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EFFECTS OF ANTIOXIDANTS ON ACROLEIN-INDUCED OXIDATIVE PROTEIN DAMAGE IN THE YEAST *SACCHAROMYCES CEREVISAE*

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Abstract
Acrolein is an important environmental and endogenous toxin. It is an electrophile showing high reactivity with biological nucleophiles leading to a decrease in the level of glutathione as well as protein and DNA modification. Our previous results showed that 1-h incubation of both wild-type and Δ*sod1* mutant cells with allyl alcohol (precursor of acrolein) caused concentration-dependent increase in the level of carbonyl groups. The aim of this study was to determine if antioxidants are able to protect proteins from oxidation. We discovered that several thiol antioxidants such as glutathione, *N*-acetylcysteine, cysteine and dithiothreitol can decrease the level of carbonyl groups in yeast cells exposed to acrolein. Similar effect wasn't observed in case of ascorbic acid and Tempo.

Key words: acrolein, allyl alcohol, oxidative stress, protein carbonyls, antioxidant, yeast

Abbreviations: AA, allyl alcohol; ASC, ascorbic acid; CYS, cysteine; DTT, dithiothreitol; GSH, glutathione; NAC, *N*-acetylcysteine; Tempo, 2,2,6,6-tetramethylpiperidine-1-oxyl

Introduction

Acrolein is the simplest, unsaturated aldehyde commonly occurring in the environment. It is formed during incomplete combustion of petrol, coal, wood, cotton and plastic materials. It is a by-product in many branches of the chemical industry and is also a constituent of tobacco smoke. Acrolein can be also formed endogenously as a product of lipid peroxidation, during degradation of threonine and polyamines as well as during activation of cytostatic drugs. Being electrophilic, acrolein shows high reactivity with biological nucleophiles leading to a decrease in the level of glutathione as well as protein and DNA modification **(Stevens and Maier, 2008)**. The studies on the effect of acrolein on cells are carried out using various experimental models. Our team has proposed a simple eukaryotic model for such studies, viz. the baker's yeast *Saccharomyces cerevisiae* **(Bilinski *et al.*, 2005)**. A definite advantage of this model is the lack of occurrence of lipid peroxidation, which is the main endogenous source of acrolein. This allows to track acrolein and its metabolites in the cell and better define the toxic and lethal doses for cells.

Our previous results **(Bilinski *et al.*, 2005; Kwolek-Mirek *et al.*, 2009; Kwolek-Mirek and Bartosz, 2011)** show that the yeast strains defective in antioxidant defense: Cu,Zn-superoxide dismutase (Δ*sod1*), γ-glutamylcysteine ligase (Δ*gsh1*), thioredoxins 1 and 2 (Δ*trx*1Δ*trx2*) and transcription factor Yap1p (Δ*yap1*) are hypersensitive to acrolein which is formed in cells from allyl alcohol. The proposal of replacement of direct introduction of acrolein with its metabolic precursor allyl alcohol has been put forward by our team and allows to eliminate extracellular effects of acrolein resulting from its high reactivity. The data obtained demonstrate that incubation of yeast cells with allyl alcohol causes a range of negative effects: a decrease of glutathione content, a decrease of total antioxidant capacity and an increase of the content of reactive oxygen species. The metabolic activation of allyl alcohol causes also activation and relocation of Yap1p from cytoplasm to the nucleus, which evidences a stress reaction. Other

acrolein effects in a cell include alteration in actin cytoskeleton, morphological changes and disintegration of mitochondria, an increase in the level of thiobarbituric acid-reactive substances (damage of lipids) and an increase in the level of carbonyl groups (damage of proteins). The consequence of that damage was a reduction in metabolic activity, arrested cell cycle and decreased survival of yeast cells (Fig. 1).

