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Physiological and Biochemical Role of Brassinosteroids and Their Structure-Activity Relationship in the Green Alga *Chlorella vulgaris* Beijerinck (*Chlorophyceae*)

A. Bajguz* and R. Czerpak

Institute of Biology, Bialystok University, Swierkowa 20 B, PL-15-950 Bialystok, Poland

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Abstract. This paper studies the influence of the 7-oxalactone type of brassinosteroids (BRs) and 6-ketone upon the biological activity of the alga *Chlorella vulgaris* (*Chlorophyceae*). The results of the study indicate significant differences in the growth and metabolism of *C. vulgaris* cells caused by the different chemical structures of the BRs used. The most significant differences in the stimulation of the growth of the biomass and metabolites contained in it were caused by structural differences in the B ring of BRs. It was found that in *C. vulgaris* 7-oxalactone type of BRs [brassinolide (BL) and its derivatives] are more active than 6-ketone type of BRs [castasterone (CS) and its derivatives]. It was found that BRs used within the range of concentration of 10^{-12} to 10^{-8} M stimulate two- to threefold the growth and division of *C. vulgaris* cells. The most stimulating influence upon the number of the algal cells and the phosphorus, chlorophyll, and monosaccharides contained in the alga, as well as the intensity of the photosynthesis, and sugar and glycolate excretion was demonstrated by BL at a concentration 10^{-8} M in the 36th h of cultivation. HomoCS was characterized by the lowest biological activity. In turn, after the 48th h an inhibition of the rate of growth and development of the alga takes place. In the range from 10^{-7} to 10^{-6} M the inhibition of growth and development of the alga was manifested by BRs. During the further toxic activity of BRs the cells of *C. vulgaris* undergo complete degradation. In turn, in concentrations lower than 10^{-12} M, BRs do not exert any biologically significant influence upon *C. vulgaris* cells.

On the basis of the study, the biological activity of BRs was arranged in the following order: BL > 24-epiBL > homoBL > CS > 24-epiCS > homoCS.

Key Words. *Chlorella vulgaris*—Brassinosteroids—Chlorophylls—Glycolate—Growth—Phosphorus—Photosynthesis—Structure-activity relationship—Secretion—Sugar

Minerals are among the most important groups of plant nutrients. Mineral metabolism is of crucial importance to the growth and development of plants. Inorganic phosphate (P_i) is one of the most important components of plant nutrients, and it is also unique in the sense that phosphate exists either as inorganic ions or organic derivatives. P_i is also an important structural component of nucleic acids and phospholipids. In plant cells, P_i is important in photosynthesis; P_i acts both as a substrate and as a controlling factor in photosynthesis-related metabolism (Mimura 1995, Smirnoff 1995). P_i consumed in the photosynthetic reaction is regenerated during starch formation within the chloroplasts. It is also released in the cytosol during the synthesis of saccharose and then transferred to the stroma of the chloroplast by triose phosphate-P_i translocator (Furbank and Taylor 1995, Smirnoff 1995).

The major oxygen-consuming process associated with photosynthesis is the oxygenase reaction of ribulose-1,5-bisphosphate carboxylase (Rubisco), which is the initial reaction of the photorespiratory pathway, in which P_i is liberated during the conversion of phosphoglycolate to glycolate in the stroma of chloroplasts (Foyer et al. 1994, Furbank and Taylor 1995).

A number of green algae may liberate organic sub-

Abbreviations: BRs, brassinosteroids; BL, brassinolide; 24-epiBL, 24-epibrassinolide; homoBL, 22(*S*),23(*S*)-homobrassinolide; CS, castasterone; 24-epiCS; 24-epicastasterone; homoCS, 22(*S*),23(*S*)-homocastasterone; P_o, organic phosphorus; P_{io}, inorganic phosphorus; P_i, inorganic phosphate; Rubisco, ribulose-1,5-bisphosphate carboxylase.

*Author for correspondence.

stances into the medium in which they grow. Extracellular products are defined as soluble substances liberated from healthy cells, as distinct from substances set free by injured cells or by autolysis or decomposition of the dead ones. Algae, especially unicellular and colonial ones, excrete large amounts of various organic substances of a complex ecological function in their surrounding environment. Secretions of low molecular weight substances, particularly sugars and organic acids, predominate quantitatively in algae. Algae of the *Chlorella* genus can excrete to their aquatic environment large amounts of monosaccharides, even 15–30% of their dry weight. Excretion of products of photosynthesis has been estimated as 8–55% of total carbon assimilated by algae (Fogg 1973, 1983, Lewin 1962, Stewart 1974). Among amino acids excreted by algae, aspartic and glutamic acids, alanine, leucine, lysine, serine, and arginine predominate (Watanabe 1980).

