PROOXIDANT-ANTIOXIDANT BALANCE IN THE METHYLOTROPHIC YEAST *OGATAEA POLYMORPHA* EXPOSED TO SPERMIDINE

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Background. Many molecular compounds used by eukaryotic cells as antioxidant agents can directly interact with reactive oxygen/nitrogen species. Among them are polyamines – natural substances with pronounced antioxidant properties. The main polyamines are putrescine, spermidine, spermine. Spermidine is a positively charged polyamine that is synthesized from putrescine and serves a precursor of spermine. In particular, spermidine is distinguished by its anti-aging properties. When administered to several model organisms, it prolonged their lifespan and provided resistance to stress. Spermidine also limits overproduction of reactive oxigene species and reduces age-related oxidative protein damage, which accounts for its antioxidant activity.

The aim of our study was to investigate the effects of exogenous spermidine in different concentrations on the prooxidant-antioxidant balance in the methylotrophic yeast *Ogataea polymorpha* naturally adapted to stressful conditions such as growth on toxic methanol-containing medium and cultivation at high temperatures (42–45 °C).

Materials and Methods. In this work, *O. polymorpha* wild-type strain was used. Yeast cells were cultivated in the mineral medium with 0.1 % glucose for creating stressful conditions. Spermidine was added in 1 and 2 mM concentrations immediately before the start of yeast cultivation. Reactive oxygen species, the level of oxidative modifications of proteins and lipids, the concentration of reduced gluthatione, superoxide dismutase and catalase activities were assayed to examine the prooxidant-antioxidant state of the yeast cells.

Results. In this study, we analyzed the effects of exogenous spermidine in different concentrations on the enzymatic (superoxide dismutase and catalase activity) and non-enzymatic (reduced glutathione) antioxidant defense systems and markers of oxidative injury (products of proteins and lipids oxidation) in the methylotrophic yeast *O. polymorpha* starved for glucose. It was revealed that 1 mM spermidine had a protective effect on *O. polymorpha* cells and decreased the content of products of the oxidative modification of proteins. At the same time, the superoxide dismutase and catalase activities and content of the reduced glutathione remained almost unchanged in the cells cultivated in the glucose-depleted medium with 1 mM spermidine compared to the medium without spermidine. Adverse effects of 2 mM spermidine (increased levels of carbonyl groups of proteins, lipid peroxidation products, disregulated superoxide dismutase and catalase activities, reduced glutathione levels, growth inhibition and cells vacuolization) were observed in the exponential growth phase of the yeast culture. During a long-term cultivation, these effects diminished, and the corresponding values approximated those of the cells grown in the control medium with the low concentration of glucose without spermidine.

Conclusions. The data suggest a concentration-dependent effect of spermidine on the physiology of *O. polymorpha* that can be used in further studies on compounds able to mitigate negative effects of the oxidative stress in this yeast and other model organisms. At 1 mM concentration, spermidine had an apparent protective effect, whereas at the elevated 2 mM concentration this polyamine exacerbated stress load in this yeast.

Keywords: methylotrophic yeast, *Ogataea polymorpha*, spermidine, oxidative stress

INTRODUCTION

There are many low molecular compounds used by eukaryotic cells as antioxidant agents. Antioxidants can be both intracellularly synthetized or utilized as external compounds and can directly interact with reactive oxygen/nitrogen species. Among them are polyamines – natural substances with pronounced antioxidant properties. These compounds are aliphatic polycations ubiquitously present in all tissues and all cell types in animals and plants. Polyamines play specific and diverse roles in multiple cellular processes including cell division and differentiation, DNA and protein synthesis, gene expression, signal transduction and apoptosis [11, 26]. They interact readily with negatively charged molecules, such as DNA, RNA and proteins, toxic aldehydes and act, therefore, as free radical (oxygene/nitrogene reactive species) scavengers [11, 26]. Polyamines also have a chelating ability towards metal ions, thereby affecting mechanisms of oxidative stress and activity of antioxidant enzymes [21, 32] probably by regulation of the corresponding genes expression [33]. These polyamine properties extend from bacteria to yeasts and metazoans [11].

The main polyamines are putrescine (Put), spermidine (Spd) and spermine (Spm) and their precursor – Agm. Polyamine metabolism and three most common polyamines, Put, Spd and Spm, were demonstrated to be involved in stress response [37]. Spd is a positively charged polyamine that is synthesized from Put and serves as a precursor of Spm. In particular, Spd is distinguished by its anti-aging properties. When administered to several model organisms, it prolonged their lifespan and provided resistance to stress. It also reduces overproduction of reactive oxigene species (ROS) and age-related oxidative protein damage, which accounts for its antioxidant activity. [12]. In the bakers' yeast *Saccharomyces cerevisiae*, polyamines Spd and Spm stimulated the expression of proteins essential for the effective antioxidant response. Microarray studies on *Saccharomyces cerevisiae* Spd- and Spm-deficient mutants revealed that exogenous Spm altered the expression of nearly 500 genes more than 2-fold, including several oxidative stress-response genes. [7] These mutants also required exogenous polyamines for protection against ROS, and their viability could not be rescued by overexpression of the antioxidative stress enzyme superoxide dismutase (SOD, EC 1.15.1.1) [4, 6]. In addition, Spd induces autophagy in cultured yeast and mammalian cells, as well as in nematodes and flies. Genetic inactivation of genes essential for autophagy abolishes the life span-prolonging effect of spermidine in yeast, nematodes and flies. These fndings complement the expanding evidence that autophagy mediates cytoprotection against a variety of noxious agents and can confer longevity when induced at the whole-organism level [22].

