Effect of Plant-Growth Stimulating *N*-Cyclohexylbenzothiazol-2-sulfenamide and its Photodegradation Products on Photosynthetic Electron Transport in Spinach Chloroplasts

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The effects of *N*-cyclohexylbenzothiazol-2-sulfenamide (CBS) and its photodegradation products on inhibition of photosynthetic electron transport in spinach chloroplasts and growth of roots and shoots of *Sinapis alba* L. were investigated. The solution of CBS in CCl_4 was irradiated with a mercury lamp for 1.0—7.5 h without and with application of ultrasound (25 kHz, 16 W cm^{-2}). Using GC the presence of following benzothiazole derivatives was confirmed: benzothiazole, benzothiazole-2-thiol, 2-(cyclohexyldisulfanyl)benzothiazole, di(benzothiazol-2-yl)disulfane, and 2-chlorobenzothiazole. In the investigated concentration range (1.5—148.5 mg dm^{-3}) CBS and its photodegradation products showed pronounced stimulating effects on the growth of *Sinapis alba* L. On the other hand, the tested reaction mixtures containing photodegradation products of CBS inhibited oxygen evolution rate in the suspension of spinach chloroplasts and the photosynthesis-inhibiting activity of the reaction mixtures depended on the time of irradiation as well as on the application of ultrasound.

Many environmental pollutants undergo photodegradation and the products of this photodegradation could exhibit harmful effects on photosynthesizing organisms. From the viewpoint of the environment pollution as dangerous could be regarded mainly toxic compounds produced and applied in great quantities in the industry and agriculture. These compounds represent potential environmental hazard for aquatic and terrestrial ecosystems. Benzothiazoles appear in the environment mainly as a result of their production and use as accelerators of rubber vulcanization and agrochemicals. The stability and the transformation of these compounds in the natural matrices (air, soil, water) are also affected by their photoreactivity.

In the presence of electron donors the halogenated organic compounds irradiated with the light of a suitable wavelength undergo dehalogenation. Dehalogenation is a bimolecular process, which is performed by excimers [1, 2], electron donors, *i.e.* amines [3], dienes [4], or sulfides [5]. These reactions can be carried out by photoexcitation of the compound acting as electron donor [6, 7] or by the photoexcitation of the halogenated compound [8].

Nowadays the attention of the researchers is focused on the effects of ultrasound on the reaction processes. The activation of the reaction by ultrasound could be successfully applied mainly in heterogeneous [9] and electrochemical reactions [10—12]. It could be assumed that ultrasound could play significant role at degradation of the pollutants as well as at the removal of the garbage.

The toxic effects of 2-sulfanylbenzothiazole towards microorganisms were attributed to its metal chelating properties and/or its interference with membrane-bound (co)enzymes in particular [13]. It was found that benzothiazole is a potent inducer of P450s and phase II metabolizing enzymes [14]. Benzothiazole derivatives substituted in the position 2 were found to exhibit antifungal activity against *Saccharomyces cerevisiae* and the clinical pathogen *Candida krusei* [15, 16] and they inhibit the photosynthetic electron transport in the photosynthesizing organisms [17—19]. Many benzothiazole derivatives have plant-growth regulating activity. Higher concentrations of some benzothiazolium salts reduced chlorophyll content in freshwater alga *Chlorella vulgaris* whereas in the presence of low concentrations of these compounds the stimulating effects were observed [20]. Pronounced stimulating effect on growth of *Zea mays* L. showed 3-alkyl-6-nitro-2-benzothiazolinones [21, 22]. As a result of an expansive research many other benzothiazole derivatives were found to exhibit interesting plant-growth regulating activity [23—28].

This study was focused on the investigation of the effects of *N*-cyclohexylbenzothiazol-2-sulfenamide (CBS) and its photodegradation products obtained without and with application of ultrasound on the growth of mustard (*Sinapis alba* L.) and on the inhibition of photosynthetic electron transport in spinach chloroplasts.