Algal excretion is correlated mainly with physiological and metabolic status and cell development phase, reaching a maximum level during mitotic division (Kaplan et al. 1987, Moroney et al. 1986). Growth substances of natural or artificial origin stimulate cellular excretion in algae such as auxins, cytokinins, and gibberellins (Burkiewicz and Kentzer 1984, Czerpak and Bajguz 1993). However, extracellular secretion stimulated by brassinosteroids (BRs) has not been observed in algae.

In this study, we present BRs stimulation of growth (number of cells, contents of organic and inorganic phosphorus, content of chlorophylls and monosaccharides) and photosynthetic oxygen exchange of *Chlorella vulgaris* cells. We also present the comparative effect of BRs on the content of extracellular products in a green alga *C. vulgaris*. We describe the structure-activity relationship between two kinds of BRs, 7-oxalactone and 6-ketone type of BRs, in the green alga *C. vulgaris* Beijerinck (*Chlorophyceae*).

Materials and Methods

Culture Conditions

C. vulgaris Beijerinck (*Chlorophyceae*) was grown under controlled conditions at 25 ± 0.5°C. Illumination was supplied during a 16-h photoperiod (8-h dark period) by a bank of fluorescent lights yielding a photon flux of 50 μmol m^{-2} s^{-1} at the surface of the tubes. Permanent synchronous growth was established according to the method of Pirson and Lorenzen (1966), in the conditions developed by Sayegh and Greppin (1973). The culture medium used was modified Knop's medium. The pH of the medium was adjusted to 6.8 with 1 N NaOH. The *Chlorella* cells were cultured in an Erlenmeyer flask (500 mL) containing 250 mL of medium and shaken at 50 rpm in a rotary shaker.

Brassinosteroids

Brassinolide (BL) was obtained from Dr. Shozo Fujioka (RIKEN, Japan). 24-Epibrassinolide (24-epiBL) and 22(*S*),23(*S*)-homobrassinolide (homoBL) were purchased from Sigma (St. Louis, MO; USA). Castasterone (CS), 24-epicastasterone (24-epiCS), and 22(*S*),23(*S*)-homocastasterone (homoCS) were purchased from Beak Technol. Inc. (Canada). Fig. 1 shows the structures of BRs that were used in the experiment. BRs were prepared as 1 μM ethanolic stock solutions and stored at −20°C. The appropriate amount of BRs for the strongest solution was transferred directly into culture medium, and weaker solutions were prepared by serial dilution. Equal amounts of ethanol were added to the controls.

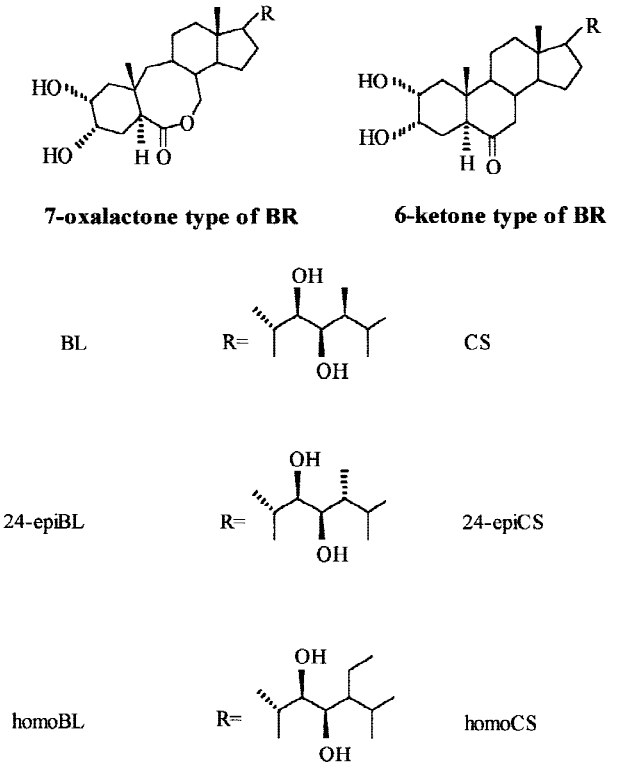

Fig. 1. Structures of BRs used.

Analysis of Plant Material

Growth of the culture was estimated by direct counting of cells in the growth medium using the Bürker chamber. Determination of the chlorophyll content followed homogenization of the algal fresh weight in methanol. The absorbance of the extract was measured with a Beckman DU-640 spectrophotometer at 653 and 666 nm. The amounts of chlorophyll *a* and *b* present in the extract were calculated according to the equation of Wellburn (1994). Phosphorus of organic phosphates and inorganic orthophosphate was assayed by the Fiske-Subbarow method (1925). For sugar secretion determination, the algae were first collected by centrifugation of 10 mL-culture samples, and the resulting supernatant was analyzed quantitatively. Sugar concentration in the cell and its excretion were determined using the anthrone method (Hewitt 1958, Yemm and Willis 1954). Glycolate was isolated from the medium using ion exchange resin Dowex 1 × 10 (OH^-) 200–400 mesh (Serva, UK). An aliquot of 20 mL of medium was applied to the resin column (8 × 90 mm). The column was washed thoroughly with distilled water, and glycolic acid was eluted with 4 N acetic acid. The eluates were

