INCUBATION OF DENATURATED SAMPLES INCREASED REPRODUCIBILITY AND QUALITY OF PROTEOMIC PROFILE OF SELDI-TOF MS

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Abstract

Protein profiling with high-throughput proteomic technology, SELDI-TOF, is a new potential tool for diagnosis of human diseases. This advanced technique has increasingly been used for the detection of disease biomarker. However, analytical reproducibility is a significant challenge in SELDI-TOF profiling in order to have confidence in the results. Here, we showed a simple step to improve its analytical performance. IMAC 30-Cu Protein Chip was used to incubate denaturated samples to increase the number of peak detection and decrease peak intensity coefficient of variation. Incubation of denaturated samples overnight at 4^{o}C increased significantly reproducibility and quality of proteomic profile of SELDI-TOF MS for IMAC30-Cu ProteinChip. This strategy could be applied to address reproducibility issue in protemictechnology in protein profiling.

Keywords: Proteomic, reproducibility, SELDI-TOF

1. Introduction

Protein profiling with surface-enhanced laser desorption-ionisation time-of-flight mass spectrometry (SELDI-TOF MS) is a promising approach for biomarker discovery. The resolution of this advanced technology has been improved by incorporating fractionation and a variety of affinity capture technique. [1] Recently, SELDI-TOF protein profiling has been applied in many proteomic biomarker researches such as cancer and infectious disease that reveal high diagnostic sensitivities and specificities. Although it is a robust platform for protein profiling, some sources of technical and non-technical variation make reproducing and validating potential biomarker challenging.[2,3] As with any proteomic analysis, it is still required for optimization of experimental procedure to ensure consistent data output. Confidence that differences in SELDI-TOF profile pattern specifically reflect changes in protein concentration is dependent in part on spectra reproducibility and quality, and these parameters are affected by multiple variables.[4] Sample preparation procedure is one of important factor to give a better quality, reproducible and robust output of proteomic profile of SELDI-TOF MS.[5] Here, we perform a simple step to increased robustness of the technique. We showed that by using IMAC30-Cu ProteinChip, incubation of denaturated samples increased significantly the number of peak detection and decreased peak intensity coefficient of variation (CV). This strategy could be used as an alternative technique to address reproducibility issue in this system.

2. Methods

Serum Samples. Blood from 5 healthy volunteers were collected in Vacutainer tube without additive, let it clot in 30 minutes at room temperature and centrifuges for 30 minutes at 3000 rpm. Serums were recovered, pooled and frozen at minus 80^{o}C until used.

SELDI Protein Profiling. For SELDI analysis, we use two ProteinChips Arrays (Ciphergen Biosystems, Inc), immobilized affinity capture (IMAC30) and weak cation exchage (CM10). Serum samples are processed as previously described with minor modification.[6] Samples were prepared in ice by mixing 20 uL serum with 30 uL U9 buffer (9 M urea, 2% CHAPS, in 100 mM Tris pH 9) in a 1.5 mL microfuge tube at 4^{o}C for 10 minutes and were shaked for 30-45 minutes at room temperature. Then, 2 uL serum/U9 buffer was diluted with 98 uL corresponding binding buffer (PBS 1X + 0.5 M NaCl for IMAC30 and 100 mM Na acetate pH 4 +/- Triton X for CM10). Denaturated samples were then stored (incubated) in 4^{o}C overnight. For control (treatment without incubation), denaturated samples

were spotted directly to the arrays. The ProteinChips Arrays were assembled into a deep-well type bioprocessor (Ciphergen Biosystems, Inc.). Prior to sample loading, IMAC30 were coated with $CuSO_4$ by adding 50 µL of 100 mM $CuSO_4$ to each arrays. After 10 min agitation on platform shaker at a speed of 250 rpm for 10 minutes, the arrays were quickly rinsed with DI water to removed unbound metal. To remove unbound copper, 50 µL sodium acetate solution pH 4 was added to each array and shaken for 5 minutes and rinsed again with DI water. The arrays were then equilibrated with 150 µL of binding buffer (PBS). Prior to sample loading, CM10 arrays were equilibrated with 150 µL of corresponding binding buffer (50 mM sodium acetate pH 4.0). Arrays were spotted with 50 µL of diluted sample for 30 min on a shaker at 250 rpm and washed three times with 150 µL corresponding binding buffer. After rinsing with DI water, the arrays were removed from the bioprocessor assembly and air dried. CHCA (Ciphergen Biosystems, Inc, Cat. No. C300-0002) as energy absorbing matrix was prepared according to the manufacturer's instruction in 50% v/v acetonitrile/5% v/v trifluoroacetic acid and 0.5 µL of the saturated solution applied twice to each spot on the chip. We used CHCA because this matrix gave best proteomic profile in molecular mass 3-15 kDa which become our and many other studies' concern in identification of biomarker. ProteinChip arrays were analyzed in the ProteinChip Biology System reader (Model PBS II, Ciphergen Biosystem) and the data were analyzed by ProteinChip Software version 3.0.