S. cerevisiae is a well-known model organism actively used for investigation of key cell processes, including oxidative stress. However, a convenient object for the study of stress resistance in yeasts is methylotrophic yeasts – specific unicellular eukaryotic organisms able to obtain both carbon and energy needed for growth from one-Carbon compound methanol, which is oxidized to toxic formaldehyde and hydrogene peroxide. These yeasts belong to several genera: *Candida*, *Pichia*, *Ogataea*, *Kuraishia* and *Komagataella* [19]. All species of methylotrophic yeasts are obligate aerobes and facultative methylotrophs. However, these yeasts can readily utilize other carbohydrates, including glucose as a preferred substrate.

The methylotroph *O. polymropha* is a biotechnologically important yeast well known as an efficient expression platform for heterologous proteins, governed primarily by glucose-repressible promoters [16, 33, 36]. In addition, this thermotolerant yeast has been proposed as an organism for high-temperature fermentation of sugars derived from lignocellulose hydrolysates, glucose and xylose [18].

It has been found that depletion of glucose in the cultural medium causes a stressful condition for *O. polymropha* cells and induces a specific cellular response. For instance, low 0.1 %, versus 1 % w/v considered as optimal, glucose concentration limits the growth of the culture [35] and causes overproduction of reactive oxygen species (ROS) in *O. polymorpha* cells [10]. The same situation was observed in bovine aortic endothelial cells cultivated in a low glucose-containing medium. It was shown that in these cells hypoglycemia was linked to increased ROS production. Low glucose increased mitochindrial ROS generation in the electron transfer chain that was suppressed by overexpression of Mn-SOD. A comprehensive metabolic analysis using capillary electrophoresis-mass spectrometry and oxygen consumption rate assessment showed that the pathway from fatty acids to acetyl-CoA through fatty acid oxidation was upregulated under low glucose conditions. In addition, etomoxir, a specific inhibitor of a free fatty acids transporter, decreased the low glucose-induced mitochindrial ROS production.

These results suggested that low glucose increased mitochindrial ROS generation through activation of fatty acid oxidation. [13].

Similarly to other organisms, *O. polymorpha* cells harbor enzymatic and non-enzymatic antioxidant molecules which efficiently protect this yeast against ROS under normal conditions. However, when yeast cells suffer a prolonged or sudden acute exposure to harmful doses of ROS, their pro-survival response mechanisms may fail [17]. The failure of antioxidant defenses that impede ROS accumulation results in oxidative stress, a condition broadly defined as an imbalance between cellular prooxidants and antioxidants, in favor of the former.

The aim of this study was to investigate how exogenous polyamine spermidine affects prooxidant-antioxidant balance in *O. polymorpha* cells starved for glucose, and whether this compound may be potentially utilized to mitigate effects of the acute oxidative stress.

MATERIALS AND METHODS

Strains, media and microbial techniques. *O. polymorpha* strain used in this work was a prototrophic wild-type strain NCYC 495 *pr* (*leu1-1 ScLEU2*). Yeast cells were cultivated on standard liquid or solid media at 37 °C: rich medium YPD (1 % yeast extract, 2 % bacto-peptone, 1 % glucose) or synthetic YNB medium (0.17 % yeast nitrogen base without amino acids and ammonium sulfate (YNB, Difco), 0.5 % ammonium sulfate). Solid media contained agar at 2 % concentration. The concentration of carbon source (glucose) was 0.1 % ($w \times v^{-1}$), unless indicated otherwise. Cell density was determined by absorbance at 600 nm. Yeast cells for biochemical experiments were cultivated in the media with 0.1 % glucose for 15 h, which corresponds to the exponential growth phase and 40 h, which corresponds to the early stationary growth phase. Spermidine was supplemented into the medium at 1 and 2 mM concentration immediately before yeast cultivation started.

Cell-free extracts preparation. Yeast cells were washed by 50 mM potassium-phosphate buffer (PPB) (pH 6.5) with 1 mM PMSF (phenylmethylsulfonyl fluoride, protease inhibitor) to remove the components of the growth medium. The harvested cells were disrupted in 50 mM PPB pH 6.5 with 1 mM PMSF with 0.5 mm glass beads using a homogenizer (1000 rpm, r = 10 cm) at 4 °C during 15 min. To prepare a cell-free extract, the homogenate was centrifuged at 15 000 g for 20 min (4 °C). Supernatants were transferred into fresh tubes and used for biochemical analysis.

Biochemical Methods

Superoxide Dismutase. The activity of yeast superoxide dismutase (SOD, EC 1.15.1.1) was measured by the method based on reducing nitroblue tetrazolium (NBT) using a superoxide radical, as described previously [14] with modification. The absorbance of the reaction mixture was read at λ = 540 nm against the blank probe. The results were expressed as % of inhibition of NBT reduction per 1 mg of protein. Protein concentration was determined with Folin reagent [20].