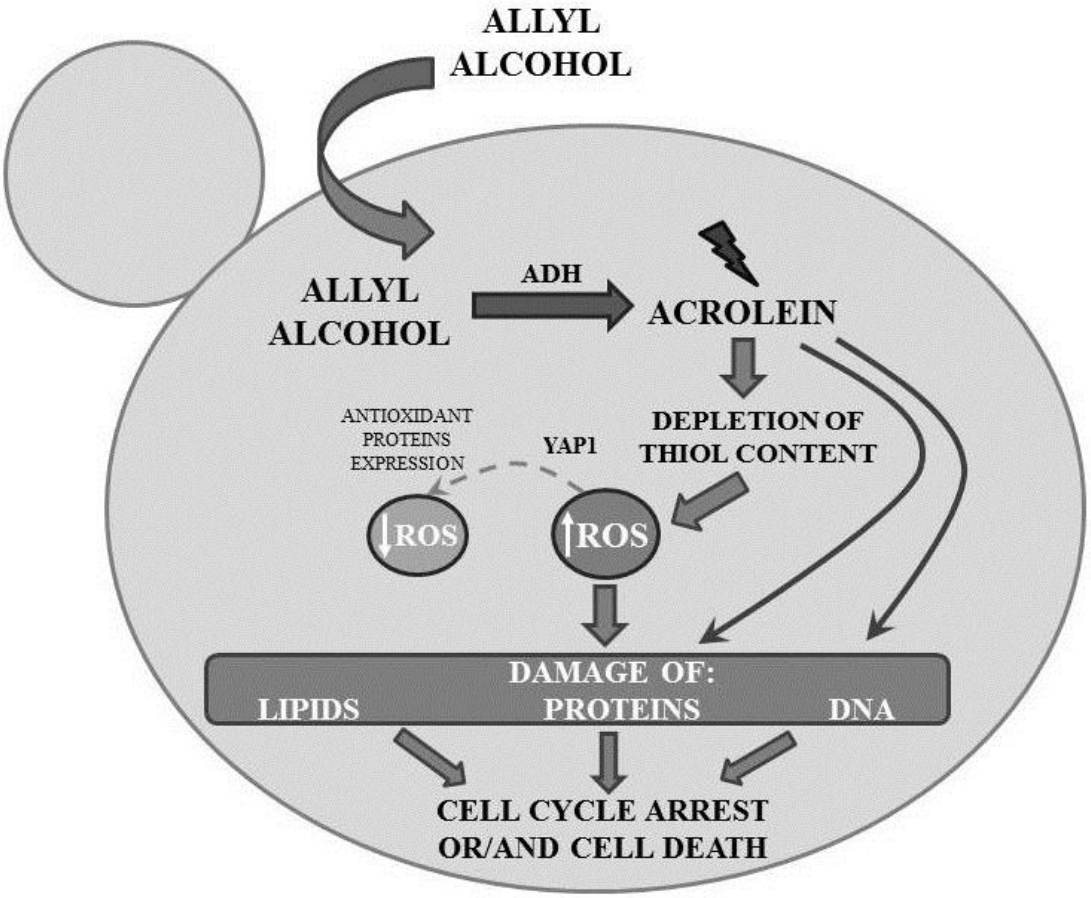

Figure 1.

Mechanisms of acrolein toxicity in yeast cells.

1-h incubation of both wild-type and Δ*sod1* mutant cells with allyl alcohol (precursor of acrolein) causes concentration-dependent increase in the level of oxidative protein damages **(Kwolek-Mirek and Bartosz, 2011)**. In this study we demonstrate that several thiol antioxidants such as glutathione, *N*-acetylcysteine, cysteine and dithiothreitol can definitely decrease the level of carbonyl groups in yeast cells exposed to acrolein.

Material and methods

Yeast strains and growth conditions

The following yeast strains were used: wild-type SP4 MATα *leu1 arg4* **(Bilinski *et al.*, 1978)**, and Δ*sod1* mutant, isogenic to SP4, MATα *leu1 arg4 sod1::natMX* **(Koziol *et al.*, 2005)**. The yeast was grown in a standard liquid YPD medium (1% yeast extract, 1% yeast bacto-peptone, 2% glucose) on a rotary shaker at 150 rpm, at a temperature 28°C.

Incubation conditions and preparation of cell extracts

Cells from exponential phase culture were centrifuged, washed and suspended in 100 mM phosphate buffer, pH 7, containing 0.1% glucose and 1 mM ethylenediaminetetraacetic acid

(EDTA) with 0.4 mM allyl alcohol and various concentration of antioxidants, viz. 25 mM ASC, 5 mM GSH, 5 mM NAC, 5 mM CYS, 5 mM DTT or 1 mM Tempo at a density of 10^8 cells/ml. After 1 h incubation the cells were centrifuged and washed twice with sterile cold water. The cell extracts were prepared according to the manufacturer's protocols (Cell Biolabs, Inc.). Protein concentration was determined using the Bradford method **(Bradford, 1976)**.

Determination of protein carbonyls

Quantification of carbonyl groups content in the yeast cell extracts was performed using OxiSelect™ Protein Carbonyl Fluorometric Assay (Cell Biolabs, Inc.) according to the manufacturer's protocols. The fluorescence was measured using a TECAN Infinite 200 microplate reader at λ_{ex} = 480 nm and λ_{em} = 530 nm.

Immunodetection of protein-bound 2,4-dinitrophenylhydrazones in the yeast cell extracts was conducted as described by **Levine *et al.* (1994)** with modification **(Kwolek-Mirek and Bartosz, 2011)**. The anti-2,4-dinitrophenyl antibody (rabbit, ab6306, Abcam) was used at a 1:20 000 dilution. The secondary antibody (goat anti-rabbit conjugated with horseradish peroxidase, 111 035 003, Jackson Immuno Research) was used at a 1:20 000 dilution.

