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BRIEF COMMUNICATION

Effect of some benzothiazolium salts on chlorophyll production in *Chlorella vulgaris*

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Abstract

Effects of benzothiazolium salts on chlorophyll (Chl) production in *Chlorella vulgaris* (inhibition or stimulation) are closely connected with the applied effector concentration and with the character of the substituents.

Benzothiazole derivatives belong to the compounds showing several biological effects, *e.g.* antimicrobial (Sutoris *et al.* 1983), antialgal (Šeršeň *et al.* 1993a) or plant growth regulating (Sutoris *et al.* 1983, Blanáriková *et al.* 1992). Previous results confirmed that 6-substituted 2-alkylthiobenzothiazoles also inhibit oxygen evolution in plant chloroplasts in the Hill reaction, the site of their action being the donor side of PS 2 before the site of action of DPC, *i.e.* the oxygen evolving complex (Šeršeň *et al.* 1993a, b).

Benzothiazolium salts (BS) representing a special water soluble group of benzothiazole derivatives also affect growth and chlorophyll synthesis in *Euglena gracilis*, with some of them inducing white mutants, probably by inhibiting the synthesis of some enzymes vitally important for the replication of chloroplasts, with subsequent decrease in the number, or complete elimination of chloroplasts from dividing cells (Foltínová *et al.* 1986).

The twenty two 3- and/or 2-substituted BS studied were prepared using procedures described in Sutoris *et al.* (1983). Their effect on Chl production in *C. vulgaris* was

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Abbreviations: BS - benzothiazolium salts; Chl - chlorophyll; DCPIP - 2,6-dichlorophenolindophenol; DPC - diphenylcarbazide; pI_{50}- negative logarithm of concentration causing 50 % inhibition with respect to untreated control samples; PS - photosystem.
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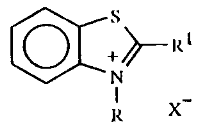
studied over a wide concentration range. Stationary cultivation techniques for *C. vulgaris* (7 d, 16 h photoperiod, irradiance at about 12 W m^{-2}, room temperature) and methods used for determination of Chl content in algae are described in Kráľová *et al.* (1991). The inhibitory activity of the compounds studied is expressed as pI_{50} values, the effects of BS applied in low concentrations were determined for the concentration range of 10^{-5} - 10^{-13} M and expressed as % of the control (Table 1).

Table 1. Effect of benzothiazolium salts on chlorophyll production in *Chlorella vulgaris*. For calculations of pI_{50}, values, experimental results obtained at 4 different BS concentrations (3 repetitions for each concentration) were used. The correlation coefficients were in the range of 0.98-0.99. Effect of BS on Chl production in the concentration range 10^{-5} - 10^{-13} M is expressed as % of the control. For confidence intervals evaluated for 95 % probability, all results obtained for concentrations 10^{-5}, 10^{-7}, 10^{-9}, 10^{-11} and 10^{-13} M were taken into account.



N.	R¹	R	X	pI_{50}	[% of control] ± σ
1	H	CH_2COOCH_3	Br	3.426	119.4 ± 6.3
2	H	$CH_2COOC_2H_5$	Br	3.540	113.9 ± 5.1
3	H	$CH_2COOC_3H_7$	Br	3.548	123.2 ± 7.2
4	H	$CH_2COOCH(CH_3)_2$	Br	3.533	120.1 ± 5.4
5	H	CH_2-CH=CH_2	Br	4.232	104.3 ± 7.3
6	H	$CH_2C_6H_5$	Br	3.817	93.6 ± 5.0
7	H	CH_3	I	4.432	108.4 ± 7.9
8	H	H	HSO_4	3.223	102.4 ± 6.2
9	H	$CH_2CON(CH_3)_2$	Br	2.833	108.8 ± 5.8
10	H	$CH_2CON(C_2H_5)_2$	Br	3.507	98.7 ± 7.1
11	H	$CH_2CONHCH_2CH$=CH_2	Br	3.093	98.8 ± 7.5
12	H	$CH_2CONHCH_2C_6H_5$	Br	3.435	100.7 ± 9.6
13	H	CH_2CONH-	Br	3.557	102.3 ± 8.8
14	H	$CH_2CON(CH_2CH$=$CH_2)_2$	Br	3.760	101.4 ± 6.5
15	$SCH_2COOC_2H_5$	CH_3	Br	3.617	129.2 ± 9.7
16	SCH_3	CH_3	CH_3SO_4	3.768	93.9 ± 7.4
17	SCH_3	CH_3	I	3.795	101.7 ± 5.9
18	$CH_2C_6H_5$	CH_3	I	4.000	97.0 ± 6.6
19	$CH_2C_6H_5$	$CH_2COOCH_2C_6H_5$	Br	-	110.3 ± 5.1
20	CH=CHC_6H_5	$CH_2COOC_3H_7$	Br	-	111.7 ± 7.4
21	CH=CHC_6H_5	$CH_2COOCH(CH_3)_2$	Br	3.480	109.5 ± 5.0
22	CH=CHC_6H_5	C_4H_9	I	3.842	94.2 ± 6.2

Due to intense interactions of BS with DCPIP (Kráľová and Mitterhauszerová 1988), the effect of BS on Hill reaction activity of plant chloroplasts could not be studied by the method using DCPIP as electron acceptor which has been applied to some amphiphilic effectors (Kráľová *et al.* 1991, 1992, Šeršeň *et al.* 1993a, b).

These compounds with an alkyl substituent in position 3 (compounds 5, 6, 7, 16, 17, 18, and 22) showed a more pronounced inhibitory activity (Table 1). Compounds having a more polar esteric substituent in position 3 (1 - 4, 21) or 2 (15) showed a lower inhibitory activity than those with an alkyl substituent. BS with the *N*-alkylcarbamoylmethyl substituent in position 3 (11, 12) were less active than the corresponding 3-alkoxycarbonylmethyl derivatives (5, 6). As shown by the 3-isopropoxycarbonylmethyl derivatives (compounds 4 and 21), the replacement of hydrogen in position 2 of BS by a styryl substituent partly decreased the inhibitory activity of the compound.

Lower concentrations (10^{-5} - 10^{-13} M) of 3-R-substituted BS with R = alkyl, benzyl, N-alkyl- or N,N-dialkylcarbamoylmethyl has little or no effect on the Chl synthesis in algae. However, compounds with an esteric substituent (1 - 4, 15) showed a stimulating effect on Chl synthesis in *C. vulgaris*, whereby the replacement of hydrogen with a styryl substituent in position 2 of BS led to suppression of stimulatory activity (19 - 21 and 6, 2, and 4, respectively). The importance of the presence of an esteric substituent in the BS molecule for stimulation of plant growth (Sutoris *et al.* 1983), of Chl content in *E. gracilis* (Foltínová *et al.* 1986) and in the sugar beet leaves (Sutoris *et al.* 1988) has thus been confirmed.

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