Catalase. Catalase (CAT, EC 1.11.1.6) activity was measured by following color intensity of the complex formed by H_2O_2 with molybdenum salts [1]. The reaction mixture consisted of 0.05 M Tris-HCl buffer (pH 7.8), 0.03 % solution of H_2O_2, and the test sample. The reaction was stopped by adding 4 % solution of ammonium molybdate after 10 min of incubation. Measurements were carried out by spectrophotometry at a wavelength of λ = 410 nm. Units of CAT activity were calculated as nmole of $H_2O_2 \times min^{-1} \times mg^{-1}$ of protein.

Reduced Glutathione. The concentration of acid-soluble thiols including reduced glutathione (GSH) was measured in cell-free extracts by the colorimetric method with Ellman's reagent (5,5-dithio-bis-(2-nitrobenzoic) acid, DTNB) – a non-enzymatic method for determining acid-soluble thiols. SH-groups of thiols reduced DTNB to yellow-colored anionic product whose optical density was measured at λ = 412 nm by spectrophotometry [29]. A molar extinction coefficient of 13,600 $M^{-1}{\times}cm^{-1}$ was used for the nitro-benzoate ion. The concentration of acid-soluble thiols, denoted as GSH concentration, was expressed in nmol per 1 mg of protein in the cell-free extract.

Lipid Peroxidation. Thiobarbituric acid reactive substances (TBA-reactive substances) in yeast cell-free extracts were assayed via formation of a colored complex with thiobarbituric acid at a high temperature [17], and the concentration of TBARS was expressed in nmoles per 1 mg of protein. The extinction coefficient of TBARS adduct at 532 nm was 155,000 $M^{-1}{\times}cm^{-1}$. The reaction mixture consisted of 10 mM phosphate buffer (pH 7.4), 1 mM potassium permanganate, and the tested sample. 10 mM ferrous sulfate was added to start the reaction and 20 % trichloroacetic acid was added to stop the reaction.

Oxidative Modifications of Proteins. The level of oxidative modifications of proteins (OMPs) was assayed via spectrophotometric detection of aldehyde and ketone groups of aliphatic amino acid residues reacting with the 2,4-dinitrophenylhydrazine reagent to form protein-conjugated 2,4-dinitrophenylhydrazones with a characteristic absorption spectrum at λ = 370 nm (OMP_{370}, represented by neutral carbonyl groups) and at λ = 430 nm (OMP_{430}, represented by basic carbonyl groups) [23] with a minor modification. The reaction mixture consisted of 0.85 % sodium chloride, 0.1 M 2,4-dinitrophenylhydrazine, 2 M hydrochloric acid, 10 % trichloroacetic acid, 8 M urea solution, and the tested sample. The level of OMP_{370} was expressed using molar absorption coefficient of 22,000 $M^{-1}{\times}cm^{-1}$, and level of OMP_{430} using molar absorption coefficient of 16,800 $M^{-1}{\times}cm^{-1}$.

Fluorescence microscopy

ROS detection in vivo by fluorescence microscopy. ROS were detected by fluorescence microscopy using 2,7-dichlorohydrofluorescein diacetate (DCFH-DA) [8]. Cell suspension was prepared in 1 mL of YNB without carbon source. In parallel, a control sample containing tert-butyl hydroperoxide (TBHP) was prepared. For this, 10 μL of 100 mM TBHP was added to the suspension. 10 μL of distilled water was added to the experimental sample. The samples were incubated at 37 °C for one hour. After incubation, the cells were washed twice in liquid YNB without a carbon source, resuspended in 1 mL of distilled H_2O and 10 μL of 2 mM DCFH-DA was added to the resulting suspensions. Then cells were incubated for 30 min in the dark at 37 °C. Images were captured on fluorescence microscope (Axio Imager A1; Carl Zeiss MicroImaging, Jena, Germany) coupled with a monochrome digital camera (Axio Cam MRm; Carl Zeiss MicroImaging) and processed using the AxioVision 4.5 (Carl Zeiss MicroImaging) and Adobe Photoshop CS5 software (Adobe Systems, Mountain View, CA). The mean fluorescence intensity per cell was calculated as the sum of intensity of fluorescence signals of the cells in the field of view (in conventional units) divided by the number of the cells in the same field of view under light microscopy. The average number of cells in the field of view was 25–30. At least five fields of view were used for the analysis.

Statistical Analysis. Statistical analysis of the results was performed using Microsoft Excel 2016. The calculation of the main statistical indicators was performed on the basis of the direct quantitative data obtained as a result of the research (arithmetic mean value – M; arithmetic mean standard error – m).

To assess the probability of the difference between the statistical characteristics of the three alternative data sets (data of three independent experiments), the Student's ratio was calculated. The difference in the indications of probability p ≥ 0.95 (significance level) was considered probable P < 0.05 after calculating t according to the table of Student's *t*-distribution. Designations were as follows: * P < 0.05 compared to NCYC 495 *pr* (wild-type strain) grown on the medium with 0.1 % glucose (control condition).

RESULTS AND DISCUSSION

As a favorite carbon substrate, glucose exerts numerous strong and well-coordinated effects on the physiological state of yeast cells. They include gene-specific regulation of transcription and mRNA stability as well as regulation at the posttranslational level, e.g., catabolite inactivation of certain enzymes [5, 9, 15, 36]. We used glucose limitation in the growth medium (0.1 %) to model conditions of glucose hypometabolism, which is associated in higher eukaryotes with increased oxidative stress, cell demage and aging [3, 13]. We also evaluated spermidine as a potential compound modulating prooxidant-antioxidant balance and thus counteracting negative effects of glucose limitation in *O. polymorpha* cells.