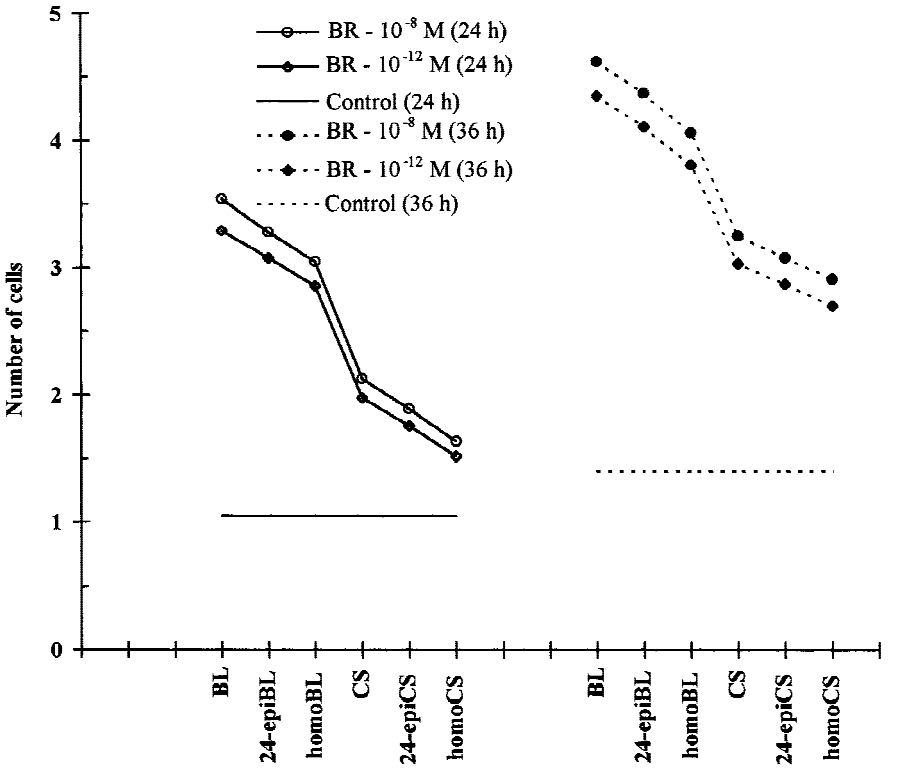

Fig. 2. Effect of BRs on the number of *C. vulgaris* cells in the culture (expressed as 10^6 ml^{-1}; S.E. < 5%).

dried under vacuum. The glycolate content of the residue was determination by Calkin's (1943) method.

Measurement of Photosynthetic Oxygen Exchange

Photosynthesis capacity was determined by measuring the amounts of oxygen released by the cells using a Clark type oxygen electrode (Hansatech Ltd., UK). A 2-mL algal suspension was incubated at 25°C and 1,000 μmol m^{-2} s^{-1} PAR. The evolution of oxygen in the medium was calculated to represent the activity of photosynthesis of *Chlorella* cells.

Replication and Statistical Analysis

Each treatment consisted of five replicates, and each experiment was carried out at least twice at different times. A minitab statistical package was used to carry out a one-way ANOVA. Significance was determined using *t*-tests and LSD values based on the ANOVA data.

Results

During the experiments BRs was used within the range of concentrations 10^{-15} to 10^{-6} M. However, this paper presents results of the most effective concentrations, 10^{-12} and 10^{-8} M, indicating the stimulating impact of BRs upon physiological and metabolic processes in *C. vulgaris.* In the range from 10^{-7} to 10^{-6} M, inhibition of the algal growth and development was recorded at the 36th h of cultivation. During the next consecutive 12 h of cultivation the cells undergo complete degradation. In turn, in concentrations lower than 10^{-12} M BRs do not exert significant influence upon the growth and metabolism of *C. vulgaris.*

Growth of C. vulgaris

The most stimulating influence upon the number of *C. vulgaris* cells was shown by BL at a concentration of 10^{-8} M at the 24th and 36th h of cultivation, within the limits 330–337% with respect to the control (100%). The lowest activity was shown by homoCS, under the influence of which, at the above concentrations, the growth in the number of cells was 156.2–207.8%, compared with the control. The data provided indicate that under the influence of BRs, at the concentrations of 10^{-12} and 10^{-8} M, a significant increase in the number of *C. vulgaris* cells occurs. Two- to threefold stimulation of the growth of cells during 36 h cultivation of algae was demonstrated (Fig. 2).

