Effects of 2-Alkylthio-6-aminobenzothiazoles and their 6-N-Substituted Derivatives on Photosynthesis Inhibition in *Chlorella vulgaris* and Spinach Chloroplasts

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Abstract. 2-Alkylthio-6-aminobenzothiazoles and their 6-N-substituted derivatives 3-(2-alkylthio-6-benzothiazolylaminomethyl)-2-benzothiazolinethiones and 3-(2-alkylthio-6-benzothiazolylaminomethyl)-6-bromo-2-benzothiazolinones inhibit photosynthetic processes in spinach chloroplasts and the chlorophyll production in *Chlorella vulgaris*. The inhibitory activity depends on the alkyl chain length of the thioalkyl substituent. The site of action of the effectors studied is on the donor side of photosystem 2 before the site of action of diphenylcarbazide. The highest antialgal effects were exhibited by compounds containing bromine.

Key words: Photosynthesis inhibition — Spinach chloroplasts — Benzothiazoles — *Chlorella vulgaris*

Introduction

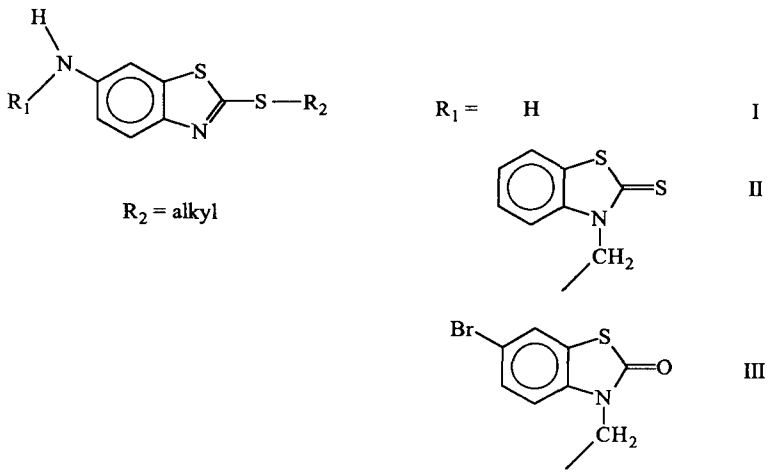
Benzothiazole derivatives show a wide variety of biological effects, including antimicrobial (Sidóová et al. 1979; Holbová et al. 1990), antimycobacterial (Sidóová and Odlerová 1985 and 1990; Sidóová et al. 1979; Holbová et al. 1976), plant growth regulating (Sidóová et al. 1992a, b), antialgal (Sidóová et al. 1992b; Kráľová et al. 1992a) and also anthelminthic (Sidóová et al. 1985). Recently, the inhibitory effects of 2-alkylthio-6-R-benzothiazoles on chlorophyll synthesis in *Chlorella vulgaris* as well as on oxygen evolution rate in plant chloroplasts have been shown to depend on the alkyl chain length of the 2-alkylthio substituent; the lipophilicity of the whole molecule plays an important role with respect to the intensity of this effect in individual derivatives (Kráľová et al. 1992a).

The present work was aimed at investigating the effect of three series, namely 6-amino-2-alkylthiobenzothiazoles and their 6-N-substituted derivatives, with re-

gard to their inhibitory activities concerning photosynthetic processes in algae and plant chloroplasts.

Materials and Methods

The synthesis of the studied compounds 2-alkylthio-6-aminobenzothiazoles (I) (alkyl = = C_2H_5 - C_7H_{15} and C_9H_{19}), 3-(2-alkylthio-6-benzothiazolylaminomethyl)-2-benzothiazolinethiones (II) (alkyl = C_2H_5 – C_9H_{19}) and 3-(2-alkylthio-6-benzothiazolylaminomethyl)-6-bromo-2-benzothiazolinones (III) (alkyl = C_3H_7 – C_8H_{17}) have been described elsewhere (Sidóová et al. 1979; 1992c (I, II), Holbová et al. (1990) (III)). The synthesis of the other benzothiazole compounds studied, namely 2-benzothiazolinone and its 6-bromo-, 6-nitro- and 6-acetamidoderivatives, have been described by Sidóová et al. (1992a, b).



scheme I

The effects of the compounds studied (see Scheme I) on chlorophyll production in stationary cultured *Chlorella vulgaris* (7 days, 16 h light / 8 h dark) were investigated according to Sidóová et al. (1992a). Because of a low solubility of the compounds studied in water, these were dissolved in N,N-dimethylformamide (DMF); the DMF content in the control samples was the same as in those containing benzothiazoles. The effects of the compounds studied on the rate of oxygen evolution in spinach chloroplasts were investigated according to Kráľová et al. (1992b). The inhibition of chlorophyll synthesis in algae as well as photosynthesis inhibition in plant chloroplasts were expressed as pI_{50} values corresponding to the negative logarithm of the respective concentration causing 50% inhibition as compared to the control. The decrease in photosynthetic activity due to the presence of DMF was taken into account.