EXPERIMENTAL

N-Cyclohexylbenzothiazol-2-sulfenamide (CBS) (Duslo Šaľa) applied for photodegradation experiments was recrystallized before the use and its purity was confirmed by ^{1}H NMR.

The photodegradation experiments were carried out in the photochemical reactor with a glass immersion finger, ultrasound (25 kHz, 16 W cm^{-2}) and 250 W mercury lamp. From the solution of CBS (0.02 mol dm^{-3}) in CCl_4 oxygen was removed before irradiation and distilled water was added to the solution for the absorption of HCl. The reaction was monitored by ITP, UV VIS spectra, and GC. At the same conditions also experiments with application of ultrasound were made. Duration of irradiation of CBS was 1.0 h, 2.5 h, 4.0 h, 5.5 h, and 7.5 h.

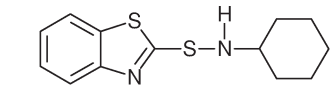
Chloroplasts were prepared from market spinach and the inhibitory activity of the studied compounds concerning oxygen evolution rate (OER) in spinach chloroplasts was investigated spectrophotometrically (Specord UV VIS, Zeiss, Jena) in the presence of the electron acceptor 2,6-dichlorophenol-indophenol (DCPIP). The rate of photosynthetic electron transport, which is proportional to OER, was monitored as photoreduction of DCPIP according to the method described in [17]. The chlorophyll (Chl) content in these experiments was 30 mg dm^{-3}. This photochemical assay was carried out under saturating irradiance of white light from a 250 W halogen lamp ($\approx$ 900 μmol m^{-2} s^{-1} PAR). For low solubility of the studied compounds in water, these were dissolved in dimethyl sulfoxide (DMSO). The applied solvent content (up to 4 vol. %) did not affect the photochemical activity in spinach chloroplasts. IC_{50} values were calculated from OER inhibition at 6—8 concentrations and tests in all concentrations were triplicated.

The seeds of *Sinapis alba* were placed in Petri dishes with a 14 cm diameter and filter paper on the bottom. In each Petri dish 58 seeds were evenly displayed on the surface of filter paper and the amount of solution used was 10 cm^3 per dish. Each concentration was duplicated. After 72 h exposure at room temperature (25 °C) the length of roots and shoots was measured. Due to low aqueous solubility of CBS this as well as the mixtures of its reaction products were dissolved in DMSO. In all experiments (including control) the concentration of DMSO was the same (1 vol. %). The applied concentration range of benzothiazole products was 1.5—150 mg dm^{-3}.

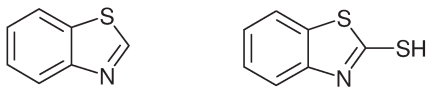
RESULTS AND DISCUSSION

Photochemical reactivity of some benzothiazole derivatives in methanol was described previously [29, 30]. In this study the photolysis of CBS was carried out in CCl_4 and the light-absorbing reaction component was electron donor.

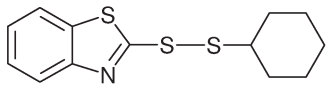
During photolysis of CBS in CCl_4 destruction of CCl_4 occurred. As a result of CCl_4 destruction following products were formed: Cl^-, C_2Cl_6, $CHCl_3$, CH_2Cl_2, small amount of organic acids – mainly derivatives of acetic acid and further compounds with lower chlorine content in the molecule in relation to CCl_4. In this reaction CBS acting as an electron donor undergoes degradation during photolysis. At the same time new benzothiazole derivatives were formed. These compounds absorbed light applied for photolysis and they could also participate in the catalytic degradation of CCl_4. The decrease of CBS concentration (determined by GC) was practically the same in experiments carried out in the absence of ultrasound as well as with its application and the determined differences were within experimental error. Using GC and comparison with the standards the presence of following benzothiazole derivatives was proved: benzothiazole (*I*), benzothiazol-2-thiol (*II*), 2-(cyclohexyldisulfanyl)benzothiazole (*III*), di(benzothiazol-2-yl)disulfane (*IV*), and 2-chlorobenzothiazole (*V*).