Intensity of Net Photosynthesis of C. vulgaris

Cultures of *C. vulgaris* treated with BRs after 24 h are characterized by a slight stimulation of the intensity of net photosynthesis (Fig. 3). A difference becomes no-

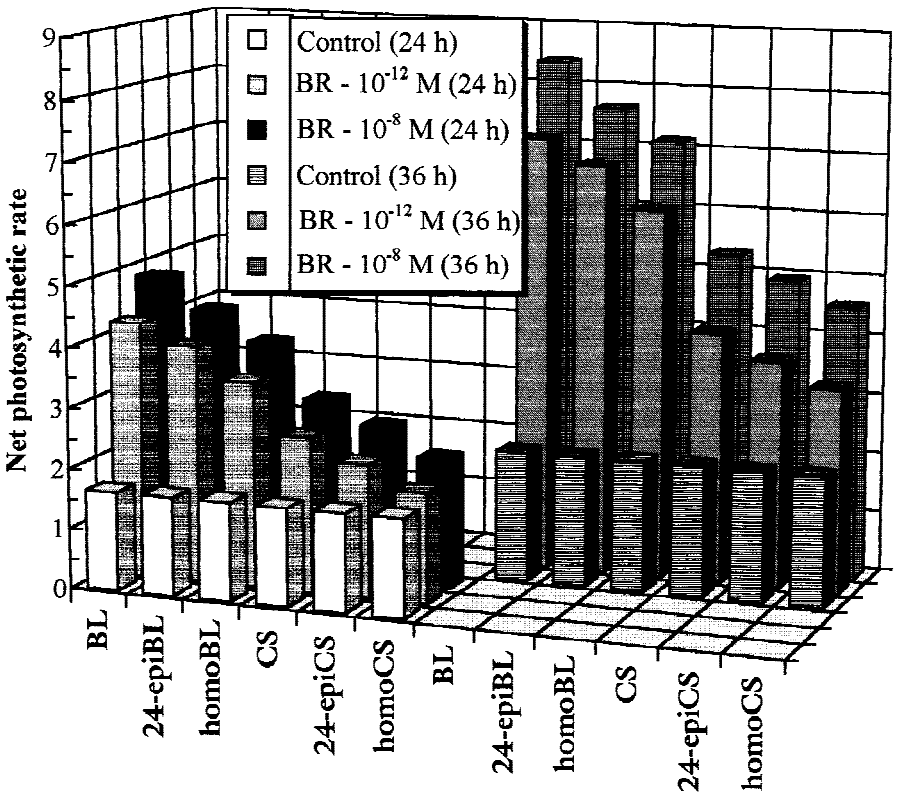

Fig. 3. Effect of BRs on the photosynthetic O_2 exchange of *C. vulgaris* (expressed as 10^{-6} nM O_2 h^{-1} $cell^{-1}$; S.E. < 5%).

ticeable between the activity of BL (as the most stimulating from the group of 7-oxalactone) and CS (a compound from the group of 6-ketone). BL at a concentration of 10^{-8} M increases the intensity of photosynthesis by 298.1% compared with the control. In turn, stimulation by CS, at the same concentration, amounts to 182.8%. HomoCS demonstrates the least stimulating impact upon the intensity of photosynthesis, reaching 132.5%.

Thirty-six hours after treatment with BRs the level of photosynthesis of *C. vulgaris* increases significantly (Fig. 3). BL is biologically most active, and at a concentration of 10^{-8} M, it stimulates the process of photosynthesis around 172.6% compared with the 24th h of activity. With respect to the control, after 36 h BL increases the intensity of photosynthesis by 390%. CS is characterized by weaker stimulation of this process (246.9%), and homoCS reaches 211.6% compared with the control cultivation.

Chlorophyll a and b Content in C. vulgaris Cells

BL shows the most stimulating influence upon the chlorophyll *a* and *b* content in *C. vulgaris* cells, without regard to the concentration or length of the cultivation (Table 1). The stimulation of the chlorophyll *a* and *b* content within the range of concentrations 10^{-12} and 10^{-8} M by BL was within the limits 256.7–328.4%, compared with the control after the 24th and 36th h of cultivation. The lowest activity in stimulating chlorophyll *a* and *b* was demonstrated by homoCS.

Content of Monosaccharides in C. vulgaris Cells

The cells of algae treated with BL at the concentration 10^{-8} M contain 2.5 to 3 times more monosaccharides than the cells of the control algae. The lengthening of the side chain in BRs by one more methyl group causes a decrease in the activity of both homoBL and homoCS. In the first case, lowering the content of monosaccharides by 13% takes place, and in the second by an average of 11–23%, compared with the control, treated, respectively, with BL and CS (Table 2).

