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Effects of Benzyladenine on Photosynthetic Pigments and Oxygen Evolution in *Chlamydomonas reinhardii* and *Anacystis nidulans*

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With 2 Figures

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Summary

KADIOĞLU A. 1992. Effects of benzyladenine on photosynthetic pigments and oxygen evolution in *Chlamydomonas reinhardii* and *Anacystis nidulans*. – Phyton (Horn, Austria) 32 (1) 111–118, with 2 figures. – English with German summary.

In this study the effects of benzyladenine (BA) on the photosynthetic pigments and on oxygen evolution of *Chlamydomonas reinhardii* and *Anacystis nidulans,* which were grown under continuous light, have comparatively been investigated. In general, photosynthetic pigments in BA-treated cultures of *Chlamydomonas* showed a gradual increase from the control up to 25 μM BA, but decreased at higher concentration (50 and 100 μM). The oxygen evolution of *Chlamydomonas* was particularly affected by 25 and 50 μM BA. In *Anacystis,* the applications of 5 and 25 μM BA significantly increased the contents of photosynthetic pigments and oxygen evolution, respectively.

Zusammenfassung

KADIOĞLU A. 1992. Einfluß von Benzyladenin auf die photosynthetischen Pigmente und auf die Sauerstoff-Entwicklung von *Chlamydomonas reinhardii und Anacystis nidulans*. – Phyton (Horn, Austria) 32 (1) 111–118, mit 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

In dieser Arbeit wurde der Einfluß von Benzyladenin (BA) auf die photosynthetischen Pigmente und auf die Sauerstoff-Entwicklung von *Chlamydomonas*

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reinhardii und *Anacystis nidulans,* die unter kontinuierlichem Licht angezogen wurden, vergleichend untersucht. Der Gehalt an photosynthetischen Pigmenten in den mit BA behandelten *Chlamydomonas*-Kulturen zeigte allgemein von der Kontrolle bis zur BA-Konzentration von 25μM eine Zunahme, und bei höheren Konzentrationen wurde eine Abnahme beobachtet.

Die Sauerstoff-Entwicklung dieser Alge wurde vor allem durch die höheren BA-Konzentrationen (25 und 50μM) beeinflußt. Bei *Anacystis* erhöhten dagen die BA-Konzentrationen von 5 und 25 μM die photosynthetischen Pigment-Gehalte und die Sauerstoff-Entwicklung.

Introduction

There are a lot of studies about the effects of cytokinins on the level of photosynthetic pigments (Wozny & Szweykowska 1975, Berube & al. 1982, Jelic & Bogdanovic 1989). It has been reported that cytokinin generally increases the level of photosynthetic pigments (Adedipe & al. 1971, Krawiarz & al. 1976, Lichtenthaler & Buschmann 1978, Volfova & al. 1978). In addition, benzyladenine (BA) has been shown to increase the size and number of chloroplasts in some leaves (Borzenkova & Bortnikova 1977, Tsuji & al. 1979). Zerbe & Wild 1980 also found that kinetin enhanced the level of carotenoids in *Sinapis alba.*

Further, many workers have investigated the effects of cytokinin on the rate of photosynthesis (Adedipe & al. 1971, Dong & Arteca 1982). Buschmann & Lichtenthaler 1977 found that chloroplasts isolated from kinetin-treated *Raphanus* cotyledons exhibit a higher P-700 content and a higher Hill activity. Similarly Zerbe & Wild 1980 reported an increase in the rate of photosynthesis and in the activity of cytochrome f in kinetin-treated *Sinapis alba.* Although many investigations have been done about the effects of cytokinin on higher plants, there are a few studies about this topic on algae.

