Available online at www.sciencedirect.com
ScienceDirect
International Journal of Mass Spectrometry 262 (2007) 67–72
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

MALDI-TOF MS sample preparation by using alkanethiolate self-assembled monolayers: A preliminary application for protein sample analysis

Yu-Chang Tyan [a,*], Ming-Hui Yang [b], Pao-Chi Liao [a], Jiunn-Der Liao [c], Shiang-Bin Jong [d], Chia-Yuan Liu [a], Ming-Chen Wang [e], Michael Grunze [f]

[a] Department of Environmental & Occupational Health, National Cheng Kung University, No. 1, Ta-Hsueh Road, Tainan 701, Taiwan, ROC
[b] Department of Chemistry, Texas Christian University, 2800 S. University Drive, Fort Worth, TX 76129, USA
[c] Department of Materials Science and Engineering, National Cheng Kung University, No. 1, Ta-Hsueh Road, Tainan 701, Taiwan, ROC
[d] Department of Nuclear Medicine, Kaohsiung Medical University, 100 Shi-Chuan 1st Road, Kaohsiung 807, Taiwan, ROC
[e] Department of Biomedical Engineering, Yuanpei University, No. 306, Yuanpei Street, Hsinchu 300, Taiwan, ROC
[f] Lehrstuhl für Angewandte Physikalische Chemie, Universität Heidelberg, Im Neuenheimer Feld 253, 69120 Heidelberg, Germany

Received 4 August 2006; received in revised form 10 October 2006; accepted 10 October 2006
Available online 13 November 2006

Abstract

Samples originating from body fluids often contain a complex mixture of inorganic salts, buffers, chaotropic agents, surfactant/detergents, preservatives, and other solubilizing agents. The presence of those contaminants often precludes direct analysis by matrix-assisted laser-desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Self-assembled monolayers (SAMs) on coinage metal can provide versatile modeling systems for studies of interfacial electron transfer, biological interactions, molecular recognition and other interfacial phenomena. In this study, SAMs surface was used for MALDI-TOF MS sample cleanup application. Experimental results from MALDI-TOF MS have revealed the better S/N ratio and resolution of using functionalized SAMs surface for the demonstration of bovine serum albumin (BSA) in artificial human urine sample. This paper reports a surface modification and cleanup method that greatly simplifies this sample preparation process.

Keywords: Self-assembled monolayers (SAMs); Matrix-assisted laser-desorption ionization-time of flight mass spectrometry (MALDI-TOF MS); Artificial human urine; Sample cleanup

1. Introduction

This paper describes a strategy for utilizing self-assembled monolayers (SAMs) as a biological sample cleanup method for matrix-assisted laser-desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis. Mass spectrometry is an important technique for characterizing the structures of surfaces and has several characteristics that are especially valuable in the bioanalytical application. MALDI-TOF MS, one of the most sensitive MS approaches, is probably the most compatible MS procedure with biological buffers and is now routinely used for biomolecular analysis [1–4]. Compared with electrospray ionization tandem mass spectrometry (ESI-MS), MALDI-TOF MS has a higher tolerance of inorganic salts and buffers. However, extremely high concentrations of contaminants such as salts are often present in biological samples to solubilize or stabilize analytes such as proteins, and the buffers typically employed by biochemists are often present in the sample at unacceptable concentrations for the MALDI-TOF MS analysis [5,6].

Several sample preparation techniques have been designed specifically for MALDI-TOF MS analysis of contaminated biological samples. A procedure has been developed for protein sample cleanup and preconcentration in a micro column system contained with hydrophobic resin. In this method, protein sample loading is accomplished by flowing the protein solution though the micro column, where the protein adsorbs to the hydrophobic surface. The proteins are eluted for detection by MS [7,8]. Zip tip, one kind of mini-reversed phase column chromatography, is useful for cleanup and concentration of samples. As peptides and proteins have differing affinities for the C18, the Zip tip can be used for fractionation of mixtures [9]. Another

* Corresponding author at: No. 154, Hua-Shing Street, Yung-Kang, Tainan 710, Taiwan, ROC.
E-mail address: yctyan@mail.ncku.edu.tw (Y.-C. Tyan).