Content of Phosphorus in C. vulgaris Cells

Cells of *C. vulgaris*, treated by chemically different BRs after 24 h of growth, demonstrate two- to threefold growth in the content of organic (P_o) and inorganic phosphorus (P_{io}) (Fig. 4). Significant differences were observed between BL and CS and their derivatives. BL, at a concentration 10^{-8} M, increases the content of P_o by

Table 1. Effect of BRs on the content of chlorophyll *a* and *b* in the cells of *C. vulgaris*. Values are expressed as 10^{-8} μg cell^{-1}; S.E. < 5%.

Time of culture	Concentration	7-Oxalactone type of BR			6-Ketone type of BR			Control
		BL	24-EpiBL	HomoBL	CS	24-EpiCS	HomoCS	
24 h	10^{-12}	2.76	2.58	2.35	1.57	1.36	1.19	1.07
	10^{-8}	2.95	2.76	2.57	1.79	1.59	1.38	
36 h	10^{-12}	3.67	3.46	3.21	2.55	2.42	2.23	1.18
	10^{-8}	3.89	3.68	3.42	2.74	2.60	2.45	

Table 2. Effect of BRs on the content of monosaccharides in the cells of *C. vulgaris*. Values are expressed as 10^{-8} μg cell^{-1}; S.E. < 5%.

Time of culture	Concentration	7-Oxalactone type of BR			6-Ketone type of BR			Control
		BL	24-EpiBL	HomoBL	CS	24-EpiCS	HomoCS	
24 h	10^{-12}	12.75	11.83	10.97	7.59	6.39	5.37	3.72
	10^{-8}	13.92	13.11	12.12	8.45	7.51	6.54	
36 h	10^{-12}	17.13	16.18	14.97	11.79	11.05	10.38	5.56
	10^{-8}	18.35	17.37	16.12	12.90	12.23	11.56	

286.8%, and the P_{io} content by 273.8%, compared with the control. In turn, cells treated by CS, at the same concentration, contain 86.8% more P_o and 66.7% more P_{io} compared with the control cells. The weakest influence upon the content of P_o is exerted by homoCS within the limits of 134.2–127.7% of P_o and P_{io} compared with the control.

After 36 h, (Table 3) BL, most active biologically, at a concentration 10^{-8} M, increases the content of P_o and P_{io} within the limits of, respectively, 273.8% and 329.6% CS, a 6-ketone, is characterized by a decreased content of phosphorus (P_o 231.8% and P_{io} 232.4%), and the lowest homoCS, within the limits of 206.8–208.4%, compared with the control.

Secretion of Sugar and Glycolate into the Medium

BRs stimulate sugar (Table 4) and glycolate (Fig. 5) secretions in *C. vulgaris* in the concentration range 10^{-12} to 10^{-8} M, reaching the maximum at a concentration of 10^{-8} M. In *C. vulgaris* the sugar secretion is stimulated most intensively at a concentration of 10^{-8} M BL after 36 h in 296% with reference to the control. The cells of algae treated with BL at a concentration of 10^{-8} M excrete three times more glycolate than control after 36 h of growth. CS is characterized by decreased glycolate excretion (208.5%) compared with the control. In stimulating extracellular products secretion, BL is more active than CS.

Discussion

BRs are now regarded as a new class of plant hormones ubiquitous in the plant kingdom in addition to auxins, gibberellins, cytokinins, abscisic acid, and ethylene. BRs are steroidal plant hormones having growth-promoting activities in plants. At present, more than 40 BRs have been shown to occur naturally, including dicots, monocots, gymnosperms, an alga (*Hydrodictyon reticulatum*), and a pteridophyte. BRs have a common 5α-cholestane skeleton, and their structural variations come from the type and position of functionality on the skeleton (Adam and Petzold 1994, Fujioka and Sakurai 1997, Mandava 1988, Sakurai and Fujioka 1993, Sasse 1991, Yokota 1997).

These studies demonstrate that BRs stimulate cell divisions intensively, at the same time leading to an increase in the number of cells of *C. vulgaris*. The stimulating influence of BRs is most noticeable within the concentration range 10^{-12} to 10^{-8} M. Among BRs applied the highest activity is demonstrated by BL at a concentration 10^{-8} M after 36 h from the moment when the algae were treated with it. The main factor influencing the biological activity of BRs appears to be a different structure of ring B BL (7-oxalactone type) and CS (6-ketone type) (Fig. 1). It was demonstrated that the oxygen built into B ring increases the biological activity of BL compared with CS deprived of oxygen. Additionally, the change in the orientation of the methyl group at C-24 and the lengthening of the side chain by one methyl group added at C-24 lead to a significant decrease of the biological activity of BRs. It was found that BRs of the 7-oxalactone type (BL and derivatives) are more active biologically than 6-ketone compounds (CS and derivatives). Changes in the biological activity occurring under the influence of BRs were also observed in vascular plants, i.e. tomato (*Lycopersicon esculentum*) and rice (*Oryza sativa*). It was demonstrated that, with the addition of the next methyl groups in position C-28 in the

