EFFECT OF AUXIN PRECURSORS AND CHEMICAL ANALOGUES ON THE GROWTH AND CHEMICAL COMPOSITION IN *CHLORELLA PYRENOIDOSA* CHICK.

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ABSTRACT

In this paper the authors present studies on the effect of auxin precursors and chemical analogues on the growth and biochemical composition in *Chlorella pyrenoidosa* (Chlorophyceae). Among auxin precursors tryptamine exhibited slightly higher stimulative activity in regard to fresh and dry weight, mineral substances, chlorophylls, carotenoids, monosaccharides (aldohexoses) and water-soluble proteins content in *Ch. pyrenoidosa* cells as compared to anthranilic acid. Among auxin analogues used phenoxycetic acid and naphthyl-3-acetic acid had the strongest stimulative effect of the above-mentioned parameters. Their activity was significantly higher than that of auxin precursors. The activity of naphthyl-3-sulphonic acid was slightly lower than that of tryptamine, whereas the stimulation by 2,4-dichlorophenoxyacetic acid was similar to that of anthranilic acid. In *Ch. pyrenoidosa* cells 2,4-dichlorophenoxyacetic acid and naphthyl-3-sulphonic reached their maximum activity at the latest (between the 15th or 16th day) of the culturing, whereas tryptamine, phenylacetic acid, naphthyl-3-acetic acid and indolyl-3-acetic acid – at the earliest (between the 8th or 12th) day.

KEY WORDS: *Chlorella pyrenoidosa*, chlorophylls, carotenoids, proteins, aldohexoses, auxin precursors and analogues.

INTRODUCTION

The effect of auxin precursors and chemical analogues on metabolism and growth processes of plant cells is similar to that of auxin itself. Tryptamine is one of the immediate auxin precursors, as its chemical conversion into indolyl-3-acetic acid (IAA), has been found in many species of vascular plants and algae (Ahmad and Winter 1969, Ahmad 1972, Augier 1977, Czerpak 1979, 1980, 1982, 1990, Rybicka 1980, Czerpak et al. 1983, 1986). In many algae, tryptamine stimulates growth processes and content of metabolites similarly to auxin, although to a less degree and at higher concentrations (Ahmad and Winter 1969, Czerpak 1982, 1990).

Anthranilic acid, a product of the shikimate acid cycle and one of more remote auxin precursors, also exhibits auxin-like properties, although weaker than auxin itself (Ahmad 1972, Czerpak et al. 1986, Czerpak 1990). Phenylacetic acid (PAA) is a naturally occurring auxin which has initiated and supported growth of plants. PAA showed activity in indolyl-3-acetic acid (IAA) bioassays but was active only at much higher concentrations than IAA. PAA has been shown to influence morphoregulation and it also has effects on enzymatic activity (Leuba and LeTourneau 1990). The latest work has demonstrated that PAA may be involved in the regulation of auxin transport (Johnson and Morris 1987). Phenylacetic acid and p-hydroxyphenylacetic acid as phenylalanine metabolites also exhibit similar although a little weaker metabolic activity in algae and vascular plants when compared to that of auxin (Millborow and Purse 1964, Fries, Aberg 1978, Letham et al. 1978, Czerpak et al. 1986, Czerpak 1990). Moreover, indole

and tryptophane exhibit weak auxin-like properties and are likely the natural precursors of IAA (Beauchesne 1974, Heerkloss and Libbert 1976, Augier 1977, Letham et al. 1978, Rybicka 1980).

Conversion of phenylacetic acid into phenoxycetic acid due to the introduction of one oxygen atom results in an increase in auxin-like activity (Letham et al. 1978, Czerpak 1990). Furthermore, weak auxin-like activities have been shown in chlorinated derivatives of phenoxycetic acid such as 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid (Erickson et al. 1955, Osborne and Hallaway 1964, Kurancowa 1966, Kim and Bidwell 1967, Gamburg 1978, Colombo and Ferrari 1982, Czerpak et al. 1983, Pandey and Tiwari 1986, Pelekis et al. 1987, Stenlid, Engvild 1987, Andr, Scherer 1991, Scherer 1992).