Therefore the main aim of our study is to investigate comparatively the effects of benzyladenine on photosynthetic pigments and on oxygen evolution in *Chlamydomonas* and in *Anacystis.* The aim of using of these both algae is a comparative investigation of the effects of BA on the photosynthetic pigments and on oxygen evolution between an eukaryotic alga, *Chlamydomonas,* and a prokaryotic alga, *Anacystis.*

Materials and Methods

1. The Growth of Algae and Application of Benzyladenine

The cultures of *Chlamydomonas reinhardii (Chlorophyceae)* and *Anacystis nidulans (Cyanophyceae)* were obtained from the Biological Research Center at the Hungarian Academy of Sciences. *Chlamydomonas reinhardii* was grown in TAP medium (Gorman & Levine 1965). *Anacystis nidulans* was grown in ASM medium (Borcakli 1986). The cultures were grown at 30–35° C on a shaker under continuous light (6 × 40 watt lamps). After inoculation, the cultures of *Chlamydomonas* and

Anacystis were incubated for two and six days, respectively. Then these cultures were distributed to the other flaks in a volume of 100 ml each. We replicated the experiment at least four times and in all replications we started the experiment with an equal number of cells. For this purpose, the cell number of *Chlamydomonas* was counted by a hemocytometer and brought to a number of 5×10^5 cells /ml. The cell number of *Anacystis* was also counted by turbidimetric method and brought to an absorbance of A_{560} : 0.20 (BORCAKLI 1986).

Under steril conditions, BA dissolved in distilled water (after a first dissolution in a small amount of ethyl alcohol) was applied to the cultures at 5, 25, 50 and 100 µM concentrations. These were the final BA concentrations of the culture. No application was done to the control cultures.

2. Determination of Chlorophyll and Carotenoids

Chlorophyll and carotenoids were extracted with 80 % acetone from the algae after precipitation of the cells by centrifugation. Absorbances of the extracts were measured spectrophotometrically at 450, 645 and 665 nm; The formules of ARNON 1949 and JASPARS 1965 were used for the estimation of chlorophyll and carotenoids, respectively. Measurement of the pigments was done once a day for three days.

3. Determination of Photosynthetic Oxygen Evolution

Photosynthetic oxygen evolution of both algae were measured by using a modified clark type oxygen electrode. For this purpose, the alga cells were exposed to a light intensity of 1500 $\mu E.m^{-2}.s^{-1}$ after the incubation of alga cells for 5 min in the dark. Oxygen evolution was expressed as µmol O_2 per mg chlorophyll per hour. The measurements of photosynthetic oxygen evolution were made at 25° C (SAMUELSSON & al. 1985) and were done once a day for three days.

Results

1. The Effects of Benzyladenine on the Photosynthetic Pigments

At the first and second day, the photosynthetic pigments in BA-treated culture of *Chlamydomonas* showed a gradual increase from the control up to 25 µM concentration of BA, but decreased at higher concentrations (Table 1). At the third day, 25 and 50 µM BA caused the highest increase in chlorophyll contents. The carotenoid level was also affected by BA similar to the chlorophyll contents of the algae.

Along the experiment the highest level of chlorophyll a was observed in the application of 25 µM BA in *Anacystis* (Table 2). In our experiment, all concentrations of BA increased the level of carotenoids in different ratios. In Table 3 was given the effects of BA on cell number of the algae in same experiment.

2. The Effects of Benzyladenine on the Oxygen Evolution

At the first day, 50 µM BA only increased the photosynthetic oxygen evolution in *Chlamydomonas* (Fig. 1). At the second day, 25 µM BA also

Table 1

The effects of benzyladenine on the content of photosynthetic pigments in *Chlamydomonas*.