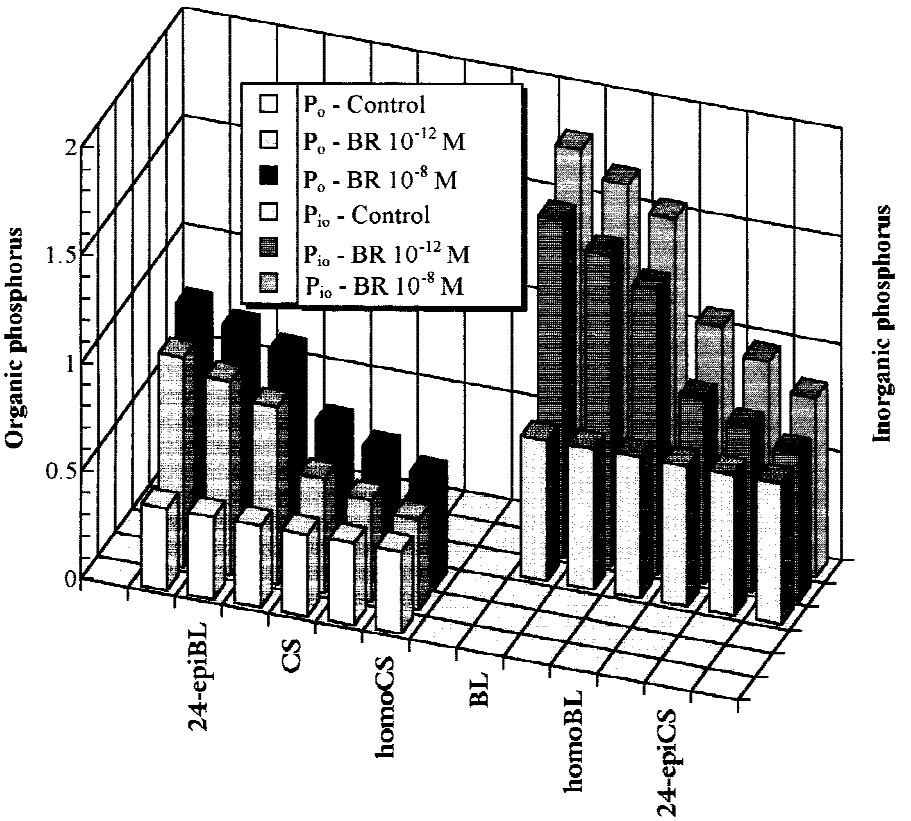

Fig. 4. Effect of BRs on the content of organic (P_o) and inorganic phosphorus (P_{io}) in the cell of *C. vulgaris* in 24 h of culture (expressed as 10^{-9} μg cell^{-1}; S.E. < 5%).

Table 3. Effect of BRs on the content of organic and inorganic phosphorus in the cells of *C. vulgaris* in 36 h of culture. Values are expressed as 10^{-9} μg cell^{-1}; S.E. < 5%.

	7-Oxalactone type of BR			6-Ketone type of BR			
Concentration	BL	24-EpiBL	HomoBL	CS	24-EpiCS	HomoCS	Control
Organic phosphorus							
10^{-12}	1.32	1.26	1.17	0.91	0.83	0.78	0.44
10^{-8}	1.45	1.37	1.28	1.02	0.97	0.91	
Inorganic phosphorus							
10^{-12}	2.12	2.01	1.87	1.46	1.33	1.25	0.71
10^{-8}	2.34	2.22	2.06	1.65	1.56	1.48	

side chain, a greater decrease in the activity of BL occurs. The inhibitory activity of BRs on the root growth of tomato occurred in the order BL > 24-epiBL > 22,23,24-trisepiBL > homoBL (Roddick 1994). The very much reduced activity of homoBL with a 24*S*-ethyl group confirms the importance of the C-24 substituent. HomoBL also showed much reduced activity in the radish and tomato seedling shoot growth assay (Takatsuto et al. 1983a). The orientation of the C-24 methyl group is very important as demonstrated by the reduction in activity of 24-epiBL with a 24*R*-configuration. The activity of 24-epiBL was about one-tenth that of BL in the radish, tomato, rice, and bean internode (Takatsuto et al. 1983a, 1983b). McMorris et al. (1994) have made a comparative study of the biological activity of BL, 24-epiBL, (22*S*,23*S*)-24-epiBL, 28-homoBL, and (22*S*,23*S*)-28-homoBL in the soybean epicotyl assay. All BRs except (22*S*,23*S*)-28-homoBL exhibited a similar biological activity, but the last compound was substantially less active. Also, Wang et al. (1994) showed that lactone types of BRs (e.g. BL) are more active than the ketone type of BRs (e.g. CS). In general, the 7-oxalactone stuctures possess a greater biological activity than the 6-ketone forms.

