Large Scale Purification of Adeno-associated Virus (AAV) with Continuous Flow Ultracentrifugation

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Abstract

Since its first approval in Europe as a gene therapy drug in human use in 2012, adeno-associated virus (AAV) vectors have gained more and more attention in the field of gene therapy research.

So far AAV vectors have usually been purified through either density gradient ultracentrifugation in small volume centrifuge tubes or column chromatography. Though these purification methods have their unique benefits, there is still a need for technology that can process large volumes of lysate with high AAV recovery rates. We reasoned that continuous flow ultracentrifugation could meet these requirements.

We tested the Alfa Wassermann AW Promatix 1000™, a research scale continuous flow ultracentrifuge, as a proof of concept for AAV vector purification. In the initial experiments, we tested cesium chloride (CsCl) solution as density gradient media for AAV vector purification but found out that CsCl solution was not stable enough to form a linear gradient even when sucrose was added to increase its viscosity for AAV purification. We then tested iodixanol solution as a density gradient media and got satisfactory purification of AAV vectors.

Our results indicate that we can obtain near-purified AAV vectors in a single-step of centrifugation with an AAV recovery rate exceeding 50%. Further experiments indicate that minor impurities associated with the purified AAV vectors could be removed by adding salts to the iodixanol solution such as CsCl to increase the ionic strength of the density gradient.

The data presented here indicate that continuous flow centrifugation can be used for large scale purification of AAV vectors and it should provide an additional tool to facilitate the translation from research to the clinic.

AAV vector production and purification

Fig. 1 Schematic illustration of AAV vector production and purification.

(A) Illustration of AAV vector production in Sf9 cells with WAVE Bioreactor; (B) Image of AW Promatix 1000TM continuous flow ultracentrifuge used in this study.

CsCl gradient formation in AW Promatix 1000™

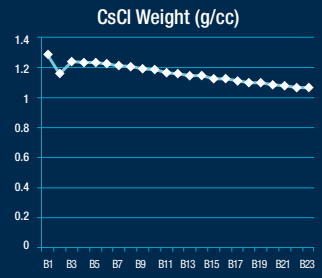

Fig. 2 Testing of CsCl gradient formation in the AW Promatix 1000™ centrifuge.

A 120-ml rotor was loaded with 30ml of 1.32g/cc and 38ml of 1.5g/cc of CsCl solution containing 30% sucrose and slowly accelerated to 26,000rpm. After centrifugation for 10min at 26,000rpm the speed was slowly decreased and fractions collected. The CsCl density was determined and plotted against the fractions. The result indicates that the CsCl density didn't reach the level that can separate AAV vectors (~1.4g/cc) even though 1.5g/cc CsCl solution was used at the bottom as a cushion.

Iodixanol gradient solution for purification of AAV in Promatix 1000™

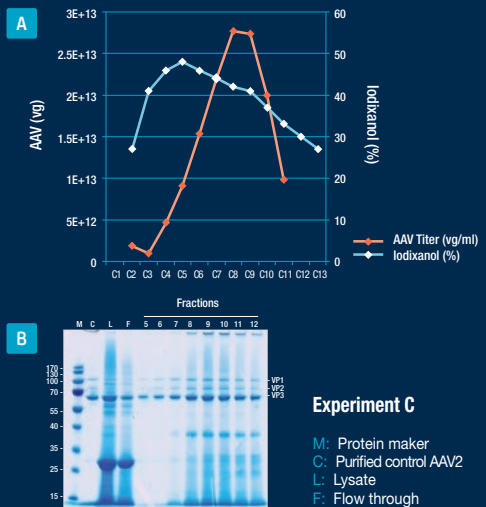

Fig. 3 Testing of iodixanol gradient solution for AAV purification with continuous flow ultracentrifugation.

A 120-ml rotor was loaded with 58ml of 50% iodixanol. After the speed reached 35,000rpm, a total of 100ml cleared cell lysate was loaded at 5ml/min continuously into the rotor. After centrifugation at 35,000rpm for 2 hours, the speed was slowly decreased, fractions collected, and AAV titer determined. The results indicate that a good formation of iodixanol gradient curve was reached (A) and that the AAV vectors were separated from the bulk cellular proteins as shown by the SDS-PAGE and SimplyBlue Staining (B). However the recovery rate was low.

