Rapid Chromatography Optimization by pH Scouting Using the New BioLogic Maximizer™ Valve System

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Introduction

Fast and efficient protein purification is essential to explore the structure and function of proteins in the human proteome. Successful protein purification strategies use experimental conditions where proteins of interest are stable and have chromatographic properties different from contaminating proteins. Significant time is often required to optimize chromatography parameters, such as buffer pH and gradient characteristics. Furthermore, purification strategies need to be easily scaled as the amount of protein required increases from milligram to gram quantities.

The new Maximizer buffer blending system, a component of the BioLogic DuoFlow™ chromatography system, significantly decreases the time and resources required for chromatography method development. With a single set of three salt solutions, the system can perform several chromatography experiments, varying the pH or gradient characteristics, or both, for each run. It is not necessary to prepare separate buffers for each chromatography experiment, and the system performs the experiments unattended. Data from the experiments are easily viewed and compared using the new Trace Compare feature of the BioLogic DuoFlow software.

Application

We were interested in purifying a recombinant protein in milligram quantities, anticipating future scale-up to preparative quantities. The 25 kD protein of interest is overexpressed as an *E. coli* inclusion body. Information from similar proteins suggested a stability range of pH 6.0–9.0. The protein was solubilized and clarified from cell paste using standard techniques. We used the pH scouting capability of the BioLogic DuoFlow Maximizer™ system to determine the pH that provides the best resolution.

Methods

We selected a Bio-Scale™ Q2 column for the chromatography, and Bis-Tris/Tris from the menu of buffer systems because its buffering range matched the stability range of the protein. We decided to run separations at pH 6.0, 7.0, 8.0 and 8.9, using a 50 ml, 0–75% buffer B (1 M NaCl) gradient for our preliminary experiments. The protein solution was diluted 1:10 with Bis-Tris/Tris loading buffer. The 5 ml sample loop was filled using an Econo™ gradient pump controlled by the automatic loop fill/inject function. Samples were collected by a BioFrac™ fraction collector, using Threshold collection to minimize the

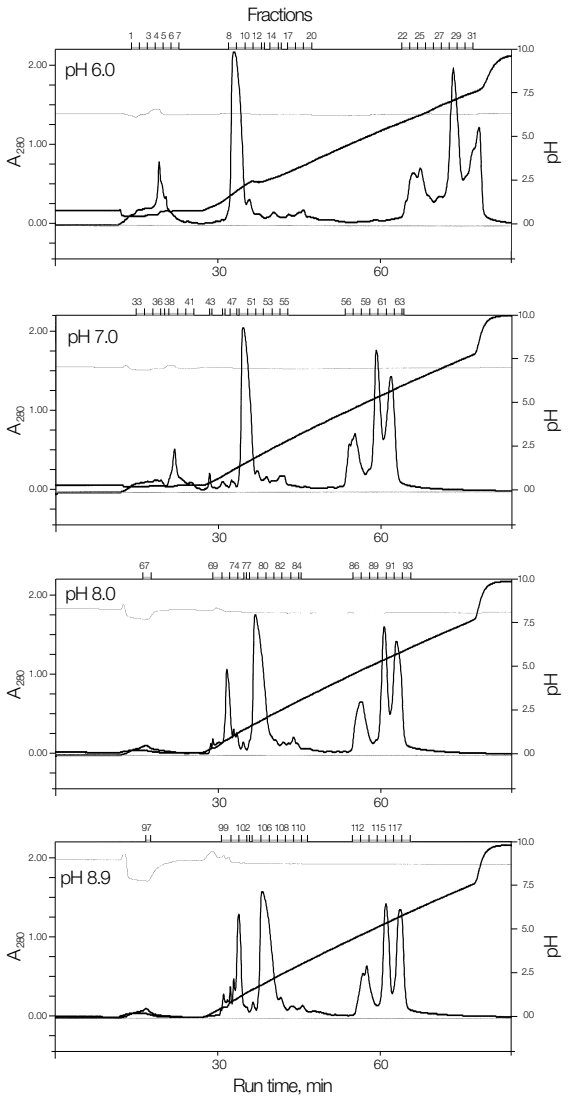

Fig. 1. Chromatograms from four scouting runs, pH 6.0 to 8.9.

number of tubes required. As coldroom space is limited in the lab, the collected samples were kept on ice using the BioFrac fraction collector's ice bath/microplate rack feature. Between experiments, the system was equilibrated to a new pH using the Hold Until function of BioLogic DuoFlow version 4.0 software. This function monitors the pH of the column effluent, and continues the run as soon as the desired pH is reached, reducing run time and volume of buffer required.