It was found that 1 mM Spd in the culture medium only slightly affected growth of *O. polymorpha* wild-type strain in the exponential and early stationary growth phase, whereas at the elevated 2 mM concentration this negative effect became more pronounced (**Fig. 1**).
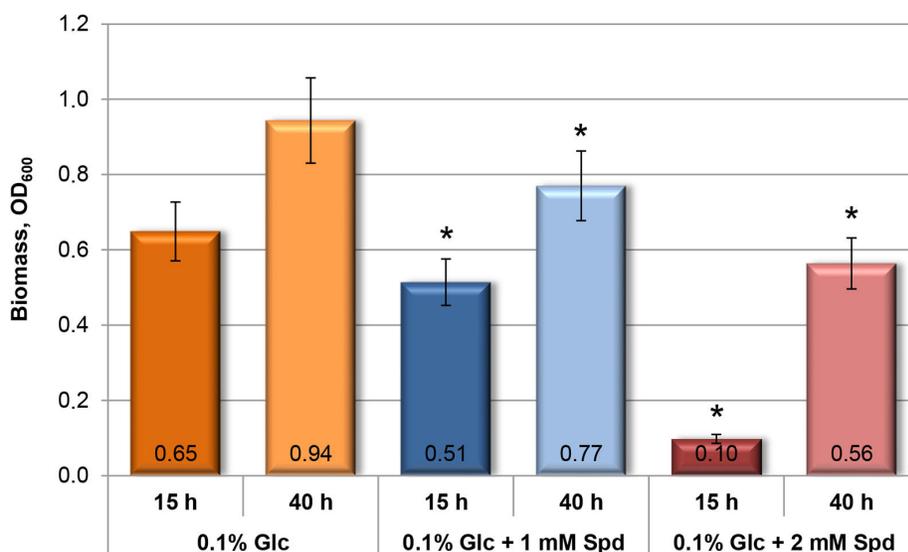

Fig. 1. Growth of *O. polymorpha* wild-type NCYC 495 *pr* strain cultivated in glucose-depleted (0.1 %) medium and different concentrations of exogenous Spd (1 and 2 mM). (* – P < 0.05; * – the difference is significant as compared to the cells grown in the medium with 0.1 % glucose without Spd)

Рис. 1. Ріст штаму дикого типу *O. polymorpha* NCYC 495 *pr* під час культивування у глюкозо-дефіцитному (0,1 %) середовищі з різними концентраціями екзогенного спермідину (1 і 2 мМ). (* – P < 0,05; * – різниця достовірна, порівняно з клітинами, вирощеними у середовищі з 0,1 % глюкози без спермідину)

However, this negative effect of 2 mM Spd was transient and diminished after a long-term cultivation, probably indicating a depletion of this compound from the growth medium (data not shown).

ROS generation in *O. polymorpha* cells cultivated at different concentrations of Spd in growth media. ROSs are various derivatives of molecular oxygen formed in actively metabolizing cells, which include hydrogen peroxide, hydroxyl radicals and superoxide anions. We exploited the ability of *O. polymorpha* cells to transport glucose by facilitated diffusion to model the conditions for ROS overproduction. We previously observed that glucose limitation (0.1 %) in the culture medium led to the accumulation of ROS in *O. polymorpha* cells. Thus, cells of the wild-type strain (NCYC 495 *pr*) were pre-grown during 15 hours on mineral medium with different concentrations (0.1, 1.0, 2.5 and 10 %) of glucose as a carbon source and 2,7-DCFH-DA was utilized for the detection of oxidative species. We found that glucose increase above 1.0 % (which is assumed to represent "physiological conditions") in culture medium did not lead to the elevation of cellular ROS content, whereas glucose deprivation upon cultivation on 0.1 % glucose for 15 hours did so. [10]

It was also reported that polyamines deficiency promoted ROS accumulatation in *S. cerevisiae* cells [6]. Our task, therefore, was to analyze how exogenous Spd at different concentrations affects the content of ROS in *O. polymorpha* cells depleted of glucose.

For this, yeast cells were pregrown during 15 and 40 hours on the mineral medium with different concentrations of Spd (1 and 2 mM) and 0.1 % glucose as a limiting growth carbon source. 2,7-DCFH-DA was utilized for the detection of oxidative species as described in Materials and Methods (**Fig. 2*A***). Mean fluorescence intensity per cell was digitized by GelPro software (**Fig. 2*B***). Fluorescence microscopy data confirmed excess ROS generation in *O. polymorpha* cells cultivated without Spd under our experimental conditions.

We also found that both 1 and 2 mM Spd reduced content of ROS in the cells, especially at an early (15 hours) growth phase. After 40 hours of incubation, the proportion of oxidative species increased in the cells exposed to 1 mM Spd. Nevertheless, ROS content in the cells incubated without polyamine was still much higher (**Fig. 2*A*, *B***). This observation is in favor of Spd acting as ROS scavenger in *O. polymorpha* cells. It can be also assumed that the inhibiting effect of Spd on cell growth (see **Fig. 1**) is not due to its interference with ROS production.