1387-3806/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2006.10.004

procedure for sample cleanup and preconcentration was using a size exclusion chromatography, such as multiscreen filter plate with Ultracel-10 membrane [10]. In this study, the strategy created SAMs surface that was designed to cleanup modify body fluid.

Many reports on the application of SAMs surface have appeared in recent years, such as the preparation of biosensor with modified Au surface as an enzyme immobilized electrode [11]. SAMs are formed spontaneously by chemisorptions and self-organization of thiolate organic molecules onto a surface of an appropriate substrate. The SAMs surface was usually prepared by immersing a substrate into a solution containing a ligand that was reactive to the surface or by exposing the substrate to the reactive species. This technique has received a great deal of attention for its fascinating potential, technical applications such as non-linear optics and device patterning on SAMs surface [12–14]. SAMs surface was also used as an ideal model to investigate the effect of intermolecular interactions in the molecular assembly system [15,16]. It was very convenient to introduce functional structure as a tail group on SAMs to investigate molecular interactions [17,18].

Previous studies also indicated that SAMs was a practical solution for analyzing of peptide/protein samples contaminated with high levels of inorganic salts, buffers, detergents, and other solubilizing agents [19,21]. Shen et al. reported a simple method for preparation of a nitrilotriacetic acid (NTA) self-assembled monolayer surface on gold. The modified surface showed strong selectivity for oligohistidine-tagged peptides or oligohistidine-tagged recombinant proteins, which provided an easy solution for sample preparation of these targeted analytes for MALDI-TOF MS analysis [19]. Xu et al. developed a hexadecanethiol SAMs surface using sample droplets to concentrate samples during solvent evaporation [20]. In our experiment, SAMs technique was used to complete a preliminary study for an artificial human urine sample cleanup strategy for MALDI-TOF MS analysis. A gold-coated MALDI-TOF MS sample plate was modified with a 1-dodecanethiol SAMs to create a hydrophobic surface that can concentrate protein samples. The hydrophobic SAMs surface is similar to C18 stationary phase commonly employed in the trap column for HPLC. This type of SAMs surface can also be successfully used to develop a protein sample cleanup surface. This strategy affords a simple, feasible and general method of sample cleanup for analyses by MALDI-TOF MS.

2. Materials and methods

2.1. Formation of SAMs

Au was the most frequently used coinage metal, as it is not easily oxidated under ambient conditions. A 100 nm thick Au film was prepared by electron beam evaporation onto a 10 × 10 MALDI-TOF MS sample plate surface (P/N: V700401, Applied Biosystems). Prior to each experiment, all containers, equipments and supplies were carefully cleaned by first rinsing with absolute ethanol, then with 30% hydrogen peroxide, again with absolute ethanol, and finally with the pure solvent to be used in the adsorption experiments. The sample plate was cleaned by hydrogen peroxide solution for 15 s followed by rinsing with high-purity ethanol (RDH 32205, Riedel-de Haën), and then immersed into 0.5 mM ethanolic alkanethiol solution at room temperature for 12 h [22]. The alkanethiols adsorb spontaneously onto the Au surface. The chemical for SAMs preparation was 1-dodecanethiol: $C_{12}H_{26}S$ (44130, Fluka). The functionalized thiol groups were chemisorbed onto the Au surface via the formation of thiolate bonds [23].

2.2. Contact angle measurement

The contact angles (θ) were measured in air using a goniometer (Krüss apparatus). The Milli-Q grade water (Millipore Co. Inc.) was used to contact with the sampling dimension by the sessile drop method. For this measurement, a 2 μl droplet was placed gently on the specimen with the needle of a syringe. The value of θ was determined as the volume of the droplet increased slowly [24,25].