Time (days)	Applications	The content of pigments (µg/ml) Chl a	Chl b	Car
1	Control	6.09 a*	3.70 a	0.95 a
	5 µM	7.32 b	4.37 b	1.18 b
	25 µM	7.14 b	4.30 b	1.13 b
	50 µM	6.67 ab	4.02 ab	1.05 ab
	100 µM	6.39 a	3.79 a	0.91 a
2	Control	9.66 a	6.02 a	1.66 a
	5 µM	11.04 b	7.32 b	1.82 b
	25 µM	11.52 b	7.38 b	1.89 b
	50 µM	11.38 b	7.03 b	1.87 b
	100 µM	10.06 a	6.15 a	1.64 a
3	Control	10.90 a	7.24 a	1.80 a
	5 µM	10.64 a	7.24 a	1.74 a
	25 µM	12.79 b	8.65 b	2.16 b
	50 µM	13.35 b	8.75 b	2.11 b
	100 µM	12.53 b	8.16 b	2.21 b

* Within columns, data followed by the same letter are not significantly different at 5 % level (Duncan's Multiple Range Test, n: 4).
Chl: Chlorophyll Car: Carotenoid

Table 2

The effects of benzyladenine on the content of photosynthetic pigments in *Anacystis*.

Applications	Time (days) 1		2		3	
	The content of pigments (µg/ml)					
	Chl a**	Car	Chl a	Car	Chl a	Car
Control	0.34 a*	0.15 a	0.58 a	0.19 a	0.80 a	0.29 a
5 µM	0.75 b	0.25 b	0.84 b	0.27 ab	0.98 a	0.30 a
25 µM	0.90 b	0.25 b	0.93 bc	0.35 b	1.31 c	0.35 a
50 µM	0.55 ab	0.21 ab	0.77 ab	0.26 ab	1.05 ab	0.31 a
100 µM	0.79 b	0.25 b	1.05 c	0.28 ab	1.24 bc	0.34 a

* Within columns, data followed by the same letter are not significantly different at 5 % level (Duncan's Multiple Range Test, n: 4).
** Chlorophyll a was only measured in *Anacystis* because it does not contain chlorophyll b.
Chl: Chlorophyll Car: Carotenoid

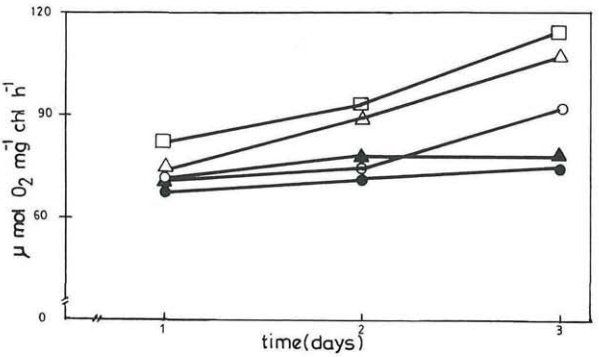

Fig. 1. The effects of benzyladenine on the oxygen evolution in *Chlamydomonas*. Every measurement was replicated four times. ● : control; ▲ : 5 μM BA; △ : 25 μM BA; □ : 50 μM BA; ○ : 100 μM BA.

enhanced the oxygen evolution of *Chlamydomonas* as well as 50 μM BA. At the third day, all concentrations of BA (except the 5 μM BA) affected the oxygen evolution of *Chlamydomonas*.

In the *Anacystis* culture, the highest increase in photosynthetic oxygen evolution was observed in the application of 5 μM BA at the second and third day. In addition, at the third day, 25 μM BA also increased the oxygen evolution of *Anacystis* (Fig. 2).

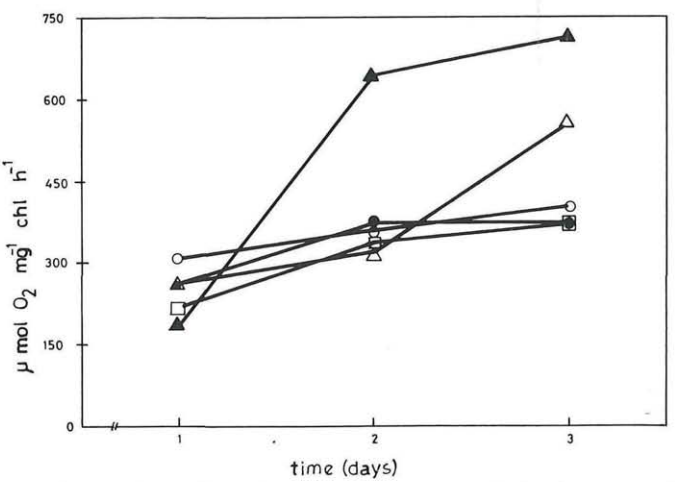

Fig. 2. The effects of benzyladenine on the oxygen evolution in *Anacystis*. Every measurement was replicated four times. ● : control; ▲ : 5 μM BA; △ : 25 μM BA; □ : 50 μM BA; ○ : 100 μM BA.