In addition, we observed that especially at its higher concentration (2 mM) Spd caused morphological alterations in *O. polymorpha* cells increasing their volume and cell vacuolization (**Fig. 2*A***). Formation of enlarged vacuoles is one of the main hallmarks of upinduced autophagy [1], and Spd is known to induce this process in a number of organisms [24]. Whether the same occurs in *O. polymorpha* and may negatively affect growth upon glucose limitation requires a separate study.

Comparative analysis of content of the oxidized proteins and lipids. Products of oxidative modifications of the biomolecules such as protein carbonyls, nitrated proteins, malondialdehyde, acrolein, isoprostanes and some others can be used as markers of oxidative and nitrosative stresses. When tyrosine and lysine residues in proteins are oxidized, the modified products are relatively stable in the cells and their accumulation may lead to the cell death [28]. Lipid peroxidation in turn yields complex products, including hydroperoxides, aldehydes, and polymeric materials which also exert cytotoxic and genotoxic effects [31]. Therefore, we addressed the question how the analyzed concentrations of Spd and intracellular ROS levels affect the cellular content of oxidized proteins and lipids under our experimental conditions.
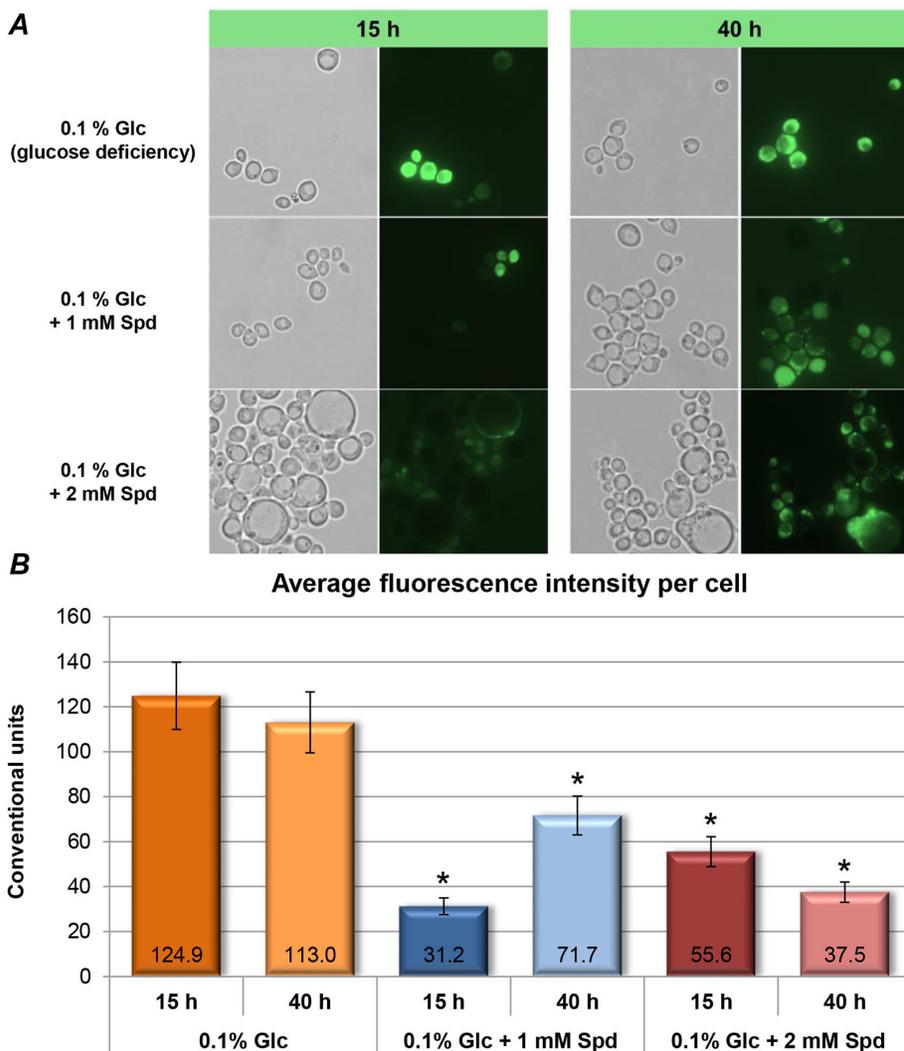
Fig. 2. *In vivo* ROS detection in cells of *O. polymorpha*: **A** – fluorescence images of *O. polymorpha* cells treated with DCFH-DA as described in Materials and Methods; **B** – average fluorescence intensity per cell digitized by GelPro software. (* – P < 0.05; * – the difference is significant as compared to the cells grown in the medium with 0.1 % glucose without Spd)

Рис. 2. *In vivo* детекція АФК у клітинах *O. polymorpha*: **A** – флуоресцентні мікрофотографії клітин *O. polymorpha*, оброблених DCFH-DA, як описано у Матеріалах і Методах; **B** – середня інтенсивність флуоресценції на одну клітину в полі зору, оцифрована за допомогою програмного забезпечення GelPro. (* – P < 0,05; * – різниця достовірна, порівняно з клітинами, вирощеними у середовищі з 0,1 % глюкози без спермідину)

