Continuous-Elution Electrophoresis Purification Of The Alpha And Beta Subunits From (Na,K)-ATPase

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Introduction

The integral membrane enzyme, (Na,K)-ATPase, is responsible for maintaining the concentration gradients of Na^+ and K^+ ions across the cell membrane. All active (Na,K)-ATPase preparations consist of two subunits, designated alpha and beta.[1] Typically, membrane proteins are not easy to obtain in large quantities. We have therefore examined Bio-Rad's Model 491 Prep Cell continuous elution electrophoresis apparatus as a method for providing the quantities of purified alpha and beta subunits from (Na,K)-ATPase required for further structural studies.

This report describes a purification procedure for the alpha and beta subunits which utilizes the high resolution capabilities of gel electrophoresis. This simple and fast method provides approximately 1.8 milligrams of each purified subunit from 24 milligrams of crude lamb kidney microsomes.

Methods

Isolation of (Na,K)-ATPase and Preparation of its Subunits

The (Na,K)-ATPase enzyme was isolated in crude lamb kidney microsomes using the NaI procedure of Lane *et al.*[2] The alpha and beta subunits of (Na,K)-ATPase were then separated from the microsomes by continuous elution electrophoresis using Bio-Rad's Model 491 Prep Cell. A 7% polyacrylamide separating gel, 4.5 cm in length, and a 4% stacking gel, 1 cm in length, were cast in the 37 mm gel tube of the Model 491 Prep Cell. Electrophoresis buffers were prepared according to the procedure of Laemmli.[3]

The crude (Na,K)-ATPase (24 mg) microsomes were mixed with an equal volume of reducing sample buffer (containing 87.5 mM Tris-Cl, pH 6.8, 20 mM dithiothreitol and 5% sodium dodecylsulfate) and heated for 10 minutes at 60 °C. The sample (4 ml of total volume) was loaded directly onto the stacking gel. The apparatus was run at 12 watts constant power for 8 hr with continuous (recirculating) cooling. Fractions were collected every 5 min (2.5 ml total volume) using 0.1% SDS as the eluant. Aliquots of every fifth fraction were analyzed using a 9% analytical SDS-PAGE gel. To assure complete purification of the glycoprotein beta subunit, Model 491 Prep Cell fractions containing the beta subunit were pooled and further purified by affinity chromatography.

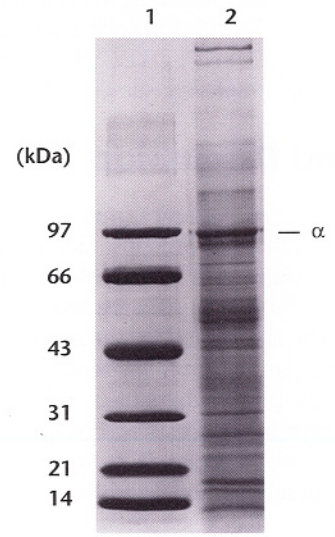

Fig. 1. SDS-PAGE analysis of the crude (Na,K)-ATPase microsomes. Analytical gels consisted of a 0.75 mm thick 4% stacking gel and 9% separating gel run in a Mini-PROTEAN® II system and stained with Coomassie® Blue. **Lane 1**, Bio-Rad low molecular weight standards. **Lane 2**, 9 µg of total protein from the crude (Na,K)-ATPase microsomes. The alpha subunit is labeled.

Affinity Chromatography

Six Model 491 Prep Cell fractions containing the glycoprotein beta subunit were pooled and further purified by lectin affinity chromatography.[4] The purified beta subunit was collected in ten 3 ml fractions. These fractions were pooled and concentrated, and purity was assessed by analytical SDS-PAGE. Results are shown in Figure 2.

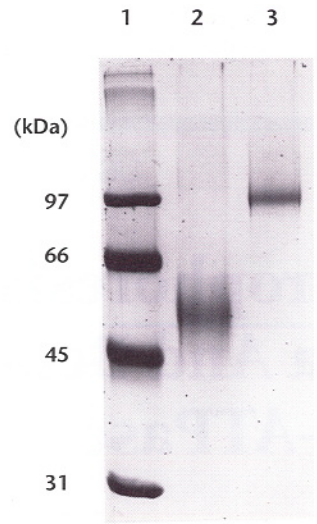

Fig. 2. SDS-PAGE Analysis of the purified alpha and beta subunits of (Na,K)-ATPase. Lane 1, Bio-Rad low molecular weight standards. **Lane 2**, 4 µg of the concentrated beta subunit after isolation on the Model 491 Prep Cell and lectin affinity chromatography. **Lane 3**, 2 µg of the concentrated alpha subunit after combining Model 491 Prep Cell fractions 34, 35, and 36.

Analytical Gel Electrophoresis

SDS-PAGE was performed according to Laemmli.[3] The analytical gels consisted of 0.75 mm-thick 4% stacking gels and 9% separating gels run in a Mini-PROTEAN II system and stained with Coomassie blue. Results are shown in Figure 2.

Results and Discussion

Crude (Na,K)-ATPase microsomes from lamb kidney consist of a considerable number of proteins. (See Figure 1, lane 2). After running the (Na,K)-ATPase microsomes in the Bio-Rad Model 491 Prep Cell, fractions were analyzed by slab gel electrophoresis. The alpha subunit was isolated in only three fractions following purification in the Model 491 Prep Cell. In Figure 2, lane 3, the pooled alpha fractions were assessed for purity. A single protein band was observed at approximately 97 kDa. The N-terminal sequence (Gly-Arg-Asp-Lys-Tyr) identified it as the alpha subunit and no additional sequences were detected.

The beta subunit was isolated in six Model 491 Prep Cell fractions. To assure complete purification of the beta subunit, a glycoprotein, from any contaminating proteins, these six fractions were pooled and applied to a lectin affinity column. In Figure 2, lane 2, the eluted beta subunit was assessed for purity. A single broad protein band at approximately 57 kDa was observed. The N-terminal sequence (Ala-Arg-Gly-Lys-Ala) identified it as the beta subunit and no additional N-terminal sequences were detected.

Amino acid analysis was also used to confirm the identity of the purified alpha and beta subunits (data not shown). Amino acid analysis indicated that 1.7 mg of the beta subunit and 1.9 mg of the alpha subunit were recovered from 24 mg of crude microsomes; *i.e.*, 81% and 28% recovery, respectively, of the estimated total beta and alpha subunits in the starting microsomes.[5] The recovery of the alpha subunit was lower than that of the beta component since the Model 491 Prep Cell fractions pooled for analysis were limited to the peak of the alpha subunit distribution to insure purity.

In summary, continuous elution electrophoresis coupled with a lectin purification of the beta subunit has been utilized as a rapid approach for purifying milligram quantities of the individual subunits of (Na,K)-ATPase.

References

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