and 1 M ammonium formate (aq.) adjusted to pH 2.8 with TFA, and elution solvents (25, 50, 100, 150, 200, and 500 mM) were prepared by mixing them. For concentration and desalting, SCX fractions were individually injected into a peptide CapTrap cartridge (2.0 mm × 0.5 mm i.d., Michrom) located on the second injector valve. After desalting with 0.1% TFA (aq.) containing 2% acetonitrile, the sample was loaded into a reversed-phase column, MAGIC C18 (3 μm, 200 Å, 50 mm × 0.2 mm i.d., Michrom) for the second dimensional separation. The solutions of 2 and 90% acetonitrile (aq.) were used as the mobile phases A and B, respectively, and both contained 0.1% formic acid. The gradient conditions in the chromatographic run were set up as follows: B 5% (0 min) → 65% (20 min) for analysis of BSA digests and B 5% (0 min) → 45% (40 min) → 95% (45 min) for the other analysis. The effluent from the HPLC at a flow rate of 80 μL/min was split using a MAGIC variable splitter (R2 position, Michrom), and the effluent at 1.0–1.2 μL/min was introduced into the mass spectrometer by the NSI interface via an injector valve with the CapTrap cartridge and the column. Almost all analytical conditions of mass spectrometry were the same as those described previously [12].

2.5. Database searches

All MS–MS data were investigated using the MASCOT search engine (Matrix Science Ltd., London, UK) against the SWISS-PROT database. The data acquired for bovine protein digests were investigated against other mammalian subsets of the sequences. The MS–MS data of human 26S and yeast 20S proteasome samples were investigated against the *Homo sapiens* (human) and *Saccharomyces cerevisiae* (baker's yeast) subsets of the sequences, respectively. The database searches were performed allowing for a fixed modification on cysteine residue (carbamidomethylation, +57 u), variable modification on methionine residue (oxidation, +16 u), peptide mass tolerance at ±2.0 u, and fragment mass tolerance at ±0.8 u.

3. Results and discussion

3.1. Configuration of online 2D-SCX/RP μLC–NSI-MS–MS system

Fig. 1 illustrates the configuration of our online two-dimensional peptide separation system consisting of two valves with a SCX cartridge, a peptide CapTrap cartridge, and a reversed-phase column. The valve switching scheme is shown in Table 1. The system, except for valve-1 with the SCX cartridge, is the same as that previously reported

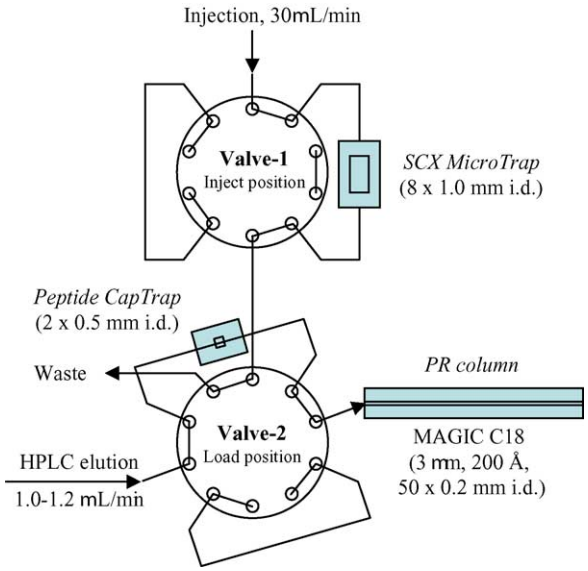

Fig. 1. Two-valve switching system configuration for two-dimensional peptide separation.

