Increased sensitivity by desalting protein samples prior to analysis on the Agilent 2100 bioanalyzer

Application

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Introduction

Ion exchange chromatography (IEX, IEC) is frequently used in the separation of proteins. As in all forms of liquid chromatography, conditions are employed that permit the interaction of sample components with the solid and mobile phase. Differences in these interactions allow separation of sample components. In IEX, charge-charge interactions bind components to a chromatographic support. A mobile phase of increasing ionic strength is then applied to the column and sample components are separated according to differences in their ionic characteristics. IEX is an effective and powerful tool for the separation of charged molecules; however, the varying amounts and high concentrations of salts required for elution can be problematic in downstream experiments.

When working with an analysis tool that requires the electrokinectic injection of samples, such as the Agilent 2100 bioanalyzer, the high salt concentration can affect the sensitivity of the system. Due to a greater ionic strength, salt is preferentially injected over proteins in the sample. Removing excess salt from samples prior to analysis on the 2100 bioanalyzer greatly improves sensitivity and consistency. The Pierce Biotechnology Protein Desalting Spin Columns allow for the quick removal of excess salts from samples in an easy to use format.

Experimental

Desalting columns were provided by Pierce Biotechnology (product number 89849). Proteins, 2-mercaptoethanol and Trizma-HCl pH 7.0 were purchased from Sigma Aldrich, St. Louis Mo. The Agilent 2100 bioanalyzer and the Protein 200 Plus LabChip kit were obtained from Agilent Technologies GmbH (Waldbronn, Germany).

Protein desalting

Samples were desalted and processed per the Pierce Protein Desalting Spin Column protocol (figure 1). For these experiments 50 µl of each sample was processed with an individual desalting column at a total protein load of 62.5 µg each. The Pierce Protein Desalting Spin Columns fit into a standard 1.5 - 2.0 mL microcentrifuge tube and are designed to desalt or exchange buffer of protein samples with volumes from 30 to 120 µl. Each column contains approximately 700 µl of desalting resin buffered in 10 mM Tris pH 7.5.

Protein 200 Plus assay

The chip-based protein analysis was performed on the Agilent 2100 bioanalyzer using the Protein 200 Plus LabChip® kit and dedicated Protein 200 Plus software assay. Samples were denatured as specified in the Reagent Kit Guide using the Protein 200 Plus Sample Buffer with added beta-mercaptoethanol. All chips were prepared

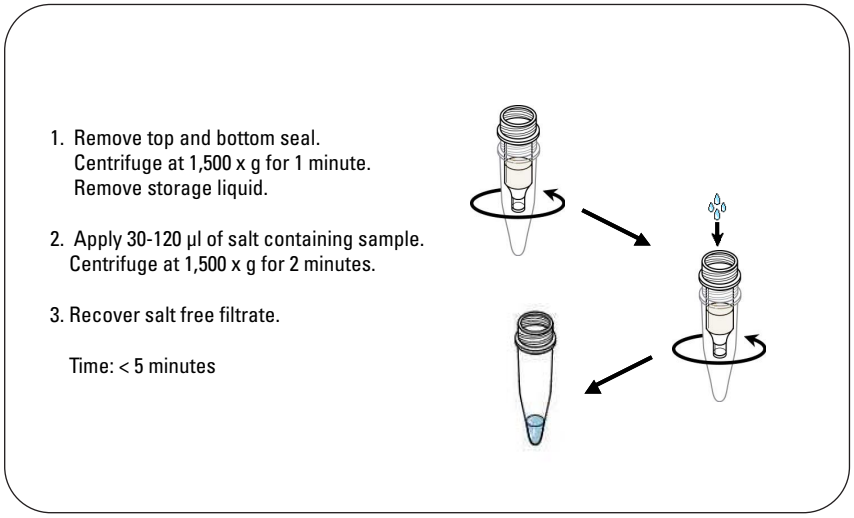

Figure 1
Pierce Desalting Spin Column protocol

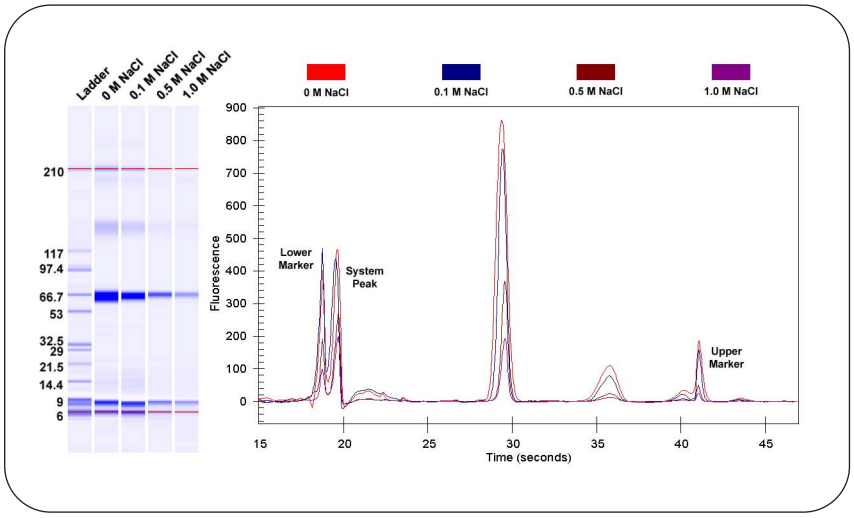

Figure 2
A 1.25 mg/mL sample of BSA was prepared in 10 mM Tris pH 7 with varying amounts of NaCl, ranging from 0 to 1 M. Although less sample is injected as amount of salt increases, concentration measurements remain accurate because the upper marker serves as an internal standard for quantitation.

according to the instructions provided with the Protein 200 Plus LabChip kit. This kit includes 25 chips, a syringe, 4 spin filters and all required reagents except for reducing agent. Data is collected in real-time and stored in a digital format.

