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CATALASE ACTIVITY AND ULTRASTRUCTURE CHANGES OF *CHLAMYDOMONAS REINHARDTII* CELLS INDUCED BY METHANOL

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Methanol at low concentrations stimulates the growth of autotrophic batch culture *Chlamydomonas reinhardtii*. The effect is maximal in the cultural medium with 0.2% methanol. In order to clarify the mechanism we studied the effects of methanol on the ultrastructure of the *C. reinhardtii*. To test the hypothesis on the participation of catalase in the metabolization of methanol a cytochemical study of localization and activity of catalase in the cell compartments of *C. reinhardtii* was carried out. Cytochemical reaction for catalase was performed with 3,3'-diaminobenzidine-tetrahydrochloride (DAB). Ultrastructure of cells was investigated by transmission electron microscopy and morphometric analysis. Biochemical study of catalase activity was determined by a Clarck oxygen electrode. Methanol-induced quantitative changes in ultrastructure of *C. reinhardtii* cells are detected: the volume of *C. reinhardtii* cells and partial volume of chloroplasts decreased, partial volumes of vacuoles, mitochondria, and plastoglobules increased. Methanol addition has also resulted in increase in catalase activity of the mitochondria. The obtained data confirm that catalase is localized in mitochondria of *C. reinhardtii* cells. In the presence of methanol, the activity of mitochondrial catalase increases, suggesting its involvement in the oxidation and metabolization of the solvent.

Key words: *Chlamydomonas reinhardtii, methanol, cell ultrastructure, catalase*

Methanol is the simplest monatomic alcohol that is highly dissolved in water and organic solvents. It rapidly penetrates through cell membranes and evenly distributes in cells (Gout et al., 2000). In contrast to bacteria, where the metabolic pathway of methanol is well studied, there is only fragmentary data about its metabolism in higher plants and unicellular algae. Experiments with labeled methanol suggest that in higher plants it is oxidized through a four-step pathway with the successive formation of formaldehyde, formic acid and carbon dioxide ($CH_3OH \rightarrow HCHO \rightarrow HCOOH \rightarrow CO_2$) (Brunner et al., 2007). Free methanol, which presents in plant tissues in a norm (about 1 μg per g of wet weight), can involve in secondary metabolism and protect plants from damages induced by reactive oxygen and nitrogen species (ROS and NOS). Variations in the intracellular concentration of methanol affect the expression of a number of plant genes responsible for metabolic and regulatory processes. Methanol acts as signaling molecule under various stresses on plants, such as hypoxia, injury, dehydration.

Metabolism of methanol in microalgae is of particular interest, since it has been shown that low concentrations of exogenous methanol can significantly increase biomass of some microalgal species (*Chlorella vulgaris*, *Selenastrum capricornutum*, *Skeletonema costatum* and *Prorocentrum minimum*) (El Jay 1996; Okumura et al., 2001). Recently, we showed that methanol affects positively growth, photosynthesis and respiration of *C. reinhardtii*, unicellular green alga (Stepanov, Zolotareva, 2011).

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The stimulating effect of methanol is observed exclusively under autotrophic (photosynthetic) conditions, while under heterotrophic growth this alcohol is toxic as for the algae as for other heterotrophic organisms (Theodoridou et al., 2001). The light-dependent pathways of methanol assimilation, metabolization, and its detoxification, are poorly understood. Nonomura and Benson (1992) proposed that a photorespiration has required metabolizing methanol. In *C. reinhardtii*, photorespiratory metabolism differs from such processes in higher plants. In particular, *C. reinhardtii* has another type of peroxisomes than that in leaves (Kehlenbeck et al., 1995) where catalase and other enzymes of the photorespiratory pathway are located in these organelles of higher plants. Photorespiration in *Chlamydomonas* occurs in the mitochondria and is catalyzed by glycolate dehydrogenase (Nakamura et al., 2005; Tural, Moroney 2005). In algal peroxisomes, peroxide is not formed, and electrons enter the mitochondrial respiratory chain by reduction of the ubiquinone pool. So far, the localization of catalase in *Chlamydomonas* cells is not entirely clear. Kato et al. (1997) has received data that a catalase is most likely located in mitochondria. However, in their earlier work, Giraud and Czaninski (1971) used DAB as test on catalase activity did not find the enzyme in *C. reinhardtii* cells. The aim of our work was to investigate the effect of methanol on the ultrastructure of the *C. reinhardtii* cells and to determine intracellular localization and the level of catalase activity.

