Effect of Chromone-Substituted Benzothiazolium Halides on Photosynthetic Processes

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The effects of 3-R^2-2-[2-(6-R^1-chromon-3-yl)ethenyl]benzothiazolium halides (CBH) on photosynthetic electron transport in spinach chloroplasts and in the algal suspension of *Chlorella vulgaris* were investigated. Using EPR spectroscopy it was confirmed that these compounds containing in their molecules two heterocyclic skeletons, namely benzothiazole and chromone, interact with the intermediate D^+, corresponding to the tyrosine radical Tyr_D situated in D_2 protein on the donor side of photosystem 2. Consequently, higher concentrations of CBH inhibited oxygen evolution rate in *Chlorella vulgaris* and the inhibitory effectiveness depended on the lipophilicity of the compound.

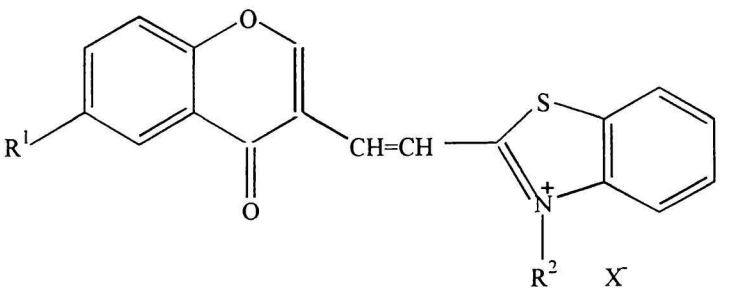
Benzothiazolium salts (BS) are biologically active compounds showing a wide spectrum of activity. Their antimicrobial [1, 2], plant-growth regulating [2], and antialgal [3, 4] effects have been reported previously. It has been shown that BS affect the growth and chlorophyll synthesis in *Euglena gracilis* [5] and *Chlorella vulgaris* [3]. The inhibitory effects of BS upon oxygen evolution rate in *Chlorella vulgaris* have been also confirmed [3, 4]. Using EPR spectroscopy it has been shown that the site of inhibitory action of 3-alkylcarbonylmethyl-substituted BS is the donor side of photosystem (PS) 2. These BS interact also with the water-splitting complex situated on the donor side of PS 2, which is reflected in the release of manganese ions into interior of thylakoid membranes [4]. BS with esteric substituent in position 2 or 3 applied in the range of lower compound concentrations stimulated chlorophyll production in *Chlorella vulgaris* [3, 4] and *Euglena gracilis* [5].

Several natural and synthetic chromone derivatives have been found to exhibit biological, *e.g.* antianaphylactic [6, 7], antiasthmatic [8], antiallergic [7, 9], antimicrobial [9, 10], and insect antifeedant activity [11]. Some natural and semisynthetic chromone alkaloids showed activity against human immunodeficiency virus (HIV) and herpes simplex virus [12]. Photosynthesis-inhibiting activity of the condensation products of 6-R^1-3-formylchromone with 4-aminosalicylic acid and of the adducts of 6-R^1-3-formylchromone with alcohols and aminosalicylic acids has been also investigated and it was found that these compounds interact with the intermediates Z^+/D^+ which are situated in D_1 and D_2 proteins on the donor side of PS 2 [13].

EXPERIMENTAL

The studied 3-R^2-2-[2-(6-R^1-chromon-3-yl)ethenyl]benzothiazolium halides (CBH) (Formula 1) were synthesized by condensation reaction of benzothiazolium salts with 3-formylchromones by heating in acetonitrile [14, 15]. The samples were submitted to elemental analysis and the differences between the calculated and found values were within 0.3 % (for C, H, and N) and 0.5 % (for S, Br, and Cl), respectively. The structures of the studied compounds were verified by ^{1}H NMR spectra. The logarithms of partition coefficients of the studied compounds (log *P*) were calculated by the Crippen method [16].