2.3. Fourier-transformed infrared reflection-absorption spectroscopy

All infrared (IR) spectroscopy optical benches were acquired with a conventional Fourier-transformed (FT) Spectrometer (FTS-175C, Bio-Rad) equipped with a KBr beam splitter and a high-temperature ceramic source. Win-IR, Win-IR Pro (Bio-Rad) and Origin 6.0 (Microcal Software Inc.) were used for the data acquisition and analysis. The IR spectra were obtained using a p-polarized beam incident at a grazing angle of around 80° with respect to the surface normal. The spectra were measured by a liquid-nitrogen cooled, narrow band MCT detector. The spectra were recorded with a resolution of 4 cm^{-1} using about 500 scans and an optical modulation of 15 kHz filter.

2.4. MALDI-TOF MS instrumentation

The MALDI-TOF MS spectra were acquired on a Voyager DE-PRO Biospectrometry Workstation (Applied Biosystems) using a nitrogen laser (VSL-337, 337 nm). Mass spectra were collected in the positive-ion mode using an acceleration voltage of 25 kV and a delay of 300 ns. The grid voltage and guide wire voltage were set to 90.0 and 0.15%, respectively. Each mass spectrum was collected and represented the sum of the data from 100 laser shots.

2.5. Formula of artificial human urine

The following reagents were necessary for the preparation of normal artificial human urine: bovine serum albumin powder (BSA, A3311, Sigma), creatinine (C4255, Sigma), distilled water, potassium chloride (P3911, Sigma), sodium chloride (31434, Riedel-de Haën), sodium phosphate, monobasic (S0751, Sigma), and urea (113563, Usb Corporation).

To 1.5 L of distilled water, 36.4 g of urea, 15.0 g of sodium chloride, 9.0 g of potassium chloride and 9.6 g of sodium phosphate were mixed until all the crystals were dissolved. The pH value was then checked to ensure the pH was within the 5–7 pH

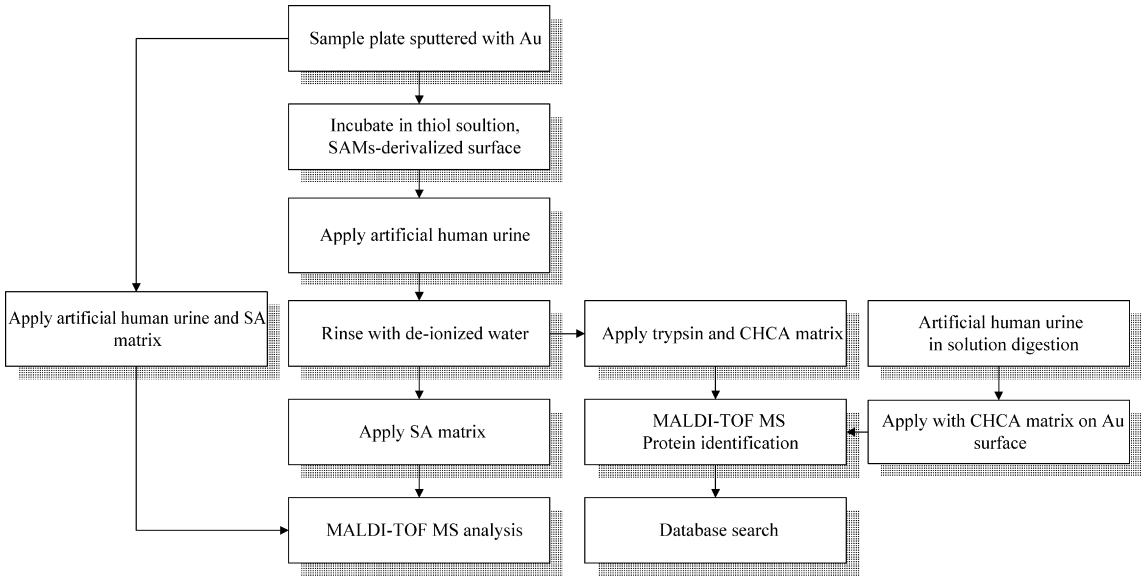

Fig. 1. Scheme of the overall experiment design developed to use alkylthiolate self-assembled monolayers as a cleanup method and analyze BSA in artificial human urine for this study.

range. The pH of the solution was adjusted with 1N hydrochloric acid or 1N sodium hydroxide. Next, a urine hydrometer was used to regulate the specific gravity into the range of 1.015–1.025 with distilled water. Then, to ensure a similarity to human urine, 4.0 g of creatinine and 100 mg of BSA were slowly mixed into the so-called normal artificial urine solution. The final volume of artificial urine solution was about 2 L [26,27].