Among chemical analogues of auxins, naphthyl- β -acetic acid (NAA) exerts strong stimulative effect in algae and vascular plants similar to that of natural auxins (Gamburg 1978, Letham et al. 1978, Pain 1981, Czerpak 1982, 1990, Czerpak et al. 1983). Metabolic activity of NAA is higher than that of its isomer: α -naphthylacetic acid. Moreover, β -naphthylsulphonic acid (NSA) – a chemical analogue of NAA possesses significant although weaker auxin-like activity when compared to IAA and NAA (Cohen and Bandurski 1982).

It has been shown that as 2,4-D used a herbicide or as an auxin. It has been shown to affect cell division and stored carbohydrate content. Studies with *Chlorella* species have shown that high levels of 2,4-D inhibit growth and interfere with the metabolic processes (Pelekis et al. 1987).

Indolyl-3-acetic, 2,4-dichlorophenoxyacetic acid activated phospholipase A which is a primary reaction in the signal transduction leading from hormone – binding to the growth response. The phospholipase A exhibits the properties expected from the primary response to auxin as an antagonist. It is triggered by a receptor protein for auxin since an antibody that binds to the membrane-associated auxin-binding protein from plant blocked the phospholipase A (Venis et al. 1990, André and Scherer 1991, Mennes et al. 1992, Scherer 1992).

Considering the lack of existing comparative analyses of metabolic activity of auxin precursors (anthranilic acid and tryptamine) and chemical analogues of auxin (phenylacetic, 2,4-dichlorophenoxyacetic, naphthyl-3-acetic and naphthyl-3-sulphonic acids) in algae, attempts were made to evaluate their stimulative effect on the fresh and dry weight, the ash content, photosynthetic pigments and water-soluble proteins and carbohydrates in *Chlorella pyrenoidosa* in relation to their chemical structure. In addition the optimal concentration range and the effective action period of the above-mentioned auxin-like substances on the algal growth and the biochemical parameters was studied.

MATERIAL AND METHODS

General methodological principles

The experiments were carried out on the fresh homogenous culture of *Chlorella pyrenoidosa*. The algae were grown for 20 days in 500 cm³ conical flasks with bacteriological stoppers on the modified Knop medium for the intensive culturing of *Chlorophyceae*. The culturing were illuminated with 250 W mercury lamp supplying 25 W/m² during 16 (±0.5) h per day at 27 °C. Total volume of the algal culture in the experimental flasks was 250 cm³. The dry weight of *Chlorella pyrenoidosa* cells in each flask on the 1st day of the culturing was about 0.1 (±0.002) g/dm³. Beginning from the 5th day of the experiment 10 cm³ samples of *Ch. pyrenoidosa* suspension were collected to determine the fresh and dry weight, total chlorophylls and carotenoids, total pool of water-soluble proteins and reducing saccharides (aldohexoses). Following chemical auxin analogues and precursors were used in the experiments: anthranilic acid (AA), tryptamine (Trp-NH₂), phenylacetic acid (PAA), 2,4-dichlorophenoxyacetic acid (2,4-D), naphthyl-3-acetic acid (NAA) and naphthyl-3-sulphonic acid (NSA), as well as indolyl-3-acetic (IAA) obtained from BDH Chemicals and Colnbrook LTD (England), and from Lachema (Czechoslovakia).

All these substances were used at the concentration range of 10⁻³-10⁻⁶ M. No growth substances were added to control algal cultures.

Fresh and dry weight, and ash content determination

Gravimetric method commonly used in laboratory practice was used. Briefly, 10 cm³ samples of the homogenous algal suspension were collected, centrifuged and the resulting pellet was dried with a filter paper and weighted. To determine the dry weight the algal fresh weight was dried in melting pots at 105 °C for a few hours to a constant weight. The dry weight was expressed in g% of the algal fresh weight.

The total of mineral substances as the ash content was determined by combustion in muffle furnace at 500-550 °C for 0.5-1 h with a few drops of 2% ammonia nitrate added and expressed as percentage of the algal dry weight. The

above-mentioned biochemical parameters were determined mainly after Hallegraef 1977 and Czerpak 1980.

Total chlorophylls and carotenoids determination

Total chlorophylls (a+b) and carotenoids were determined in the fresh weight and then converted into the dry algal weight using methods and formulas cited by Allen et al. 1960, Goodwin 1976, Czerpak 1977, Lichtenthaler and Welburn 1983. For this purpose 10 cm³ samples of *Ch. pyrenoidosa* suspension were collected from each flask. After centrifugation the resulting pellet was homogenized, the photosynthetic pigments were extracted and determined quantitatively using adsorption and column chromatography, and spectrophotometry. Their contents were expressed in milligrams per g of the dry algal weight.