The oxygen evolution rate (OER) in algal suspensions (*Chlorella vulgaris*) was measured at 24 °C by a Clark-type electrode (SOPS 31 atp, Chemoprojekt, Prague) in a chamber constructed according to *Bartoš et al.* [17]. Prior to the OER measurements the suspensions were exposed in the dark (4 h). The samples were then illuminated with a 250 W halogen lamp through a water filter (0.05 m width) from 0.3 m distance. The irradiance of the sample was $\approx$ 0.45 mmol m^{-2} s^{-1} PAR. The composition of the algal cultivation medium was as follows: 20 mmol KNO_3, 2.5 mmol KH_2PO_4, 4.0 mmol $MgSO_4$ $7H_2O$, 7.0 μmol $CaCl_2$ $6H_2O$, 5.0 μmol $CuSO_4 \cdot 5H_2O$, 34.6 μmol $FeSO_4$, 34.6 μmol Na_2EDTA, 50 μmol H_3BO_3, 5.0 μmol $ZnSO_4$ $7H_2O$, 5.0 μmol $MnCl_2 \cdot 4H_2O$, 5.0 μmol $CoCl_2 \cdot 6H_2O$,



	R^2	R^1	X
I	CH_3	Cl	I
II	C_2H_5	H	I
III	$CH_2—C_6H_5$	H	Br
IV	$CH_2—C_6H_5—4\text{-}NO_2$	H	Cl
V	$CH_2—CH=CH_2$	Cl	Br
VI	$CH_2—C\equiv CH$	H	Br
VII	$CH_2—C\equiv CH$	CH_3	Br
VIII	C_8H_{17}	H	I

	R^2	R^1	X
IX	C_8H_{17}	Cl	I
X	$CH_2CH_2—C_6H_5$	H	Br
XI	$CH_2CH_2—C_6H_5$	Br	Br
XII	$CH_2COOC_2H_5$	Cl	Cl
XIII	$CH_2CH_2CH_2CH_2COOCH_3$	Cl	Br
XIV	$CH_2CH_2CH_2COOC_2H_5$	Cl	Br
XV	$C_2H_5COO—CH—COOC_2H_5$	Cl	Br

Formula 1

and 1.5 μmol $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 1 dm^3 of solution (pH = 7.2). The chlorophyll (Chl) content in the samples was 20 mg dm^{-3} The inhibitory activity of the compounds has been expressed by IC_{50} values, *i.e.* by concentration of the inhibitor causing a 50 % OER decrease with respect to the untreated control sample.

Spinach chloroplasts applied for the study of CBH effects upon photosynthetic centres were prepared by a partly modified procedure of current preparation methods described by *Walker* [18] using TRIS buffer (20 mmol dm^{-3}, pH 7.0) containing 0.4 mol dm^{-3} saccharose and 0.2 mmol dm^{-3} $MgCl_2$ (for details see *Šeršeň et al.* [19]).

The effects of the studied compounds on photosynthetic centres of chloroplasts were investigated by studying of fluorescence of chlorophyll *a* using excitation wavelength λ_{ex} = 436 nm. The measurements were carried out with a fluorescence spectrophotometer F-2000 (Hitachi, Japan) at room temperature, using excitation and emission slits of 10 nm. The Chl content in the samples was 10 mg dm^{-3} The chloroplast suspensions used for the study of Chl_a fluorescence were exposed in the dark for 10 min prior to the measurements.

EPR measurements were carried out with the instrument ERS 230 (WG, Akademie der Wissenschaften, Berlin, Germany) operating in X-band at 5 mW of microwave power and 0.5 mT modulation amplitude. EPR spectra of untreated spinach chloroplasts and in the presence of studied compounds (0.05 mol dm^{-3}) were recorded in the dark and on the light. The Chl content in the samples was 4 g dm^{-3} The samples were irradiated directly in the resonator cavity with a 250 W halogen lamp from 0.5 m distance through a water filter in order to avoid warming of the samples. The irradiance of the samples was $\approx$ 0.35 mmol m^{-2} s^{-1} PAR.

Due to a lower aqueous solubility of the studied compounds these were dissolved in dimethyl sulfoxide. The effect of dimethyl sulfoxide on OER in the algal suspensions and on EPR and fluorescence spectra was in the range of experimental error and it could be neglected.