2.6. MALDI-TOF MS sample preparation

The artificial human urine sample was prepared for MALDI analysis using a conventional dried droplet protocol. This protocol used sinapinic acid (SA, S8313, Sigma) and α-cyano-4-hydroxycinnamic acid (CHCA, C8982, Sigma) as the matrixes. The CHCA matrix was prepared as a saturated, aqueous solution containing 50% (v/v) acetonitrile (B15466, J.T. Baker) and 0.01% (v/v) trifluoroacetic acid (TFA, 302031, Aldrich). SA dissolved in 70% (v/v) acetonitrile with 0.01% TFA was used as the matrix.

In the direct MALDI-TOF MS analysis of artificial human urine, 2 μl of the artificial human urine sample described above was mixed with 2 μl methanol and then deposited on the SAMs surface. After the sample was dried at room temperature, the sample was rinsed twice with deionized water, dried, and then 1 μl of SA matrix was added. For the Au surface, 2 μl of artificial human urine with methanol, and 1 μl of SA matrix were applied at the same time. Each surface had six sample spots, respectively; each spot was measured twice.

We also experimented the artificial human urine using trypsin digestion. The sample on the SAMs surface, prepared as described above, was applied with 1 μl of 0.1 μg/μl of modified trypsin digestion buffer (V511A, Promega) in 25 mM ammonium bicarbonate solution and allowed to shake slowly at 37 °C for 30 min using an incubator for digestion. Then 1 μl of CHCA matrix was added to air-dry at room temperature. Fig. 1 contains the scheme of the overall experiment design developed to analyze BSA in the artificial human urine for this study. Generally 100 laser shots were used, and the data of MALDI-TOF MS were collected at different positions of each crystallized sample spot.

2.7. Mass spectral analysis

The data in each mass spectrum were visualized using the software (Data Explorer V 4.0, Applied Biosystems) provided by the manufacturer. The resulting peptide mass fingerprints were compared with databases using the program MS-Fit, available at the World Wide Web site at the University of California at San Francisco (http://prospector.ucsf.edu/).

3. Results and discussion

3.1. Surface characterization

The contact angle measurement for the 1-dodecanethiol/Au surface was an indicative of a well-ordered and homogeneous layer with methyl group at the monolayers/ambient interface. Contact angle measurements of the 1-dodecanethiol/Au surface using water as a liquid probe was about $91 \pm 1.32^\circ$ (Au surface was about $78 \pm 1.94^\circ$). The SAMs surface with the alkyl tail group was therefore hydrophobic [28].

The monolayer assembly was routinely characterized with FTIR-RAS upon preparation. Fig. 2 shows the alkane FTIR-RAS spectra of the SAMs. The position of the C–H stretching bands of the methylene groups from the alkyl chains indicates the order of the alkyl chains within SAMs. In the spectrum of the SAMs surface, two absorption bands at 2918 and 2850 cm^{-1} were assigned to asymmetric (d^-) and symmetric (d^+) C–H

