Comparative studies on the analysis of glycoproteins and lipopolysaccharides by the gel-based microchip and SDS-PAGE

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In order to determine time efficiency between the gel-based microchip (LabChip) and traditional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), glycoproteins and lipopolysaccharides were analyzed in this study. After 90 min of gel electrophoresis, glycoproteins (bovine serum albumin, lysozyme, ovalbumin, and apo-transferrin) and fluorescent lipopolysaccharides (LPS-O and LPS-S) under reducing conditions could be analyzed by SDS-PAGE, and it would take (including imaging and analyzing) more than 3 h. The same sample could also be assayed on a Bioanalyzer in combination with the LabChip, and it would only need 30 min from start to finish. The assay software automatically calculated the size and concentration of each separated peak and displayed the results in real time, thus eliminating time-consuming procedures such as imaging and analyzing. Compared to the traditional reducing SDS-PAGE, LabChip has a faster turnaround time. © 2006 American Institute of Physics.
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I. INTRODUCTION

Glycoproteins and lipopolysaccharides have recently attracted more attention in the biochemical field. These are widely distributed in the tissues of animals and microorganisms. Traditionally, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a technique employed by biochemists to detect and characterize proteins.¹ It is a method to resolve proteins in a mixture based on their molecular size. Negatively charged SDS binds with proteins in the sample buffer. The proteins then migrate toward the positive pole in an electrical gradient, impeded by the polymerized and cross-linked polyacrylamide. The gels were stained with Coomassie brilliant blue G-250, Sypro[®] Ruby or silver nitrite.^{2,3} The lower detection limit of conventional Coomassie Blue and colloidal Coomassie Blue stains are 10 and 50 ng, respectively.⁴ Moreover, Novex[®] Tris-Glycine polyacrylamide gel has been used to analyze the lipopolysaccharide (LPS) samples.^{5,6} This gel is a kind of polyacrylamide gel, which is based on the Laemmli system with minor modifications for maximum performance in the precast format. LPS is separated in this gel with the presence of SDS and stained in the silver nitrate solution.

SDS-PAGE, however, is a time-consuming method, which consists of a number of necessary manual steps such as staining, destaining, imaging, and analyzing. Recently, a novel approach

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based on microfluidics technology (Agilent 2100 Bioanalyzer, Agilent Technologies) and the Bioanalyzer (with LabChip) to analyze proteins has been introduced.^{7–9} Bioanalyzer contains 16 individually programmable high-voltage power supplies. It is a compact system for rapid and automated analysis of proteins, integrating multiple experimental procedures, such as sample handling, detecting, and analysis in a single process. The LabChip utilizes a network of channels and wells that are etched onto glass chips to build mini laboratories.^{10,11} The electrokinetic force then can move samples in a finely controlled manner through the channels, and the detection was based on laser-induced fluorescence by the Bioanalyzer. It is capable of fluorescence detection, in which the red laser has a maximum excitation at approximately 630 nm (maximum detection at 680 nm), whereas the maximum excitation of the blue LED is at 470 nm (maximum detection at 525 nm).^{12,13} LabChip can analyze DNA, RNA, and protein samples. It automatically calculates the size and concentration of each separated peak and displays the results in real time. One of the limitations of LabChip is not being able to analyze lipopolysaccharides.

Compared with LabChip, SDS-PAGE is used for detecting and the characterizing of proteins and lipopolysaccharides; however, it is not suitable for sample quantification. Therefore, the primary objective of this study is to analyze glycoproteins and lipopolysaccharides and compare the data obtained by LabChip and SDS-PAGE.

II. MATERIALS AND METHODS

A. Chemicals and reagents

The Protein 200 Plus kit was obtained from Agilent Technologies (Palo Alto, CA, USA), while the Novex[®] Tris-glycine gels (4%–20%, 1.0 mm × 10 well) and XCell SureLock[™] Mini-Cell were the products of Invitrogen Co. (Carlsbad, CA, USA). Bovine serum albumin, lysozyme, ovalbumin and apo-transferrin were purchased from Pierce Biotechnology Co. (Rockford, IL, USA). Lipopolysaccharides (LPS from *E. coli* serotype 055:B5 and *Salmonella minnesota*) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). LPS-Alexa Fluor[®] 488 conjugates (fluorescent LPS from *E. coli* serotype 055:B5 and *Salmonella minnesota*) and CandyCane glycoprotein standards were obtained from Molecular Probes, Inc. (Eugene, OR, USA). PageRuler[™] prestained protein ladder was obtained from Fermentas Inc. (Hanover, MD, USA).

B. Protein quantification

A modified Bradford protein quantification assay was utilized to quantify the commercial glycoproteins.¹⁴ Standard curve was prepared using commercially available ovalbumin (Sigma, St. Louis, MO, USA). Each sample (100 μ L) and Bradford reagents (1 mL) were added to the 1.5 mL microcentrifuge tube, and the stand was left at room temperature for 2 min. Absorbance was determined at 595 nm by a UV spectrophotometer (Beckman DU640; Beckman Instruments, Palo Alto, CA, USA).