Discussion

In this study, it has been found that BA increased the contents of photosynthetic pigments and oxygen evolution in different ratios for both algae. These findings are similar to previous studies which were generally made on higher plants (ADEDIPE & al. 1971, WOZNY & SZWEYKOWSKA 1975, BORZENKOVA & BORTNIKOVA 1977, JELIC & BOGDANOVIC 1989). In addition, in accordance with our results, BERUBE & al. 1982 found that the chlorophyll content in BA-treated *Lemna minor* showed a gradual increase from the control up to 0.6 ppm concentration of BA, but decreased at 2 ppm and 5 ppm concentrations. Therefore our results might support the idea that the various concentrations of plant hormones have different effects on the photosynthetic pigments and on oxygen evolution.

On the other hand, investigations about the effect of cytokinin on the rate of photosynthesis (BUSCHMANN & LICHTENTHALER 1977, ZERBE & WILD 1980) are also similar to our findings. For example, DONG & ARTECA 1982 investigated the changes in photosynthesis of tomato plants with a concentration of 0.47 µM BA and they found a 30–35 % increase in photosynthetic activity after 8 days. The stimulating effects of BA on the photosynthetic pigments and on oxygen evolution might also be based on the continuous light used in our experiment. Already STRAUB & LICHTENTHALER 1973 have suggested that indoleacetic acid, kinetin and light have a synergistic influence on *Raphanus*-plants.

The increasing contents of photosynthetic pigments at certain concentrations of BA in our experiment could indirectly be mediated by BA as a result of regulation of some metabolic events or by a direct effect of BA on the photosynthetic reactions for both algae. The increase of the pigments may also be a result by the increase of cell number per ml of the cultures because of the effect of BA on cell division of both algae. In accordance with this suggestion we found an increase in the number of the cells in these experiments (see Table 3).

Table 3

The effects of benzyladenine on the growth rate of *Chlamydomonas* and *Anacystis*.

	Growth rate	
Applications	*C. reinhardii* (cell number/ml) × 10^5	*A. nidulans* A_{560}
Control	10.2*	0.38*
5 µM	11.3	0.48
25 µM	14.6	0.47
50 µM	14.3	0.42
100 µM	12.1	0.41

* The values of third day were only given.

Our various observations about the effect of BA on oxygen evolution of the algae suggested that oxygen evolution, like so many other physiological process, may be influenced by BA. Therefore, it can be speculated that BA affects the oxygen evolution complex by an unknown mechanism. On the other hand, however, some investigators have suggested that the increase in the rate of photosynthesis are direct effects of phytohormones on the activity of ribulose biphosphate carboxylase and other dark reactions of photosynthesis (TURNER & BIDWELL 1965, TAMAS & al. 1972, ZERBE & WILD 1980). However, there is no information about the effect of BA on the oxygen evolution complex. Therefore it would be of interest to test the effect of BA on the oxygen evolution complex proper in further experiments too.

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References

ADEDIPE N. O., HUNT L. A. & FLETCHER R. A. 1971. Effects of benzyladenine on photosynthesis, growth and senescence of the bean plant.-Physiol. Plant. 25: 151–153.

ARNON D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. – Plant Physiol. 24: 1–15.

BERUBE R., BEAUMONT G. & GRENIER G. 1982. Effects physiologiques de la benzyladenine sur *Lemna minor* I. Influence sur la composition lipidique. – Can. J. Bot. 60: 758–764.