[12]. Considering the capacity of the trap cartridge (maximum 2 μg), the elution volume, and the number of SCX fractionations, a SCX cartridge with 20 μg capacity was adapted for the online system. Before sample injection into the SCX cartridge, the SCX resin is activated with a high-salt concentration solution and then with a no-salt solution. As shown in Fig. 1, valve-1 is in the inject position and valve-2 is in the load position of the rotator (#1 in Table 1). After washing the injector and lines (#2), the tarp cartridge on valve-2 is rinsed with 0.1% TFA (aq.) containing 2% acetonitrile in order to activate the peptide trapping resin to provide a high adsorption before sample loading (#3). Subsequently, sample injection into the SCX cartridge is performed at the inject position for both valves-1 and -2 (#4). The sample flows through the trap cartridge situated next to the SCX cartridge to bind the pass-through peptides, which have no interaction with the SCX resin. A SCX separation with stepwise elution is carried out in the same manner and the peptides eluted by each salt concentration solution are also kept on the trap cartridge (#4). Pass-through and SCX separation fractions are desalted and concentrated by loading them on the CapTrap cartridge, followed by the step #5. In this step, a solvent rinse is performed onto the trap cartridge prior to RP column separation. Finally, valve-2 is switched from the inject position to the

Table 1
Scheme of valve switching for online SCX separation

	Action	Valve-1[a]	Valve-2[a]
#1	Activation of SCX cartridge	Inject	Load
#2	Injector and line wash	Load	Load
#3	Activation of trap-cartridge	Load	Inject
#4	SCX separation (sample injection)	Inject	Inject
#5	Washing of trap-cartridge	Load	Inject
#6	Start of LC–MS analysis	Load	Load

[a] Refer to Fig. 1 for valve position.

load position, and the HPLC system and mass spectrometer are simultaneously triggered to start analysis (#6). From sample injection (pass-through fraction) to the last SCX fraction, the steps #2–6 are repeated, and all fractions are analyzed by the μLC–NSI-MS–MS system with the RP column on valve-2. The sample with HPLC elution, which is split into a microflow rate of 1.0–1.2 μL/min, is electro-sprayed by our NSI interface with FortisTip attached to the RP column end via a trap cartridge [12,13]. Sample injection and application of SCX elution solvents are performed by the HTS PAL auto sample injection system, and all the solvents for washing and activating cartridges are maintained in a stack cooler in the system. The SCX separation is carried out during the equilibration of RP chromatography and the processing of all online 2D μLC–NSI-MS–MS analyses was fully automated. The analyses are completed in 4 h based on fraction number (six fractionations) and running time for the second dimensional RP chromatography (40 min). Furthermore, 1D μLC–NSI-MS–MS analysis with only RP separation could also be automatically carried out without any disturbances, such as changing of the tubing and stopping of the system, before and after the 2D μLC–NSI-MS–MS analysis.

As a preliminary experiment, elution volume and salt concentration for the SCX separation were examined, and it was suggested that the elution of a fraction can be achieved with over four times the column volume (>20 μL), and that all samples absorbed with SCX could be eluted with a 500 mM salt concentration (data not shown). Therefore, it was determined that the stepwise elution would be carried out using six fractionations, each with a 40 μL elution volume, using salt concentrations of 25, 50, 100, 150, 200, and 500 mM. In order to maintain the reproducibility and good resolution of the first fraction, the pass-through sample held on the trap cartridge at the time of sample injection is analyzed along with a 25 mM salt concentration fraction, because the contents and separation behavior of the pass through fraction is susceptible to the salt concentration and injection volume of the sample that is injected.

