Public Health Res Perspect 2011 2(3), 171—177
doi:10.1016/j.phrp.2011.11.041
pISSN 2210-9099 eISSN 2233-6052

ORIGINAL ARTICLE

Cyclophilin A Cpr1 Protein Modulates the Response of Antioxidant Molecules to Menadione-induced Oxidative Stress in *Saccharomyces cerevisiae* KNU5377Y

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Received: July 6, 2011
Revised: September 27, 2011
Accepted: October 27, 2011

KEYWORDS:
ascorbate-like reductant,
cyclophilin,
glutathione,
menadione,
Saccharomyces cerevisiae KNU5377Y

Abstract
Objectives: The cellular function of cyclophilin A (CypA) differs between organisms, even though CypA is conserved in both prokaryotes and eukaryotes. The purpose of this study was to elucidate the role of activated CypA isoform CPR1 in the antioxidative mechanisms of *Saccharomyces cerevisiae* under menadione (MD)-induced oxidative stress.
Methods: Four *S. cerevisiae* strains, KNU5377Y (kwt) and BY4741 (bwt), and their isogenic *cpr1Δ* mutant strains (kc1 and bc1), were treated with MD, at a concentration ranging between 0.25 and 0.4 mM. Cpr1-mediated antioxidative effects were analyzed by measuring the levels of cellular glutathione (GSH) and ascorbate (AsA)-like molecules in yeast.
Results: GSH and AsA-like reductant molecule concentrations were more reduced in the presence of MD in the kc1 strain than in the kwt strain; whereas, there was no significant difference between the bwt and bc1 strains under the same conditions. In kc1 strain samples, we observed a reduction in the expression of proteins related both to GSH synthesis and the recycling system, and simultaneously, downregulation of GSH synthetase and GSH reductase activities were also evident. Oxidative stress in the kc1 strain was alleviated by the application of the GSH and AsA analog.
Conclusion: These results indicate that activated Cpr1 modulates the response of antioxidant molecules involved in cellular redox homeostasis of KNU5377Y during oxidative stress induced by MD.

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1. Introduction

Oxidative stress results from the imbalance of stressful oxidants and homeostatic antioxidant systems [1]. To overcome the negative effects of transient or continuous reactive oxygen species (ROS) exposure, cells have evolved a variety of enzymatic and nonenzymatic antioxidant defense systems that are capable of removing free radicals and their byproducts to protect the integrity of cellular structures and molecular chaperone systems [1,2]. Among the many antioxidative mechanisms, oxidative stress can be buffered by the activation of chaperone-like proteins, such as cyclophilins (Cyps). Menadione (MD; 2-methyl-1,4-naphtoquinone; vitamin K3) has been used as a model for studies on oxidative stress [3].

CypA has been isolated from a range of organisms, including bacteria and humans, and is the founding member of a class of ubiquitous and highly conserved enzymes collectively known as peptidyl *cis−trans* isomerases, or prolyl isomerases. These enzymes catalyze the *cis−trans* isomerization of the peptide bonds preceding proline residues [4]. Cyps have been reported in a wide range of metabolic processes, such as: cell division, transcriptional regulation, protein trafficking, signaling and pre-mRNA splicing [5]; molecular chaperoning, stress tolerance, receptor expression, modulation of receptor activity and, proinflammatory cytokine-like behavior [6]; bacterial effectors during animal and plant pathogenesis [7]; and cell growth, mating and virulence, and cyclosporin toxicity in the pathogenic fungus *Cryptococcus neoformans* [8]. Furthermore, Cyp expression in plant tissues increases response to different types of stress, such as heat shock and infection by pathogens [9]. In vascular smooth muscle cells, oxidative stress leads to increased expression and secretion of CypA, where the peptidyl *cis−trans* isomerases activity of CypA is thought to be necessary for inhibiting nitric oxide (NO)-induced apoptosis and for activating extracellular signal-regulated kinase 1/2 [9]. However, the role of Cpr1 p as a component of cellular protection in yeast under oxidative stress has not been extensively studied because there is no noticeable difference in the response of BY4741 wild-type and *cpr1* mutant cells to H_2O_2 stress [10].

In this study, we compared the concentrations of antioxidant molecules containing glutathione (GSH) and ascorbate (AsA)-like reductants in KNU5377Y and BY4741 wild-type strains and in their isogenic *cpr1Δ* mutants, to investigate further the origin of the sensitivity of the *cpr1Δ* mutant to MD-induced oxidative stress.

2. Materials and Methods

2.1. Strains and growth conditions

The strains used in this study are listed in Table 1. Precultures, grown aerobically at 30°C overnight in YPD medium (1% yeast extract, 2% peptone, and 2% dextrose), were transferred to fresh YPD medium, and further cultured at 30°C and shaking at 160 rpm. Cells, upon reaching the mid-log phase (OD_{600} = 1.5), were exposed directly to 0.4 mM MD for 1 hour at 30°C. To analyze the effects of antioxidant compounds, antioxidant molecules with 10 mM N-acetyl-cysteine (NAC), 10 mM GSH, and 10 mM AsA were pretreated for 1 hour at 30°C. Subsequently, cells were washed twice with a phosphate-buffered saline solution (pH 7.0) to remove residual molecules. The washed cells were resuspended in YPD medium, exposed to 0.4 mM MD for 1 hour, and serially diluted to 10^{-4}. 5 μL samples of the diluted solutions were spotted onto YPD agar plates and incubated at 30°C for 2−3 days. In the stress sensitivity assay, the wild-type and the isogenic mutant strains, upon reaching the mid-log phase, were exposed to 0.25 mM MD for 1 hour. 5 μL samples of the diluted solutions were spotted onto YPD agar plates.

2.2. Determination of GSH levels

Oxidized GSH (GSSG) and reduced GSH levels were determined by the formation of thio-nitrobenzoic acid, via the recycling assay using glutathione reductase

Table 1. *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype	Reference
KNU5377		[12]
kwt	*MATa*	[13]
kc1 (*cpr1Δ*)	*MATa, YDR155C:: kanMX4*	[13]
BY4741		
bwt	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0*	EUROSCARF
bc1 (*cpr1Δ*)	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, YDR155C:: kanMX4*	EUROSCARF
glr1Δ	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, YPL091 W::kanMX4*	EUROSCARF
gsh1Δ	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, YJL101C::kanMX4*	EUROSCARF
gsh2Δ	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, YOL049 W::kanMX4*	EUROSCARF
ara2Δ	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, YMR041C::kanMX4*	EUROSCARF

EUROSCARF = European Saccharomyces Cerevisiae Archives for Functional Analysis.