Conformational Changes of Membrane-Bound (Na\(^+\)–K\(^+\))-ATPase as Revealed by Trypsin Digestion

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Summary. To distinguish ligand-induced structural states of the (Na\(^+\)–K\(^+\))-ATPase, the purified membrane-bound enzyme isolated from rat kidneys was digested with trypsin in the presence of various combinations of Na\(^+\), K\(^+\), Mg\(^++\) and ATP. It was found that first the large and then the small polypeptide chain of the (Na\(^+\)–K\(^+\))-ATPase was degraded, indicating that the lysine and arginine residues of the large chain are more exposed than are those of the small one. The (Na\(^+\)–K\(^+\))-ATPase activity was inactivated in parallel with the degradation of the large polypeptide chain. After the degradation of the large polypeptide chain, about 75% of the (Na\(^+\)–K\(^+\))-ATPase protein remained bound to the membrane, demonstrating that the split protein segments were only partially released.

It was found that the combinations of ATP, Mg\(^++\), Na\(^+\) and K\(^+\) present during trypsin digestion influenced the time course and degree of degradation of the (Na\(^+\)–K\(^+\))-ATPase protein. The degradations of the large and the small polypeptide chain were affected in parallel. Thus, certain ATP and ligand combinations influenced neither the degradation of the large nor the degradation of the small polypeptide chain, whereas by other combinations of ATP and ligands the degree of susceptibility of both polypeptide chains to trypsin was equally increased or reduced.

In the absence of ATP the time course of trypsin digestion of the (Na\(^+\)–K\(^+\))-ATPase was the same, whether Na\(^+\) or K\(^+\) was present. With low ATP concentrations (e.g., 0.1 mM), however, binding of Na\(^+\) or K\(^+\) led to different degradation patterns of the enzyme. If a high concentration of ATP (e.g., 10 mM) was present, Na\(^+\) and K\(^+\) also influenced the degradation pattern of the (Na\(^+\)–K\(^+\))-ATPase, but differentially compared to that at low ATP concentrations, since the effects of Na\(^+\) and K\(^+\) were reversed. Furthermore, it was found that the degradation of the small chain was only influenced by certain combinations of ATP, Mg\(^++\), Na\(^+\) and K\(^+\) if the large chain was intact when the ligands were added to the enzyme.

The described results demonstrate structural alterations of the (Na\(^+\)–K\(^+\))-ATPase complex which are supposed to include a synchronous protrusion or retraction of both (Na\(^+\)–K\(^+\))-ATPase subunits. The data further suggest that ATP and other ligands primarily alter the structure of the large (Na\(^+\)–K\(^+\))-ATPase subunit. This structural alteration is presumed to lead to a synchronous movement of the small subunit of the enzyme. The structural state of the (Na\(^+\)–K\(^+\))-ATPase is regulated by binding of Na\(^+\) or K\(^+\) to the enzyme-ATP complex. The effects of Na\(^+\) and K\(^+\) on the (Na\(^+\)–K\(^+\))-ATPase structure are modulated by the ATP binding to “high affinity” and to “low affinity” ATP binding sites.
Conformational changes of the \((\text{Na}^+ - \text{K}^+)\)-ATPase protein have been studied with different methods \cite{review Ref. 14}. One of these, which utilizes the reaction between the purified \((\text{Na}^+ - \text{K}^+)\)-ATPase and specific antibodies to distinguish between conformational states of the enzyme, has been recently applied in our laboratory \cite{14}. Thus, different conformational states which depend on the presence of certain ATP and ligand combinations could be distinguished. It was found that \(\text{Na}^+\) and \(\text{K}^+\) can alter the antibody inhibition of the \((\text{Na}^+ - \text{K}^+)\)-ATPase—which means, in our interpretation, that \(\text{Na}^+\) and \(\text{K}^+\) altered the enzyme structure—provided \(\text{Mg}^{++}\) and ATP were also present. This result is different from data presented by Jørgensen \cite{10, 11}, who studied structural states of the \((\text{Na}^+ - \text{K}^+)\)-ATPase by measuring the characteristics of the trypsin degradation of the membrane-bound enzyme and reported that the \((\text{Na}^+ - \text{K}^+)\)-ATPase structure was different when only \(\text{Na}^+\) or only \(\text{K}^+\) was present.

This discrepancy may be explained by the different methods which were used to detect structural alterations of the \((\text{Na}^+ - \text{K}^+)\)-ATPase and which may each only detect some of the enzyme conformations. As the method employed to remove the nucleotides, present during the \((\text{Na}^+ - \text{K}^+)\)-ATPase preparation, is not described by Jørgensen \cite{9, 10} \cite{11}, another explanation could be the presence of tightly bound nucleotides, which in our preparation were completely removed by gel filtration in the presence of glycerol and \(\text{Mg}^{++}\). Thus we performed trypsin digestion experiments on the membrane-bound \((\text{Na}^+ - \text{K}^+)\)-ATPase isolated from rat kidneys, including the conditions tested by Jørgensen. Furthermore, the influence of small amounts of nucleotide on the \(\text{Na}^+\) and \(\text{K}^+\) effects on the trypsin digestion was investigated.

If ATP was completely removed from the \((\text{Na}^+ - \text{K}^+)\)-ATPase, \(\text{Na}^+\) and \(\text{K}^+\) had no specific effects on the trypsin digestion. If the tightly bound nucleotides, ATP and ADP, remain associated with the enzyme, the \(\text{Na}^+\) and \(\text{K}^+\) effects, which were described by Jørgensen, could be partially reproduced. Also in the presence of high concentrations of ATP or \(\text{Mg}^{++}\) plus ATP, \(\text{Na}^+\) and \(\text{K}^+\) had different effects on the trypsin digestion of the enzyme, but these effects were opposite to those observed in enzyme preparations, which contained tightly bound nucleotides, when no ATP was added.

\section*{Materials and Methods}

\textit{Preparation of \((\text{Na}^+ - \text{K}^+)\)-ATPase}

Membrane-bound \((\text{Na}^+ - \text{K}^+)\)-ATPase was purified from the outer medulla of rat kidneys by incubation of a microsomal fraction with sodium dodecyl sulfate and ATP.