EPR spectra of spinach chloroplasts (chlorophyll content: 4 $g.dm^{-3}$) in the dark and in the light without and in the presence of the studied compounds (0.05 $mol.dm^{-3}$) were recorded using an ERS 230 apparatus (WG, Akad. Wiss., Berlin, Germany) operating in X-band at 5 mW of microwave power. The compounds studied were added to chloroplast

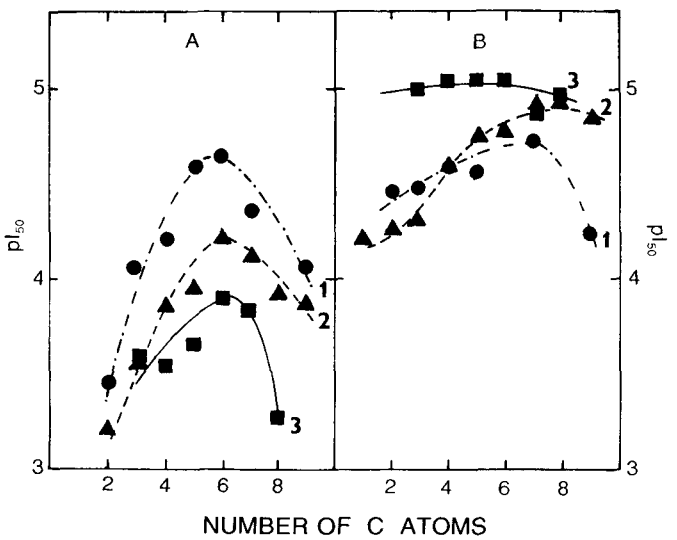


Figure 1. The dependence of pI_{50} values of the studied compounds concerning inhibition of oxygen evolution rate in spinach chloroplasts (A) as well as inhibition of chlorophyll production in *Chlorella vulgaris* (B) on the number of carbon atoms in the alkylthio substituents (1: 2-alkylthio-6-aminobenzothiazoles; 2: 3-(2-alkylthio-6-benzothiazolylaminomethyl)-2-benzothiazolinethiones; 3: 3-(2-alkylthio-6-benzothiazolylaminomethyl)-6-bromo-2-benzothiazolinones).

suspensions dissolved in dimethyl sulfoxide. The irradiation of the samples was carried out in the resonant cavity by an halogen lamp (250 W).

Results and Discussion

The dependences of pI_{50} values of the compounds studied on the number of carbon atoms in the alkylthio substituent are presented in Fig. 1*A* (pI_{50} values for oxygen evolution rate in spinach chloroplasts), and in Fig. 1*B* (the corresponding values for chlorophyll synthesis in *Chlorella vulgaris*). The most intensive increase of the inhibition of photosynthetic activity of spinach chloroplasts with the increasing length of the alkyl chain showed the derivatives of the homologous series I. 6-N-substitution of derivatives from series I decreases the inhibitory activity of the substituted effectors (see the corresponding pI_{50} values for compounds of series II and III and compare them to those of series I). The less active derivatives are those from series III, i.e. compounds containing bromine in the 6-N-substituent. The decrease of the inhibitory activity at heptyl, octyl and nonyl derivatives of series I and II, as well as of the octyl derivative of series III, is probably connected with the too high lipophilicity of these compounds causing a limited penetrability through

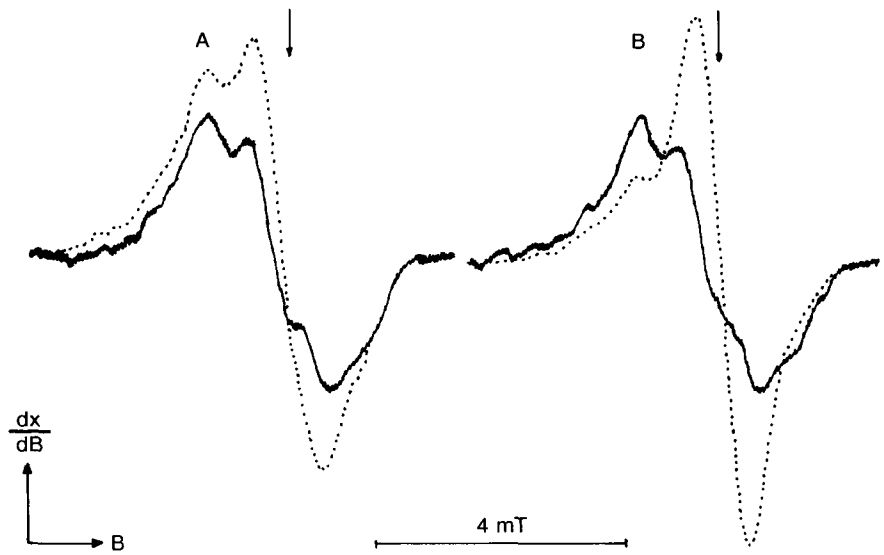


Figure 2. EPR spectra of untreated spinach chloroplasts (lines A) and chloroplasts treated with 0.05 mol.dm^{-3} 6-amino-2-hexylthiobenzothiazole (lines B); spectra recorded in the dark (solid lines) and in the light (dotted lines). The dotted line in B was recorded at 0.5 amplification. The arrows correspond to the g factor value of 2.0026.