3.2. Evaluation of the newly-constructed online 2D-SCX/RP μLC–NSI-MS–MS system

In order to evaluate the performance of the fully automated online 2D μLC–NSI-MS–MS (2D-LC) system that we constructed, we first checked the separation behavior of online SCX separation, which is the first dimensional chromatography. Fig. 2 shows the base-peak chromatograms of BSA digests obtained by 2D-LC and 1D μLC–NSI-MS–MS (1D-RP) analyses. The fully automated processing including 2D-LC (six fractionations) and 1D-RP analyses is completed in 3.5 h based on 30 min of running time for the RP chromatography. Almost all peptides analyzed by the 2D-LC system were fractionated in a high yield into respective fractions of different salt concentrations, indicating that the optimized conditions for the offline 2D-LC system, as previously reported [12], can be successfully applied to the automated online system. Subsequently, we investigated the performance of our system in the detection and identification of proteins, as a multi-dimensional protein profiling system by comparison with 1D-RP analysis. Six authentic proteins covering a wide range of molecular weights (18–128 ku) were used, and 1D-RP and 2D-LC analyses were carried out for the mixed protein sample after in-solution digestion of the proteins. The identification of the proteins was carried out using the MASCOT database search software for the resulting MS and MS–MS spectra from these analyses [14]. The number of peptide fragments identified as those of the six proteins and with a score higher than 20 for the MS–MS spectra is shown in Fig. 3. Under the analytical and database search thresholds, for MS–MS score higher than 20 on the MASCOT database search results, their total statistical identification confidence levels were over 90% [15]. In the experiment of the 1D-RP analysis, about 120 peptide fragments were detected and identified, providing approximately 40% sequence coverage for each protein, whereas about 80% coverage was obtained in the 1D-RP analysis for only the individual protein. On the other hand, in the 2D-LC analysis, approximately 55 peptide fragments were detected and identified on an average in each SCX fraction (Fig. 3). The total number of non-duplicated peptide fragments detected was found to be approximately twice that in the 1D-RP analysis. The sequence coverage in each protein increased two-fold, indicating that almost all peptide fragments in the mixed protein sample could be detected and identified by 2D-LC analysis.

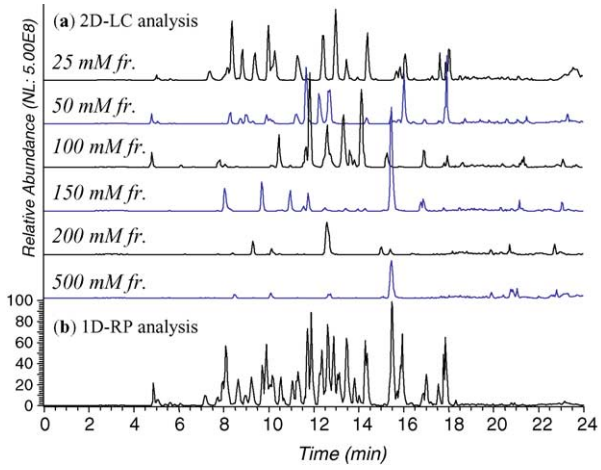

Fig. 2. Base–peak chromatograms of the digested bovine serum albumin sample analyzed by the: (a) online 2D-SCX/RP; and (b) 1D-RP μLC–NSI-MS–MS systems.

3.3. Application of the system to proteome analysis of 20S and 26S proteasome samples

Furthermore, in order to confirm the efficacy of our automated multi-dimensional protein profiling system, we analyzed more complex protein mixtures, and the results for both detection and protein identification were compared with those obtained by 1D-RP analysis. The available yeast 20S and hu-

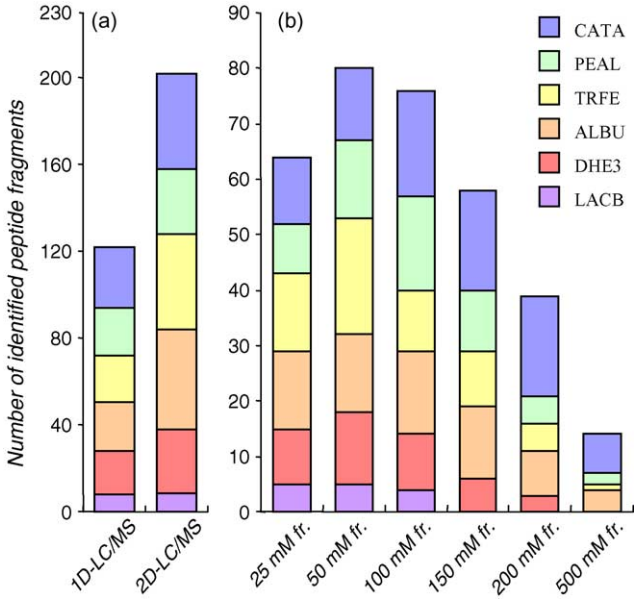

Fig. 3. Database search results for the digest mixture of six standard proteins analyzed by the 2D μLC–NSI-MS–MS system using MASCOT protein identification. CATA, catalase; PEAL, lactoperoxidase; TRFE, apotransferrin; ALBU, bovine serum albumin; DHE3, glutamic dehydrogenase; LACB, β-lactoglobulin.

