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ORIGINAL PAPER

The effect of natural and synthetic auxins on the growth, metabolite content and antioxidant response of green alga *Chlorella vulgaris* (Trebouxiophyceae)

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Abstract The effect of exogenously applied natural [indole-3-acetic acid (IAA), phenylacetic acid (PAA), indole-3-butyric acid (IBA)] and synthetic [1-naphthaleneacetic acid (NAA)] auxins on the growth and metabolism of green microalga *Chlorella vulgaris* was examined. Exogenous auxins acted in a concentration-dependent manner on algal growth. Phytohormones at concentration of 100 μM inhibited algal growth expressed as the number of cells. IAA and IBA displayed the highest biological activity at 0.1 μM, whereas PAA and NAA were characterized by the greatest stimulatory effect on the number of cells at 1 μM. Treatment with IAA and IBA at 0.1 μM or NAA and PAA at 1 μM increased the concentration of photosynthetic pigments, monosaccharides and soluble proteins in *C. vulgaris*. Moreover, all auxins stimulated enzymatic (ascorbate peroxidase, catalase, superoxide dismutase) and non-enzymatic antioxidant (ascorbate, glutathione) systems in *C. vulgaris*, and therefore, suppressed lipid peroxidation and hydrogen peroxide accumulation. The data supports the hypothesis that auxins play a central role in the regulation of *C. vulgaris* growth and metabolism and the components of cellular redox systems that are thought to have a prominent role in the regulation of auxin-dependent processes.

Keywords Antioxidants · Auxins · Growth · Monosaccharides · Photosynthetic pigments · Proteins

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Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
DTT	Dithiothreitol
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
MDA	Malondialdehyde
NAA	1-Naphthaleneacetic acid
NBT	Nitroblue tetrazolium
NEM	*N*-ethylmaleimide
PAA	Phenylacetic acid
PVP	Polyvinylpyrrolidone
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid

Introduction

Auxins are a class of phytohormones involved in numerous aspects of plant growth and development at the molecular and whole-plant level. Decades of research have shown that natural auxins, such as indole-3-acetic acid (IAA), phenylacetic acid (PAA) and indole-3-butyric acid (IBA) regulate cell division, cell growth, ethylene biosynthesis, root development, leaf formation, apical dominance and differentiation of vascular tissues and fruit setting (Finet and Jaillais 2012). Synthetic auxins such as 1-naphthaleneacetic acid (NAA) induce similar physiological responses as natural auxins in bioassays (Imin et al. 2005). Auxins were detected in higher plants and algae. IAA is the natural auxin commonly occurring in all vascular plants

and in green algae from the *Chlorella* and *Scenedesmus* genus (Stirk and Van Staden 1997; Mazur et al. 2001; Stirk et al. 2004; Bajguz 2011). However, the concentration of this hormone in algal cells is somewhat lower than in vascular plants (Lau et al. 2009).

The research on the effect of plant growth regulators on algae lags far behind work with other terrestrial plants. Traditionally, plant hormones and synthetic plant growth regulators are used as valuable research tools to elucidate physiological responses of plants or to probe biochemical control mechanisms. In some algal species, auxin stimulates rhizoid formation (Basu et al. 2002) similarly to mosses (Sakakibara et al. 2003). In *Chara globularis* (Charophyta), auxin treatment resulted in similar changes in the cytoskeleton as in angiosperms (Jin et al. 2008). In the apical and intercalary zones of the thallus of the red alga *Grateloupia dichotoma*, the phytohormone stimulated cell division and elongation and/or suppressed branching, which also resembles processes characteristic of angiosperms (Yokoya and Handro 1996). Auxins determined zygote polarization in fucoid algae, with a disturbance of normal zygote development in the presence of inhibitors of IAA polar transport (Basu et al. 2002; Polevoi et al. 2003). However, the use of auxins could also be extended to the field of algae production to enhance the potential viability of commercial applications of alga-based renewable biomass production (Hunt et al. 2011).

Synchronous and homogenous cell population of *Chlorella vulgaris* is an especially promising experimental system for examining the effect of auxins on growth and metabolism of green algae. *C. vulgaris* is commonly found in freshwater and seawater and has a short growth cycle, which makes it ideal for biochemical studies and it can be used to directly observe phytohormone response at the cellular level, because observation of the signalling molecule and biochemical response takes place within the same cell under controlled conditions (Piotrowska-Niczyporuk et al. 2012).

Reactive oxygen species (ROS) are emerging as important regulators of plant growth, development and plant responses to environmental stresses. There is abundant evidence that ROS play a role in cell growth and physiological processes and that spatial regulation of ROS production is an important factor controlling plant development (Hirt 2000). In plants, various ROS, including H_2O_2, are involved in signalling pathways leading to alternations in ion fluxes, activation of kinases and changes in gene expression (Hancock et al. 2006). ROS can interact with other signal molecules, including phytohormones in the regulation of these physiological responses. It is suggested that plant growth regulators can modify the synthesis of antioxidants and the activity of basic antioxidant enzymes, and some of these enzymes are also implicated in phytohormone catabolism (Synková et al. 2006). Our knowledge about the physiological and molecular aspects of interaction between auxin and ROS is rapidly expanding. Auxin and H_2O_2 possesses antagonistic effects on cell cycle progression and gene activation (Hirt 2000). In addition, ROS are probably involved in typical auxin-mediated phenomena like root gravitropism (Joo et al. 2001). Auxins promote increases in the activity of antioxidant enzymes regulating ROS levels which could be associated with the activation of embryo/organogenesis (Pasternak et al. 2002, 2005). Moreover, exogenous natural (IAA) and synthetic (2,4-D) auxins can stimulate the activities of antioxidant enzymes in wheat (*Triticum aestivum* L.) tissue (Szechyńska-Hebda et al. 2007).