METHODS

Unicellular green alga *Chlamydomonas reinhardtii* was obtained from the microalgal collection of Department for Membranology and Phytochemistry in M.G. Kholodny Institute of Botany, NAS of Ukraine (IBASU-B - 163). Batch autotrophic cultures were grown on liquid Kessler's medium (Bishop, Senger, 1971) in 0.5 l flasks with magnetic stirrer agitation at room temperature. White fluorescent light with 100 μmol photons • m^{-2} • s^{-1} intensity of photosynthetic active radiation (PAR) on the surface of flasks was used round-the-clock. When the culture were grown to mid-exponential phase 0.2% (v/v) methanol was added to cultural medium and the culture exposed to the alcohol for 8 hours. Then cells were concentrated by centrifugation at 3000 rev/min for 2 min. Concentrated culture were used for ultrastructural and cytochemical studies.

The cells were embedded inside agaric blocks and specimens were prefixed with 1.5% formalin in 0.2 M phosphate buffer, pH 7.0, for 20 h at 4ºC and were fixed with 2.5% glutaraldehyde (GA) in the same buffer for 2 h at room temperature. Then samples were rinsed and post-fixed with 1% OsO4 in 0.1 M sodium cacodylate buffer, pH 7.0, for 3 h at 4 ˚C. Specimens were then dehydrated with increasing concentrations of ethanol and acetone and soaked in mixes of acetone and epone-araldite resin and in the pure resin. The samples were put in molds and filled with fresh-prepared resin and polymerized for 48 h at 60ºC in a thermostat. Ultra-thin sections (70-80 nm) were cut with a LKB-V ultramicrotome (LKB, Sweden) and post-stained in 2% aqueous uranyl acetate and viewed with a JEM 1300 transmission electron microscope (JEOL, Japan) at 80 kV. Images were recorded on an EB19H TEM film (AGFA, Belgium).

Localization of catalase in cells of *C. reinhardtii* was determined by cytochemical reaction with 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma, USA). Samples of microalga were fixed with 3% GA in 0.05 M phosphate buffer, pH 7.0, rinsed in the same buffer and 0.1 M Glycine-NaOH buffer, pH 10.5, and then distributed in 3 sets with the following incubation media: (A) 20 mg DAB + 2 ml 3% H_2O_2 + 8 ml 0.1 M Glycine-NaOH buffer, pH 10.5 (complete medium); (B) 20 mg DAB + 10 ml 0.1 M Glycine-NaOH buffer, pH 10.5 (control medium without substrate); (C) 20 mg DAB + 2 ml 3% H_2O_2 + 8 ml 0.1 M Glycine-NaOH buffer, pH 10.5 + 3 mg sodium azide (Sigma, USA) (control medium with catalase inhibitor).

All sets were incubated in the dark at 37ºC for 1 hour. Before the beginning of the incubation in the C medium, cells were preincubated with 3 mg of sodium azide in Glycine-NaOH buffer for 30 min. Following the incubation, all sets (A, B, and C) were rinsed in 0.05 M phosphate buffer and postfixed with 2% OsO_4 in the same buffer, pH 7.0. Then the procedure performed according to the general scheme of sample preparation for electron microscopic study.

Determination of electron density index for mitochondria was carried out using a «Line profile» tool of ImageTool 3.0 computer program. Index of mitochondria electron density (I_m) indicating what portion of the mitochondria matrix density (D_m) is due to catalase activity is expressed as a ratio of the difference between mitochondria matrix density and the cytoplasm one (as a control without catalase) to mitochondria matrix density and determined by the formula:

$I_m = (D_m - D_{cyt}) / D_m$,

where D_{cyt} – the main grey intensity of the cytoplasm in pixels, D_m – the main grey intensity of mitochondrial matrix in pixels.

For morphometric analyses, the cells on random sections were photographed and scanned on an Epson Perfection 3200 Photo scanner. Photos were prepared using Adobe Photoshop 7.0 and Corel Photo-Paint 11 computer programs.