man 26S proteasome samples, the components of which are already known in detail [16–22], were used in this experiment as examples of highly complex protein mixtures. The yeast 20S proteasome is composed of a total of 14 components classified into seven proteasome subunits of the α-type (PSA1–7) and seven subunits of the β-type (PSB1–7), as shown in Table 2. The human 26S proteasome was finally confirmed to consist of a total of 31 components classified as 6 regulatory subunits of the 26S protease (PRS), 7 proteasome subunits of α-type (PSA), 8 subunits of β-type (PSB) and 10 non-ATPase regulatory subunits of the 26S protease (PSD), as shown in Table 3. After the in-solution digestion of 20S and 26S proteasome samples, 1D-RP and 2D-LC analyses of their digests were simultaneously performed using the same amount of the samples. To improve identification results using the database search, data acquisition was carried out by the data-dependent double MS–MS method, in which the full MS acquisition is followed by two MS–MS scans of the two most intense precursor ions from the full MS scan, and the resulting data were evaluated using a MASCOT database search against the SWISS-PROT database.

On the basis of all peptide threshold scores higher than 20 by the MASCOT search, Table 2 summarizes the numbers of the identified peptides, the total protein score, and the coverage for each protein of the yeast 20S proteasome in comparison to those obtained by 1D-RP analysis [15]. The total number of peptide fragments and the total protein score were found to be approximately twice that in 1D-RP analysis. The sequence coverage in 20S proteasome subunits increased 1.6 times on an average (1D-RP, 26%; 2D-LC analysis, 42%). Fig. 4 shows the base–peak chromatograms of human 26S

Table 2
Number of the peptide fragments identified as 20S proteasome components from *Saccharomyces cerevisiae*

Protein name	1D-RP analysis[a]			2D-LC analysis[a]		
	Peptide	Score	Coverage (%)	Peptide	Score	Coverage (%)
Proteasome subunit alpha types						
PSA1	9	353	47	13	636	59
PSA2	4	262	24	10	570	38
PSA3	6	294	18	10	519	25
PSA4	9	507	26	14	792	40
PSA5	16	790	62	26	1420	75
PSA6	7	403	28	16	913	59
PSA7	7	336	20	13	631	43
Proteasome subunit beta types						
PSB1	4	305	20	9	459	37
PSB2	3	160	14	7	366	33
PSB3	3	78	23	5	256	27
PSB4	4	168	22	6	269	24
PSB5	4	222	14	7	353	29
PSB6	5	260	20	10	597	45
PSB7	5	283	26	11	664	51
	86[b]	316[c]	26[c]	157[b]	603[c]	42[c]

[a] Number of the identified peptides: score of MS–MS spectra ≥20.
[b] Total number of peptide fragments.
[c] Averages of score and coverage.

proteasome digests obtained by 2D-LC and 1D-RP analyses, and Table 3 summarizes the same contents for the search results of the 2D-LC analysis. It was found that the 2D-LC analysis could detect 723 peptide fragments that could be identified as 26S proteasome subunits. From these identified peptide fragments, it was evident that 31 components related to proteasomal proteins (sequence coverage, 63% on an average) were detected in the 26S proteasome sample purchased. Additionally, since it is known that proteasome subunits are highly acetylated at the *N*-terminus, all MS–MS

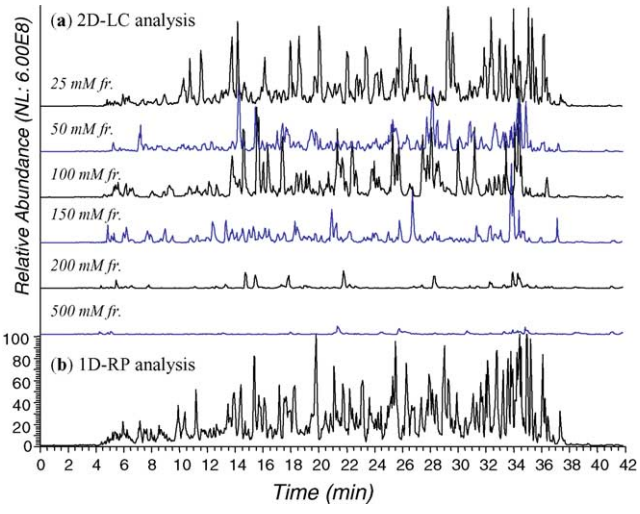

Fig. 4. Base–peak chromatograms of the digested human 26S proteasome sample analyzed by the: (a) online 2D-SCX/RP; and (b) 1D-RP μLC–NSI-MS–MS systems.

