Purification of Amyloidogenic Transthyretin Protein Using Nuvia™ Q Anion Exchange Resin

Sébastien Penninckx, Vincent Raussens, and Stéphanie Deroo
Center for Structural Biology and Bioinformatics, Université Libre de Bruxelles, Blvd du Triomphe, 1050 Brussels, Belgium.

Process Separations

Bulletin 6711

Abstract

Purification of recombinant amyloidogenic proteins can sometimes prove challenging due in part to their aggregation-prone character. Here, we demonstrate how we were able to use Bio-Rad's Nuvia Q Resin to successfully purify one such protein, transthyretin (TTR).

Introduction

TTR is involved in both senile and familial forms of amyloidosis. Over 100 amyloidogenic mutations have been reported and they represent the most common cause of hereditary amyloidosis. Purification of recombinant TTR can be problematic due to its aggregation properties, which prevent the use of acidic buffers or denaturant agents. Purification of recombinant TTR is typically performed by salting out TTR using an ammonium sulfate precipitation procedure. This procedure requires an extra purification step to isolate TTR from contaminating DNA and coprecipitating proteins. Since TTR has an isoelectric point of 5.7, we used high-capacity Nuvia Q Anion Exchange Resin to effectively purify it under neutral buffer conditions.

Materials and Methods

Recombinant TTR was produced in BL21(DE3)pLysS *Escherichia coli* cells (Merck Millipore Corporation). The cells were harvested by centrifugation (Avanti J20-XPI, Beckman Coulter, Inc.) and lysed using thermal shock and sonication (Bioruptor UCD-200, Diagenode, Inc.). Cell debris was removed by centrifugation and the lysate was submitted to the salting out procedure. The protein pellet was collected at 40–60% ammonium sulfate saturation by ultracentrifugation (Optima L-70 Ultracentrifuge, Beckman Coulter, Inc.), resuspended in buffer A (50 mM Tris-HCl, pH 7.5), and dialyzed overnight (Slide-A-Lyzer G2 Dialysis Cassettes, 20K MWCO, Thermo Fisher Scientific Inc.).

Purification of TTR was carried out by anion exchange chromatography on an NGC™ Chromatography System (Bio-Rad Laboratories, Inc.) using a 5 ml Foresight™ Chromatography Column prepacked with Nuvia Q Resin (Bio-Rad Laboratories, Inc.). The column was equilibrated using 2 column volumes (CV) of buffer A. Dialyzed TTR was then applied to the Nuvia Q Column at a linear velocity of 300 cm/hr and the column was washed with 2 CV of buffer A. TTR was eluted using a segmented NaCl gradient between buffer A and buffer B (50 mM Tris-HCl, 2 M NaCl, pH 7.5). In the first step, a 7 CV linear gradient up to 17% of buffer B was established (corresponding to 340 mM NaCl) followed by a 3 CV hold in 17% buffer B to elute TTR. In the second step, a 5 CV linear gradient up to 100% of buffer B was used followed by a 1 CV hold in buffer B to elute and eliminate the contaminating DNA. The column was then re-equilibrated in buffer A by the application of a 2 CV linear gradient from buffer B to buffer A and a 2 CV hold in buffer A.

Protein fractions were analyzed on a 12% Coomassie-stained SDS-PAGE gel using a prestained protein standard (Thermo Fisher Scientific Inc.). Absorbance spectra were measured on a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc.). Mass spectrometry (MS) analysis of the purified proteins was performed on a Q-Tof Ultima API Spectrometer (Waters Corporation). Size exclusion chromatography (SEC) was carried out on an NGC Chromatography System (Bio-Rad Laboratories, Inc.) using a Superose 6 10/300 GL Column (GE Healthcare).

Results

Following salting out of recombinant TTR from the cell lysate, purification of TTR from DNA and coprecipitating proteins was accomplished using a Nuvia Q Column in a simple one-step procedure using a segmented NaCl gradient (Figure 1A). First, application of 0–0.34 M NaCl allowed the separation of TTR from coprecipitating proteins. The protein contaminants were eliminated both in the flowthrough and by the NaCl gradient, allowing the recovery of TTR in a well-resolved peak. Following this, the host-cell DNA contaminants that were bound to the Nuvia Q Resin were eliminated by application of 0.34–2 M NaCl, as confirmed by the absorbance spectra of the pooled fractions (Figure 1B).