For the analysis, median sections of cells contained clearly distinguishable cup-shaped chloroplast with the pyrenoid and the nucleus were selected. Measurements of organelles' areas for determining partial volumes of cellular components (i.e., the ratio of total area of all organelles of the certain type to the area of whole cell) were performed on scanned micrographs using an UTHSCSA ImageTool 3.0 software program (the University of Texas, Health Science Center, USA). The area of the cytoplasm was calculated as the difference between the total area of the cell and the sum of the areas of all organelles and intracellular inclusions. For each experimental set and control, 30-39 median longitudinal cell sections were analyzed on micrographs with a total magnification of ×8000 or ×10000.

Linear dimensions of cell, length (L) and width (W), were assessed as the longest and shortest axis of the cell on the section by ImageTool 3.0 software program. The ratio of width to length in % was also determined. The main thickness of the cell wall was calculated as the distance from the plasmalemma to the outside of the cell wall. Volume of cell was calculated as volume of an ellipsoid:

$V = \pi \cdot W/2 \cdot (L/2)^2$,

where V – volume, W – width, L – length of cell.

Biochemical determination of catalase activity was carried out to estimate a rate of O_2 production by microalga suspension after addition of H_2O_2. O_2 evolution was measured with the Clarck electrode method. Samples were taken after 1, 6 and 24 h incubation with 0.2% methanol and without methanol (as a control). Then microalgal culture was concentrated to 60 μg/ml chlorophyll content. To investigate the rate of O_2 production 3% H_2O_2 (20 μl) and concentrated suspension of microalga (200 μl) were added in oxygen measuring cell with 4.5 ml culture medium. Each experiment was repeated several times and the mean values calculated for each treatment.

RESULTS AND DISCUSSION

Since *C. reinhardtii* cultures grew at round-the-clock illumination, their cells were at different stages of their life cycle. Cell morphology and *C. reinhardtii* culture contamination were evaluated in a preliminary study under the light microscope. Only algologically and bacteriologically pure cultures were used for further studies. Light microscopy showed that the culture consisted in mobile and immobile individual cells and tetrads of cells. Loss of flagella by some cells was characteristic for *C. reinhardtii* during prolonged cultivation in the laboratory under constant stirring (Andersen, 2004). Conventionally, cells of non-synchronized culture could be divided into two types according to their life stage – young (up to 10 μm^2), and mature (over 10 μm^2). Generation by cell division was observed in mature cells. The cells of tetrads with a broken cell wall of maternal cell were attributed to the young type. Because old cells were rarely met in our cultures, we did not determine the effect of methanol on them.

No significant qualitative changes in organelles of the algal young and mature cells treated with 0.2% methanol were observed (fig. 1). It indicates that the methanol concentration stimulated productivity of the culture does not cause damaging effects on the ultrastructure of *C. reinhardtii* cells.

At the same time, our morphometric studies have showed that the addition of methanol can affect the size of *C. reinhardtii* cells (table 1). Young cells responded to 0.2% methanol by a decrease in cell length but their width was not changed, while in mature cells methanol treatment resulted in a reduction in both their length and width. According to the changes in linear dimensions of young and mature cells, their area and volume also decreased after adding the alcohol (table 1).

Methanol did not affect the thickness of the cell wall of *C. reinhardtii*. We can assume that reducing the size and volume of cells is due to the influence of methanol on the expression of genes responsible for growth of cell wall, since it is known that methanol reduces the expression of the gene of expansin consisted in plant cell wall (Downie et al., 2004).

Methanol resulted in an increase in partial volume of vacuoles in young cells almost twice, and in mature cells – 2.3 times (table 2). Such an expansion of vacuoles under the influence of methanol can be explained by changes in osmotic potential of these cells. Formate formed in the pro-

Table 1. Effect of methanol on dimensions of *Chlamydomonas reinhardtii* young (1) and mature (2) cells

Variant	Length, μm	Width, μm	Ratio W/L, %	Square, μm^2	Volume, μm^3	Cell wall thickness, μm
Control 1	3.77 ± 0.06^{b}	2.97 ± 0.04^{a}	79.71 ± 1.75^{a}	8.40 ± 0.20^{b}	16.67 ± 0.56^{b}	0.067 ± 0.002^{ab}
Experiment 1	3.56 ± 0.08^{a}	3.01 ± 0.06^{a}	84.86 ± 2.22^{b}	7.87 ± 0.25^{a}	15.12 ± 0.84^{a}	0.068 ± 0.006^{a}
Control 2	4.74 ± 0.07^{d}	4.08 ± 0.06^{b}	85.31 ± 1.36^{b}	14.10 ± 0.31^{d}	36.38 ± 1.26^{d}	0.061 ± 0.002^{a}
Experiment 2	4.42 ± 0.08^{c}	3.73 ± 0.06^{b}	84.78 ± 1.34^{b}	12.59 ± 0.32^{c}	29.12 ± 1.32^{b}	0.064 ± 0.004^{a}