For that reason, the objective of the present study was to compare the effect of natural and synthetic auxins at a range of concentrations (0.01–100 μM) on the growth and the level of cellular components (photosynthetic pigments, monosaccharides, soluble proteins) in *C. vulgaris*. We also tested the hypothesis that auxin-induced changes in the growth and metabolism may be connected with its influence on the oxidative response, i.e. lipid peroxidation, hydrogen superoxide (H_2O_2) level, ascorbate and glutathione content and the activity of superoxide dismutase (SOD), catalase and ascorbate peroxidase. The obtained results may be important for elucidation of the plant hormone role in the physiology of green microalgae.

Materials and methods

Plant material, culture conditions and treatments

The wild-type *C. vulgaris* Beijerinck (SAG211-12) (Trebouxiophyceae) used in this study was obtained from cultures cultivated by the Institute of Biology at the University of Bialystok. The axenic cultures of *C. vulgaris* were grown in modified Knop's medium under the conditions of 50 μmol m^{-2} s^{-1} light intensity and 16:8-h light/dark cycle at 25 °C (Bajguz 2010, 2011; Piotrowska-Niczyporuk et al. 2012). Synchronization of the culture was controlled by studying cell division and the diagrams of cell size distribution. The cell size of the control culture was estimated at 4–6 μm in diameter. Growth of cultures was initiated by introduction of inoculums containing about 10^6 algal cells. The algal cells of stock cultures were always in the same physiological state (exponential growth phase) at the start of each experiment. The cell suspension was bubbled by atmospheric air at 1 L min^{-1} using air pumps to provide the necessary CO_2. The pH of the medium was adjusted to 6.8 with 1 M NaOH at the beginning of each experiment. *C. vulgaris* cells were cultured in Erlenmeyer flasks (500 mL) containing 250 mL medium and shaken in a rotary shaker.

Determination of optimum auxin concentrations for algal growth

For the determination of the optimum IAA, IBA, NAA and PAA (Sigma-Aldrich Co., USA) concentrations for *C. vulgaris* growth, auxins dissolved in 50 % ethanol were applied at 5 concentrations: 0.01, 0.1, 1, 10 and 100 μM. Each treatment consisted of two replicates and each experiment was repeated at least four times at different times. An equal amount of 50 % ethanol was added to the control. The final concentration of ethanol in the culture media did not exceed 1 % v/v and this amount did not affect algal growth. The starting density of algal suspension (day 0) was 20×10^5 cells mL^{-1}. Growth profile of the cells was determined as follows: 100 μL of algal samples were taken after 24, 48 and 72 h of culture at the beginning of each light period and the cell number was counted using a Bürker chamber. Algal growth was expressed as the number of cells in the cultures.

Effect of optimum auxin concentrations on metabolite content and antioxidant responses

The above experiment showed that 0.1 μM IAA and IBA and PAA and 1 μM NAA were the most effective in inducing culture growth, because cell numbers reached the maximum in these cases. Therefore, 0.1 μM IAA and IBA, 1 μM PAA and NAA and a control were used in this experiment where cultures grown for 72 h. Samples were collected every 24 h and analyzed for their metabolite content and antioxidant activity using the methods outlined below. There were two replicates and the experiment was repeated at least four times.

Determination of the number of cells and the content of proteins, monosaccharides and photosynthetic pigments

The number of cells was determined by direct counting of cells in the growth medium using a Bürker chamber (Bajguz 2010, 2011). The content of protein in algal cells was determined following the Bradford (1976) method, using bovine serum albumin as the standard. The monosaccharide content was estimated according to the Somogyi (1952) method. The Wellburn (1994) method was used for the determination of the content of photosynthetic pigments (chlorophyll *a*, total carotenoids) in *C. vulgaris*.

Stress markers determination

Lipid peroxidation was determined by measuring the amount of total malondialdehyde (MDA) (Heath and Packer 1968). Algal cells were harvested by centrifugation at 10,000×*g* for 10 min and the resulting pellet was treated with 0.25 % (w/v) thiobarbituric acid (TBA) in 10 % (w/v) trichloroacetic acid (TCA). After heating at 95 °C for 30 min, the mixture was cooled and centrifuged. The absorbance of the supernatant at 532 nm was recorded and corrected for unspecific turbidity by subtracting the value at 600 nm.

The level of hydrogen peroxide in *C. vulgaris* cells was measured spectrophotometrically at 390 nm by reaction with 1 M KI. The results were calculated using a standard curve prepared with fresh hydrogen peroxide solutions (Alexieva et al. 2001).

