

PHYSICAL SCIENCES

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A Single Amino Acid Substitution in the C-Terminal Tail of the *Ca*²⁺-ATPase Alters the Kinetics of the Phosphorylation Cycle and the Effect of the Cationic Lipid Cardiolipin on the Phosphorylation State of the Enzyme

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ABSTRACT

The *Ca*²⁺-ATPase is a transmembrane protein that is responsible for the active transport of Ca^{2+} out of the cell. The enzyme is a member of the P-type ATPase family, which is characterized by the presence of a conserved phosphorylation site in the cytoplasmic tail. The kinetics of the phosphorylation cycle and the effect of the cationic lipid cardiolipin on the phosphorylation state of the enzyme were studied. A single amino acid substitution in the C-terminal tail of the *Ca*²⁺-ATPase, from Serine to Alanine at position 368, altered the kinetics of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme. The results suggest that the C-terminal tail of the *Ca*²⁺-ATPase is involved in the regulation of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme.

INTRODUCTION

The *Ca*²⁺-ATPase is a transmembrane protein that is responsible for the active transport of Ca^{2+} out of the cell. The enzyme is a member of the P-type ATPase family, which is characterized by the presence of a conserved phosphorylation site in the cytoplasmic tail. The kinetics of the phosphorylation cycle and the effect of the cationic lipid cardiolipin on the phosphorylation state of the enzyme were studied. A single amino acid substitution in the C-terminal tail of the *Ca*²⁺-ATPase, from Serine to Alanine at position 368, altered the kinetics of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme. The results suggest that the C-terminal tail of the *Ca*²⁺-ATPase is involved in the regulation of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme.

MATERIALS AND METHODS

The *Ca*²⁺-ATPase was purified from the plasma membrane of the *Ca*²⁺-ATPase-expressing cells. The enzyme was reconstituted into lipid vesicles containing cardiolipin. The kinetics of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme were studied using a stopped-flow apparatus. The phosphorylation state of the enzyme was determined by measuring the fluorescence of a fluorescently labeled ATP analog. The effect of cardiolipin on the phosphorylation state of the enzyme was determined by measuring the fluorescence of a fluorescently labeled ATP analog in the presence and absence of cardiolipin.

RESULTS AND DISCUSSION

The kinetics of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme were studied. A single amino acid substitution in the C-terminal tail of the *Ca*²⁺-ATPase, from Serine to Alanine at position 368, altered the kinetics of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme. The results suggest that the C-terminal tail of the *Ca*²⁺-ATPase is involved in the regulation of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme.

CONCLUSION

The C-terminal tail of the *Ca*²⁺-ATPase is involved in the regulation of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme. A single amino acid substitution in the C-terminal tail of the *Ca*²⁺-ATPase, from Serine to Alanine at position 368, altered the kinetics of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme. The results suggest that the C-terminal tail of the *Ca*²⁺-ATPase is involved in the regulation of the phosphorylation cycle and the effect of cardiolipin on the phosphorylation state of the enzyme.

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