



Separation of His-tag Protein Aggregation Variants on the New Analytical ProPac® IMAC-10 Column

Patrick McCarthy, Andrea Heckenberg, and Chris Pohl
Dionex Corporation

Immobilized-metal affinity chromatography (IMAC) stationary phases are typically used as capture / release cartridges for enrichment of peptides and proteins (e.g., surface-exposed histidines or phosphopeptides/phosphoproteins) or for the purification of His-tag recombinant proteins. The capture / release approach captures all proteins with an affinity for the immobilized metal and therefore further purification is generally required. To address this problem, we developed the high-resolution ProPac IMAC-10 column, using state-of-the-art nanotechnology. The design of the stationary phase is such that a single protein can interact with a single nanoparticle on the surface of the stationary-phase bead. In this manner, the captured protein is isolated from all other components in the matrix and from components bound to neighboring nanoparticles. Conceptually, this design should be optimal for the separation of proteins or peptides within a class, on-column refolding, on-column protein-protein interaction studies, and on-column site-directed reactions (e.g., site-directed biotinylation).

There are generally two stages to recombinant-protein purification; purification of the His-tagged proteins and assessment of product purity. Proteins are often purified using a copper-loaded IMAC cartridge with product-purity assessment by SDS PAGE. Protein aggregation can affect protein function, activity, and ease of crystallization, thus it is important to be able to detect and fractionate protein-aggregate variants during the IMAC-purification process. The current IMAC protocols lack of UV monitoring and de-aggregation during SDS PAGE makes it very difficult to detect the presence of protein-aggregation variants during purification. The IMAC-purification example described in this application note demonstrates the advantage of the ProPac IMAC-10 column for separation, detection, and fractionation of His-tagged protein aggregation variants.

Experimental

We used a Dionex BioLC® system, including a GP50 micro bore quaternary pump, an AD20 UV-Vis detector, a Wyatt Technologies (Santa Barbara, California) DAWN® EOS detector, Dionex Chromleon®, and Wyatt Technologies ASTRA® software. A 4 × 250 mm ProPac IMAC-10 column was prepared by loading it with a solution of 50 mmol/L copper sulphate and 1 mol/L sodium chloride at 1 mL/min.

Results

The separation of purified His-tagged protein aggregation variants is shown in Figure 1. The blue trace is the UV trace at 280 nm. The red trace is the light-scattering trace. The four major peaks observed in the UV trace are verified as monomer, dimer, trimer, and tetramer aggregation variants by light scattering. Seven fractions were collected. SDS PAGE of the fractions showed that the His-tag

protein was the major component in each fraction, which confirms that the peaks are His-tag protein aggregation variants. Interestingly, the light-scattering trace showed a peak not visible by UV detection. Because the light-scattering response is molecular-weight dependent, we expect that this peak is a very large aggregate at low concentration.

Conclusions

The Dionex analytical ProPac IMAC-10 column offers increased resolution for separation and fractionation of His-tag proteins. This column can be used to detect and remove His-tag protein aggregates and impurities which is especially important for protein crystallization and activity assays. The stationary-phase substrate and column formats allow for easy automation and are compatible with UV and light-scattering detectors. The nano-scale architecture of the stationary-phase surface was tuned to provide the highest resolution for His-tag protein separations, but should also provide an optimal surface for on-column refolding experiments, and site-directed reactions.

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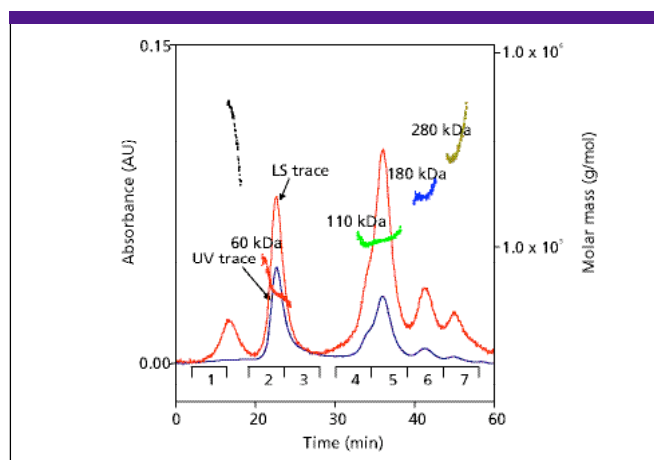


Figure 1: His-tagged protein aggregation variants using IMAC-HPLC-MALLS. IMAC separations were carried out using: Column: ProPac IMAC-10 (4 × 250 mm); eluents: A. 20 mM HEPES + 0.5 M NaCl, pH 7.5 and B. 20 mM HEPES + 0.5 M NaCl + 500 mM imidazole, pH 7.5; gradient: 0–100%B over 80 min; flow rate: 1 mL/min; detection: UV at 280 nm and DAWN EOS; injection volume: 100 µL; sample: His-tagged protein; flow rate: 0.5 mL/min.

Dionex Corporation

1228 Titan Way, P.O. Box 3603, Sunnyvale, CA 94088

tel. (408) 737-0700, fax (408) 730-9403

www.dionex.com