Salt Effects on the Kinetics of the Electrogenic Na^+ Transport in the Na,K-ATPase

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The kinetics of electrogenic Na^+ transport through the Na,K-ATPase is decelerated in concentrated salt solutions. This effect is anion specific, and the effectiveness of the anions is $\text{I}^- > \text{Br}^- > \text{Cl}^-$, which agrees with the Hofmeister series of chaotropic ions. It can be explained by an effect on the conformation transition, $\text{E}_1\text{-E}_2$.^{1,2} Here, we show how different salts affect the apparent activation energy of the rate-limiting $\text{E}_1\text{-E}_2$ conformation transition. Experiments were performed with Na,K-ATPase-containing membrane fragments adsorbed to lipid membranes (BLMs). Transient currents were induced by ATP release from caged ATP¹ and were fitted by three exponentials. The rate constant k of the exponential reproducing the falling phase of the current represents the rate-limiting step.¹ According to the Arrhenius equation, $k = A \cdot \exp(-E_a/RT)$, the activation energy E_a and the preexponential term A were obtained from the temperature dependency. To study the salt effects on both sides of the protein, two types of experiments were carried out: (1) choline salts with different anions were added into the cell before adsorption of the membrane fragments to the BLM so that they could affect the Na,K-ATPase on both sides of the membrane; (2) salts were added after adsorption and could affect then only the cytoplasmic side.

Without iodide or bromide salts, the activation energy was 63 kJ/mol. As shown in FIGURE 1A, choline iodide decreased the activation energy E_a and the factor A . Comparable results were found with bromide (not shown). The decrease of rate constant k with ion concentration was dominated by A . This effect was more significant in experiments of type 1, which indicates that it is caused preferentially by action of the anions on the extracellular side.

As found in experiments with varying ATP concentration, the effect of iodide could not be explained by altered ATP-binding constants. Changes of k with temperature due to addition of the salts differed from those induced by a decreased amount of ATP released from caged ATP. At high ATP concentrations, the temperature dependence of k remained linear, regardless of the presence of choline salts. In contrast, at low ATP concentrations, it was nonlinear, with an activation energy E_a of 125 kJ/mol at temperatures above 21°C; at decreased temperatures, $\ln(k)$ bent to a lower value of $E_a \approx 25$ kJ/mol (FIG. 1B).

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FIGURE 1. (A) Dependence of the activation energy E_a (triangles) and preexponential term A (squares) of the Arrhenius equation (see text) on choline iodide concentration obtained from type 1 (solid symbols) and type 2 (open symbols) experiments. BLMs were formed as described elsewhere.¹ (B) Arrhenius plot for different UV-flash intensities: 100% (circles) and 44% (squares) intensity. The solid, dashed, and light dotted lines correspond to activation energies of 63, 125, and 25 kJ/mol, respectively. Solutions are without choline salts.

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