Results and discussion

The Agilent 2100 bioanalyzer, while compatible with a large range of buffer components, can be sensitive to salt concentrations outside of the recommended specifications. To demonstrate the influence of salts on the Agilent 2100 bioanalyzer, samples were prepared to mimic those from a typical ion exchange experiment and then analyzed with the Protein 200 Plus kit. Samples were prepared in 10 mM Tris buffer with final salt concentrations of: 0 M, 0.01 M, 0.5 M or 1.0 M respectively, at a final protein concentration of approximately 1.25 mg/mL. As shown by the overlay of electropherograms in Figure 2, signal intensity decreases as the salt concentration of a sample is increased. However, while sensitivity is affected, quantitation is not. All samples processed on the 2100 bioanalyzer are prepared in a denaturing solution containing a lower and upper marker. The upper marker is used not only to align the samples with the ladder for accurate sizing but also as an internal standard for accurate quantitation measurements. The upper marker of the Protein 200 Plus assay is a protein and is injected at the same ratio as the unknown sample proteins, thereby correcting for injection effects due to varying salt concentrations in the samples (final peak figure 2). A quantitation measurement can then be derived relative to the known concentration of the upper marker [1,2].

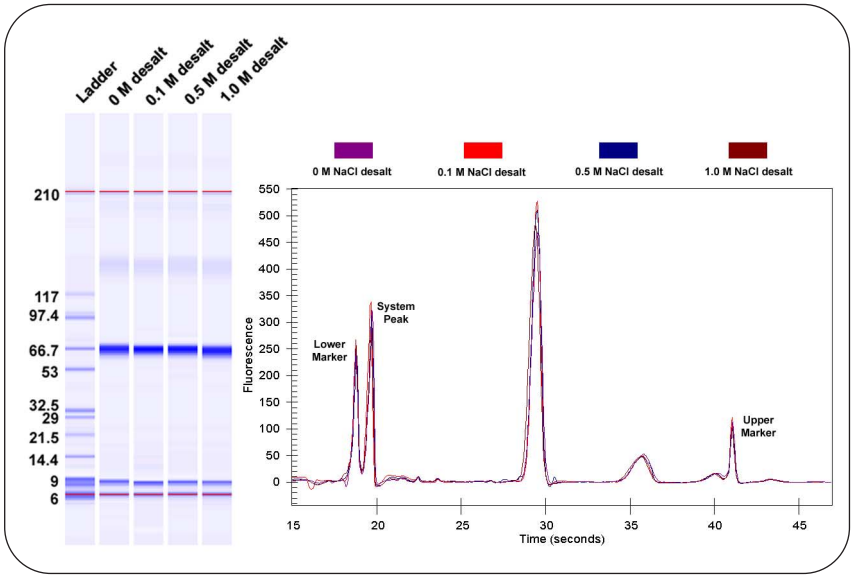

Figure 3
After using the Pierce desalting columns, samples have a similar buffer composition and are therefore injected at the same ratio.

The influence of salts on the sensitivity of the 2100 bioanalyzer can be eliminated by desalting samples prior to analysis. Each of the four samples containing differing NaCl concentrations was desalted according to instructions and again analyzed on the Agilent 2100 bioanalyzer. The Protein Desalting Spin Columns are based on size exclusion or molecular sieve chromatography. Salts and small molecules in the sample enter pores within the size exclusion resin and will therefore move slower through the column resin. Larger molecules are excluded from the beads and move at a faster rate through the column. Using the spin format, excess salts remain trapped in the resin bed. After desalting, the samples have a similar buffer composition and salt concentration and run reproducibly with no suppression of sensitivity as shown by the overlay of the traces in the electropherograms (figure 3). Only small differences between the samples were observed due to sample handling and preparation.

Conclusion

The Agilent 2100 bioanalyzer can analyze and provide real-time data on composition, molecular weight and concentration, for ten protein samples in less than 45 minutes, from sample preparation to analysis. However, high concentrations of salt within a sample can affect the amount of material injected into the microfluidic channels of the chips. Desalting of samples prior to analysis significantly improves the sensitivity. The Protein Desalting Spin Columns from Pierce provide a quick and efficient way of desalting protein samples. These columns are capable of removing 95 % of the sample salt content and achieve a protein recovery of greater than 90 % in approximately 5 minutes[3] (table 1). The speed and ease-of-use of the desalting columns make them the perfect compliment for the Agilent 2100 bioanalyzer. The Agilent 2100 bioanalyzer and Pierce products continue to make protein research quick and easy.

	Rel. Concentration		
NaCl	Prior	After	% Recovered
0 M	1163.4	1150.6	99 %
0.1 M	1230.7	1247.2	101 %
0.5 M	1679.1	1450.1	86 %
1.0 M	1763.7	1435.7	81 %

Table 1
Relative concentration measurements before and after desalting of the samples. Initial Relative Concentration measurement is slightly elevated at higher salt concentrations, 0.5 M and 1.0 M. This affects the calculation of the % recovered after desalting

References

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