Determination of the contents of non-enzymatic antioxidants

For extraction of total ascorbate, *C. vulgaris* cells were harvested by filtration and quickly homogenized in liquid N_2 and 5 % (w/v) TCA (Kampfenkel et al. 1995). The homogenate was centrifuged for 5 min at 15,600×*g* (4 °C) and the supernatant was assayed for ascorbate content in a reaction mixture with 10 mM dithiothreitol (DTT), 0.2 M phosphate buffer (pH 7.4), 0.5 % *N*-ethylmaleimide (NEM), 10 % TCA, 42 % H_3PO_4, 4 % 2,2′-dipyridyl, and 3 % $FeCl_3$.

Determination of glutathione was as described (De Kok et al. 1986). Glutathione was extracted from algal cells in an extracting buffer (2 % sulfosalicylic acid, 1 mM Na_2EDTA, and 0.15 % ascorbate) and homogenized. The homogenate was centrifuged at 12,000×*g* for 5 min. An aliquot of supernatant was then used for the measurement of glutathione content with a glutathione assay kit (Sigma Chemical Co. USA).

Determination of the activities of antioxidant enzymes

The antioxidant enzymes were extracted in 50 mM phosphate buffer, pH 7.0, containing 1 mM EDTA, 0.05 % (v/v) Triton X-100, 2 % (w/v) PVP, and 1 mM ascorbic acid. SOD activity of *C. vulgaris* was determined by measuring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm as suggested by Beauchamp and Fridovich (1971). One unit of SOD (per mg protein) was defined as the amount causing 50 % inhibition of the photochemical reduction of NBT. Catalase (CAT) activity was estimated by recording the decrease in absorbance of H_2O_2 at 240 nm (Aebi 1984). One unit of CAT activity (U) was assumed as the amount of enzyme that decomposes 1 μmol of H_2O_2 per mg of soluble protein per minute at 30 °C. The method given by Nakano and Asada (1981) was followed for determining ascorbate peroxidase (APX) activity of *C. vulgaris*. The enzyme activity (U) was calculated as the amount of the enzyme

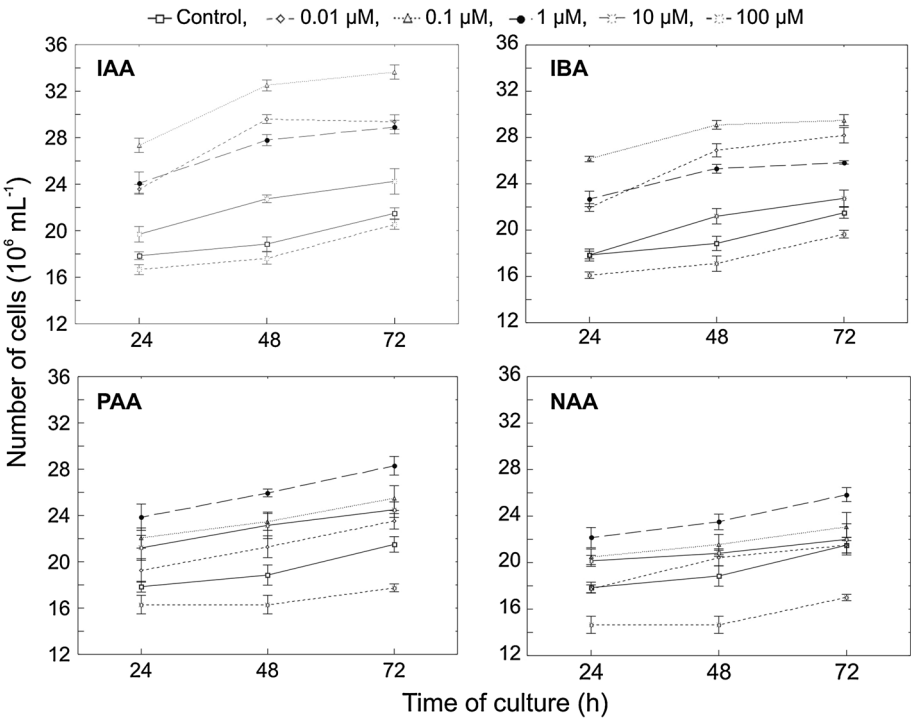

Fig. 1 The effect of different concentrations of auxins (IAA, IBA, NAA, PAA) on the growth expressed as cell number of *C. vulgaris* after 24, 48 and 72 h of cultivation. Data are the means of four independent experiments ± SD

that oxidizes 1 μmol of ascorbate consumed per mg of soluble protein per min at 30 °C.

Replication and statistical analysis

Each treatment consisted of two replicates and each experiment was carried out at least four times at different times. The data was analyzed by a one-way analysis of variance (ANOVA) and the means were separated using Duncan's multiple-range test (IBM SPSS Statistics Version 21). The level of significance in all comparisons was $p < 0.05$.

Results

Dose–effect of auxins in *C. vulgaris* growth

The experiment showed that IAA and IBA at 0.1 μM, and PAA and NAA at 1 μM were the most effective in inducing culture growth after 48 h of treatment because the number of cells reached a maximum (Fig. 1). IAA at 0.1 μM induced the highest increase in the number of cells by 53 %, 0.1 μM IBA by 46 %, 1 μM PAA by 34 % and 1 μM NAA by 24 % in comparison with the control after 48 h of cultivation. However, all auxins tested at 100 μM suppressed *C. vulgaris* growth, significantly inhibiting cell proliferation. Therefore IAA and IBA at 0.1 μM, and PAA and NAA at 1 μM were used in the following experiment.

