

2D Blue native SDS-PAGE analysis of multiprotein complexes of human erythrocyte membrane

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Introduction

Patients with chronic kidney disease in haemodialysis present low quality erythrocytes, showing higher rigidity. We hypothesize that membrane structure may play a role in the flexibility of erythrocytes. Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) is an electrophoretic technique for high resolution separation of membrane protein complexes in the mass range of 10KDa to 10MDa. Here we describe a methodology to perform BN-PAGE of erythrocyte membrane complexes followed by a second dimension of tricine-SDS page for isolating and identifying individual proteins forming these complexes.

Methods

Erythrocytes were isolated from plasma after maturation, washed in 5mM Na₂HPO₄ pH 8, 1mM EDTA and 0.9% NaCl and lysed in 5mM Na₂HPO₄ pH 8, 1mM EDTA and 1mM PMSF. Membrane pellets were frozen at -80° C in 50 mM imidazole / HCl and 10% glycerol (pH 7) or immediately analyzed. For BN-PAGE, samples were centrifuged and pellets were solubilized in 50 mM sodium chloride, 50 mM imidazole, 2 mM 6- aminohexanoic acid, 1mM EDTA (pH 7) and the appropriate detergent. After centrifugation, 5 µl of 5% glycerol and Coomassie G-250 in a detergent/dye ratio of 8:1 were added to the supernatant. Membrane complexes were run in 4-13% acrylamide gradient gels. The second dimension was run in 10% tricine-SDS/PAGE and a silver staining protocol with modifications was used for tricine gels [1, 2].

Results

Membrane proteins complexes solubilization was carried out by testing two different detergents (digitonin and Triton X-100) at various concentrations (0.1-10% and 0.5-2 %, respectively). Solubilization was always performed for 1 h. 4% Digitonin resulted to be the best option in terms of first dimension resolution, as it was the lowest detergent concentration at which the highest number of distinct bands can be observed without disturbance from the detergent in excess. Prior to the second dimension, protein complexes disruption into individual proteins was optimized by treating the strips with SDS/β-mercaptoethanol solution tested at various compositions (1-20% SDS; 1-5% β-mercaptoethanol). 12% SDS treatment for 1h prior to load the strip on the second dimension provided a higher number of protein spots detected; however, further increase of SDS amount negatively affected resolution. 1% β-mercaptoethanol was used, as its variation did not show any changes. Identification of erythrocyte membrane protein complexes isolated and analyzed by 2D-BN-PAGE is being carried out by mass spectrometry.

References

- [1] Wittig I, Braun H-P & Schaumligger H. Blue native PAGE. *Nature protocols* 2006; 1:418–428.
- [2] Hermann Schägger. Tricine- SDS-PAGE. *Nature protocols* 2006; 1:16-22.