Note. Here and in Table 2 means within a column flanked by the same letter are not significantly different at $P \leq 0.05$

Table 2. Effect of methanol on partial volumes of *Chlamydomonas reinhardtii* components in young (1) and mature (2) cells,%

Variant	Vacuole	Chloroplast					Nucleus	Mitochondria	Polyphosphate granules	Cytoplasm
		Overall	Pyrenoid	Pyrenoid coating	Starch	Plastoglobules				
Control 1	1.23 ± 0.35^{b}	57.53 ± 2.25^{bc}	20.29 ± 1.05^{ab}	10.40 ± 0.72^{ab}	1.90 ± 0.16^{a}	1.42 ± 0.32^{bc}	16.68 ± 1.04^{b}	2.26 ± 0.37^{a}	9.50 ± 1.28^{a}	12.80 ± 1.96^{a}
Experiment 1	2.42 ± 0.28^{c}	54.51 ± 2.05^{b}	20.79 ± 1.80^{ab}	9.82 ± 0.69^{a}	2.40 ± 0.43^{a}	1.17 ± 0.27^{b}	12.17 ± 0.84^{a}	2.77 ± 0.51^{a}	8.19 ± 1.33^{a}	19.94 ± 1.92^{b}
Control 2	0.65 ± 0.10^{a}	54.21 ± 1.29^{b}	17.31 ± 0.79^{a}	8.61 ± 0.46^{a}	3.33 ± 0.45^{b}	0.74 ± 0.14^{a}	10.36 ± 0.64^{a}	2.20 ± 0.26^{a}	7.84 ± 0.73^{a}	24.74 ± 1.48^{b}
Experiment 2	1.48 ± 0.28^{b}	48.83 ± 1.44^{a}	18.65 ± 0.81^{a}	9.82 ± 0.95^{a}	4.19 ± 0.84^{b}	1.32 ± 0.25^{c}	11.61 ± 0.46^{a}	3.18 ± 0.50^{b}	8.11 ± 0.73^{a}	26.79 ± 1.39^{bc}

cess of the intracellular metabolism of methanol, apparently, causes an increment of osmotic pressure and enhancement of water flow into cell that induces the growth of partial volume of vacuoles. Following methanol treatment the partial volume of chloroplasts in mature cells was reduced by 10%.

Methanol did not affect the partial volumes of chloroplast components in young and mature cells, besides plastoglobules, partial volume of which increased by 78% in mature cells (table 2). Plastoglobules are particles inside chloroplasts containing lipids, carotenoids, plastoquinone, and tocopherol (Austin et al., 2006). It is proposed that plastoglobules are also involved in stress response, and their number has been shown to increase in diverse plant species under various abiotic and biotic stresses. In addition, increased numbers of plastoglobules have been found in aged leaves where thylakoids break down (Ghosh et al., 2001) and also in thylakoid biogenesis of mutants which had smaller chloroplasts and decreased thylakoid accumulation (Rudella al., 2006). We report here that plastoglobules also increased in response to methanol treatment.

The partial volume of nuclei in young cells reduced by 27% after methanol adding (table 2). There was an increase in the partial volume of mitochondria by 22% in young cells and by 45% in mature ones compared with controls suggesting an intensified activity of respiration. The partial volume of cytoplasm increased in young methanol-treated cells too, apparently, due to reducing the relative volume of the chloroplasts and the nucleus.

Oxidation of methanol in plants can occur non-enzymatically through H_2O_2 oxidation or due to peroxidase activity of catalase:

$$CH_3OH + H_2O_2 \rightarrow HCOH + 2H_2O.$$

It was proposed that additional carbon supply provided with methanol causes the photorespiration to be shifted from catabolism to anabolism (Zbiec et al., 2003). As mentioned above, the presence of catalase in *Chlamydomonas* cells and its participation in the process of photorespiration is questioned.