CBS



I *II*



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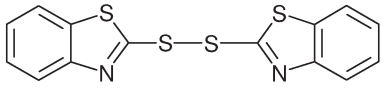
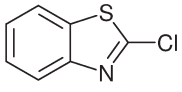


IV



V

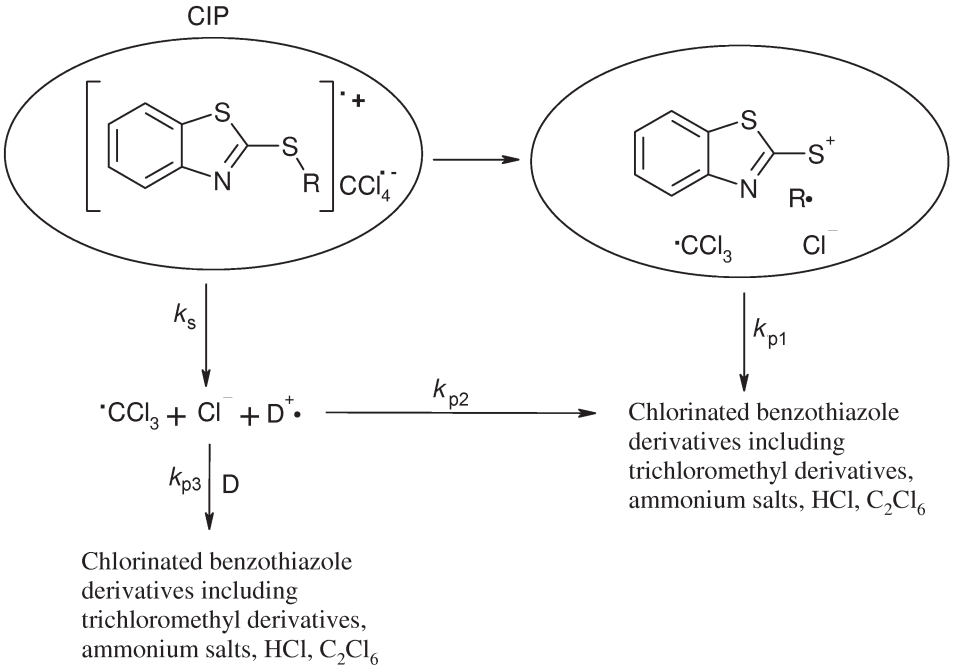


Table 1. IC_{50} Values Concerning Inhibition of Photosynthetic Electron Transport in Spinach Chloroplasts by the Mixture of Photodegradation Products of CBS

Time of irradiation	IC_{50}/(mg dm^{-3})	
h	Irradiation	Irradiation + ultrasound
0	19.7 ± 4.7	–
1	20.7 ± 3.5	34.9 ± 5.6
2.5	44.9 ± 5.0	41.3 ± 2.1
4.0	41.8 ± 1.9	20.4 ± 1.0
5.5	43.9 ± 3.7	34.5 ± 4.4
7.5	42.0 ± 4.1	32.7 ± 2.1

The ultrasound did not affect the rate of CBS loss. The decreased C_2Cl_6 content in the reaction mixture at the application of ultrasound can be explained by the degradation of the contact ion pair "CIP" by ultrasound.

Sonochemical degradation of this ion pair facilitated the formation of chlorinated products with different chlorine content inside of solvent cage. Such action of ultrasound resulted in decreased probability of the formation of Cl^- ions.

N-Cyclohexylbenzothiazol-2-sulfenamide and its photodegradation products inhibited photosynthetic electron transport in spinach chloroplasts. The effects of CBS and of the mixtures of its photodegradation products obtained after irradiation in the absence as well as with the application of ultrasound are shown in Table 1.