Determination of water-soluble proteins and aldohexoses

The total of water-soluble proteins and aldohexoses were determined as g% of *Ch. pyrenoidosa* dry weight. The protein fraction was analyzed spectrophotometrically using biuretic reaction after homogenization of the algal cells and water extraction of the protein. The similar preparation procedure was employed for spectrophotometric determination of aldohexoses except for o-tholuidine used in the coloured reaction (Ostrowski and Filipowicz 1980).

RESULTS

Among the auxin precursors applied to *Chlorella pyrenoidosa* cultures, tryptamine exerts a little higher effect on the biochemical parameters analyzed when compared to that of anthranilic acid. It is probably due to more distant position of anthranilic acid in auxin biosynthesis precursors pathway when compared to tryptamine. Moreover, tryptamine is characterized by a much higher structural homology with indolyl-3-acetic acid (IAA) – the auxin commonly encountered in plant world.

Anthranilic acid and tryptamine at the concentration 5×10⁻⁵ M exert the maximum stimulatory effect on the fresh weight (210.2% and 214.2%, respectively), chlorophylls (a+b) (212.9% and 240.3%, respectively), water-soluble proteins (191.3% and 200%, respectively), when compared to the control culture (100%) (Figs 1-7, Table 1). Among the auxin analogues analyzed the highest activity in *Ch. pyrenoidosa* cultures, have been found for phenylacetic acid (PAA) and naphthyl-3-acetic acid (NAA), similar to that of IAA. This activity level reflects a high similarity in chemical structure between the both substance and IAA.

IAA, PAA and NAA at the concentration 5×10⁻⁵ M, had a maximum stimulatory effect on the fresh weight (286.7%, 251.8% and 297.1%, respectively), the dry weight (172.3%, 165.6% and 191.3%, respectively), chlorophylls (a+b) (273.5%, 262.8% and 270.2%, respectively), carotenoids (258.2%, 247.9% and 231.6%, respectively), water-soluble proteins (270.9%, 211.2% and 192.7%, respectively), the ash content (182.2%, 201% and 187.9%, respectively) and aldohexoses (183.7%, 196.3% and 191%, respectively) in *Ch. pyrenoidosa* cells, when compared to the control (100%) (Figs 1-7, Table 1).

On the other hand, naphthyl-3-sulphonic acid (NSA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were characterized by significantly lower activity in the biochemical parameters stimulation in *Ch. pyrenoidosa* when compared to NAA and

TABLE 1. Intensity of the analyzed biochemical parameters stimulation at the optimal concentration of auxin precursors and chemical analogues in *Chlorella pyrenoidosa* cells.

Auxin precursors and analogues used	Optimal molar concentration	Stimulation in particular days of the culturing in absolute numbers ¹						
		Fresh weight (g/dm ³)	Dry weight (% of the fr. wt.)	Ash content (% of the dry wt)	Chlorophylls (a+b) (mg/g of the dry wt.)	Carotenoids (mg/g of the dry wt.)	Water-soluble proteins (g% of the dry wt.)	Aldohexoses (g% of the dry wt.)
Anthranilic acid (AA)	5×10 ⁻⁵	5.76 ± 0.31 15 day	13.52 ± 0.78 12 day	12.86 ± 0.76 18 day	6.92 ± 0.4 15 day	1.61 ± 0.1 20 day	16.68 ± 0.92 19 day	6.72 ± 0.36 12 day
Tryptamine (Trp-NH ₂)	5×10 ⁻⁵	5.87 ± 0.36 13 day	14.54 ± 0.83 12 day	14.02 ± 0.8 18 day	7.81 ± 0.51 15 day	2.05 ± 0.12 18 day	17.41 ± 0.95 18 day	7.17 ± 0.42 11 day
2,4 -dichloro-phenoxyacetic acid (2,4-D)	5×10 ⁻⁶	5.06 ± 0.28 18 day	16.02 ± 0.87 16 day	13.62 ± 0.77 20 day	7.63 ± 0.5 19 day	1.87 ± 0.11 20 day	17.1 ± 0.93 20 day	7.02 ± 0.47 20 day
Phenylacetic acid (PAA)	5×10 ⁻⁵	6.9 ± 0.38 15 day	18.96 ± 0.97 12 day	15.84 ± 0.85 16 day	8.54 ± 0.59 15 day	2.43 ± 0.17 16 day	18.42 ± 0.99 15 day	9.64 ± 0.55 14 day
Naphthyl-3-acetic acid (NAA)	5×10 ⁻⁵	8.14 ± 0.56 12 day	21.9 ± 1.18 15 day	14.81 ± 0.81 17 day	8.78 ± 0.62 16 day	2.27 ± 0.16 18 day	16.8 ± 0.93 16 day	9.38 ± 0.51 18 day
Naphthyl-3-sulphonic acid (NSA)	10 ⁻⁴	6.32 ± 0.35 15 day	18.14 ± 0.93 18 day	13.28 ± 0.75 20 day	8.06 ± 0.56 18 day	1.92 ± 0.13 20 day	15.86 ± 0.87 17 day	9.13 ± 0.5 20 day
Indolyl-3-acetic acid (IAA)	5×10 ⁻⁵	7.86 ± 0.44 12 day	19.73 ± 1.04 13 day	14.36 ± 0.8 15 day	8.89 ± 0.87 13 day	2.53 ± 0.19 16 day	18.13 ± 0.91 14 day	9.02 ± 0.47 10 day
Control	—	2.74 ± 0.18 8-16 day	11.45 ± 0.66 10-20 day	7.88 ± 0.45 15-20 day	3.25 ± 0.22 10-16 day	0.98 ± 0.07 15-20 day	8.72 ± 0.5 15-20 day	4.91 ± 0.26 8-18 day