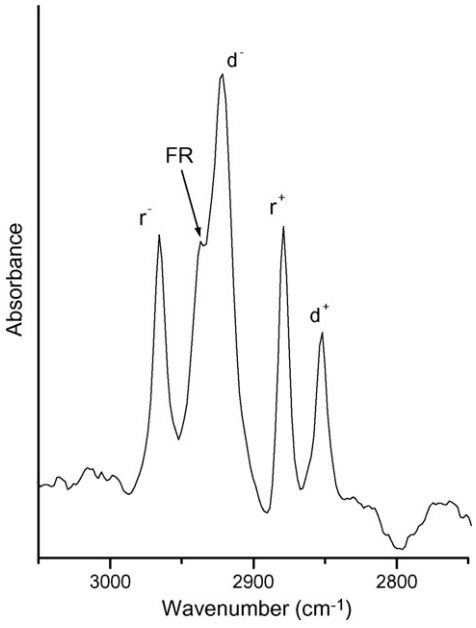

Fig. 2. FTIR-RAS spectra show the high-frequency region: 3050–2750 cm^{-1} of the 1-dodecanethiol SAMs surface.

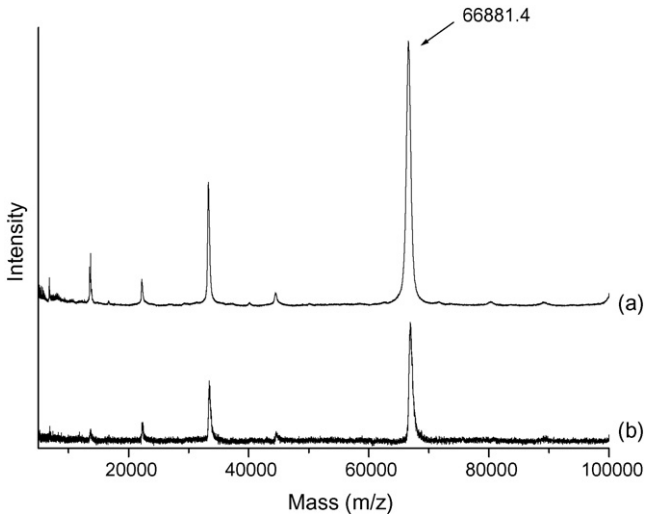

Fig. 3. Analysis of artificial human urine samples by MALDI-TOF MS: (a) sample cleanup by 1-dodecanethiol SAMs surface and (b) sample deposited with SA matrix on the Au surface.

stretching bands of the methylene groups, respectively [29]. The peak positions of CH_3 stretching modes were consistent with the presence of a dense crystalline-like phase: r^+, 2876 cm^{-1}; FR, 2935 cm^{-1}; r^-, 2963 cm^{-1}.

3.2. MALDI-TOF measurement

Using SAMs technique to modify MALDI-TOF MS sample plate was allowed for cleanup of protein in bio-fluid sample. The results of an artificial human urine sample cleanup by SAMs surface and MALDI-TOF MS experiment were shown in Fig. 3. In this experiment, 2 μl of the artificial human urine sample with methanol was dropped on the SAMs modified sample plate followed by air-drying and rinsing with deionized water to remove salt or non-protein contaminants. After those procedures, the SA matrix was applied, vacuum-dried, and the sample plate was loaded into the MALDI-TOF mass spectrometer. The spectra were obtained from the artificial human urine sample on the SAMs modified surface (Fig. 3a) and the Au surface (Fig. 3b). The S/N ratios of the spectra were around 1952.2:1 and 76.3:1 at spectra peak 66881.4, and the resolutions at mass 66881.4 calculated with 50% centroid were around 226.63 and 58.27, respectively (Table 1). Analyte signals were obtained with better S/N ratio and signal resolution by using SAMs modified surface. This would also improve the detection limit and prevent the salt or contamination affection.

3.3. Protein digestion and identification

The purpose of this work was to develop a SAMs surface cleanup device for the MALDI-TOF MS sample plate. After applying the cleanup method to the SAMs surface, the artificial human urine was applied with trypsin and CHCA matrix. The analysis of the artificial human urine digestion sample can be performed using MALDI-TOF MS for determination of the protein identification. The database search with MS-Fit was performed using the recorded peptide mass fingerprint data sets.