We observed a decreased level of protein carbonyls in the cells treated with 1 mM Spd in comparison to those incubated in polyamine-free medium after a short (15 h) or prolonged (40 h) treatment (**Fig. 3A**, **B**). However, 2 mM Spd caused an opposite effect significantly increasing the oxidized proteins level. Apparently, these protein modifications did not strictly correlate with the measured intracellular ROS values (see **Fig. 2**).
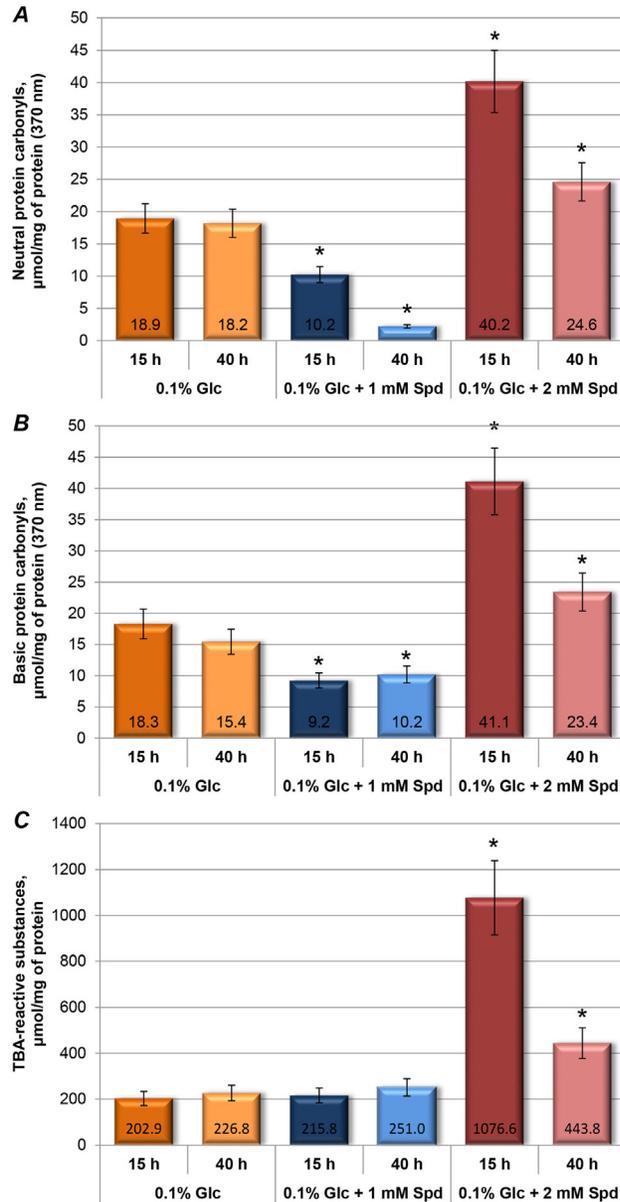
Fig. 3. Content of the oxidized proteins and lipids in *O. polymorpha* cells depleted of glucose and exposed to Spd: **A –** content of neutral protein carbonyls (λ = 370 nm); **B –** content of basic protein carbonyls (λ = 430 nm); **C –** content of TBA-reactive substances. Assays were conducted as described in Materials and Methods (* – P < 0.05; * – the difference is significant as compared to the cells grown in the medium with 0.1 % glucose without Spd)

Рис. 3. Вміст продуктів окиснення білків і ліпідів у клітинах *O. polymorpha*, культивованих у середовищі з дефіцитом глюкози та різними концентраціями спермідину: **A –** вміст карбонільних груп білків нейтрального характеру (λ = 370 нм); **B –** вміст карбонільних груп білків основного характеру (λ = 430 нм); **C –** вміст ТБК-позитивних продуктів. Визначення відповідних показників описано у розділі Матеріали та Методи (* – P < 0,05; * – різниця достовірна, порівняно з клітинами, вирощеними у середовищі з 0,1 % глюкози без спермідину)

The same concerns the measured intracellular levels of oxidized lipid forms (TBA-reactive substances, **Fig. 3*C***). 2 mM Spd significantly increased their content, while 1 mM polyamine did not produce a visible effect. It should be noted, however, that an increased cellular content of TBA-reactive substances usually indicates an excessive ROS production [17]. Apparently, the situation in *O. polymorpha* and effects of exogenous Spd are more complex.

Effect of Spd on *O. polymorpha* antioxidative defense systems. If increased above the physiological limits free radicals, including ROS are removed by cellular anti-oxidant defenses that involve enzymatic (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)), or non-enzymatic components, such as glutathione (GSH) [3, 10, 28]. In our previouse study, we revealed that SOD and CAT activities were highest in the *O. polymorpha* cells cultured under glucose limitation (0.1 % glucose in growth medium) that evokes ROS overproduction, whereas in the cells grown on the media with elevated glucose concentrations (higher then 1 %), activities of these enzymes were significantly lower and did not exceed the values of the wild-type cells cultured under physiological conditions (1 % glucose-containing medium) (see **Fig. 1, 2**). [10]

In the present study, we observed that 1 mM Spd had a minimal effect on SOD or CAT activities as well as the intracellular level of the reduced GSH (**Fig. 4**). However, the effects of the elevated 2 mM Spd were disctinct and well pronounced. Although SOD and CAT are functional partner enzymes, 2 mM Spd had an opposite effect on their activities in the glucose-depleted *O. polymorpha* cells. It apparently inhibited CAT in the early exponential growth phase, but upinduced its activity after 40 h (stationary phase), as compared to cells exposed to 1 mM Spd and control cells incubated without polyamine (**Fig. 4*A***). At the same time, 2 mM Spd profoundly elevated SOD activity in the early (15 h), but not later (40 h), growth phase (**Fig. 4*B***). It is known that Spd can regulate SOD at the transcriptional level [6]. In turn, the modulation of CAT activity by 2 mM Spd may be related to its own catabolism that involves hydrogen peroxide production [25].