Results and discussion

Protein oxidation is defined as the covalent modification of a protein, induced either directly by reactive oxygen species (ROS) or indirectly by reaction with secondary by-products of oxidative stress. The most sensitive to damage are: cysteine, methionine, lysine, proline, histidine, tryptophan, phenylalanine, tyrosine and arginine residues of proteins **(Bartosz, 2003; Lushchak, 2006)**. The formation of carbonyl groups in the proteins leads to irreversible damage and loss of their biological activity. The accumulation of this damage in the cell may impair its function and it is associated with aging, several diseases and cell death **(Berlett and Stadtman, 1997; Stadtman and Levine, 2000; Nystrom, 2005)**.

In the yeast *Saccharomyces cerevisiae* a higher level of carbonyl groups occurs in case of growth on fructose as a carbon source **(Semchyshyn *et al.*, 2011)**, and under aerobic condition (aerobic respiration) **(Cabiscol *et al.*, 2000)**. Many compounds such as hydrogen peroxide, menadione **(Cabiscol *et al.*, 2000)**, crotonaldehyde, and acrolein **(Trotter *et al.*, 2006)**, also cause increase of proteins oxidation in the yeast cells.

Our results show that 1-h incubation of both wild-type and Δ*sod1* mutant cells with allyl alcohol causes concentration-dependent increase in the level of carbonyl groups **(Kwolek-Mirek and Bartosz, 2011)**. In this study we demonstrate that several thiol antioxidants such as 5 mM glutathione, 5 mM *N*-acetylcysteine, 5 mM cysteine and 5 mM dithiothreitol can protect against proteins oxidation caused by 1-h incubation with 0.4 mM allyl alcohol (Fig. 2 and 3). We have observed that the most powerful protective effect in case of cysteine, the level of carbonyl groups reaches control level (in absence of allyl alcohol) (Fig. 2 and 3). 25 mM ascorbate and 1 mM Tempo doesn't have a protective effect (Fig. 2 and 3). The results obtained using both methods (fluorometric and immunoblotting assay) are comparable (Fig. 2 and 3).

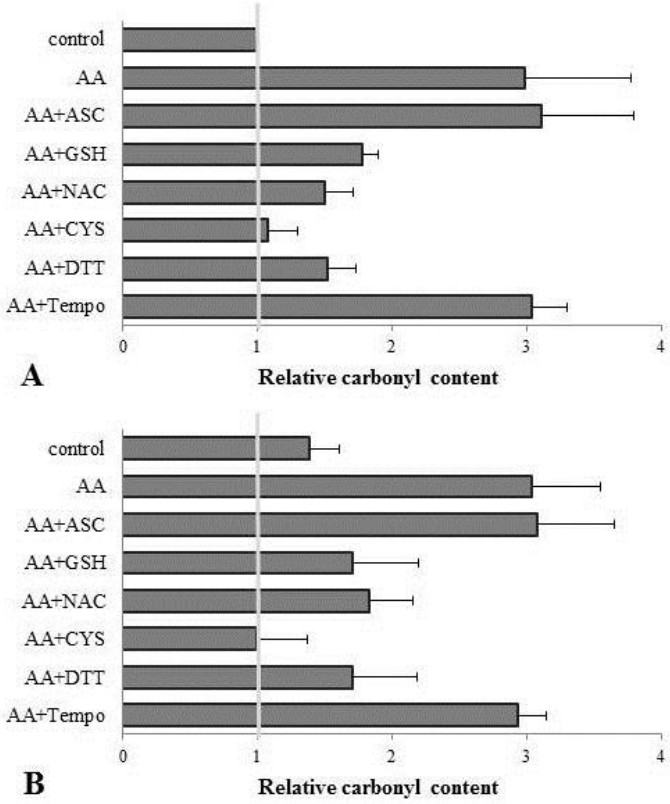

Figure 2.

The effect of antioxidants on the level of carbonyl groups in yeast cells exposed to acrolein. Quantification of carbonyl groups content for wild-type (A) and Δ*sod1* mutant (B) cell extracts was performed using OxiSelect™ Protein Carbonyl Fluorometric Assay. The fluorescence was measured using a TECAN Infinite 200 microplate reader at λ_{ex} = 480 nm and λ_{em} = 530 nm. Data represent mean ± SD from three independent experiments.

The effect of cellular action of acrolein is the induction of oxidative stress. The major pathway for the detoxification of acrolein in cell is its reaction with glutathione and the formation of GS-acrolein conjugates. Acrolein can reacts not only with glutathione but also with other sulfur-containing compounds such as cysteine, *N*-acetylcysteine, 2-mercaptoethane sulfonate, lipoic acid or 2,6-dithiopurine **(Zhu *et al.*, 2011)**. Acrolein can react also with nitrogen (amino)-containing compounds, naturally occurring polyphenols as well as ascorbic acid **(Zhu *et al.*, 2011)**. When this pathway for the detoxification is exhausted, it occurs the formation of ROS and modification of macromolecules (Fig. 1; **Kwolek-Mirek *et al.*, 2009; Kwolek-Mirek and Bartosz, 2011)**.