To test catalase localization and the effect of methanol on it we examined the intensity of staining with DAB reagent of the algal mitochondria by calculating a mitochondrial electron density index. Following treatment with DAB reagent, membranes of mitochondria were seen better in A set cells than in cytochemical control cells (B and C sets) (fig. 1 d, e). Comparison of indices of mitochondrial electron density in A, B, and C sets in growth control and after methanol addition al-

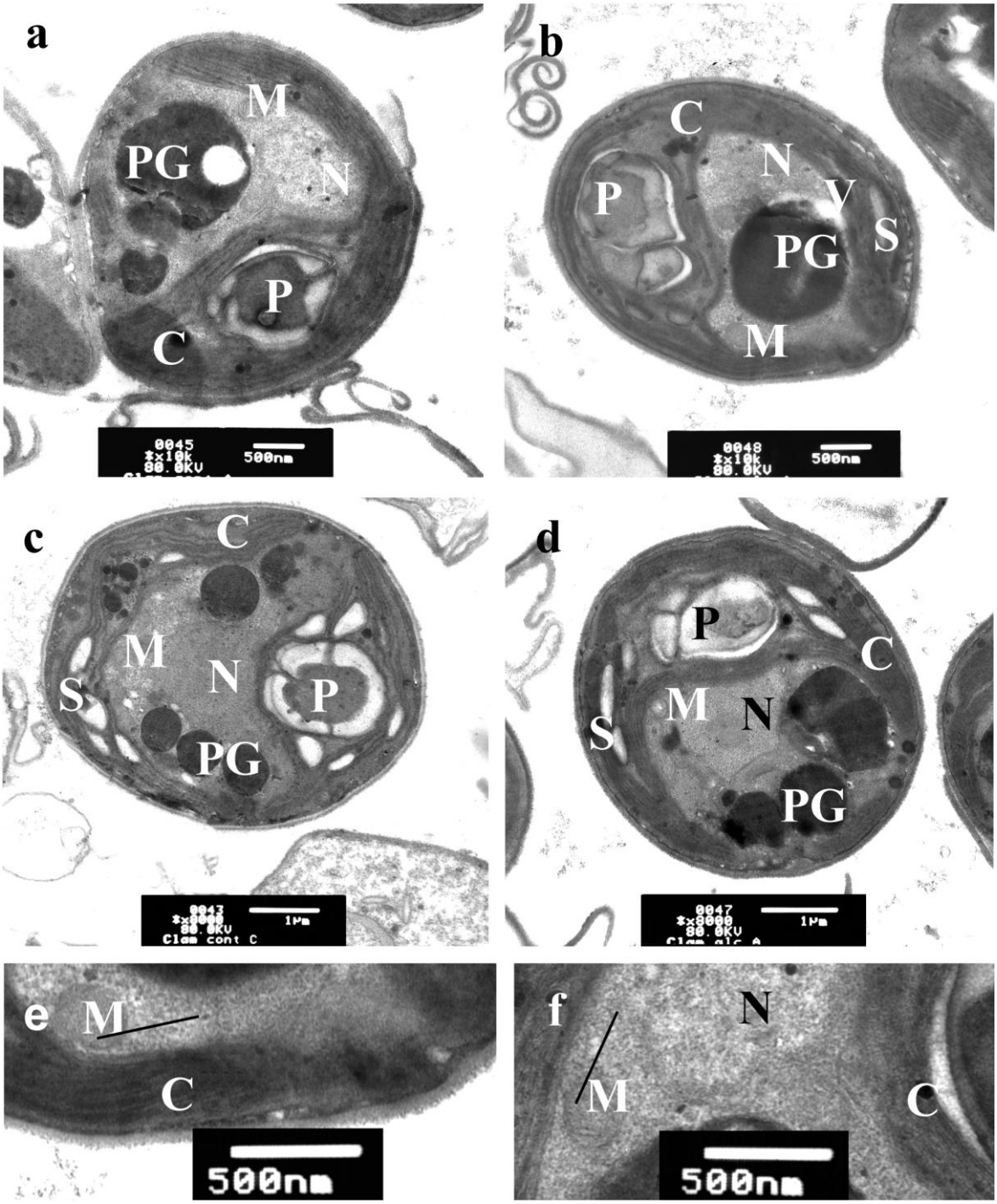

Fig. 1. Cells of *C. reinhardtii*.
a, b – young cells, c, d – mature cells, and a, c – control, b, d – experiment (treatment in 0.2 % methanol). Cytochemical reaction for catalase (cell fragments): e – full medium, f – control with catalase inhibitor; lines indicate the sites of density determination. Abbreviations: C – chloroplast, M – mitochondria, N – nucleus, P – pyrenoid, PG – polyphosphate granule, S – starch grain, V – vacuole.

lowed to characterize quantitatively the effect of methanol on activity of catalase in *C. reinhardtii* mitochondria (fig. 2).

