Multimeric Species in Equilibrium in Detergent-Solubilized Na,K-ATPase

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INTRODUCTION: Na,K-ATPase (NKA) is a cation transporter present in the plasma membrane of all mammalian cells. It consists of two main polypeptide chains: the α and β-subunits. NKA uses ATP hydrolysis to transport three Na^+ ions out of the cell, and pumps two K^+ ions into the cell, against their concentration gradients. Although NKA is well known, there is a debate whether the enzyme native state in cell membranes is a αβ-monomer or $(\alpha\beta)_n$ oligomers. **OBJECTIVES:** Identify oligomeric species in NKA solubilized with a non-ionic detergent $C_{12}E_8$ after purification. **MATERIAL AND METHODS**: Purification of NKA and enzymatic activity were carried out as described in Santos et al. (2002)[1]. Dynamic Light scattering (DLS), Analytical Ultracentrifugation (AUC), Small Angle X-Ray Scattering (SAXS), Spectrophotometry were used to perform the determination of oligomeric species. **RESULTS AND DISCUSSION:** The NKA sample forthwith chromatography purification presented seven different populations as identified by AUC, with monomers and tetramers amounting to ~ 55% of the total protein mass in solution. These two species constituted less than 40% of the total protein mass after increasing the NKA concentration. Removal of higher-order oligomer/aggregates from the NKA solution using 220 nm-pore filter resulted in an increase in specific enzymatic activity. Nevertheless, new large aggregates were formed over an elapsed time of 20 h. Increasing $C_{12}E_8$ concentration avoided the re-aggregation, nonetheless the protein function was lost, probably because of the separation of the α and β subunits and/or modification in structure involved in its activity. **CONCLUSIONS**: The results show that $C_{12}E_8$-solubilized NKA is in a dynamic equilibrium of monomers, tetramers and high-order oligomers/aggregates. It is still unclear which is the functional unit of NKA *in vivo*, and it cannot be ruled out that oligomerization could be a natural process involved in metabolic regulation of various membrane cellular processes.

[1]Santos et al., Braz. J. Med. Biol. Res. 35: 277-288, 2002.

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