Microfluidic Technology Applied to Protein Sizing and Quantitation

P. Barthmaier*

*Agilent Technologies, 3500 Deer Creek Rd. Palo Alto, CA 94304 USA

ABSTRACT

The current standard method for analyzing proteins is denaturing gel electrophoresis (SDS-PAGE). Researchers rely on this traditional method that has not substantially changed in the past 30 years. There is, however, a strong demand for automation and higher throughput.
The development of microfluidics offers an alternative for protein analysis. The first commercial microfluidic system, the Agilent 2100 bioanalyzer, allows for rapid and automated separation of proteins.
The chip-based protein analysis is comparable in sensitivity, sizing accuracy and reproducibility to SDS-PAGE stained with Coomassie. Resolution and linear dynamic range are improved. Absolute quantitation accuracy and reproducibility is superior to SDS-PAGE and is comparable to Lowry or Bradford. Agilent 2100 bioanalyzer offers significant advantages to SDS-PAGE such as: fast analysis times, ease of use, automated data analysis, and good reproducibility.

Keywords: Microfluidic, Protein, 2100 Bioanalyzer, SDS-PAGE.

INTRODUCTION

The use of recombinant proteins has increased greatly in recent years. With the advances in protein expression and the molecular techniques used to detect, characterize and purify those proteins researchers can now begin to assign molecular and cellular functions to numerous proteins. Protein tags such as HIS, FLAG or GST make it possible to use one protein purification strategy for different proteins allowing a move to high throughput protein expression and purification. With these advances there is a need for more automated and faster analysis of proteins that cannot be achieved using traditional protein analysis methods, such as SDS-PAGE. The Agilent 2100 bioanalyzer in combination with the Protein LabChip® kits provides an automated and easy way to analyze proteins on a microfluidic platform. The Agilent 2100 bioanalyzer was developed in collaboration with Caliper Technologies and is the first commercial instrument based on lab-on-a-chip technology. The system is compact and provides a rapid, automated and reproducible method for the analysis of proteins. Multiple manual experimental steps have been integrated such as: sample handling, separation, staining, de-staining, detection and sample analysis.[1] LabChip kits are developed depending on the application requirements. Currently two Protein lab chip kits are available. The Protein 50 assay is complementary to the well-established Protein 200 Plus assay. Much like the 2100 bioanalyzer DNA assays, each protein assay has a specified size range. The Protein 200 Plus assay can analyze proteins from 14-200 kDa in size while the Protein 50 assay has a size range from 5-50 kDa. Both the Protein 200 Plus and the Protein 50 assays offer significant time-savings and more reliable, reproducible data in a digital format compared to SDS-PAGE analysis.

Sizing and Quantitation

The Agilent 2100 bioanalyzer is a user-friendly system that has integrated the complexities of SDS-PAGE onto a microfluidic chip. Each chip has a designated ladder well and ten individual sample wells. To achieve accurate sizing on the Protein 200 Plus LabChip a lower and upper marker are incorporated into the ladder and each sample. The software automatically generates a standard curve of the migration time of the ladder proteins versus their known molecular weights. This standard curve is used to determine the size of the unknown proteins in the samples once the samples have been aligned to the upper and lower markers in the ladder. A sizing resolution of 10% or better is achieved on the chip comparable to a 4-20% gradient slab gel.
The relative concentration of protein samples are determined using a one-point calibration to the upper marker included in each sample. The Agilent 2100 bioanalyzer compares the peak areas of the unknown sample to the peak area of the upper marker. The relative concentration of the unknown proteins within the sample is calculated based on the known concentration of the upper marker. The inclusion of the upper marker in each sample corrects for differences in sample injection into the separation channel and allows for reproducible quantitation. Usually a quantitation reproducibility of ±30% or better is achieved. The relative quantitation accuracy of the Protein 200 assay depends on the staining efficiency of the protein. Each of the commonly used total protein quantitation methods, such as Lowry, Bradford or BCA assays, exhibits some degree of varying color response when assaying different proteins. The staining efficiency depends upon the characteristics of the specific protein and its interaction with the dye. These differences are related to variations among proteins in amino acid sequence, isoelectric point (pI), structure and the presence of certain side chains or

prosthetic groups. The variations in staining efficiency may result in the under or overestimation of protein concentrations. Similar effects are observed when staining polyacrylamide gels with protein stains such as Coomassie or silver stain. The Agilent 2100 bioanalyzer detection is based on laser-induced fluorescence of an intercalating dye, which interacts with the protein/SDS complexes. The staining efficiency of different proteins also varies with this method.[2]

More accurate, absolute quantitation can only be obtained by using a calibration curve generated with identical proteins and known concentrations. Figure 1 shows a calibration curve generated by the software for a 200 µg/ml sample of carbonic anhydrase with both its relative and absolute quantitation values shown.