C. Gel-based capillary electrophoresis (LabChip)

The LabChip analysis was performed by using the Protein 200 Plus LabChip kit. All chips were prepared according to the protocol provided with the kit. Samples (4 μ L) were denatured by mixing with sample buffer (2 μ L) and were denatured in boiling water for 3 min. The sample buffer contained 4% SDS, the proprietary fluorescent dye and two protein markers. After the denaturing step, samples were diluted with deionized water (84 μ L) and loaded (6 μ L) onto a primed chip (LabChip). The primed chip was performed according to the manual provided with the kit. Samples were then separated in this LabChip. Detection was based on laser-induced fluorescence of a fluorescent dye, which was added to the polymer, and noncovalently bound to protein-SDS micelles. Finally, the software automatically calculated the size and concentration of each separated peak and displayed the results in real time.

FIG. 1. Protein 200 Plus LabChip used in the Agilent 2100 Bioanalyzer. This LabChip is capable of delivering size, purity, and concentration information for ten different protein samples. The top side of the chip shows layout of Ladder and sample wells. Sample wells were designated as 1–10. Gel-dye mix (12 μ L) was added into the “G” well. Destaining solution (12 μ L) was added into the “DS” well. Protein marker (6 μ L) was added into the “Ladder” well.

D. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE analysis was performed by using the precast Novex[®] Tris-glycine gels. Samples (6.5 μ L), reducing agent (1.0 μ L), and sample buffer (2.5 μ L) were denatured in boiling water for 3 min. The sample buffer containing 10% glycerol, 70 mM Tris-HCl (pH 6.8), 2% SDS, and 0.02% bromophenol blue. Protein Ladder and samples (10 μ L) were then loaded into the separate wells. After an electrophoretic run, gels were stained with Coomassie brilliant blue G-250.¹⁵ The stained gels were digitized using the EPSON perfection 1270 image scanner. For the fluorescent samples, gels were digitized by a Typhoon 9200 image scanner (Amersham Pharmacia Biotech). The image acquisition station was carried out at a wavelength of 488 nm.¹⁶

III. RESULTS AND DISCUSSION

A. LabChip and Protein 200 Plus Ladder

The LabChip was used to conduct analysis on glycoproteins and lipopolysaccharides in this study. The separation, detection, and quantification of protein samples were performed by Bioanalyzer and its assay software.¹⁷ As indicated in Fig. 1, the Protein 200 Plus Ladder and ten samples could be analyzed in the Protein 200 Plus LabChip, and it took 30 min from start to finish. The size, concentration, and protein content of Protein 200 Plus Ladder are shown in Fig. 2. The sample buffer provided lower and upper markers serving as internal controls, and the amount of protein in a sample is expressed as percentage of the total protein. There were 11 peaks, which consist of the lower marker (6.0 kDa), system peak (9.0 kDa), upper marker (200.0 kDa), and eight protein peaks. The molecular weights (MW) of these eight protein peaks were 14.4, 21.5, 29.0, 32.5, 53.0, 66.7, 97.4, and 117.0 kDa, respectively. The concentrations of these proteins were 75.5, 50.1, 65.3, 114.0, 104.6, 56.5, 88.0, and 59.8 μ g/mL, while protein content was 7.0, 4.6, 6.0, 10.5, 9.7, 5.2, 8.1, and 5.5%, respectively. These results of the size and concentration of each separated peak were automatically displayed in real time by assay software.

B. Comparison of glycoproteins by LabChip and SDS-PAGE

Commercial glycoproteins (bovine serum albumin, lysozyme, ovalbumin, and apo-transferrin) were separated electrophoretically by LabChip, and it took 30 min from start to finish. Bousse *et al.*¹⁸ suggested that analyzing one sample within 1 min, and a set of ten samples on one LabChip takes approximately 25–30 min.¹⁸ The peak number, molecular weight (present as kDa), migration time (present as second), and protein content of these proteins are shown in Figs. 3 and

FIG. 2. Analysis of the Protein 200 Plus Ladder. This Ladder consists of lower marker, system peak, upper marker, and eight protein peaks.

4(a). The apparent molecular mass of bovine serum albumin (A), lysozyme (L), ovalbumin (O), and apo-transferrin (T) were 65.5, 13.5, 42.7, and 85.1 kDa, respectively. These results were in good agreement with the theoretical MW for those purified proteins. The concentrations of A, L, O, and T were 1701.0, 1657.4, 771.2, and 2611.9 μ g/mL, and protein content was 69.4, 97.4, 93.3, and 90.8%, respectively. In addition, two impurity peaks in the bovine serum albumin sample were found, and the MW at these peaks were at 157 and 190 kDa. Similar results were also generated in other glycoprotein samples.