¹ Mean values from twenty analyses.

Data in the table are supplemented by standard deviation.

Fig. 1. Effect of auxins and their precursors, and chemical analogues on the fresh weight content in *Chlorella pyrenoidosa*. Bars – mean values (n = 12).

Fig. 2. Effect of auxins and their precursors, and chemical analogues on the dry weight content in *Chlorella pyrenoidosa*. Bars – mean values (n = 12).

Fig. 3. Effect of auxins and their precursors, and chemical analogues on the ash content in *Chlorella pyrenoidosa*. Bars – mean values (n = 12).

Fig. 4. Effect of auxins and their precursors, and chemical analogues on the chlorophylls (a+b) content in *Chlorella pyrenoidosa*. Bars – mean values ($n = 12$).

Fig. 5. Effect of auxins and their precursors, and chemical analogues on the carotenoids content in *Chlorella pyrenoidosa*. Bars – mean values ($n = 12$).

Fig. 6. Effect of auxins and their precursors, and chemical analogues on the water-soluble protein content in *Chlorella pyrenoidosa*. Bars – mean values (n = 12).

Fig. 7. Effect of auxins and their precursors, and chemical analogues on the aldohehexoses content in *Chlorella pyrenoidosa*. Bars – mean values (n = 12).

PAA. Mainly due to their chemical structure, as NSA carries sulphonic moiety instead of carboxyl group of NAA, whereas in 2,4-D aromatic phenyl ring has been replaced by phenyl ring with two chlorine atoms and one oxygen atom substituted. This kind of chemical modification is responsible for a marked decrease of NSA and NAA metabolic activities, when compared to those of NSA and 2,4-D, respectively.

NSA and 2,4-D exerted the maximum stimulatory effect on *Ch. pyrenoidosa* culture at the concentrations 5×10^{-5} and 5×10^{-6} M on the fresh weight (230.7% and 184.7%), chlorophylls (a+b) (248% and 234.8%), carotenoids (195.9% and 190.8%) and water-soluble proteins (181.9% and 196.1%) respectively, when compared to the control (100%) (Figs 1-7, Table 1).

DISCUSSION

Existing data from fragmentary studies on algae of *Cyanophyceae* and *Chlorophyceae* groups carried out by Manos 1945, Erickson et al. 1955, Kurancowa 1966, Ahmad and Winter 1969, Ahmad 1972, Augier 1977, Fries and Aberg 1978, Czerpak et al. 1983, Pandey and Tiwari 1986, Pelekis et al. 1987, André and Scherer 1991, indicate high metabolic activity of tryptamine, anthranilic acid, phenylacetic acid, p-hydroxyphenylacetic acid, naphthyl-3-acetic acid and 2,4-dichlorophenoxyacetic acid, similar to that of IAA – the natural auxin commonly found in vascular plants.