It remains to be investigated how the observed elevated GSH levels (**Fig. 4*C***) in the cells exposed to 2 mM Spd for 15 h are mechanistically or regulatory connected with the oxidative forms of proteins and lipids (**Fig. 3**) and intracellular ROS levels (**Fig. 2**).

In our previous work, we noted that changes in the intracellular GSH levels in *O. polymorpha* wild type strain generally resembled those in SOD activity. The level of GSH increased by 35 % in the wild type cells grown on glucose-deficient medium compared to the cells grown under physiological conditions (1.0 % glucose in the culture medium). The GSH content decreased with increasing glucose concentration in the medium similarly to SOD activity [10]

In this work, we showed that 1 mM Spd did not effect GSH content in the yeast cells as compared to the cells grown on the medium with 0.1 % glucose without this polyamine. At the same time, 2 mM Spd promoted increase in GSH in 15 hours' culture of *O. polymorpha* (**Fig. 4*C***).

It is of note that a drop in GSH level in 40 hours' culture positively correlated with a decrease in the content of products of protein and lipid oxidation (**Fig. 3**). These changes may be tentatively caused by a gradual Spd depletion from the medium.
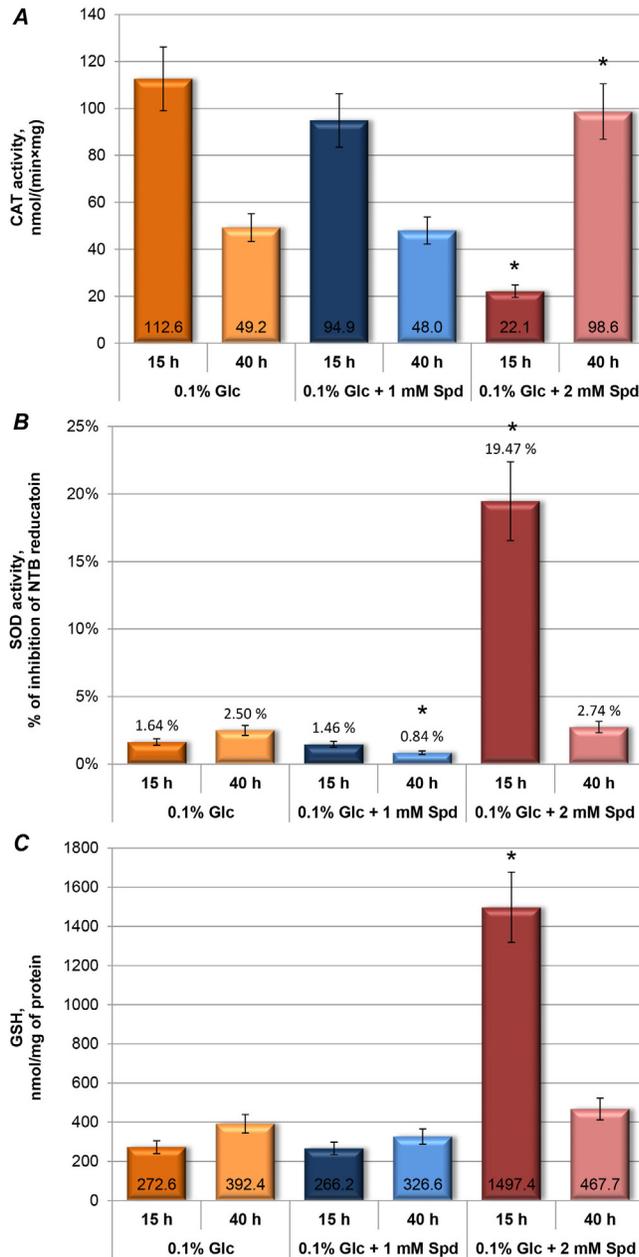
Fig. 4. Components of the antioxidant defense system in *O. polymorpha*: **A** – activity of catalase (CAT); **B** – activity of superoxide dismutase (SOD); **C** – content of the reduced gluthatione (GSH). Yeast cells were grown in the medium with 0.1 % glucose with or without Spd. (* – P<0.05, * – the difference is significant as compared to the cells grown in the medium with 0.1 % glucose without Spd)

Рис. 4. Компоненти антиоксидантної системи захисту клітин *O. polymorpha*: **A** – активність каталази (CAT); **B** – активність супероксиддисмутази (SOD); **C** – вміст відновленого глутатіону (GSH). Клітини дріжджів вирощували у середовищі з 0,1 % глюкози із додаванням чи без екзогенного спермідину (* – Р < 0,05; * – різниця достовірна, порівняно з клітинами, вирощеними у середовищі з 0,1 % глюкози без спермідину)