The data presented in fig. 2 have confirmed the localization of catalase in the algal mitochondria since the mitochondrial index of electron density in A set was always greater than in the B and C sets (controls to the cytochemical reaction). Also, the presence of methanol in the A set cells resulted in increased density of mitochondria by 28% compared to that of methanol-free cells, while these indices of cells in B and C sets were not significantly different. Thus, the 0.2% methanol can enhance a catalase activity in the mitochondria of *C. reinhardtii* cells or increase biosythesis of the enzyme.

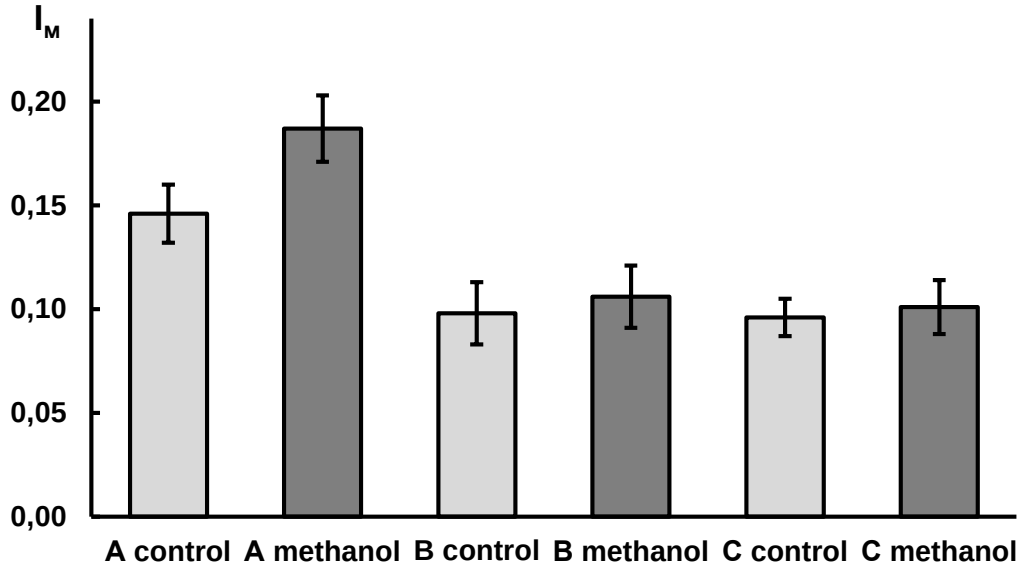

Fig. 2. Mitochondrial indices of electron density in A, B and C sets with 0.2% methanol and without it (control).
Standard errors of 3 experiments are indicated.