data obtained by 2D-LC analysis of the 26S proteasome sample were investigated using the database search including the *N*-terminal acetylation (+42 u) as a variable modification to identify the acetylated *N*-terminal peptide fragments of proteasome subunits. Subsequently, from 15 peptide fragments at the *N*-terminus, it was found that that 9 proteasome subunits (PRS6, 8, PSA1, 3, 5, PSB3, PSD1, 2, and PSDB) were acetylated in the human 26S proteasome sample (Table 3) [21–23]. Furthermore, it was found that PRS4 was modified by myristoylation at the *N*-terminus [22,23]. Additionally, through database searches of the results of 2D-LC analyses of these proteasome samples, we discovered other proteins namely, fatty acid synthase α- and β-subunits (FAS1 and 2) in the yeast 20S proteasome sample and tripeptidyl-peptidase II (TPP2) in the human 26S proteasome sample.

Thus, it was demonstrated that our online 2D μLC–NSI-MS–MS system has a capability of high resolution peptide mapping, which is sufficient to identify protein components in a highly complex protein mixture.

Table 3
Number of the peptide fragments identified as human 26S proteasome components and the *N*-terminus detected by 2D μLC–NSI-MS–MS analysis[a]

Protein name	Peptide	Score	Coverage (%)	*N*-terminus[b]
26S protease regulatory subunits				
PRS4	31	1650	67	Myristoylation
PRS6	26	1416	72	Acetylation
PRS7	35	1876	78	Free
PRS8	26	1674	67	Acetylation
PRSA	29	1672	67	ND
PRSX	28	1448	70	ND
Proteasome subunit alpha types				
PSA1	27	1415	86	Acetylation
PSA2	17	906	70	ND
PSA3	21	971	62	Acetylation
PSA4	18	974	79	ND
PSA5	15	733	59	Acetylation
PSA6	17	995	57	ND
PSA7	19	1053	72	ND
Proteasome subunit beta types				
PSB1	18	1155	64	ND
PSB2	18	953	76	ND
PSB3	14	745	54	Acetylation
PSB4	11	640	52	Free
PSB5	19	1106	85	ND
PSB6	8	590	50	ND
PSB7	11	562	40	ND
PSB8	3	163	16	ND
26S proteasome non-ATPase regulatory subunits				
PSD1	43	2422	57	Acetylation
PSD2	57	2799	69	Acetylation
PSD3	43	2239	74	ND
PSD4	12	716	36	Free
PSD6	34	1784	68	Free
PSD7	16	848	56	Free
PSD8	12	536	46	ND
PSDB	30	1667	60	Acetylation
PSDC	37	1968	71	ND
PSDD	28	1445	74	Free
	723[c]	1262[d]	63[d]	

[a] Number of the identified peptides: score of MS–MS spectra ≥20.
[b] ND: not detected, acetylation: *N*-acetylated modification. Free: no modification.
[c] Total number of peptide fragments.
[d] Averages of score and coverage.

4. Conclusion

For high throughput proteome profiling of biological samples, we have constructed a fully automated online 2D μLC–NSI-MS–MS system that combines SCX chromatography and the μLC–NSI-MS–MS system based on RP chromatography. SCX separation has been optimized as an online system for the first dimensional chromatography using a versatile auto sampler with a two-valve switching system. This online 2D μLC–NSI-MS–MS system was evaluated using digests of the six authentic proteins and yeast 20S proteasome with regard to detection and identification of proteins, by comparing the results obtained with those by 1D μLC–NSI-MS–MS (1D-RP) analysis. The number of detected peptides and the sequence coverage for each protein and protein subunit was found to be more than double in comparison with the 1D-RP analysis. Moreover, the application of this system to proteomic analysis of human 26S proteasome could result in a comprehensive detection of 723 peptide fragments identified as 31 proteasome components. This demonstrates the high performance of this method in complex protein mixtures. In addition, all the analyses, including the 1D-RP analysis, were performed under fully automated conditions within 6 h based on 50 min running time for the second dimensional RP chromatography.