OER inhibition by photodegradation products of CBS obtained after 1 h irradiation was comparable with the effect of untreated CBS. The prolongation of the irradiation up to 2.5 h led to the decrease of inhibitory activity of CBS degradation products. However, the further prolongation of irradiation already did not affect the IC_{50} value, *i.e.* concentration of the compound causing 50 % inhibition of the studied parameter. These results indicate that with the prolongation of irradiation probably benzothiazoles characterized by lower aqueous solubility were uppermost.

The application of ultrasound caused the increase of the inhibitory activity of CBS photodegradation products obtained after 4.0 h, 5.5 h, and 7.5 h of irradiation with respect to the effects of compounds obtained at the same conditions without application of ultrasound.

These results indicated that the application of ultrasound contributed to the formation of more soluble CBS photodegradation products, which could more effectively penetrate through the aqueous regions of thylakoid membranes to their site of action.

1,5-Diphenylcarbazide (DPC) is an artificial electron donor acting in Z^+/D^+ intermediate [31]. By addition of DPC to chloroplasts inhibited by inhibitors of photosynthetic electron transport the supply of electrons to the primary electron donor of the photosystem (PS) 2 (P680) is secured. The complete restoration of the electron transport to PS 1 could be reached only in the case if the electron transport chain between the Z^+/D^+ intermediate and plastoquinone is not damaged. After addition of DPC to chloroplasts inhibited by the studied compounds (CBS and its photodegradation products) the OER was restored approximately up to 95 %. This indicates that these compounds did not interact with the constituents of

Table 2. Stimulation of Root and Shoot Growth of *Sinapis alba* L. by CBS and its Photodegradation Products

Time of irradiation / h	ρ / mg dm^{-3}	Irradiation		Irradiation + ultrasound	
		w_r (control ± SE)/%			
		Root	Shoot	Root	Shoot
0	148.5	129.3 ± 7.0***	111.1 ± 5.8*	–	–
	74.6	124.9 ± 6.3***	110.9 ± 5.1*	–	–
	15.0	110.7 ± 6.7	101.4 ± 5.6	–	–
	7.5	109.5 ± 7.2	100.6 ± 5.7	–	–
	1.5	102.9 ± 6.3	105.9 ± 5.5	–	–
	0	100.0 ± 6.1	100.0 ± 5.2	–	–
1.0	148.5	77.0 ± 5.3***	73.8 ± 6.6***	109.2 ± 6.5	107.1 ± 7.9
	74.6	84.8 ± 5.6*	84.1 ± 7.0*	120.5 ± 6.4***	108.6 ± 8.9
	15.0	96.8 ± 5.7	84.7 ± 7.0*	107.3 ± 6.5	100.8 ± 8.9
	7.5	104.8 ± 5.8	93.0 ± 7.7	105.2 ± 6.0	108.6 ± 8.7
	1.5	113.0 ± 6.1*	102.9 ± 7.4	104.5 ± 6.6	110.0 ± 9.6
2.5	148.5	103.6 ± 6.1	84.4 ± 6.8*	110.4 ± 7.9	95.0 ± 8.4
	74.6	128.4 ± 6.1***	90.6 ± 6.3	105.3 ± 8.4	105.0 ± 9.0
	15.0	122.5 ± 5.6**	91.7 ± 6.6	121.5 ± 7.6	103.2 ± 9.8
	7.5	111.6 ± 5.8*	107.4 ± 7.2	109.0 ± 6.5***	106.3 ± 9.0
	1.5	114.8 ± 6.2*	102.3 ± 7.7	97.3 ± 7.0	98.9 ± 10.7
4.0	148.5	140.6 ± 8.1***	108.3 ± 9.5	135.0 ± 7.4***	91.5 ± 7.7
	74.6	139.5 ± 8.5***	103.9 ± 8.5	125.0 ± 7.3***	88.7 ± 7.9
	15.0	126.4 ± 8.2***	106.5 ± 9.2	122.8 ± 6.6***	95.2 ± 7.1
	7.5	146.5 ± 8.3***	111.8 ± 9.4	136.9 ± 6.9***	95.0 ± 7.6
	1.5	134.2 ± 8.9***	115.0 ± 8.2**	93.6 ± 6.2	90.7 ± 7.4
5.5	148.5	135.4 ± 9.2***	105.1 ± 8.8	132.9 ± 7.3***	106.5 ± 9.5
	74.6	158.7 ± 9.4***	111.9 ± 8.6	143.4 ± 7.6***	98.7 ± 9.8
	15.0	165.3 ± 9.2***	109.6 ± 8.8	130.9 ± 6.7***	113.7 ± 9.1
	7.5	166.0 ± 9.3***	112.1 ± 9.1	123.1 ± 6.8***	116.0 ± 9.9*
	1.5	122.0 ± 9.0**	122.4 ± 9.3**	102.1 ± 2.1	95.5 ± 9.7
7.5	148.5	149.0 ± 9.4***	107.0 ± 9.4	134.9 ± 6.7***	118.2 ± 9.4*
	74.6	169.7 ± 9.6***	114.1 ± 9.6	146.0 ± 7.1***	114.8 ± 8.7*
	15.0	143.7 ± 9.4***	120.2 ± 9.7*	122.8 ± 6.5***	119.3 ± 9.2*
	7.5	143.7 ± 9.4***	116.6 ± 9.6*	135.7 ± 6.7***	129.7 ± 9.4**
	1.5	141.7 ± 8.9***	109.1 ± 8.6	103.7 ± 6.4	109.2 ± 9.5