3. Results and Discussion

In biomarker study with SELDI-TOF protein profiling the aim is to identify peak intensity (or peaks area) that are different between case and control samples and thus reproducibility of peak intensities is of highest importance. Despite promising, SELDI-TOF analysis on biological specimen such as serum remain challenging due to its complexity. Much has been discussed in SELDI-TOF technology concerning its long-term robustness, the contribution of non-biological variation to the result and the need for better quality control.[7] One of the most critical issue of this technique is reproducibility of the data that is critical in biomarker identification and validation. Several studies addressed this issue have been looking at the impact of some preanalytical variables such as blood sample processing[2], and standardization analytical condition.[8,9] To ensure consistent data output, procedures or stringent protocol that have been optimized and reproducible is an urgent need. The main thrust of this work was to develop enhanced protocol to minimize variability of data caused by preanalytical variables. Here we describe a simple step to improved proteomic profiles and reproducibility of peaks detection as determined by number of peaks and signal intensity coefficient of variance (CVs) on a QC sample (pooled normal serum). To calculate CV of peak intensity, all peak detected in each experiment were used. It was shown that by incubating denaturated samples overnight in 4^{o}C, in general, robustness of the technique was increased for IMAC30-Cu as demonstrated by reproducibility and number of peak detected (Table 1 and Figure1).

The average of CV was decreased from 18.5 (without incubation) to 13.9 (o/n incubation) ($p<0,05$) and the average of number of peak was increased from 30 (without incubation) to 50 (o/n incubation) ($p<0.01$). For CM10, although incubation treatment seemed to have positive effect on proteomic profile quality, the improvement were not statistically significant. We also found that some peaks were detected higher by increasing of the incubation time (30 minutes and 4 hours h Vs Versus O/N), however contrary effect was shown in some other peaks (Figure 2 and 3). IMAC30-Cu and CM10 ProteinChip were widely used in biomarker study for infectious disease such as AfricanTrypanosomiasis,[10] SARS[11] and cancers like ovarian.[12] and lung cancers.[6] These two arrays give more peaks compared to other Chip available.

This simple step described in this work could be taken together with other strategies to address reliable proteomic data developed by some other studies. Since validation of disease biomarker relies on optimized and reproducible laboratory methods, such work is of importance. Further study, that focus on investigating the effect on other Protein Chip arrays is needed.

Table 1. Reproducibility and Quality of Proteomic Profiles in IMAC30-Cu and CM10 ProteinChip Arrays were Shown Increased with Overnight Incubation Treatment

Exp	%CV ($p<0,05$)[a]		Number of ($p<0.01$)[a]	Peak
Array IMAC30				
	w/o incubation	o/n incubation	w/o incubation	o/n incubation
I	21.4	11.6	40	49
II	13.7	11.8	39	50
III	16.8	14.0	39	51
IV	17.9	12.7	35	49
V	20.8	18.1	37	47
VI	20.6	15.5	42	50
	18.5	13.9	39	50
Array	CM10			
I	18.0	12.8	37	40
II	20.8	18.1	35	45
	19.4	10.4[b]	36	43[b]

[a]Calculated by the non-parametric One-Way Anova
[b]Statistically not significant

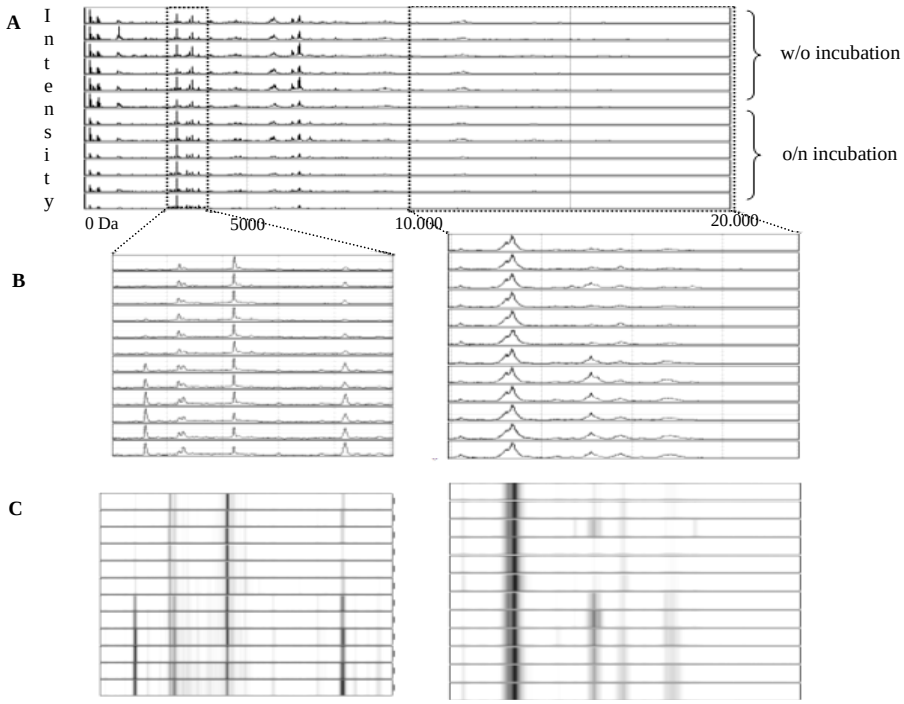

Figure 1. Effect of Overnight Incubation to Proteomic Profile in IMAC30-Cu arrays. Spectra Ranged 0-20.000 Da (A); Some Peaks (Ranged 3000-4000 Da) were Detected Higher in Incubated Samples Compared to un-Incubated Samples (B); Gel View (C)