BIO-RAD

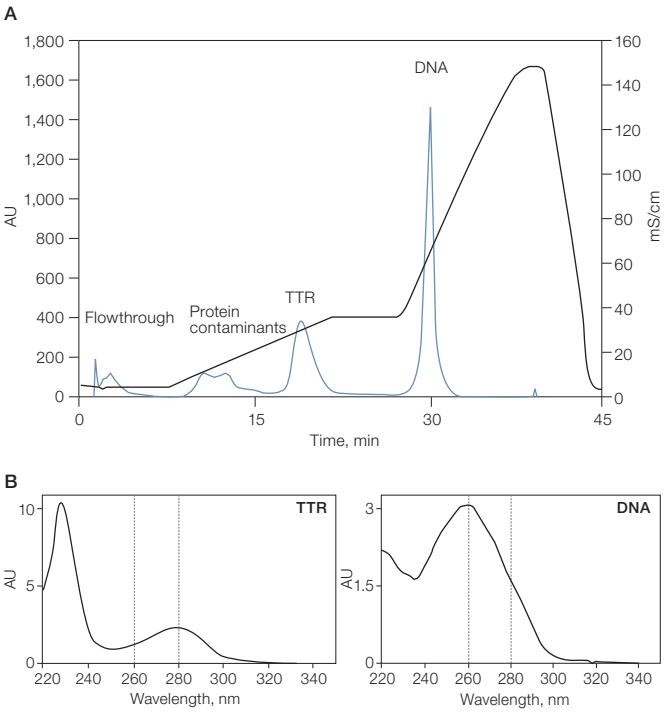

Fig. 1. One-step chromatographic purification of salted-out TTR using Nuvia Q Resin. A, chromatogram of TTR purification on a Nuvia Q Column. OD 280 (–); conductivity (–). **B**, absorbance spectra of pooled TTR and DNA fractions. AU, absorbance units.

The purity of TTR was assessed by reducing SDS-PAGE, SEC, and MS analyses (Figure 2). Under the optimized conditions, TTR was efficiently separated from the contaminating proteins as visualized by the SDS-PAGE gel and SEC chromatogram. Based on the gel, protein purity could be assessed at >95%. The mass spectrum presented a single well-resolved series of charge states corresponding in mass to 13,892.0 ± 1 Da. This value is in excellent agreement with the calculated mass of TTR and confirms its identity. The procedure described here was applied successfully to purify wild-type TTR and one of its amyloidogenic mutants (data not shown).

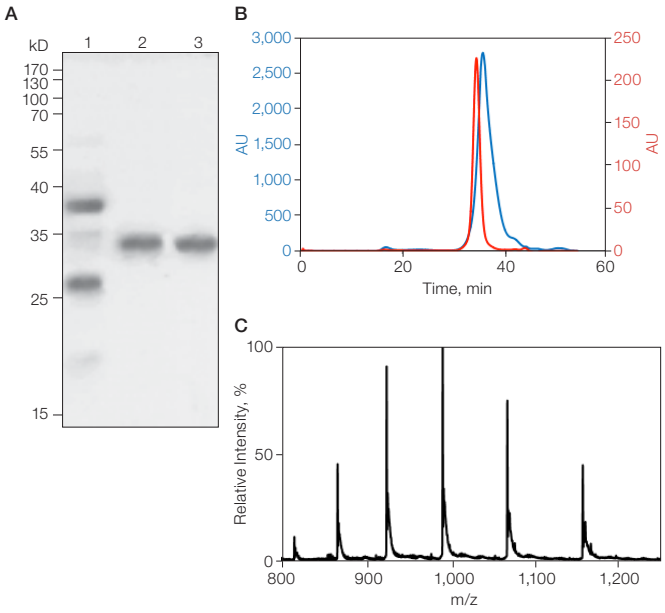

Fig. 2. Analysis of TTR purified on Nuvia Q Resin. A, fractions collected from the chromatography run shown in Figure 1 were separated by SDS-PAGE. Lane 1, protein contaminants; lane 2, TTR eluted from the Nuvia Q Column; lane 3, TTR standard. **B**, SEC comparison (OD 280) of salted-out TTR (–) and purified TTR (–). AU, absorbance units. **C**, mass spectrum of purified TTR (measured mass: 13,892.0 ± 1 Da; calculated mass: 13,892.6 Da).

Conclusions

- Nuvia Q Anion Exchange Resin was successfully used to purify TTR from contaminating DNA and proteins
- The purity of TTR was verified using three different methods — SDS-PAGE, SEC, and MS analyses — and shown to be >95%

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *43 1 877 89 01* ***Belgium*** *03 710 53 00* ***Brazil*** *55 11 3065 7550* ***Canada*** *905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *49 89 31 884 0* ***Greece*** *30 210 9532 220* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 (0) 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *026 674 55 05* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *1800 88 22 88* ***United Kingdom*** *020 8328 2000*