Mol Cell Biochem
DOI 10.1007/s11010-013-1847-8

Functional role and analysis of cysteine residues of the salt tolerance protein Sod2

Asad Ullah · Rabab Abou El-Magd · Larry Fliegel

Received: 13 August 2013 / Accepted: 27 September 2013
© Springer Science+Business Media New York 2013

Abstract Sod2 is the major salt tolerance plasma membrane protein of *Schizosaccharomyces pombe*. It functions to remove excess intracellular sodium (or lithium) in exchange for protons. We investigated the role of cysteine residues and created a cysteine-free Sod2 protein. Each cysteine residue of the ten present was individually mutated to serine and the different proteins expressed and characterized in *S. pombe*. Western blotting revealed that all the individual mutant proteins were expressed. We examined the ability of the mutant proteins to confer salt tolerance to *S. pombe* with the endogenous Sod2 protein deleted. Only proteins with C26S and C374S mutations were partially reduced in their ability to confer salt tolerance. Additionally, they showed a change in conformation in comparison to the wild-type protein, indicated by differential sensitivity to trypsin. Deletion of all the cysteine residues of Sod2 resulted in a functional protein that was expressed in *S. pombe* at levels similar to the wild type and also conferred salt tolerance. The conformation of the cysteine-free Sod2 protein was not altered relative to the wild-type protein. We examined the accessibility of amino acids of the cysteineless protein present on putative extracellular loop 2. A cysteine placed at position Ala119 was accessible to externally applied [2-(trimethylammonium)ethyl] methane thiosulfonate bromide. The results demonstrate that cysteines in the Sod2 protein can be changed to serine residues resulting in an expressed, functional protein. The utility of the cysteine-free Sod2 protein for determination of topology and amino acid accessibility is demonstrated.

A. Ullah · R. A. El-Magd · L. Fliegel (✉)
Department of Biochemistry, University of Alberta, 347 Medical Science Building, Edmonton, AB T6G 2H7, Canada
e-mail: lfliegel@ualberta.ca

Keywords Cysteines · Disulfides · Na^+/H^+ exchanger · Salt tolerance · Plasma membrane · Yeast

Introduction

Na^+/H^+ exchangers are a large superfamily of membrane proteins that function in the exchange of cations across lipid bilayers [1]. This superfamily includes eukaryotic and prokaryotic proteins. The mammalian NHE1 isoform is a well-studied member of the cation proton antiporter 1 family [2]. The cation proton antiporter 2 family includes Sod2, the *S. pombe* Na^+/H^+ exchanger which shares its origins with prokaryotic NhaA, the *E. coli* antiporter [1]. While a crystal structure has been deduced for NhaA [3], knowledge of the topology and structural analysis of all the other kinds of Na^+/H^+ exchangers has been lagging or is in dispute (reviewed in [4]). For example, for mammalian NHE1, there is discordance in models of the topology of NHE1 [5–7]. For the other members of the superfamily there is little or no experimental data on topology or structure aside from computer predictions based on hydrophobicity analysis, which still need to be confirmed.

Sod2 is a plasma membrane Na^+/H^+ exchanger, providing salt tolerance for *S. pombe* by removing excess intracellular sodium (or lithium) in exchange for protons [8]. Salt tolerance in eukaryotes is of enormous significance in agriculture [9] and Sod2 of *S. pombe* is a particularly useful model system in *S. pombe* to study mechanisms of salt transport as its knockout confers a salt-sensitive phenotype which can be complemented by return of the protein [10, 11].

In general, membrane proteins are particularly difficult to study, due to their low abundance and inherent

Published online: 09 October 2013