Addition of sodium citrate to the Iodixanol gradient improves AAV recovery with continuous flow ultracentrifugation

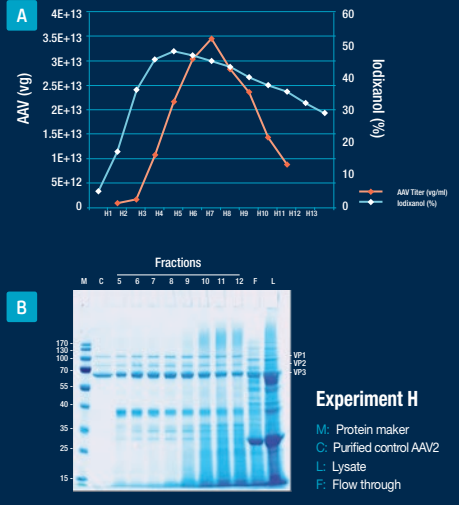

Fig. 4 Improvement of AAV recovery rate by adding sodium citrate to the iodixanol gradients and by using step gradients.

A 120-ml rotor was loaded with 25ml of 25% and 58ml of 50% iodixanol containing 100mM sodium citrate. After the speed reached 35,000rpm, a total of 95ml cleared cell lysate was loaded at 5ml/min continuously into the rotor. After centrifugation at 35,000rpm for 2 hours, the speed was slowly decreased, fractions collected, and AAV titer determined. The results indicate that AAV recovery was increased to over 50% (A) and that the AAV vectors were separated from the bulk cellular proteins as shown by the SDS-PAGE and SimplyBlue Staining (B).

Second run with continuous flow ultracentrifugation didn't increase AAV purity

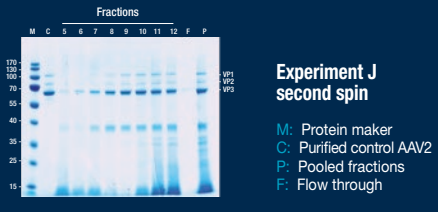

Fig. 5 Testing of AAV purification by second continuous flow ultracentrifugation.

A 120-ml rotor was loaded with 58ml of 50% iodixanol. After the speed reached 35,000rpm, a total of 43ml AAV collected from the first run was diluted 10-fold to 430ml and loaded at 5ml/min continuously into the rotor. After centrifugation at 35,000rpm for 2 hours, the speed was slowly decreased, fractions collected, and AAV titer determined. The results indicate that a repeat of the continuous flow ultracentrifugation couldn't increase the purity of the AAV vectors.

Dialysis couldn't remove the contaminants

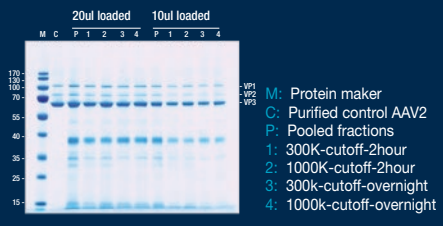

Fig. 5 Testing of dialysis method to remove contaminating proteins.

AAV2 vectors purified with the continuous flow centrifuge were pooled and used for dialysis for different time period with cassettes of different cutoff sizes. The results indicate that dialysis couldn't remove the contaminating proteins.

Addition of salt to the iodixanol gradients removed the contaminants

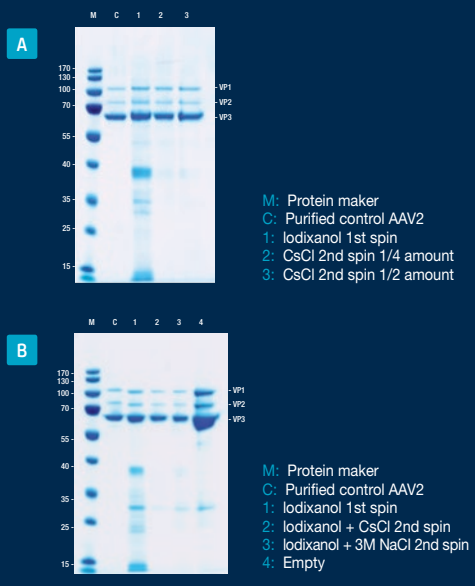

Fig. 6 Testing of different salts to remove contaminants from AAV vectors purified with the continuous flow ultracentrifugation method.

AAV vectors purified from the continuous flow centrifuge were pooled and added with CsCl to 1.38g/cc (A) or NaCl to 3M (B) and subjected to a second centrifugation overnight at 65,000rpm. Then AAV vectors were collected, dialyzed and analyzed by SDS-PAGE and SimplyBlue Staining. Th results indicate that the salts can remove contaminating proteins from the AAV vectors.

Results

Alfa Wassermann's continuous flow ultra centrifuge obtains substantially pure AAV vectors with recovery rates exceeding 50% in the presence of 100mM sodium citrate and or with iodixanol media.

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Written and researched in collaboration with VIROVEK