Protein, chlorophyll *a*, carotenoid and monosaccharide content

Chlorella vulgaris treated with IAA at 0.1 μM was characterized by the highest (81 %) increase in protein content after 48 h of cultivation (Fig. 2). Other auxins showed weaker biological activity in the algal cell suspension with a 70 % increase in protein level measured in response to 0.1 μM IBA, 54 % and a 33 % in the case of 1 μM PAA and 1 μM NAA, respectively, in comparison with the control.

IAA applied at 0.1 μM had the most stimulatory effect on chlorophyll *a* and carotenoid accumulation after 48 h of cultivation. Other auxins were characterized by lower stimulatory effects on photosynthetic pigment levels in *C. vulgaris* cells. IBA at a concentration of 0.1 μM stimulated chlorophyll *a* accumulation by 78 % and carotenoid by 76 % after 48 h of cultivation. The significant 33–50 % increase in chlorophyll *a* level was noted in response to 1 μM PAA and μM NAA. These auxins also stimulated the carotenoid content by 41–59 % after 48 h of cultivation.

The highest (73 %) enhancement in monosaccharide content was observed in *C. vulgaris* treated with 0.1 μM IAA in relation to the control after 48 h of cultivation. The application of 0.1 μM IBA caused a 56 % increase in sugar content. 1 μM PAA and 1 μM NAA were characterized by the lowest activity stimulating monosaccharide level in algal cells by 35 and 24 %, respectively.

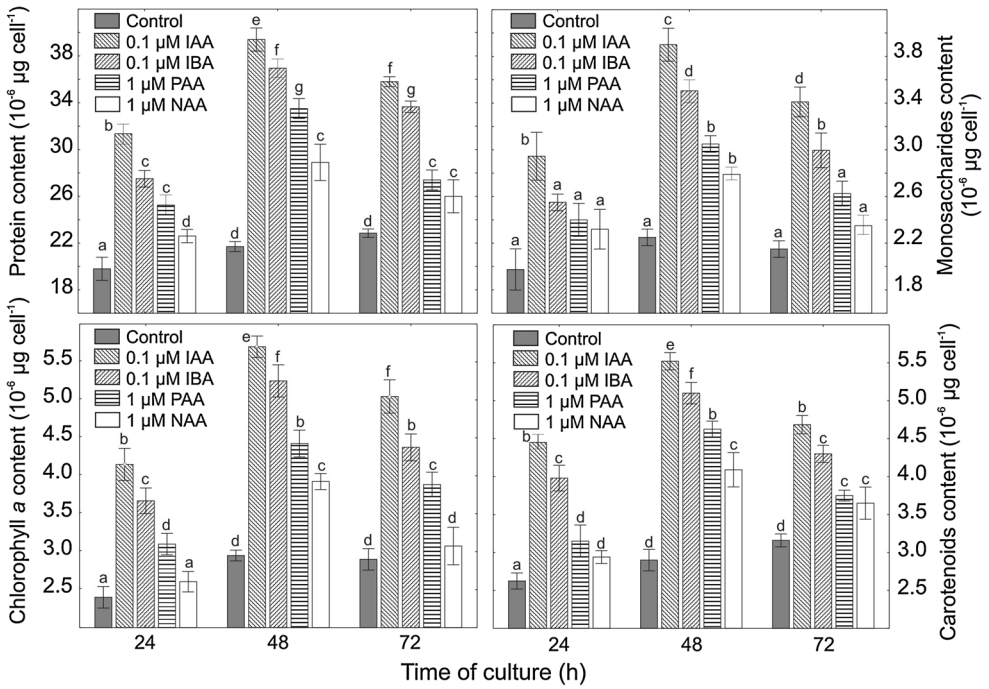

Fig. 2 The effect of auxins (IAA, IBA, NAA, PAA) on the level of proteins, monosaccharide, chlorophyll *a* and carotenoid in *C. vulgaris* after 24, 48 and 72 h of cultivation. Data are the means of four independent experiments ± SD. Treatment with at least *one letter* the same are not significantly different according to Duncan's test

Antioxidant level

Algal cells in the presence of 0.1 μM IAA produced 94 and 79 % more ascorbate and glutathione, respectively, than the control after 48 h of cultivation (Fig. 3). A significant increase in the antioxidant content (81 % in the case of ascorbate and 52 % in the case of glutathione) was also observed with 0.1 μM IBA application. In addition, exposure of *C. vulgaris* to 1 μM PAA or NAA caused a weaker, but statistically significant increase in the total ascorbate (53–70 %) and glutathione (20–35 %) level after 48 h of cultivation.