Results

Chromatograms from the four runs are shown in Figure 1, and a Trace Compare overlay of the UV traces is shown in Figure 2. The separation at pH 6 shows a single peak, which is believed to contain the protein of interest, and a second peak group containing contaminants. The separations at pH 7 and 8 show increasing resolution of the peaks of interest, with the best resolution at pH 8.9.

The run reports were printed automatically and the samples were stored in the BioFrac ice bath collection rack. The BioLogic DuoFlow software handled everything from automated sample loop loading and injection to comparison of results.

The buffer blending and pH scouting capabilities of the Maximizer system allowed execution of this experiment with minimal attendance. The total time for the set of four separations was 5 hr. The experiment was started at the end of the day, and ran unattended during the evening. Eliminating the need to make up three extra sets of buffers saved an estimated additional 1.5 hr of labor. The reduced waste from unused buffers was an additional cost savings.

Peak Identification

Samples from the first set of peaks were run on a Criterion™ gel, using Precision Protein™ prestained standards to identify the proteins by molecular weight. In the gel (Figure 3), the protein of interest appears in fractions 105–110, with the highest concentration found in fractions 106 and 107.

Scale-Up Plans

We are now scaling up the recombinant protein separation. A larger column will be required to produce sufficient protein for the next phase of development. This will require approximately 70 ml/min of flow at a total system pressure of 500 psi. We will install F40 pumpheads on our existing DuoFlow system to increase the maximum flow rate to 80 ml/min at 1,000 psi. A preparative rack for the BioFrac fraction collector will allow collection of the larger fractions involved.

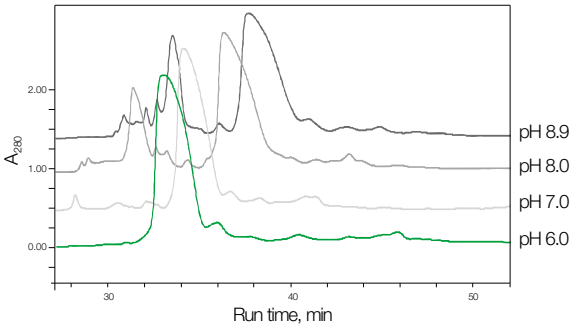

Fig. 2. Comparison of separations at different pHs using Trace Compare feature. Green trace is set to scale; other traces are offset vertically for comparison.

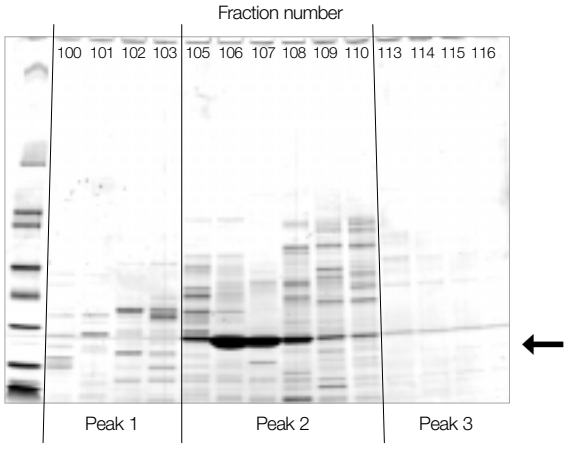

Fig. 3. Criterion™ gel separation of peaks of interest. Protein standards are in the far left lane. Arrow indicates the protein of interest in peak 2.

Conclusion

The optimum pH for this separation on the Bio-Scale Q2 was pH 8.9, at the high end of the protein's stability range. Gel electrophoresis confirmed the presence of the protein of interest. The purity of the recovered protein was good, with faint bands indicating that some other proteins were present. We will experiment with other gradient slopes, using the queuing function of the software to attempt to resolve the small shoulder peaks on each side of the main peak on the chromatogram.

The Maximizer system, with its buffer blending capability, allowed us to determine the pH for optimum separation in an automated pH scouting experiment. Using this system, we reduced the time required to purify the protein and were able to proceed to the next phase of the project more quickly and easily than we would have by mixing eight buffer solutions and moving tubing manually between runs.

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