RESULTS AND DISCUSSION

The studied CBH inhibited oxygen evolution rate (OER) in the algal suspension of *Chlorella vulgaris* and their OER-inhibiting activity expressed by IC_{50} values varied in the range of 36 (*VIII*)—229 μmol dm^{-3} (*IV*). Fig. 1 shows the dependence of OER on the partition coefficient (log *P*) of CBH. From Fig. 1 it is evident that the inhibitory activity significantly depends on the lipophilicity of the studied compounds. In the range of $5.89 > \log P > 3.38$ an increase of the inhibitory activity with increasing lipophilicity of the compound can be observed. However, further increase of lipophilicity ($\log P > 5.89$) leads to dramatic activity decrease. No pronounced effect of the anion X^- (Cl^-, Br^- or I^-) was found. Decreased inhibitory effectiveness of the compounds with lower lipophilicity can be connected with the fact that their passage through the lipophilic regions of thylakoid membranes is limited. This results in an insufficient number of inhibitors reaching the site of action in proteins situated on the inner side of thylakoid membrane. On the other hand, the activity decrease of more lipophilic compounds ($\log P > 5.89$) is probably connected with the too high lipophilicity of these compounds caus-

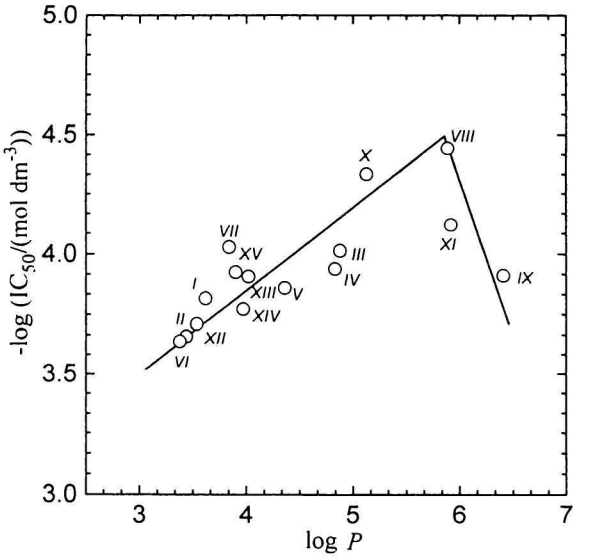


Fig. 1. The dependence of OER inhibition in *Chlorella vulgaris* on the lipophilicity of the studied compounds (P – partition coefficient).

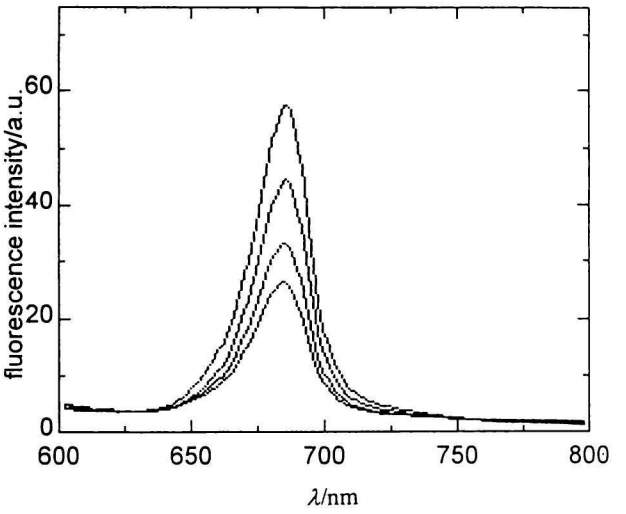


Fig. 2. Fluorescence emission spectra of untreated spinach chloroplasts and in the presence of $c(VIII)/(\mu\text{mol dm}^{-3})$: 2.0, 10.0, and 20.0 (curves from top to bottom) (λ_{ex} = 436 nm).

ing limited penetrability through the hydrophilic regions of thylakoid membranes. The most potent inhibitor of the studied set was compound *VIII* with $R^2 = C_8H_{17}$ (IC_{50} = 36 μmol dm^{-3}; log P = 5.89). Inhibition of OER in algal suspensions of *Chlorella vulgaris* has been found also at high concentrations of 3-alkylcarbonylmethyl-substituted BS [4].

The molecule of CBH consists of two biologically active heterocyclic skeletons, benzothiazole and chromone. The site and mechanism of action of several inhibitors of photosynthesis derived from these two heterocyclic compounds have been investigated previously by EPR and emission fluorescence spectroscopies [4, 13]. Both spectroscopic methods were chosen also for the study of the site of action of CBH. The experiments were carried out with partially broken spinach chloroplasts at the same conditions as applied in previous experiments with 3-alkylcarbonylmethyl-substituted BS [4] and with derivatives of 3-formylchromone [13].