Activity of antioxidant enzymes

Similarly, auxins influenced the activity of antioxidant enzymes involved in the scavenging of ROS (Fig. 4). The highest enhancement of the activity of antioxidant enzymes (55 % SOD, 89 % CAT, 75 % APX) appeared as a consequence of algal exposure to 0.1 μM IAA after 48 h of cultivation. Exogenously applied 0.1 μM IBA stimulated the activity of SOD by 42 %, CAT by 77 % and APX by 56 %. On the other hand, PAA and NAA at a concentration of 1 μM were characterized by weaker influence on the activity of antioxidant enzymes. Results indicated that, PAA stimulated SOD by 20 %, CAT by 51 % and APX by 23 %, whereas NAA enhanced the activity of SOD by 8 %, CAT by 32 % and APX by 15 % after 48 h of cultivation.

Lipid peroxidation and H_2O_2 content

The increase in the level of ROS scavenging metabolites and antioxidant enzymes activity may be linked to the decrease in lipid peroxidation and hydrogen peroxide content (Fig. 5). Therefore, the results of our experiments have shown that the content of lipid peroxides, measured as the concentration of MDA, the cytotoxic product of lipid peroxidation, was reduced in the presence of auxins in algal culture. IAA at 0.1 μM was the most effectively (by 38 %) at inhibiting the formation of lipid peroxides involved in oxidative cellular destruction. Lower activity was observed in algal cells exposed to 0.1 μM IBA, which induced a 29 % decrease in MDA content. The exogenous application of PAA and NAA at concentrations of 1 μM resulted in a 16–23 % reduction in MDA production in algal culture after 48 h of cultivation.

Similarly, H_2O_2 levels (Fig. 5) decreased in response to all exogenous natural and synthetic auxins. The lowest content of this ROS was observed in *C. vulgaris* cells treated with 0.1 μM IAA (36 % decrease in H_2O_2 content) in the 48th h of cultivation. Other auxins showed a weaker inhibitory effect on hydrogen peroxide formation in algal cells. The decrease in H_2O_2 level by 29, 25 and 18 % was obtained in the culture growing in the presence of exogenous 0.1 μM IBA, 1 μM PAA and 1 μM NAA, respectively after 48 h of cultivation.

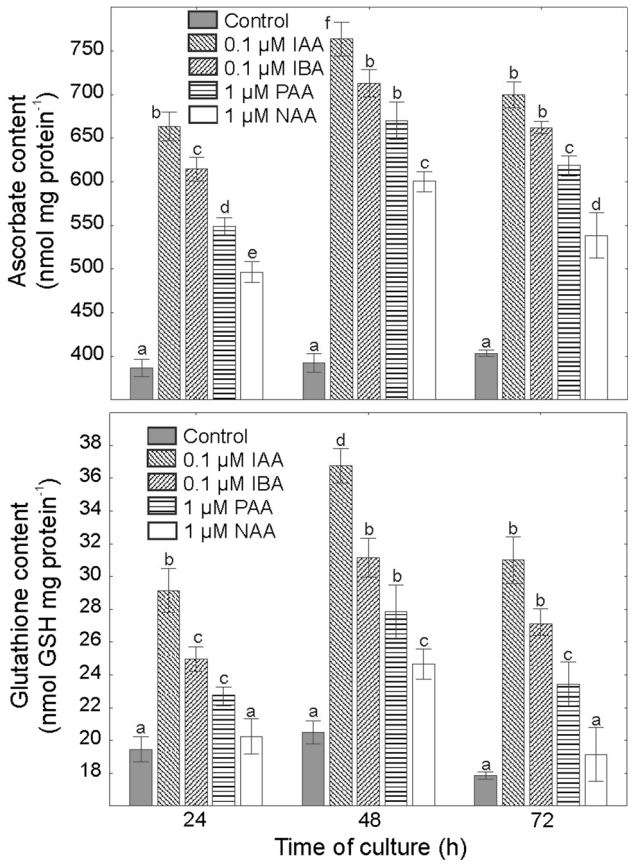

Fig. 3 The effect of auxins (IAA, IBA, NAA, PAA) on the level of ascorbate and glutathione in *C. vulgaris* after 24, 48 and 72 h of cultivation. Data are the means of four independent experiments ± SD. Treatment with at least *one letter* the same are not significantly different according to Duncan's test