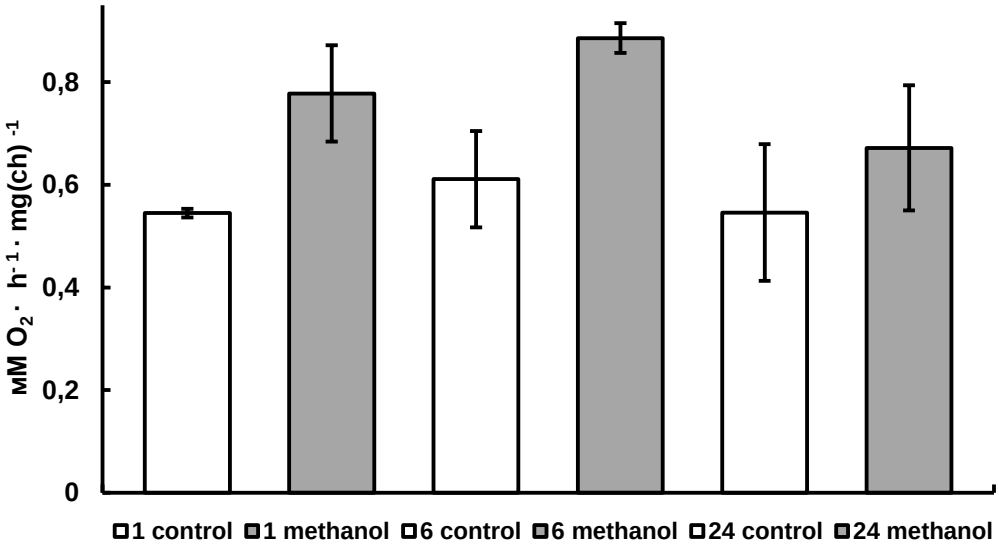

Fig. 3. The rate of O_2 production by *C. reinhardtii* culture after addition of 20 µl H_2O_2. Samples was taken after 1, 6 and 24 hour treatment in 0.2% methanol.
Standard errors of 3 experiments are indicated.

It is known that the key enzyme in the process of photorespiration in *C. reinhardtii* is the mitochondrial enzyme glycolate decarboxylase (E.C. 1.4.4.2), which does not accept molecular oxygen as end electron acceptor (Chauvin et al., 2008; Nelson, Tolbert 1970) and respectively does not produce H_2O_2. Thus, catalase is not proposed to play a role in the photorespiratory cycle of *C. reinhardtii*. Yet, genomic sequence of C. *reinhardtii* (http://genome.jgipsf.org/Chlre3/Chlre3.home.html) has a gene encoding a putative glycolate oxidase (Chauvin et al., 2008), which takes part in H_2O_2 production, and other gene encoding a typical catalase. In addition, a gene encoding a putative catalase–peroxidise homologous to enzymes found in prokaryotes, was also found (Shao et al., 2008). However, expression of the latter gene appears to be very weak as compared to the expression of the typical catalase gene, even under oxidative stress conditions.

Biochemical activity of catalase was estimated in terms of rate of O_2 production in microalga suspension after addition of 20 μl H_2O_2. A count was conducted per the amount of chlorophyll in unit volume of culture. As chlorophyll accumulation correlates with the cell concentration at exponential phase of microalga growth, then the rate count of O_2 production can be conducted per the concentration of chlorophyll. Due to low content of catalase in the cells of *C. reinhardtii*, the rate of O_2 production after addition of H_2O_2 was at the average 0.55 мМ $O_2 \cdot h^{-1} \cdot$ mg $(ch)^{-1}$ in control cells and 0.75 mM $O_2 \cdot h^{-1} \cdot$ mg $(ch)^{-1}$ in cells after addition of 0.2% methanol (fig. 3). A 30% rate increment compared to control cells was produced by methanol after 1 hour which, by a negligible margin, rose to 31% at 6 hours, finally reaching only 18% after 24 hours. A reduction in catalase activity occurred after 24 hours incubation in methanol, obviously, took place due to alcohol metabolisation.

Catalase of plants (EC 1.11.1.6), besides dismutase activity, shows peroxydase activity too. It can use methanol for H_2O_2 reduction.

Peroxydase activity	Dismutase activity
$CH_3OH + H_2O_2 \rightarrow HCOH + H_2O$	$2H_2O_2 \rightarrow O_2 + 2H_2O$

It is known that ethanol represses a catalase activity, as it is a competitive inhibitor of H_2O_2 metabolisation. However, methanol addition, on the contrary, stimulates activity of catalase in *C. reinhardtii* to increase rates of O_2 production by microalga. Such an enhancement in rate of O_2 production after methanol addition can be explained by formation of endogenous H_2O_2 due to increasing a redox potential in cells followed oxidization of methanol. Recently, induction of endogenous H_2O_2 formation was detected after adding 50 mM methanol to the culture of *Oncidium* orchid (Shen, Yeh 2008). The authors observed a double increase in concentration of H_2O_2 after 30 min incubation with methanol, then the concentration H_2O_2 slightly went down and remained at permanent level during 6 hour. However, our studies showed that a rate of O_2 production by culture without addition of 20 μl H_2O_2 was similar in both the presence and absence of methanol suggesting that endogenous H_2O_2 did not affect rate of O_2 production by culture of *C. reinhardtii*. Obviously, methanol can improves availability of exogenous H_2O_2 to catalase or methanol can directly act as inductor of catalase gene expression.

