ABSTRACT

Tuberculosis is a deadly disease caused by bacteria of the genus Mycobacterium. One-third of the world's population is infected with *Mycobacterium tuberculosis*. Two million these deaths occur each year in immunocompromised AIDS patients. *M. tuberculosis* has co-evolved with humans for many thousands of years. The bacillus has developed tactics to overcome the immune defense system and multiply in the macrophage. At the interface of the host and pathogen interactions, there is an interchange of metals and electrolytes. The host on one hand reduces the availability of metals essential for pathogen survival, like manganese and iron, in the macrophage and increases potassium ions which reduce pH in the phagolysosome. The host also generates Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), to create toxic affects through interactions with metals and metalloproteins. *M. tuberculosis* copes with the hostile environment in the macrophage by preventing the acidification of the phagolysosome, secreting antioxidant enzymes such as alkylhydroperoxidase (AhpF) and peroxiredoxin (AhpC), superoxide dismutase, SodA and SodC, and catalase KatG through the SecA system. *M. tuberculosis* contains 28 metal transporters, among them there are 12 unique P-type ATPases. This is an unusually high number of P-type ATPases in an organism. These ATPases transport several monovalent and divalent metals (Cu^{+}, Cu^{2+}, Ag^{+}, Zn^{2+}, Na^{+}, K^{+}, Ca^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Mg^{2+}, and Co^{2+}) across biological membranes, using energy from ATP hydrolysis. Our analysis has revealed that these P-type ATPases have homologs in other intracellular symbiotic/pathogenic bacteria and certain chemolithotrophic archaea and bacteria. A correlation can hence be drawn among these pumps and the capability of surviving in noxious environments and coping with adverse redox conditions. Possible substrates were identified by determining the consensus sequences in different helices of these ATPases. However, out of the 12 P-type

ATPases confirmed, transported substrate could be postulated for four of these proteins; CtpA, CtpB, CtpV and KdpB. Using bioinformatic approaches we have characterized the possible genetic environment of these genes. The transmembrane regions were analyzed for consensus sequences and the N-terminals and C-terminals were scrutinized for metal binding domains, and we were able to categorize these ATPases into P_1 type and P_2 type ATPases. In an attempt to determine the substrate specificity, two of these ATPases (CtpC and ctpG) were cloned and transformed into *Escherichia coli* cells. Cells expressing CtpC were grown in different concentrations of metals and pHs. In these experiments CtpC was found to show an interaction with copper and cadmium. Pure protein was obtained by His-tag purification and para-Nitro Phenol Phosphatase (pNPPase) assay was performed with different metals, it was found that copper and zinc activated the phosphatase activity of the enzyme; and cobalt and manganese were inhibitory. Inhibition of the pNPP assay could mean that there would be activation in the ATPase assay, meaning that cobalt and manganese could be possible substrates to this enzyme.

Keywords: P-type ATPases, Mycobacterium tuberculosis, CtpC, CtpG, macrophage.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AhpC: Peroxiredoxin

AhpF: Alkylhydroperoxidase

APS: Ammonium persulfate

ATP: Adenosine Tri Phosphate

ATPases: Adenosine Tri Phosphatases

ATP-BD: ATP Binding Domain

BLAST: Basic Local Alignment Search Tool

CDF: Cation Diffusion Facilitator

cDNA: complementary DNA

CTP: Cation Transporting P-type ATPase

DNA: Deoxyribo Nucleic Acid

DTT: Dithiothreitol

IPTG: isopropyl β-D-thiogalactopyranoside

KatG: Catalase-Peroxidase

Mtb: *Mycobacterium tuberculosis*

OD_{600}: Optical Density at a λ of 600nm

pBLAST: protein BLAST

PCR: Polymerase Chain Reaction

pNPP: p-Nitro Phenyl Phosphate

ROS: Reactive Oxygen Species

RNS: Reactive Nitrogen Species

NCI: No consensus identified

NOS: Nitric Oxide Synthase

SOD: Super oxide dismutase

SR: Sarcoplasmic Reticulum

TM: TransMembrane segment

TM-MBD: TransMembrane - Metal Binding Domain

WNDP: Wilson's Disease Protein

ZIP: Zinc-regulated transporter, Iron-regulated transporter Protein

The more you care, the stronger you can be.

Jim Rohn

If your heart acquires strength, you will be able to remove blemishes from others without thinking evil of them.

The weak can never forgive. Forgiveness is the attribute of the strong.

Mohandas Karamchand Gandhi

All too often arrogance accompanies strength, and we must never assume that justice is on the side of the strong. The use of power must always be accompanied by moral choice.

Theodore Bikel