Self-Assembly of Detergent-Solubilized Na,K-ATPase

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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 \( \alpha \) and \( \beta \)-subunits. NKA uses ATP hydrolysis to transport three \( \text{Na}^+ \) ions out of the cell, and pumps two \( \text{K}^+ \) 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 \( \alpha\beta \)-monomer or \( (\alpha\beta)_n \) oligomers. The aim of this work was to identify oligomeric species in NKA solubilized with a non-ionic detergent (\( \text{C}_{12}\text{E}_8 \)) after purification. Purification of NKA and enzymatic activity were carried out as described in Santos et al. (2002) \cite{1}. Dynamic Light scattering (DLS), Analytical Ultracentrifugation (AUC), Small Angle X-Ray Scattering (SAXS), Spectrophotometry were used to perform the determination of oligomeric species. The NKA sample forthwith chromatography purification presented seven different populations as identified by AUC, with monomers and tetramers amounting to \( \sim 55\% \) 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 \( \text{C}_{12}\text{E}_8 \) concentration avoided the re-aggregation, nonetheless the protein function was lost, probably because of the separation of the \( \alpha \) and \( \beta \) subunits and/or modification in structure involved in its activity. Concluding, the results show that \( \text{C}_{12}\text{E}_8 \)-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 \textit{in vivo}, and it cannot be ruled out that oligomerization could be a natural process involved in metabolic regulation of various membrane cellular processes.

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