In this experiment, 2 μl artificial human urine solution deposited and digested on the SAMs surface was analyzed. For the Au surface, the artificial human urine digestion was deposited with CHCA matrix. The MS-Fit search of the data yielded BSA as the top hit with MOWSE scores of 964 and 473, and the sequence coverage of ∼46 and ∼23%, corresponding to 25 and 15 peptide matches, respectively (Tables 1 and 2). This result demonstrated that BSA in artificial human urine was sufficient for protein identification. Compared with sample deposited on the Au surface directly, the sample after the SAMs surface cleanup resulted better mass accuracy (Table 3).

Samples containing salts would widen peaks and lower resolution, and thus desalting prior to MALDI-TOF MS analysis

Table 1
The S/N ratio and resolution calculated with 50% centroid of the spectra at spectra peak 66881.4, and the result of protein identification with MS-Fit search

Sample plate surface	BSA in artificial urine (peak at 66881.4)[a]		BSA digestion in artificial urine[b]		
	S/N ratio	Resolution	MOWSE score	Sequence coverage	Peptide matches
Au	76.3:1	58.27	473	23%	15
SAMs	1952.2:1	226.63	964	46%	25

[a] The results of S/N ratio and resolution were the mean of 12 measurements.
[b] Data shown in this table were the results of 1 of 12 measurements.

Table 2
Protein view and matched peptides shown in the BSA of artificial human urine sample

1	M***KWVTFISLL***	***LLFSSAYSRG***	***VFRRDTHKSE***	***IAHRFKDLGE***	***EHFK***GLVLIA
51	FSQYLQQCPF	DEHVK***LVNEL***	***TEFAKTCVAD***	***ESHAGCEK***SL	HTLFGDELCK
101	***VASLRETYGD***	***MADCCEK***QEP	ER***NECFLSHK***	DDSPDLPK***LK***	***PDPNTLCDEF***
151	***K***ADEKKFWGK	***YLYEIARRHP***	***YFYAPELLYY***	***ANK***YNGVFQE	CCQAEDKGAC
201	LLPKIETMRE	KVLASSARQR	LRCASIQKFG	ERALKAWSVA	R***LSQKFPK***AE
251	FVEVTKLVTD	LTK***VHKECCH***	***GDLLECADDR***	***ADLAK***YICDN	QDTISSKLKE
301	CCDKPLLEKS	HCIAEVEKDA	IPENLPPLTA	DFAEDKDVCK	NYQEAK***DAFL***
351	***GSFLYEYSRR***	***HPEYAVSVLL***	***R***LAKEYEATL	EECCAKDDPH	ACYSTVFDKL
401	K***HLVDEPQNL***	***IK***QNCDQFEK	***LGEYGFQNAL***	***IVR***YTR***KVPQ***	***VSTPTLVEVS***
451	***R***SLGK***VGTRC***	***CTKPESER***MP	CTEDYLSLIL	NRLCVLHEKT	PVSEKVTKCC
501	TESLVNR***RPC***	***FSALTPDETY***	***VPK***AFDEKLF	TFHADICTLP	DTEK***QIKKQT***
551	***ALVELLKHKP***	***K***ATEEQLKTV	MENFVAFVDK	CCAADDK***EAC***	***FAVEGPKLVV***
601	***STQTALA***				

Italic and bold: matched peptides on the 1-dodecanethiol SAMs surface; underline: matched peptides on the Au surface.