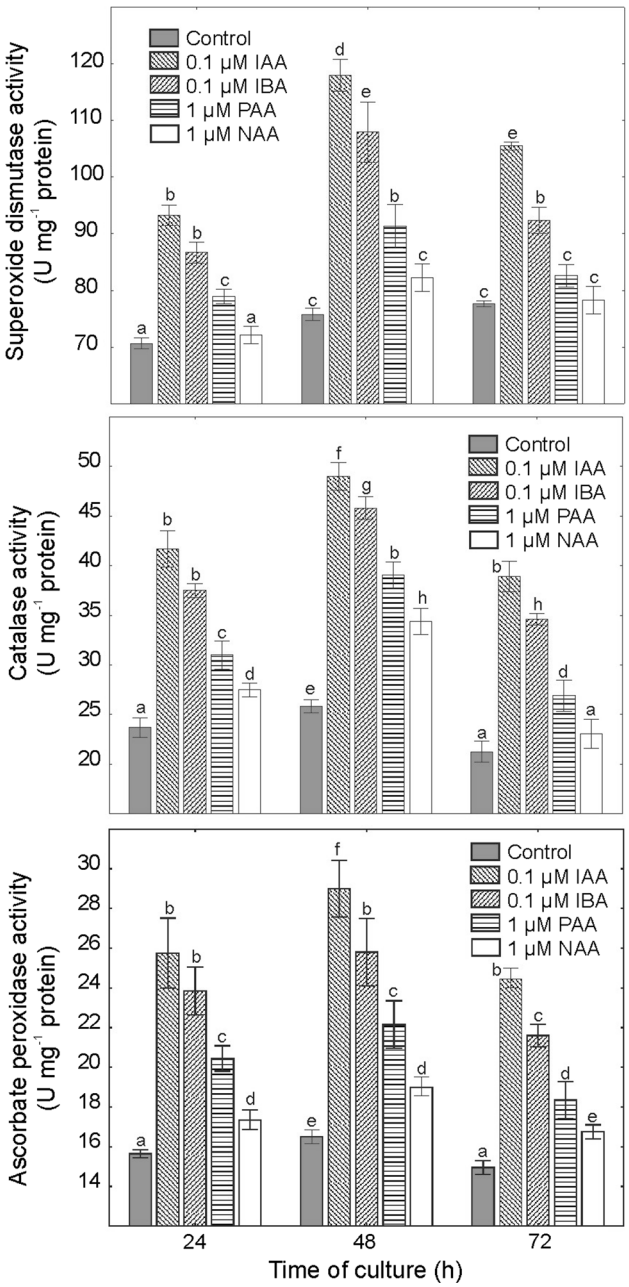

Fig. 4 The effect of auxins (IAA, IBA, NAA, PAA) on the activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in *C. vulgaris* after 24, 48 and 72 h of cultivation. Data are the means of four independent experiments ± SD. Treatment with at least *one letter* the same are not significantly different according to Duncan's test

Discussion

In many bioassays, it has been shown that auxins play a critical role in plant growth and development (Cooke et al. 2002). IAA is the natural auxin commonly occurring in all vascular and lower order plants. In addition to indolic auxins, PAA has been identified in plants and is an active auxin in most bioassays (Ludwig-Müller and Cohen 2002). Similarly, IBA, identical to IAA except for two additional methylene groups in the side chain, is effective in bioassays. IBA, originally classified as a synthetic auxin, is in fact an endogenous plant compound (Bartel et al. 2001). The most commonly used synthetic plant growth regulators with high auxin activity is NAA (Hunt et al. 2011).

Our results indicate that natural (IAA, IBA, PAA) and synthetic (NAA) auxins play a crucial role in *C. vulgaris* growth and metabolism during a 72-h period of culture. The unicellular green alga responded to exogenously applied phytohormones in a dose-dependent manner. Algal growth was suppressed in the presence of all auxins applied at 100 μM. Our findings correspond to other studies where IAA at high concentration significantly reduced *Chlorella pyrenoidosa* cell division (Vance 1987). Experiments with synchronous cultures of *Chlorella fusca* showed growth inhibition when IAA was applied at concentrations higher than 60 μmol dm^{-3} (Lien et al. 1971). Phytotoxic activity of the highest dose of auxins used in the present study may be explained by exogenous auxin-induced biosynthesis, conjugation and degradation, which allow plant cells to

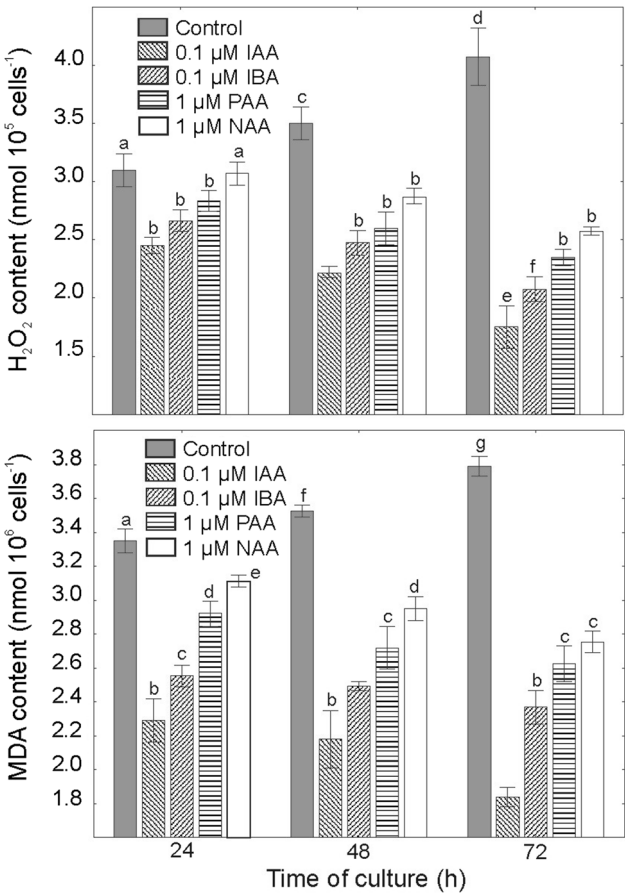

Fig. 5 The effect of auxins (IAA, IBA, NAA, PAA) on the level of hydrogen peroxide and lipid peroxidation expressed as malondialdehyde (MDA) content in *C. vulgaris* after 24, 48 and 72 h of cultivation. Data are the means of four independent experiments ± SD. Treatment with at least *one letter* the same are not significantly different according to Duncan's test

maintain precise homeostatic regulation of intracellular auxin levels (Bajguz and Piotrowska 2009; De Smet et al. 2011).