BORCAKLI M. 1986. Laboratuarda mikroalg *(Scenedesmus obliquus)* yetiştirme denemeleri, – TUBITAK MBEAE Beslenme ve Gıda Bölümü, Gebze.

BORZENKOVA R. A. & BORTNIKOVA J. F. 1977. Light dependence of kinetin action in the process of chloroplastogenesis. – Fiziol. Rast. 25: 254–261.

BUSCHMANN C. & LICHTENTHALER H. K. 1977. Hill activity and P-700 concentration of chloroplasts isolated from radish seedlings treated with indoleacetic acid, kinetin or gibberellic acid. – Z. Naturforsch 32c: 798–802.

DONG C. & ARTECA R. 1982. Changes in photosynthetic rate and growth following root treatments of tomato *(Lycopersicum esculentum)* plants with phytohormones. – Photosynthetic Res. 3: 45–52.

GORMAN D. S. & LEVINE R. P. 1965. Cytochrome f and plastocyanin: Their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardii*. – Proc. Natl. Acad. Sci. USA 54: 1665–1669.

JASPARS E. M. J. 1965. Pigmentation of tobacco crown gall tissues cultured *in vitro* in dependence of the composition of the medium. – Physiol. Plant. 18: 933–940.

JELIC G. & BOGDANOVIC M. 1989. Antagonism between abscisic acid and cytokinin in chlorophyll synthesis in Pine seedlings. – Plant Sci. 61: 197–202.

KRAWIARZ K., KARAVAIKO N. N., KOF E. M. & KULAEVA O. N. 1976. Interaction of abscisic acid and cytokinin in regulation of growth and greening of pumpkin cotyledons. – Fiziol. Rast 24: 160–167.

LICHTENTHALER H. K. & BUSCHMANN C. 1978. Control of chloroplast development by red light, blue light and phytohormones. In: Developments in Plant Biology, AKOYUNOGLU G. & AKOYUNOGLU S. H. (eds.), Biomedical Press, pp. 801–816. – Amsterdam.

SAMUELSSON G., LÖNNEBORG A., ROSENQVIST E., GUSTAFSSON P. & ÖQUST G. 1985. Photoinhibition and reactivation of photosynthesis in the cyanobacterium *Anacystis nidulans*. – Plant Physiol. 79: 992–995.

STRAUB V. & LICHTENTHALER H. K. 1973. Effect of gibberellic acid A_3 and Kinetin on the formation of photosynthetic pigments, lipoquinones and anthocyanins in *Raphanus*-seedlings. – Z. Pflanzenphysiol. 70: 308–321.

TAMAS I. A., ATKINS B. D., WARE S. M. & BIDWELL R. G. S. 1972. Indoleacetic acid stimulation of phosphorylation and bicarbonate fixation by chloroplast preparations in light. – Can. J. Bot. 50: 1523–1527.

TSUJI H., NAITO K., HATAKEYAM I. & UEDA K. 1979. Benzyladenine-induced increase in DNA content per cell, chloroplast size and chloroplast number per cell in intact bean *(Phaseolus vulgaris)* leaves. – J. Exp. Bot. 30: 1145–1152.

TURNER W. B. & BIDWELL R. G. S. 1965. Rates of photosynthesis in attached and detached bean leaves, and the effect of spraying with indoleacetic acid solution. – Plant Physiol. 40: 446–451.

VOLFOVA A., CHVOJKA L. & FRIEDRICH A. 1978. The effect of kinetin and auxin on the chloroplast structure and chlorophyll content in wheat coleoptiles. – Biol. Plant. (Praha) 20: 440–445.

WOZNY A. & SZWEYKOWSKA A. 1975. Effect of cytokinins and antibiotics on chloroplast development in cotyledons of *Cucumis sativus*. – Biochem. Physiol. Pflanzen. 168: 195–209.

ZERBE R. & WILD A. 1980. The effect of kinetin on the photosynthetic apparatus of *Sinapis alba*. – Photosynthesis Res. 1: 53–64.