Table 3
Matched peptides sorted by residue number

Start–end	Sequence	M_r (calculated)	Observed[a]	
			SAMs	Au
2–23	M.KWVTFISLLLLFSSAYSRGVFR.R	2589.45	2589.20	–
25–34	R.DTHKSEIAHR.F	1192.59	1194.45	1194.12
29–34	K.SEIAHR.F	711.37	–	712.84
29–44	K.SEIAHRFKDLGEEHFK.G	1941.97	1942.92	–
35–44	R.FKDLGEEHFK.G	1248.61	1250.35	–
66–75	K.LVNELTEFAK.T	1162.62	1164.43	–
76–88	K.TCVADESHAGCEK.S	1348.54	1348.83	–
101–117	K.VASLRETYGDMADCCEK.Q	1889.80	1890.03	–
123–130	R.NECFLSHK.D	976.44	977.99	–
139–151	K.LKPDPNTLCDEFK.A	1518.74	1519.13	–
161–167	K.YLYEIAR.R	926.49	928.23	928.02
168–183	R.RHPYFYAPELLYYANK.Y	2044.02	2046.30	–
184–204	K.YNGVFQECCQAEDKGACLLPK.I 3 Carbamidomethyl (C); 2 Deamidation (NQ)	2488.07	–	2488.92
212–220	K.VLTSSARQR.L	1016.57	–	1018.06
223–228	R.CASIQK.F Deamidation (NQ)	649.31	–	649.83
236–241	K.AWSVAR.L	688.37	–	389.85
242–248	R.LSQKFPK.A	846.50	847.68	–
264–285	K.VHKECCHGDLLECADDRADLAK.Y	2440.09	2440.77	–
347–359	K.DAFLGSFLYEYSR.R	1566.74	1568.82	1568.33
360–371	R.RHPEYAVSVLLR.L	1438.80	1440.67	1440.37
361–371	R.HPEYAVSVLLR.L	1282.70	1284.43	1284.24
402–412	K.HLVDEPQNLIK.Q	1304.71	1306.37	
	K.HLVDEPQNLIK.Q Deamidation (NQ)			1306.29
421–433	K.LGEYGFQNALIVR.Y	1478.79	1480.97	
	K.LGEYGFQNALIVR.Y Deamidation (NQ)			1480.37
437–451	R.KVPQVSTPTLVEVSR.S	1638.93	1641.31	
	R.KVPQVSTPTLVEVSR.S Deamidation (NQ)			1640.53
456–468	K.VGTRCCTKPESER.M	1464.68	1463.92	–
508–523	R.RPCFSALTPDETYVPK.A	1822.89	1825.16	–
545–557	K.QIKKQTALVELLK.H	1510.94	1512.85	–
549–561	K.QTALVELLKHKPK.A	1503.91	1503.20	–
569–580	K.TVMENFVAFVDK.C Deamidation (NQ)	1399.67	–	1400.48
588–597	K.EACFAVEGPK.L	1049.49	1051.42	1050.96
598–607	K.LVVSTQTALA.	1001.58	1002.49	–

[a] (–) Not observed.

could be reasonable. Similarly, spectra obtained in high concentrations of detergent were low in signal yield and resolution. The ability for SAMs surface to cleanup biologic samples can be helpful for direct analysis using MALDI-TOF MS. Such enhancement is particularly attractive for the analysis of complex protein mixtures with a significant difference in their individual concentrations.

4. Conclusion

Bio-fluids, from very complex matrixes, often contain large amounts of inorganic salts, buffers, and proteins. The presence of contaminants virtually ensures the failure of subsequent analysis of the sample by mass spectrometry. This paper details a procedure for depositing artificial human urine, containing BSA as a model protein. The artificial urine was deposited on a MALDI-TOF MS sample plate coated with SAMs from dodecanethiol for sample desalt and cleanup. The presented results provide an example of the 1-dodecanethiol self-assembled monolayers applications for the MALDI-TOF MS bio-sample cleanup plate. The preparation of the SAMs surface for the sample cleanup was relative to increase the S/N ratio and resolution for the MALDI-TOF MS analysis. SAMs formation provides an easy way to prepare the structure that can be further functionalized with biomolecules to yield biorecognition surfaces for biomedical sample analysis devices.

Acknowledgements

The authors thank the National Cheng-Kung University Proteomics Research Core Laboratory for the assistance in protein identification. This work was partially supported by grants (NSC-94-2113-M-006-016) from the National Science Council.