Moreover, these glycoproteins were also separated electrophoretically in the precast Novex[®] Tris-glycine gels [Fig. 4(b)]. The total running time (including imaging and analyzing) was more than 3 h. Compared with the results obtained from LabChip and SDS-PAGE, the apparent molecular mass of A (MW=66), L (MW=13), O (MW=41), and T (MW=84) was similar. In the past, scientists have reported that analysis of proteins by the LabChip has a good correlation with the SDS-PAGE.¹⁹ In our findings, glycoproteins analyzed by the LabChip also have a good correlation with SDS-PAGE.

C. LPS and fluorescent LPS comparison by LabChip and SDS-PAGE

Lipopolysaccharides (LPS, LPS-O, and LPS-S) were separated by LabChip, but no peak was observed [Fig. 5(a)]. As mentioned previously, sample detection was based on laser-induced fluorescence by the Bioanalyzer. This indicated that LPS could not bind with the fluorescent dye in LabChip. We further analyzed commercial fluorescent LPS (LPS-Alexa Fluor[®] 488 conjugates, LPS-O) by LabChip. However, components of the fluorescent LPS could be observed by LabChip [Fig. 5(b)]. A total of six peaks in LPS-O sample were found, and the molecular weights of these peaks were 11.6, 15.6, 17.6, 23.8, 115.1, and 137.2 kDa. Among these peaks, the concentrations of three peaks were higher than 90 μ g/mL. Furthermore, there were seven peaks found in the fluorescent LPS-S sample, and the concentrations of four peaks were higher than 90 μ g/mL [Fig.

FIG. 3. Analysis of the glycoproteins by the LabChip. A: bovine serum albumin; L: lysozyme; O: ovalbumin; T: apo-transferrin.

5(b)]. These results suggest that fluorescent LPS and samples conjugated with fluorescent dye could be analyzed by LabChip.

Commercial LPS (LPS-O and LPS-S) were also separated by SDS-PAGE and stained with Coomassie brilliant blue G-250. However, no peak was observed on LPS samples [Fig. 6(a)]. These results were similar with the data obtained from LabChip. We also analyzed commercial fluorescent LPS (LPS-Alexa Fluor[®] 488 conjugates, LPS-O and LPS-S) and digitized images by a

FIG. 4. Comparison of glycoproteins by LabChip (a) and SDS-PAGE (b). A: bovine serum albumin; L: lysozyme; O: ovalbumin; T: apo-transferrin.

FIG. 5. Analysis of lipopolysaccharides (LPS) and fluorescent LPS (LPS-Alexa Fluor[®] 488 conjugates) by LabChip. (a) LPS-O: LPS from *E. Coli*. Serotype O55:B5; LPS-S: LPS from *Salmonella minnesota*. (b) LPS-O: LPS-Alexa Fluor[®] 488 conjugates from *E. Coli*. Serotype O55:B5; LPS-S: LPS-Alexa Fluor[®] 488 conjugates from *Salmonella minnesota*.

Typhoon 9200 image scanner. Components of the fluorescent LPS were observed after separation by the SDS-PAGE [Fig. 6(b)]. Moreover, the LPS conjugated with fluorescent also could be detected in SDS-PAGE by a UVP transilluminator/Polaroid MP4+ system.²⁰ According to the results, fluorescent LPS could be analyzed by both LabChip and SDS-PAGE. However, fluorescent LPS could be analyzed by LabChip within 30 min. This showed that LabChip has a faster turnaround time than SDS-PAGE.

D. Other limitations of LabChip

Several limitations of LabChip were found in this investigation. LabChip was used for sizing and analyzing samples, in which the size ranges from 5 to 200 kDa; therefore, the molecular weight of samples higher than 200 kDa or smaller than 5 kDa might not be applicable to LabChip. In addition, the sensitivity and linear dynamic range of this LabChip was 0.02–4.0 μg/μL bovine serum albumin (BSA). The lower detection limit of LabChip was 0.02 μg/μL BSA with an approximate signal-to-noise ratio of 3. Moreover, the detection of the sample was based on laser-induced fluorescence in LabChip. Thus, samples bound with the Agilent dye or conjugated with fluorescent dye could be analyzed by LabChip.

FIG. 6. Analysis of lipopolysaccharides (LPS) and fluorescent LPS (LPS-Alexa Fluor[®] 488 conjugates) by SDS-PAGE. (a) LPS-O: LPS from *E. Coli*. Serotype O55:B5; LPS-S: LPS from *Salmonella minnesota*. (b) LPS-O: LPS-Alexa Fluor[®] 488 conjugates from *E. Coli*. Serotype O55:B5; LPS-S: LPS-Alexa Fluor[®] 488 conjugates from *Salmonella minnesota*.

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