The data of our study allow proposing that the light-dependent metabolism of methanol, which induces significant reorganization of pigment-protein components of photosynthetic membranes (Kotzabasis et al., 1999), activates also mitochondrial catalase, probably, involving in the oxidation of the alcohol. We suggest that methanol can be oxidized due to nonspecific peroxidase activity of mitochondrial catalase.

Thus, 0.2% methanol does not cause qualitative changes in ultrastructure of *C. reinhardtii*, but reduces the size of cells and chloroplast partial volume, and increases partial volume of vacuoles, mitochondria, and plastoglobules. Our research also confirms that the catalase in the *C. reinhardtii* cells is localized in mitochondria. In the presence of methanol, the activity of catalase in mitochondria increases, suggesting its involvement in the oxidation and metabolization of the solvent.

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ИЗМЕНЕНИЯ АКТИВНОСТИ КАТАЛАЗЫ И УЛЬТРАСТРУКТУРЫ КЛЕТОК *CHLAMYDOMONAS REINHARDTII* ПОД ВЛИЯНИЕМ МЕТИЛОВОГО СПИРТА

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Метанол в низких концентрациях стимулировал рост автотрофной накопительной культуры *Chlamydomonas reinhardtii*. Наибольший эффект наблюдался при добавлении в культуральную среду 0,2% метанола. С целью уточнения механизма его действия было изучено влияние этой концентрации метанола на ультраструктуру клеток *C. reinhardtii*. Для проверки предположения об участии каталазы в метаболизации метанола было проведено цитохимическое исследование локализации и уровня активности каталазы в компартментах клеток *C. reinhardtii*. Цитохимическую реакцию на каталазу проводили с 3,3′-диаминобензидин-

тетрагидрохлоридом (ДАБ). Ультраструктуру клеток исследовали методом трансмиссионной электронной микроскопии и морфометрического анализа. Метанол вызывал количественные изменения в структуре клеток *C. reinhardtii*. Показано, что под действием метанола уменьшался объем клеток *C. reinhardtii* и парциальный объем хлоропластов, увеличивались парциальные объемы вакуолей, митохондрий и пластоглобул. Данные подтверждают, что каталаза в клетках *C. reinhardtii* локализована в митохондриях. Добавление метанола приводило также к повышению активности каталазы в митохондриях. В присутствии метанола активность митохондриальной каталазы возрастала, что свидетельствует об ее участии в окислении и метаболизации этого спирта.

Ключевые слова: *Chlamydomonas reinhardtii, каталаза, метанол, ультраструктура клеток*

ЗМІНИ АКТИВНОСТІ КАТАЛАЗИ ТА УЛЬТРАСТРУКТУРИ КЛІТИН *CHLAMYDOMONAS REINHARDTII* ПІД ВПЛИВОМ МЕТИЛОВОГО СПИРТУ

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Метанол в низьких концентраціях стимулює ріст автотрофної накопичувальної культури *Chlamydomonas reinhardtii*. Найбільший ефект спостерігався при додаванні в середовище культивування 0,2% метанолу. З метою уточнення механізму його дії було вивчено вплив цієї концентрації метанолу на ультраструктуру клітин *C. reinhardtii*. Для перевірки припущення про участь каталази в метаболізації метанолу було проведено цитохімічні та біохімічні дослідження цього ферменту в клітинах *C. reinhardtii*. Цитохімічну реакцію на каталазу проводили з 3,3-діамінобензидин-тетрагідрохлоридом (ДАБ). Ультраструктуру клітин досліджували методом трансмісійної електронної мікроскопії та морфометричного аналізу. Під дією метанолу відбувалися кількісні зміни в структурі клітин *C. reinhardtii*. Показано, що під впливом метанолу зменшувався об'єм клітин *C. reinhardtii* і парціальний об'єм хлоропластів, збільшувалися парціальні об'єми вакуолей, мітохондрій і пластоглобул. Дані підтверджують, що каталаза в клітинах *C. reinhardtii* локалізована у мітохондріях. Додавання метанолу призводило також до підвищення активності каталази в мітохондріях. У присутності метанолу активність мітохондріальної каталази збільшувалася, що свідчить про її участь в окисненні і метаболізації цього спирту.

Ключові слова: *Chlamydomonas reinhardtii, каталаза, метанол, ультраструктура клітин*