References

[1] M.E. Warren, A.H. Brockman, R. Orlando, Anal. Chem. 70 (1998) 3757.
[2] K. Tanaka, H. Waki, Y. Ido, S. Akita, T. Yoshido, Rapid Commun. Mass Spectrom. 2 (1988) 151.
[3] F. Hillenkamp, M. Karas, R.C. Beavis, B.T. Chait, Anal. Chem. 63 (1990) 1139A.
[4] R. Wang, B.T. Chait, Curr. Opin. Biotechnol. 5 (1994) 77.
[5] L. Zhang, R. Orlando, Anal. Chem. 71 (1999) 4753.
[6] U. Kallweit, K.O. Bornsen, G.M. Kresbach, H.M. Widmer, Rapid Commun. Mass Spectrom. 10 (1996) 845.
[7] D. Craft, A. Doucette, L. Li, J. Proteome Res. 1 (2002) 537.
[8] A. Doucette, D. Craft, L. Li, J. Am. Soc. Mass Spectrom. 14 (2003) 203.
[9] H. Wei, S.L. Dean, M.C. Parkin, K. Nolkrantz, J.P. O'Callaghan, R.T. Kennedy, J. Mass Spectrom. 40 (2005) 1338.
[10] C.S. Spahr, M.T. Davis, M.D. McGinley, J.H. Robinson, E.J. Bures, J. Beierle, J. Mort, P.L. Courchesne, K. Chen, R.C. Wahl, W. Yu, R. Luethy, S.D. Patterson, Proteomics 1 (2001) 93.
[11] J.J. Gooding, V.G. Praig, E.A. Hall, Anal. Chem. 70 (1998) 2396.
[12] J.C. Horne, G.J.J. Blanchard, Am. Chem. Soc. 120 (1998) 6336.
[13] F. Morhard, J. Schumacher, A. Lenenbach, T. Wilhelm, R. Dahint, M. Grunze, D.S. Everhart, Proc. Electrochem. Soc. 97 (1997) 1058.
[14] K. Bierbaum, M. Grunze, A.A. Baski, L.F. Chi, W. Schrepp, H. Fuchs, Langmuir 11 (1995) 2143.
[15] A. Schertel, C. Woll, M. Grunze, J. Phys. IV 7 (1997) 537.
[16] C. Yan, M. Zharnikov, A. Goelzhaeuser, M. Grunze, Langmuir 16 (2000) 6208.
[17] H.J. Himmel, K. Weiss, B. Jaeger, O. Dannenberger, M. Grunze, C. Woell, Langmuir 13 (1997) 4943.
[18] C. Jung, O. Dannenberger, Y. Xu, M. Buck, M. Grunze, Langmuir 14 (1998) 1103.
[19] J. Shen, T. Ahmed, A. Vogt, J. Wang, J. Severn, R. Smith, S. Dorwin, R. Johnson, J. Harlan, T. Holzman, Anal. Biochem. 345 (2005) 258.
[20] Y. Xu, J.T. Watson, M.L. Bruening, Anal. Chem. 75 (2003) 185.
[21] J. Su, M. Mrksich, Angew. Chem. Int. Ed. 41 (2002) 4715.
[22] L. Sun, R.M. Crooks, A.J. Ricco, Langmuir 9 (1993) 1775.
[23] Y. Miura, S. Kimura, Y. Imanishi, J. Umemura, Langmuir 15 (1999) 1155.
[24] E.L. Smith, C.A. Alves, J.W. Anderegg, M.D. Porter, L.M. Siperko, Langmuir 8 (1992) 2707.
[25] M. Lestelius, M. Liedberg, P. Tengvall, Langmuir 13 (1997) 5900.
[26] R.M. Kark, J.R. Lawrence, V.E. Pollack, C.L. Pirani, R.C. Muehrcke, H. Silva, A Primer of Urinalysius, second ed., Hoeber Medical Division, Harper & Row Publishers, New York, 1964.
[27] G. Ballman, Am. Clin. Lab. 8 (1989) 10.
[28] C.E.D. Chidsey, D.N. Loiacono, Langmuir 6 (1990) 682.
[29] P.E. Laibinis, G.M. Whitesides, D.L. Allara, Y.T. Tao, A.N. Parikh, R.G. Nuzzo, J. Am. Chem. Soc. 113 (1991) 7152.