BRs within the range of concentrations 10^{-12} to 10^{-8}

Table 4. Effect of BRs on the reducing sugar excretion into medium by *C. vulgaris* cells. Values are expressed as 10^{-6} nM cell^{-1}; S.E. < 5%.

Time of culture	Concentration	7-Oxalactone type of BR			6-Ketone type of BR			Control
		BL	24-EpiBL	HomoBL	CS	24-EpiCS	HomoCS	
24 h	10^{-12}	1.55	1.43	1.31	0.87	0.75	0.63	0.52
	10^{-8}	1.73	1.61	1.50	1.05	0.93	0.81	
36 h	10^{-12}	2.05	1.98	1.82	1.43	1.35	1.26	0.69
	10^{-8}	2.28	2.15	2.00	1.60	1.52	1.44	

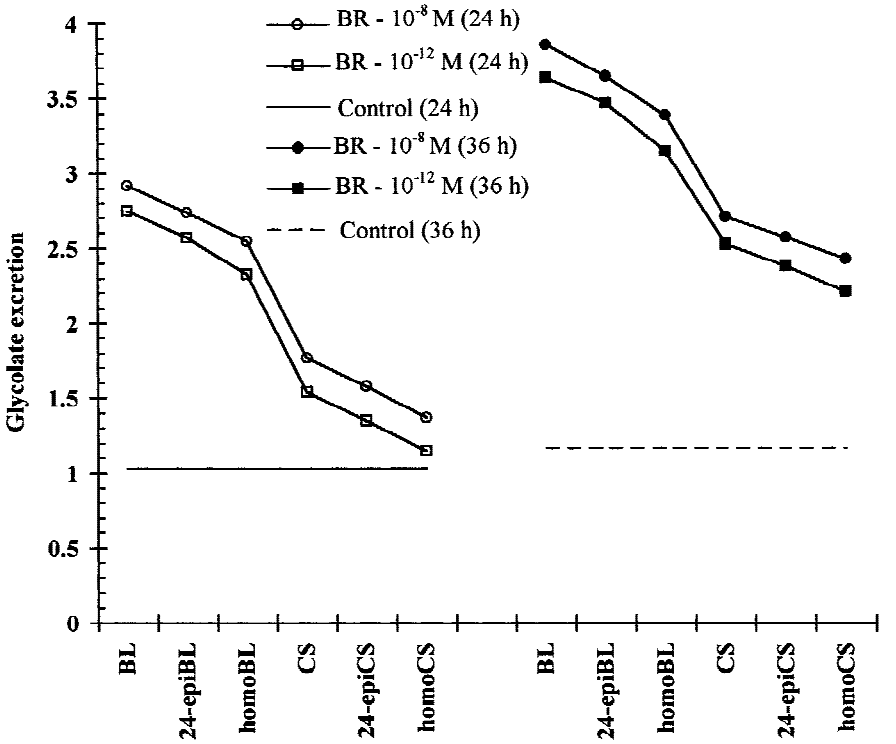

Fig. 5. Effect of BRs on glycolate excretion into medium by *C. vulgaris* cells (expressed as 10^{-6} nM cell^{-1}; S.E. < 5%).

M, in a short period of time (24–48 h), increase by two to three times the efficiency of the developmental cycle of *C. vulgaris.* It was shown that between the 24th and 36th h of cultivation BR-induced stimulation of number of cells increases by about 50% compared with the control cultivation of the algae. This unusual fact may be explained by the expansive activity of BRs upon the cell genome of *C. vulgaris*, despite the use of submicromolar concentrations. An interesting phenomenon is the fact that, 48 h after the treatment of algae with BRs, almost complete stagnation occurs in the development of *C. vulgaris.*

BRs participate in the growth of plant tissue in the processes of transcription and translation. It was demonstrated that activation of the growth of plant tissue and a higher level of polimerase RNA and DNA are manifested by an increase in the content of DNA, RNA, and proteins (Mandava 1988, Mandava et al. 1987). Our studies conducted on *C. vulgaris* confirm the stimulating role of BRs in the growth of the content of nucleic acids (unpublished results) and proteins (Bajguz and Czerpak 1996a). The shortening of the developmental cycle and the increase by two to three times its efficiency during a 36-h cultivation of the alga suggest an unusual increase in the rate of the process of transcription and translation. At the same time a diversified impact of BRs, depending on their chemical structure, upon the content of DNA, RNA, and proteins, was demonstrated. The highest growth of the content of nucleic acids (unpublished data) and proteins is observed in the case of BL at the concentration 10^{-8} M in the 36th h of the impact of the hormone upon the cells of the algae (Bajguz and Czerpak 1996a).