There are a few studies which investigated the absorption and metabolism of exogenous auxins by green algae. Experiments performed on *Caulerpa paspaloides* revealed the presence of dioxindole-3-acetic acid, an IAA catabolite, produced via oxidation pathways (Jacobs 1993). Exogenous IAA in *Nitella* was predominantly converted into degradation products and other inactive metabolites (Cooke et al. 2002). Additionally Dibb-Fuller and Morris (1992) showed that *C. pyrenoidosa* cells can take up IAA from the media and metabolize it to inactive molecules. Moreover, genes responsible for auxin biosynthesis and metabolism have been identified in some microalgal Chlorophyceae species (De Smet et al. 2011). Based on available data it can be assumed that *C. vulgaris* probably regulates free auxin levels via balance between the biosynthesis of new auxin molecules and degradation of the existing or introduced substance i.e. IAA, IBA, NAA or PAA. This interpretation is supported by the observation that IAA is synthesised endogenously by *C. pyrenoidosa* and *Scenedesmus armatus* and released to the medium influencing the growth and metabolism of other algal cells in the culture (Mazur et al. 2001).

IAA and IBA displayed the highest biological activity at 0.1 μM whereas PAA and NAA were characterized by the greatest stimulatory properties at 1 μM after 48 h of cultivation. The stimulatory effect of phytohormones on algal growth was arranged in the following order: 0.1 μM IAA > 0.1 μM IBA > 1 μM PAA > 1 μM NAA. Our results confirm previous studies indicating that among auxins, IAA has the highest biological activity in algal cultures (Czerpak et al. 1994). The activity of specific auxins is influenced by the position of the carboxyl group in the aliphatic chain. This chain has a strong negative charge while there is a positive charge in the centre of aromatic ring (Lau et al. 2009). The lengthening of the aliphatic chain at the indole ring of auxin causes a decrease in metabolic activity. Therefore IAA is more active in microalga in comparison with IBA, whose aliphatic chain has two more atoms of carbon in length. Moreover, the replacement of the indole (IAA) ring with a phenol (PAA) or naphthyl (NAA) group reduces the physiological activity in the algal culture.

The most characteristic response elicited by natural and synthetic auxins is the stimulation of cell division in higher plants and algae (Stirk and Van Staden 1997). This paper provides evidence that auxins might also have been involved in inducing mitosis in unicellular green algae. Synchronous culture of *C. vulgaris* showed a significant increase in cell number in response to an optimal dose of auxins after 48 h of cultivation. Our results confirm the data obtained in experiments performed on *Caulerpa prolifera* (Chlorophyceae) when IAA affected the optimal growth stimulation (Jacobs 1993). Moreover, application of IAA to microalgae *Scenedesmus obliquus* and *S. armatus* cultures resulted in the stimulation of cell division, growth and formation of four-celled rather than two-celled colonies (Mazur et al. 2001). Earlier studies with the unicellular desmid *Micrasterias thomasiana* (Charophyta) (Wood and Berliner 1979) and with *C. pyrenoidosa* (Chlorophyceae) (Vance 1987), auxin was demonstrated to induce cell division. Kawano (2003) suggested that auxin might promote cell growth through ROS production. A compilation of evidence indicates that ROS may be an essential component of the biochemical mechanism engaged in cell-wall loosening during IAA-induced extension growth (Kawano 2003).

Nevertheless, the evidence for a real physiological and developmental role of auxin in algae is limited and inconclusive. Our results indicate that natural and synthetic auxins stimulate the content of primary metabolites in

C. vulgaris. The observed increases in protein, chlorophyll *a*, carotenoid and monosaccharide content due to the effects of exogenously applied auxins added to the growth media would be of value in the cultivation of *C. vulgaris* for commercial production of animal feed or bioproducts. Increased metabolite production has also been widely observed in *C. pyrenoidosa* due to exogenously applied auxins (Czerpak et al. 1994; Czerpak and Bajguz 1997), auxin precursors and analogues (Czerpak et al. 1994). The application of IAA, indole-3-lactic acid (ILA) and IBA to the growth media of *C. pyrenoidosa* increased the content of chlorophyll, carotenoid, aldohexoses and water-soluble proteins, with the strongest effect observed for IAA (Czerpak et al. 1994). Natural (IAA, IBA, PAA) and synthetic (NAA) auxins were also found to have stimulatory effects on *C. vulgaris* growth and metabolite production in the present study. Considering the importance of rapid growth and high metabolite content in commercial algal cultivation, more study to gain a better understanding of these plant growth substances is warranted.