Mean values ± SE, *significant differences at $P = 0.05$, **significant differences at $P = 0.01$, ***significant differences at $P = 0.001$; SE – standard error.

the electron transport chain between the Z^+/D^+ intermediate and plastoquinone. That means, it can be assumed that the site of action of CBS derivatives is situated on the donor side of PS 2, before DPC site of action.

The results of our previous EPR study with 2,6-disubstituted benzothiazole derivatives in the photosynthetic electron transport showed that these compounds did not affect the EPR signal II_{slow} belonging to the intermediate Tyr_D, *i.e.* tyrosine radical situated at the 161st position on the D_2 protein on the donor side of PS 2. Similarly, the signal $II_{very\ fast}$ belonging to the intermediate Tyr_Z (*i.e.* tyrosine radical situated at the 161st position in D_1 protein on the donor side of PS 2) did not change in the presence of these compounds. On the other hand, a great increase of the EPR signal belonging to PS 1 was observed indicating that the photosynthetic electron transport was interrupted and the reduction of PS 1 did not occur. Thus, it could be assumed that the benzothiazole derivatives caused interruption of the electron transport in the photosynthetic chain by interaction with the oxygen-evolving complex [18, 19].

In general it can be concluded that CBS and its photodegradation products exhibit plant-growth stimulating activity (Table 2). The prolongation of the irradiation contributed to the enhancement of the stimulating effects. The stimulation of root growth was more effective than the stimulation of the shoots. The root-growth stimulating effect of CBS photodegradation products showed an increase with the duration of irradiation and the most active root-growth promoters were products obtained after irradiation for 4.0 h, 5.0 h, and 7.5 h, respectively. The CBS photodegradation products undergoing simultaneous ultrasound treatment showed similar stimulating activity as those obtained in the absence of ultrasound. Based on the above-mentioned results it can be con-

cluded that CBS and its photodegradation products can be considered as synthetic phytohormones exhibiting mainly cytokinin-like effects. Cytokinins are compounds with a structure resembling adenine which promote cell division and have other similar functions to kinetin. Cytokinins applied in physiological concentrations stimulate cell division, morphogenesis in tissue culture, growth of lateral buds-release of apical dominance as well as leaf expansion resulting from cell enlargement [32]. High concentrations of phytohormones (natural as well as synthetic) inhibit plant growth whereas in relatively large range of low concentrations of these plant-growth regulating compounds stimulating effects were observed. Plant-growth stimulating effects were previously confirmed for many benzothiazole derivatives [20—22, 25—28].

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