CONCLUSIONS

We analyzed the effects of different concentrations of exogenous Spd (1 and 2 mM) in the background of glucose deprivation on prooxidant-antioxidant balance in the cells of the methylotrophic yeast *O. polymorpha*, and whether this polyamine can counteract intracellular oxidative stress. Indeed, we observed a concentration-dependent effect of Spd on *O. polymorpha* physiology. It can be also concluded that Spd at 1 mM concentration had a lagerly protective effect on *O. polymorpha* cells by decreasing ROS content evoked by the stress of glucose limitation. Thus, upon addition of 1 mM Spd to the glucose-limited culture medium, the antioxidant defense system dominates in the yeast cells. However, when elevated to 2 mM concentration, Spd produced a multifaceted complex effect inhibiting growth and dramatically elevating cellular contect of the oxidized froms of proteins and lipids, and most probably upinducing autophagy. This effect, however, diminished with incubation time and was not due to the accumulation of free radical species that were probably counterbalanced by the observed increased GSH content. Therefore, 2 mM Spd apparently shifts the prooxidant-antioxidant balance towards the domination of prooxidants in *O. polymorpha* cells.

The observed apparent positive effect of a lower (1 mM) concentration of Spd on the prooxidant-antioxidant balance of *O. polymorpha* cells can be used in further studies with this yeast model on possible correction of pathological conditions associated with different forms of oxidative stress. For instance, it can be examined whether in addition to the oxidative state of a cell, lower concentrations of Spd, or of its mixture with other polyamines, such as spermine or agmatine, will affect cellular life span, prosurvival autophagy or apopotosis.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: This article does not contain any studies with animal subjects performed by the any of the authors.

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ПРООКСИДАНТНО-АНТИОКСИДАНТНА РІВНОВАГА В МЕТИЛОТРОФНИХ ДРІЖДЖІВ *OGATAEA POLYMORPHA* ЗА ВПЛИВУ СПЕРМІДИНУ

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Вступ. Багато низькомолекулярних сполук, які клітини використовують як антиоксиданти, можуть безпосередньо взаємодіяти з активними формами кисню/нітрогену. Серед них поліаміни, – природні речовини з яскраво вираженими антиоксидантними властивостями. Основними поліамінами є путресцин, спермідин, спермін. Спермідин – позитивно заряджений поліамін, який синтезується з путресцину і слугує попередником сперміну. Під час застосування спермідину на кількох модельних організмах було виявлено його антивікові властивості, здатність продовжувати життя та забезпечувати стійкість до стресу. Спермідин також зменшує окисне пошкодження білків, асоційоване з віком, і надпродукцію активних форм кисню, що обумовлює його антиоксидантну активність. Метою дослідження було проаналізувати вплив різних концентрацій екзогенного спермідину на баланс прооксидантів і антиоксидантів у метилотрофних дріжджах *Ogataea polymorpha*, природно пристосованих до стресових умов існування, таких як ріст на середовищі, що містить токсичний метанол, і культивування за високих температур (42–45 °C).

Матеріали та методи. У цій роботі використано штам дикого типу *O. polymorpha*. Для створення стресових умов клітини дріжджів культивували в мінеральному середовищі з 0,1 % глюкози. Спермідин додавали в концентраціях 1 і 2 мМ безпосередньо перед початком вирощування культури дріжджів. Для дослідження прооксидантно-антиоксидантного стану клітин було проведено визначення активних форм кисню *in vivo*, рівня окисних модифікацій білків і ліпідів, концентрації відновленого глутатіону, активностей супероксиддисмутази та каталази.

Результати. У дослідженні проаналізовано вплив різних концентрацій екзогенного спермідину на ферментативну (активність супероксиддисмутази і каталази) та неферментативну (відновлений глутатіон) ланки системи антиоксидантного захисту та маркери окисного пошкодження клітин (продукти окиснення білків і ліпідів)

у метилотрофних дріжджів *O. polymorpha* за умов дефіциту глюкози. Виявлено, що 1 мМ спермідин мав захисний ефект у клітинах *O. polymorpha*, зменшуючи вміст продуктів окисної модифікації білків. Водночас активність супероксиддисмутази та каталази та вміст відновленого глутатіону залишалися майже незмінними, порівняно зі середовищем без спермідину. Побічні ефекти 2 мМ спермідину (підвищений вміст карбонільних груп білків, продуктів перекисного окиснення ліпідів, змінений рівень активності супероксиддисмутази та каталази, підвищення пулу відновленого глутатіону, інгібування росту та вакуолізація клітин) спостерігали у фазі експоненційного росту культури дріжджів. Під час тривалого культивування ці ефекти зменшувалися, а значення досліджуваних показників наближалися до значень показників клітин, вирощених у середовищі з низькою концентрацією глюкози без спермідину.

Висновки. Отримані дані свідчать про залежний від концентрації вплив спермідину на фізіологію *O. polymorpha*, який може бути використаний для подальшого вивчення сполук, здатних пом'якшувати негативний вплив оксидативного стресу в цих дріжджах та інших модельних організмах. У концентрації 1 мМ спермідин чинив помітний захисний ефект, тоді як за підвищеної 2 мМ концентрації цей поліамін посилював стрес у клітинах дріжджів.