On the molecular level within a single cell the effect of auxin-like substances is probable common to all unicellular and multicellular eukaryotic photosynthesizing plants.

Among the analogues and the precursors of the auxin (IAA) studied NAA and PAA are characterized by the highest stimulation level on the analyzed parameters in *Ch. pyrenoidosa* which is likely to result from a high similarity in the chemical structure of the both substances and indolyl-3-acetic acid. Their metabolic activity is significantly higher than that of auxin precursors (anthranilic acid and tryptamine) and of the chemical auxin analogues, eg. NSA and 2,4-D. Naphthyl-3-sulphonic acid exhibits slightly lower metabolic activity, when compared to tryptamine, whereas 2,4-D is characterized by the same stimulation activity as anthranilic acid.

2,4-D at the concentration range of 10^{-4} – 5×10^{-5} M has intensive stimulated growth and activity of phospholipase A₂ and an accumulation of phosphatidylcholine in etiolated zucchini hypocotyls (*Cucurbita pepo* L.) and cultured soybean cells (*Glycine max* L.) (André and Scherer 1991, Scherer 1992). Instead of heterotrophic cultured of algae: *Polytoma uvella*, *Polytonella papillata* and *Prototheca chlorelloides*, 2,4-D at concentration 10^{-4} M inhibited but at the concentration range of 10^{-5} – 10^{-7} M has small stimulation of the cell number, the fresh and dry weight, as well as content of the stored polyglucan (Pelekis et al. 1987).

Among the IAA analogues and precursors used in *Ch. pyrenoidosa* cultures tryptamine, phenylacetic acid and naphthyl-3-sulphonic acid reached their peak activity at the earliest (between the 12th and the 16th or 18th) day of the culturing and naphthyl-3-sulphonic acid – at the latest (between the 15th or the 16th and the 20th) day. The results obtained from authors' comparative analyses: (Czerpak 1979, 1980, 1982, 1990, Czerpak et al. 1983, 1986) indicate high similarity level between the metabolic activity of the above-mentioned auxin precursors and analogues that have been used so far in experiments on vascular plants (Walrand-Bouillenne and Bouillenne 1960, Millborow and Purse 1975, Gamborg 1978, Letham et

al. 1978, Cohen and Bandurski 1982, Colombo and Ferrari 1982, Szwejkowska 1987, André and Scherer 1991).

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WPLYW PREKURSORÓW I CHEMICZNYCH ANALOGÓW AUKSYN NA WZROST I SKŁAD CHEMICZNY *CHLORELLA PYRENOIDOSA* CHICK.

STRESZCZENIE

W pracy badano wpływ prekursorów i chemicznych analogów auksyn na wzrost i skład chemiczny glonu *Chlorella pyrenoidosa* (*Chlorophyceae*). Tryptamina jako jeden z bezpośrednich prekursorów auksyn wykazuje nieco wyższą aktywność biochemiczną pod względem świeżej i suchej masy, substancji mineralnych, chlorofili, karotenoidów, aldoheksoz i białek rozpuszczalnych w wodzie w komórkach *Ch. pyrenoidosa* w porównaniu z kwasem antranilowym. Spośród badanych analogów chemicznych auksyn najsilniejszy wpływ stymulujący na analizowane parametry biochemiczne wykazują kwasy: fenoksyoctowy i naftylo-3-octowy. Aktywność metaboliczna kwasu naftylo-3-sulfonowego (NSA) jest znacznie słabsza w stosunku do wymienionych kwasów, zaś nieco mniejsza aniżeli tryptaminy. Natomiast właściwości stymulujące kwasu 2,4-dichlorofenoksyoctowego (2,4-D) są niewielkie w porównaniu z kontrolą i podobne do kwasu antranilowego. W komórkach *Ch. pyrenoidosa* 2,4-D i NSA powodują maksymalną stymulację ich wzrostu i biochemizmu najpóźniej, t.j. między 15 a 16 dniem hodowli, podczas gdy tryptamina i kwasy: fenoksyoctowy, naftylo-3-octowy i indolilo-3-octowy – znacznie wcześniej, przeważnie pomiędzy 8 a 12 dniem hodowli.

SŁOWA KLUCZOWE: *Chlorella pyrenoidosa*, chlorofile, karotenoidy, białka, aldoheksozy, analogi i prekursorzy auksyn.