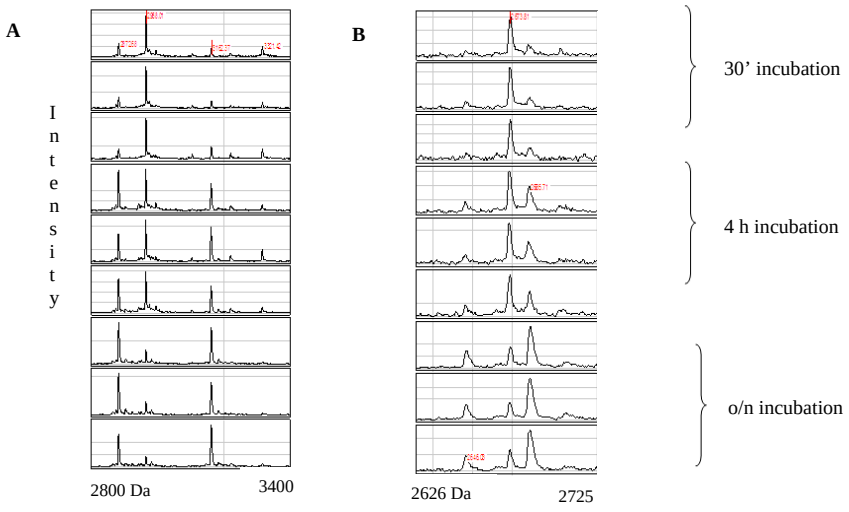

Figure 2. Effect of Incubation Time of Denatured Samples in Proteomic Profiles for IMAC30-Cu Arrays. Two Peaks (2872 and 3162) were Detected Higher in Samples Incubated for 4 h, and Over Night). However Peak of 2968 and 3321 were Decreased by Longer Incubation (4 h, and over night) (A); The same Phenomenon was also Shown some other Peaks (B)

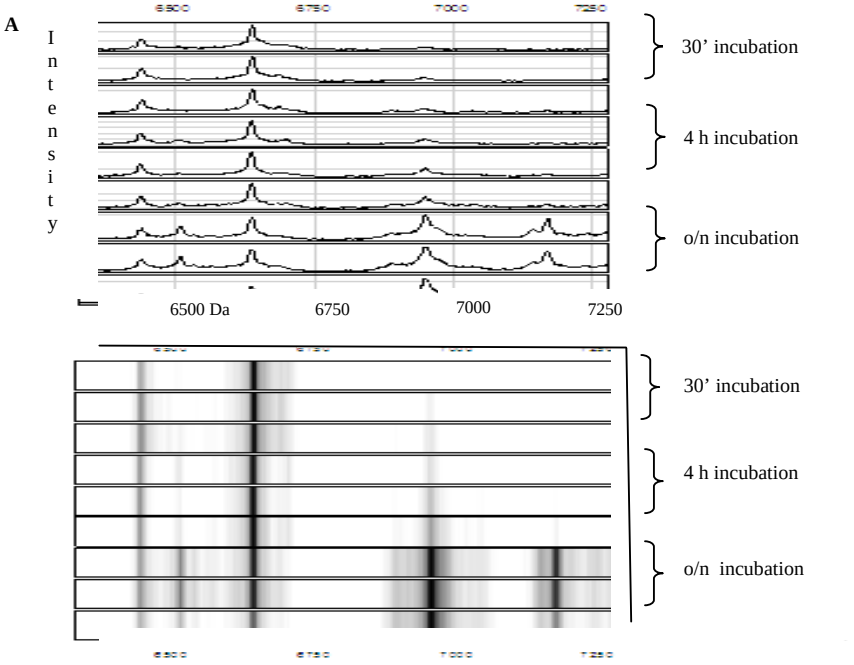

Figure 3. Effect of Incubation Time in Proteomic Profiles, Ranged 6000-7500 Da (A); Gel View (B)

4. Conclusion

For IMAC30-Cu, overnight incubation of denaturated samples at 4°C were shown to increased reproducibility and quality of proteomic profiles of SELDI-TOF MS. This were shown by significantly decreasing in peak intensity coefficient of variant (CV) and increasing number of peak detection, respectively. This strategy could become a simple step to address reproducibility issue in this proteomic technology.

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