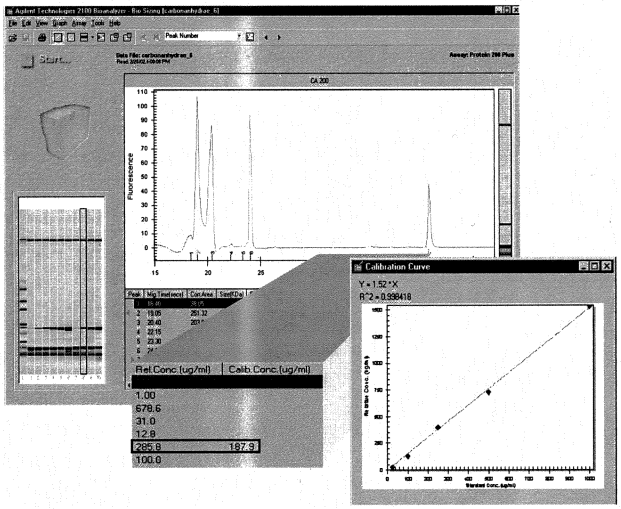

Figure 1: Agilent 2100 bioanalyzer user interface showing the relative and absolute quantitation of a carbonanhydrase (CA) sample using the Protein 200 Plus assay.

Applications

The Agilent 2100 bioanalyzer in combination with the Protein 200 Plus LabChip kit can analyze a variety of protein samples such as cell lysates, column fractions, antibodies and purified proteins. Analysis with the Protein 200 Plus assay produces comparable results to SDS-PAGE but in a much shorter time frame. Size, purity and concentration are determined in one experimental step. All of the data is acquired, analyzed and stored in real-time, in an electronic format for easy storage and retrieval. No staining, destaining, imaging or further analysis is necessary. The Protein 200 Plus assay has a linear dynamic range of 20-2000 µg/ml, for BSA in a PBS solution, and a sizing range from 14-200 kDa. Figure 2 illustrates the monitoring of the purification of GFP tagged with a Strep-tag®, a short eight amino acid peptide. Strep-tags have a highly selective binding property for streptavidin and can be fused to a protein's N- or C-terminal, making them ideally suited for the rapid purification of recombinant proteins by affinity chromatography. Using the 2100 bioanalyzer researchers can quickly assess the purity and concentration of the protein of interest. The GFP fusion protein is accurately identified with its correct size of 28 kDa and the relative concentration of this protein in elution fraction 77 was determined to be 143.4 µg/ml with a purity of 97%. In fraction 78 protein concentration is 35.8 µg/ml with a purity of 96%. The bioanalyzer automates protein analysis providing fast and easy quality control of the sample purity and yield.[3]

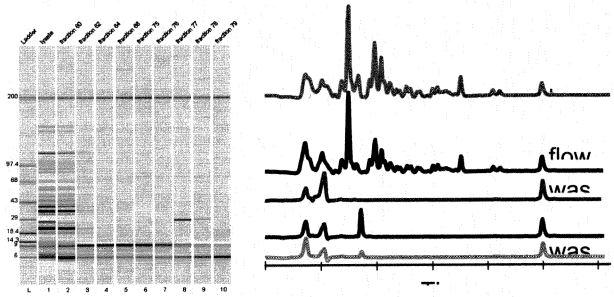

Figure 2: Monitoring the purification process of a GFP fusion protein using the Agilent 2100 bioanalyzer. The gel-like image is shown on the left and electropherograms of selected column fractions are shown on the right. (Courtesy of P. Sebastian and S.R. Schmidt GPC-Biotech AG, Martinsried, Germany)

Another benefit of the Agilent 2100 bioanalyzer is the ability to run both reduced and non-reduced samples on the same chip. All proteins are heat denatured in the presence of a sample buffer, which contains SDS and is provided with the kit. The reducing agents, dithiothreitol or beta-mercaptoethanol can be added to the sample buffer if it is desired to reduce the proteins disulfide bonds. Figure 3 shows the analysis of a reduced and non-reduced antibody (IgG from Sigma Aldrich) in the gel-like image along with their respective overlaid electropherograms. In the non-reduced sample there is a distinct peak with the intact antibody running at 157 kDa. There is also a peak at 87 kDa correlating to a single light and heavy chain. When the sample is run under reducing conditions there are only individual light and heavy chain peaks, sized at 27 kDa and 59 kDa respectively. This is a clear advantage over SDS-PAGE, as this technology does not allow running reduced and non-reduced samples sided by side due to the diffusion of the reducing agent within the gel.

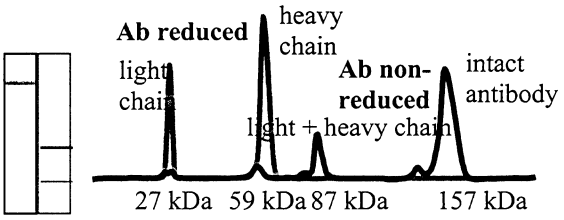

Figure 3: Gel-like image and overlayed electropherograms of a IgG antibody analyzed under reducing and non-reducing conditions.

Conclusion

The Agilent 2100 bioanalyzer lab-on-a-chip system is an ideal tool for the analysis of proteins, providing information on size, concentration and purity in one single assay. The performance of this system in terms of sizing, sensitivity, linear dynamic range and resolution is comparable or even superior to conventional SDS-PAGE, with greatly reduced analysis times. The Agilent 2100 bioanalyzer is an easy to use system with automated separation, detection and data analysis, which ensures for good reproducibility from lane to lane as well as chip to chip. The digital data format makes it simple to both store and share data, while the microscale volumes greatly reduce the use and exposure to hazardous materials.

In addition, the 2100 bioanalyzer can also analyze DNA, RNA and, allows for simple flow cytometric analysis of cell fluorescent parameters using vacuum driven flow. The Agilent 2100 bioanalyzer provides a complete solution for the laboratory.

REFERENCES

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