Low molecular weight antioxidants such as GSH, NAC, CYS and DTT, which decrease the level of carbonyl groups in the yeast cells exposed to acrolein (Fig. 2 and 3), can be both effective ROS scavengers and prevent changes in the level of glutathione.

Lack of differences in the level of carbonyl groups in the cells incubated with 0.4 mM allyl alcohol in case of both tested strains (Fig. 2) suggests that this type of protein damages is not responsible for increased sensitivity of Δ*sod1* mutant to acrolein. However, an increased level of carbonyl groups under control condition (absence of allyl alcohol) in case of Δ*sod1* mutant compared to wild-type strain (Fig. 2), confirms the protective effect of Cu,Zn-superoxide dismutase against ROS and proteins oxidation.

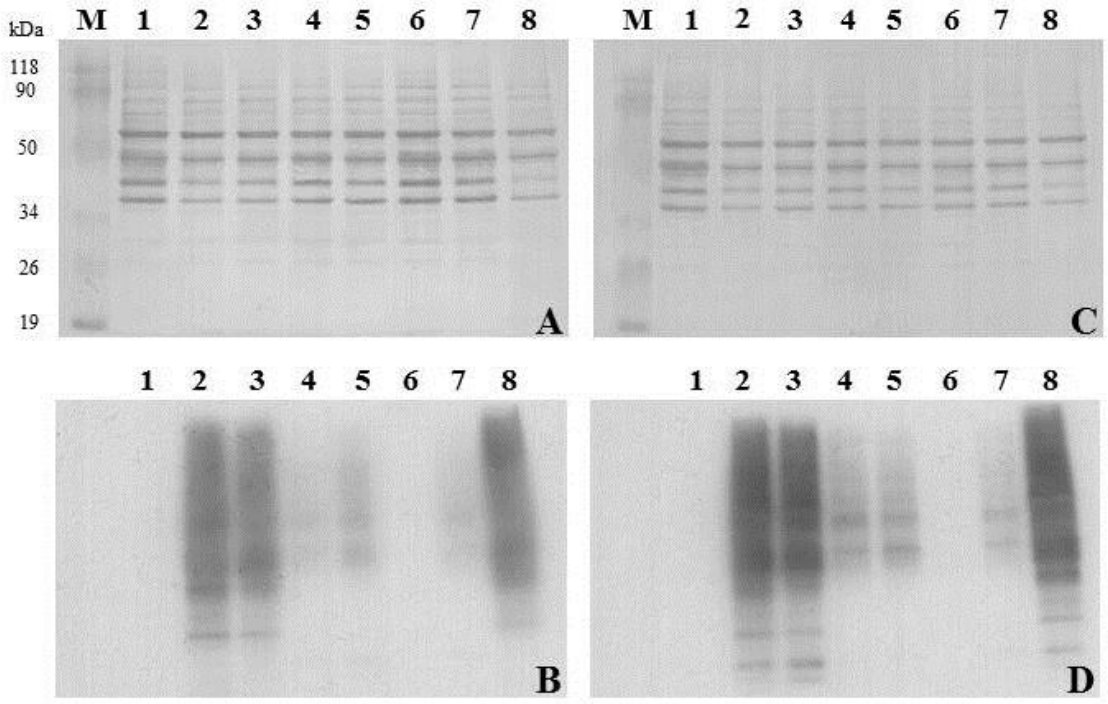

Figure 3.

The effect of antioxidants on the level of carbonyl groups in yeast cells exposed to acrolein. Image A and B presents the results for wild-type, C and D for Δ*sod1* mutant cell extracts. Line M – Prestained Protein Molecular Weight Marker, line 1 – control cells (in absence of allyl alcohol), line 2 – cells incubated with 0.4 mM AA, line 3 – cells incubated with 0.4 mM AA and 25 mM ASC, line 4 – cells incubated with 0.4 mM AA and 5 mM GSH, line 5 – cells incubated with 0.4 mM AA and 5 mM NAC, line 6 – cells incubated with 0.4 mM AA and 5 mM CYS, line 7 – cells incubated with 0.4 mM AA and 5 mM DTT and line 8 – cells incubated with 0.4 mM AA and 1mM Tempo. Image A and B Coomassie Brilliant Blue staining PVDF membranes, C and D Immunoblotting analysis using anty-2,4-dinitrophenyl antibodies. The figure shows representative images recorded during one of the two independent experiments.

Acknowledgements

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