Plant hormonal responses are often linked with ROS-induced signalling. For example, auxin and H_2O_2 produce antagonistic effects on cell cycle and gene activation (Hirt 2000). Expression of auxin-responsive genes is decreased by H_2O_2 treatment via mitogen-activated protein kinase activation (Kovtun et al. 2000). Ultimately, prolonged stress exposure leads to altered growth patterns, including more compact growth, reduced cell division and increased lateral growth (Potters et al. 2009). The results obtained in the present study indicate that natural (IAA, IBA, PAA) and synthetic (NAA) auxins reduced the accumulation of ROS, such as H_2O_2 in *C. vulgaris* cells after 48 h of cultivation. This finding is consistent with data reporting the antagonistic effect of auxins and ROS on physiological processes. Low levels of ROS have been reported to promote many cellular processes including cell cycle progression and onset of secondary cell wall differentiation (Hirt 2000). The present results suggest that auxins may regulate the cellular redox state in *C. vulgaris* and in this way, prevent oxidative degradation of photosynthetic pigments and proteins. The complex interaction of auxin and ROS during algal growth and development is not well known yet. However, our results indicate that auxins influence the algal growth and metabolism through the regulation of ROS levels.

Given that the highly ROS may cause lipid peroxides' formation the content of MDA, a cytotoxic product of lipid peroxidation, was determined. Our results revealed that IAA at 0.1 μM inhibited lipid peroxides generation as seen from the lower MDA content in *C. vulgaris*. Similarly, 0.1 μM IBA as well as 1 μM NAA and PAA had weaker negative effect on lipid peroxide formation in microalgae. Probably, auxins diminished lipid peroxidation through the stimulation of non-enzymatic (ascorbate, glutathione) and enzymatic (SOD, CAT, APX) antioxidants tightly regulating ROS homeostasis.

Ascorbate is known to operate as an antioxidant either in direct chemical interaction with ROS, or during a reaction catalyzed by ascorbate peroxidase (Kampfenkel et al. 1995). Ascorbate derives its role from its sensitivity to ROS and from the fact that its oxidation affects the redox balance of other metabolites, such as glutathione which themselves are involved in the perception of the cellular redox unbalance (Apel and Hirt 2004). Our results are supported by the data obtained by Tyburski et al. (2008) indicating that the presence of exogenous auxin in the culture induced an increase in ascorbate level in the roots of tomato seedlings.

Glutathione is an important water-phase antioxidant with proposed roles in the storage and transport of reduced sulphur, the synthesis of proteins and nucleic acids and as a modulator of enzyme activity (De Kok et al. 1986). It has been demonstrated that exogenously applied auxins may increase in glutathione content in *C. vulgaris* cells. The stimulation in glutathione level in auxin-treated plant is supported by Takahashi and Nagata (1992) indicating that a significant increase in glutathione level and glutathione *S*-transferase activity was detected in tobacco mesophyll protoplasts. The level of glutathione was also increased in roots of tomato seedlings after IAA treatment indicating that auxin may regulate root elongation through regulation of glutathione content (Tyburski and Tretyn 2010). Moreover, auxins enhanced the capacity for glutathione synthesis in *C. vulgaris* when there is no demand for this substance in our experimental system.

In this study we present evidence that exogenous auxins affect the activities of important enzymes of redox metabolism in *C. vulgaris*. It was shown that auxins transiently stimulated the activities of SOD, CAT and APX in algal culture. SOD is the first enzyme in the detoxification process, which converts superoxide anions to H_2O_2. The principal H_2O_2-scavenging enzyme in plants is CAT, which is located in peroxisomes/glyoxysomes and mitochondria. Alternative H_2O_2-scavenging mechanisms may compensate for reduced catalase activity, as shown by increased peroxidases, such as APX (Pasternak et al. 2002; Apel and Hirt 2004). The rise in SOD activity may result in increased production of H_2O_2. However, we found that H_2O_2 levels in cells were lower if plants were grown in the presence of auxins when compared to the control. The decrease in H_2O_2 levels in algal cells exposed to exogenous auxins may be explained by the increase in the activity of H_2O_2 consuming enzymes, especially CAT and APX (Mallick and Mohn 2000). Enhanced antioxidant enzymes activity in response to auxins suggests an increased rate of ascorbate and glutathione turn-over in *C. vulgaris*.

In conclusion, the data reported in the present study demonstrates that the exposition of algal cells to exogenous auxins is followed by changes in the level of oxidative stress. The effect of auxin is mediated by ROS-scavenging enzymes, which respond to the presence of the exogenous hormone with an increase in their levels of activity.

Some literature data indicates that auxins can increase in the activity of antioxidant enzymes regulating ROS levels, which could be associated with the activation of embryo/organogenesis (Synková et al. 2006). Moreover, exogenous natural (IAA) and synthetic (2,4-D) auxins can stimulate the activities of antioxidant enzymes, such as CAT, SOD and peroxidases leading to a regeneration (shoot production) process in wheat (*T. aestivum* L.) tissue (Szechyńska-Hebda et al. 2007). Our data showed that the precise control of H_2O_2 amounts in algal cells is necessary to allow optimum cell division and metabolite production. ROS under the control of cellular antioxidant machinery can mediate signalling pathways between exogenously applied auxins and the induction of physiological response in algal cells.

In conclusion, auxins play a central role in the regulation of short-term growth events and the content of primary metabolites in *C. vulgaris* culture. Moreover, the mechanism of auxin action in algal cells are associated with oxidative stress, which is under the control of cellular antioxidant machinery. However, more research on the molecular level is required to unequivocally attribute a role to auxins in microalgae in order to validate this hypothesis.

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