Reactions of photosynthesis and photorespiration as well as metabolism of glycolate in algae are known to have a course similar to that in vascular plants. The role

of Rubisco as well as P-glycolate phosphatase in the production and transformation of P-glycolate is the same as in other plants. In turn, photosynthesis in unicellular algae growing at low concentration of CO_2 shows similarity to photosynthesis in C_4 type plants (Lloyd et al. 1977). These facts and our own studies indicate that BRs activate intensively the process of algae photosynthesis (Fig. 3). Elements for the process of photosynthesis come from the environment, whereas algae use CO_2 from photorespiration (Lloyd et al. 1977), whose activity BRs increase significantly, thanks to the binding of CO_2 by ribulose 1,5-bisphosphate (Braun and Wild 1984). A condition necessary to carry out the photochemical reaction is the participation of the photoreceptor, whose role is fulfilled by protein complexes with chlorophyll and carotenoids. As a result of the studies conducted it was found that BRs stimulate about twice the content of the chlorophyll *a* and *b* in the cells of *C. vulgaris* (Table 1).

The excess of synthesized proteins and sugars from the cells of algae is secreted by the cell wall to the outside environment. As the most biologically active compound, BL not only increases the content of proteins (Bajguz and Czerpak 1996a) and sugars (Table 2) in algae cells but also increases the intensity of sugar extracellular secretion (Table 4). The differences in the chemical structure of BRs, 7-oxalactone, and 6-ketone type, caused by a change in the orientation of the methyl group by C-24 and, additionally, the lengthening of the side chain by one methyl group, bring about the decrease in the rate of secretion of monosaccharides. It was found that BRs at concentrations 10^{-12} and 10^{-8} M have a stimulating influence during the entire 36-h cultivation of algae.

One of the key factors influencing the growth and development of algae is phosphorus. Phosphorus, mostly in the form of phosphate remains, constitutes a component of many biological compounds. BR-induced accumulation of organic and inorganic phosphorus (Fig. 4 and Table 3) is useful for algae, as phosphates are substrates in the reactions of photosynthesis and also enter the transitional compounds in the Calvin cycle (Mimura 1995). Orthophosphorus is one of the factors regulating the course of the dark phase of photosynthesis through activation of a number of enzymes in the Calvin cycle, including Rubisco. Orthophosphorus is also used as a substrate in the reactions of production of the majority of transitional compounds in the process of photosynthesis. The reproduction of this substrate takes place during the synthesis of final secondary products of photosynthesis, such as starch and saccharose (Furbank and Taylor 1995).

Monosaccharides produced during photosynthesis are building substances for a plant as well as a source of energy necessary for inciting all the biochemical reactions and processes. The stimulation by BRs of the chlorophyll content (Table 1) and the process of photosynthesis (Fig. 3) cause an increase of sugars in *C. vulgaris* cells (Table 2). The change in the content of sugars in the cells of algae is connected with structural diversity of BRs, as mentioned earlier.

The algae excrete a part of photosynthetically assimilated carbon as glycolate into medium. It is known that *Chlorella* has the photorespiratory glycolate pathway and metabolizes glycolate. The amount of glycolate excreted depends on several factors: light, CO_2 and O_2 concentrations, the pH of the medium, and the age of the cells (Tolbert 1979, 1997, Tolbert and Zill 1956). The main factor causing the activation of extracellular secretion of glycolate from *C. vulgaris* cells is the acidification of the environment by BRs (Bajguz and Czerpak 1996b). The change of the value of pH from 6.8 to 6.3 supports the synthesis and secretion of glycolate by algal cells. It was found that the effect of stimulation of extracellular secretion of glycolate is inversely proportional to the decreasing pH of the environment. The highest effect of glycolate secretion was observed also in the case of BL at the concentration 10^{-8} M, and the lowest under the influence of homoCS. The lengthening of the time of cultivation of *C. vulgaris* is accompanied by an increased level of glycolate secretion (Fig. 5).

C. vulgaris cells grown in a phosphorus-deficient medium were characterized by higher glycolate excretion and also by an increased capacity for production and metabolism of glycolate (Kozlowska and Maleszewski 1994). In the experimental conditions that we applied, *C. vulgaris* cells were cultivated in complete inorganic medium. We show that BRs not only stimulated phosphorus concentration in the cells (Fig. 4 and Table 3) but also increased glycolate excretion into the medium (Fig. 5).

These facts concerning the extremely high stimulating activity of BRs upon the growth, development, and some metabolical processes of *C. vulgaris* indicate that these hormones are characterized by unusual biological activity. The growth and development of *C. vulgaris* under the influence of BRs are unusually dynamic, despite the application of submicromolar concentrations. Intensive stimulation of the biosynthesis of chlorophyll and the processes of photosynthesis and its final products demonstrates the most notable influence of BRs upon algae. Organic substances excreted by algae to water have important biochemical and metabolical functions in the shaping of proper ecological relationships and interactions among organisms in the aquatic ecosystem.

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