Thus, the fully automated 2D μLC–NSI-MS–MS system combined with online SCX separation has been successfully operated, and the results appear to indicate a capability of high resolution peptide mapping, which is sufficient to comprehensively identify protein components in a highly complex biological mixture with a reasonably high throughput. The integrated system could be used as a technical platform not only for static proteomics to catalog proteomes, but also for dynamic and clinical proteomics in the future [15].

Acknowledgment

This work was supported by the Medical ProteoScope (MPS) Co. Ltd., Tokyo, Japan.

References

[1] M. Tyers, M. Mann, Nature 422 (2003) 193.
[2] R. Aebersold, M. Mann, Nature 422 (2003) 198.
[3] D. Figeys, Anal. Chem. 75 (2003) 2891.

[4] W.S. Hancock, A.J. Apffel, J.A. Chakel, K.G. Hahnenberger, G. Choudhary, J. Traina, E. Pungor, Anal. Chem. A 71 (1999) 742A.
[5] E.P. Romijn, J. Krijgsveld, A.J.R. Heck, J. Chromatogr. A 1000 (2003) 589.
[6] S.-L. Wu, H. Amato, R. Biringer, G. Choudhary, P. Shieh, W.S. Hancock, J. Proteome Res. 1 (2002) 459.
[7] N.L. Anderson, N.G. Anderson, Mol. Cell. Proteomics 1 (2002) 845.
[8] J.N. Adkins, S.M. Varnum, K.J. Auberry, R.J. Moore, N.H. Angel, R.D. Smith, D.L. Springer, J.G. Pounds, Mol. Cell. Proteomics 1 (2002) 947.
[9] H. Wang, S. Hanash, J. Chromatogr. B 787 (2003) 11.
[10] M.P. Washburn, D. Wolters, J.R. Yates 3rd, Nat. Biotechnol. 19 (2001) 242.
[11] D.A. Wolters, M.P. Washburn, J.R. Yates 3rd, Anal. Chem. 73 (2001) 5683.
[12] K. Fujii, T. Nakano, T. Kawamura, F. Usui, Y. Bando, R. Wang, T. Nishimura, J. Proteome Res. 3 (2004) 712.
[13] H. Tojo, J. Chromatogr. A (2004) in press.
[14] http://www.matrixscience.com/.
[15] K. Fujii, T. Nakano, M. Kanazawa, S. Akimoto, T. Hirano, H. Kato, T. Nishimura, Proteomics (2004) in press.
[16] K. Tanaka, C. Tsurumi, Mol. Biol. Rep. 24 (1997) 3.
[17] M. Groll, L. Ditzel, J. Lowe, D. Stock, M. Bochtler, H.D. Bartunik, R. Huber, Nature 386 (1997) 463.
[18] B. Dahlmann, F. Kopp, P. Kristensen, K.B. Hendil, Arch. Biochem. Biophys. 363 (1999) 296.
[19] F. Kopp, B. Dahlmann, L. Kuehn, J. Mol. Biol. 313 (2001) 465.
[20] C. Wojcik, J. Cell. Mol. Med. 6 (2002) 25.
[21] Y. Kimura, M. Takaoka, S. Tanaka, H. Sassa, K. Tanaka, B. Polevoda, F. Sherman, H. Hirano, J. Biol. Chem. 275 (2000) 4635.
[22] Y. Kimura, Y. Saeki, H. Yokosawa, B. Polevoda, F. Sherman, H. Hrano, Arch. Biochem. Biophys. 409 (2003) 341.
[23] T. Shibahara, H. Kawasaki, H. Hirano, Eur. J. Biochem. 269 (2002) 1474.