The effect of CBH on the photosynthetic centres of chloroplasts was investigated by studying the Chl_a fluorescence. In the presence of CBH the intensity of the fluorescence emission band at 684 nm, belonging mainly to the pigment—protein complex in PS 2 [20], showed a decrease with the increasing compound concentration (Fig. 2), suggesting PS 2 as the site of action of the studied compounds.

The paramagnetic constituents occurring in the chloroplasts were investigated by EPR spectroscopy. The chloroplasts of higher plants exhibit in the region of free radicals ($g \approx 2.00$) EPR signals, the so-called signal I and signal II, belonging to the photosynthetic centres PS 1 and PS 2, respectively. The signal II consists of two components, namely of the signal $II_{very\ fast}$

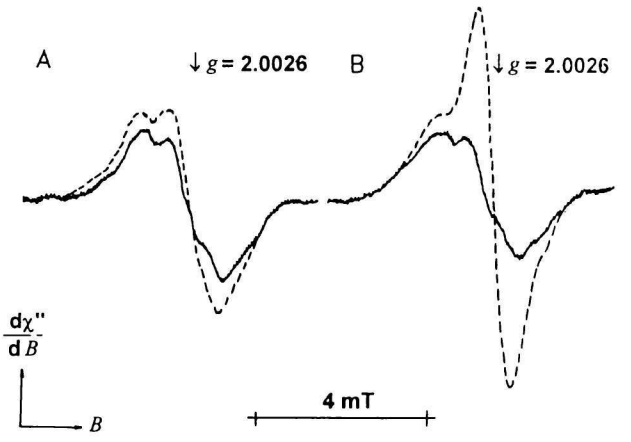


Fig. 3. EPR spectra of untreated spinach chloroplasts (A) and of chloroplasts treated with $c(VIII)$ = 0.05 mol dm^{-3} (B). The full lines correspond to chloroplasts kept in the dark, the dashed lines to the illuminated chloroplasts.

and the signal II_{slow}, belonging to the intermediates Z^+/D^+ (*i.e.* tyrosine radicals Tyr_Z and Tyr_D which are present at 161 position in D_1 and D_2 proteins) on the donor side of PS 2 [21—23].

EPR spectra of spinach chloroplasts without (Fig. 3A) and in the presence of compound *VIII* (Fig. 3B) were measured in the dark (full line) and on the light (dashed line). It is evident that in the presence of compound *VIII* a decrease of the intensity of EPR signal II_{slow} (Fig. 3B, full line) can be observed. Thus, it can be concluded that this compound interacts with the intermediate D^+ (Tyr_D) which is present in D_2 protein on the donor side of PS 2. Although at present the function of the intermediate D^+ is not exactly known, some evidences show that this interme-

diate is involved in the oxidation-reduction processes occurring in the manganese cluster and at certain circumstances it can interact with the core of PS 2 (P680), too [24, 25]. Therefore we suggest that the interaction of CBH with the intermediate D^+ can lead to restriction or interruption of the photosynthetic electron transport between PS 2 and PS 1. This damage of the electron transport results in a pronounced increase of signal I intensity in the light (Fig. 3B, dashed line) due to restricted reduction of the oxidized core of PS 1 ($P700^+$).

Summarizing it can be concluded that the site of CBH action partially differs from the site of inhibitory action of previously investigated 3-alkylcarbonylmethyl-substituted BS and also from that of 3-formylchromone derivatives (the condensation products of 6-R^1-3-formylchromone with 4-aminosalicylic acid and the adducts of 6-R^1-3-formylchromone with alcohols and aminosalicylic acids). It was shown that the site of inhibitory action of the former compounds are the intermediates Z^+/D^+ and the water-splitting complex, namely its manganese cluster [4]. The latter compounds interact only with the intermediates Z^+/D^+, however they do not damage the own core of PS 2 (P680) [13]. It can be concluded that similarly to the sites of action of the above-mentioned BS and chromone derivatives, the site of action of the studied CBH is also located on the donor side of PS 2, however it is limited only to the intermediate D^+ Thus, the CBH do not interact with the intermediate Z^+ and the water-splitting complex (the release of manganese ions was not observed).

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