the hydrophilic regions of thylakoid membranes (Baláž et al.1988).

The compounds of all the three series investigated showed a relatively high antialgal efficiency. Their inhibitory activity concerning chlorophyll production in *Chlorella vulgaris* increased with the increasing alkyl chain length in series I and II, whereas with derivatives of series III it remained practically unchanged. In contrast to the efficiency sequence determined for the series studied with chloroplasts (Fig. 1*A*), the antialgal effects showed an opposite sequence, and the most active inhibitors were compounds containing bromine in the 6-N substituent (Fig. 1*B*, curve 3). The increase of the antialgal activity of benzothiazole derivatives with the bromine atom in the molecule was confirmed with 2-benzothiazolinone and some of its 6-substituted derivatives. At 10^{-4} mol.dm^{-3} the inhibition of chlorophyll production in *Chlorella vulgaris* was 12% with 2-benzothiazolinone and only 5.4 or 5.8% with its 6-acetamido and 6-nitro derivatives, the corresponding inhibition produced by 6-bromo-2-benzothiazolinone was 74% (Sidóová et al. 1992b). The relatively strong antialgal activity of the studied derivatives of series III (i.e. of the compounds containing bromine in their molecules) practically does not depend on the alkyl chain length of the alkylthio substituent.

Comparing EPR spectra of spinach chloroplasts treated with the compounds

studied with those of untreated chloroplasts in the dark, insignificant changes of signal II_{slow} (belonging to the intermediate D^+ tyrosine 160 of the D_2 polypeptide in photosystem (PS) II (Barry and Babcock 1987, 1988; Noren et al. 1991; Noren and Barry 1992) could be observed (the solid lines in Figs. 2*B* and 2*A*). However, a great rise of the EPR signal intensity of PS I occurs in EPR spectra of treated chloroplasts under irradiation (Fig. 2*B*, dotted line). This indicates that the electron transport in the photosynthetic process is perturbated, and so no reduction of PS I can take place (Hoff 1979, 1987). Signal $II_{very\ fast}$ observable under irradiation which belongs to the intermediate Z^+ tyrosine 161 of the D_1 polypeptide in PS II (Barry and Babcock 1987, 1988; Noren et al. 1991; Noren and Barry 1992) remains practically unchanged (see dotted lines in Figs. 2*A* and 2*B*). To specify the site of action of the compounds studied, i.e. the site of the interruption of the electron flow to PS I, an experiment was designed with the artificial electron donor of PS II diphenylcarbazide (DPC). DPC is able to supply electrons to the donor side of PS II (Hauska 1977; Izawa 1980) and it can practically completely restore the electron transport between photosynthetic centres in spinach chloroplasts treated with the studied compounds (e.g. inhibition of DCPIP reduction caused by 137 μmol.dm^{-3} of 6-amino-2-heptylthiobenzothiazole - up to 85% with respect to the control sample - was restored up to 94% after addition of 2 mmol.dm^{-3} DPC). Therefore, it can be assumed that the site of action of the studied compounds is the donor side of PS II, before the site of DPC action.

Assuming the following scheme for the electron transport chain in photosynthesis

$$H_2O \xrightarrow{e^-} OEC \rightarrow Z/D \rightarrow P_{680} \rightarrow Pheo \rightarrow Q_A \rightarrow Q_B \rightarrow PQ \rightarrow Cyt_b \rightarrow PC \rightarrow P_{700} \rightarrow$$

where OEC is the oxygen evolving complex, Z is the intermediate in polypeptide D_1 (tyrosine 161), D is the intermediate in polypeptide D_2 (tyrosine 160), P_{680} is the core of PS II, Pheo is pheophytin, Q are quinone molecules, PQ is the plastoquinone pool, Cyt_b is cytochrome b, PC is plastocyanine, and P_{700} is the core of PS I, the site of the DPC action according to Jegerschöld and Styring (1991) is in Z/D. The suggestion that the site of action of the studied compounds is OEC could be confirmed by the study of S states of OEC. It seems, however, that the studied compounds exhibit a specific action upon OEC, and they might serve as modell compounds for the study of photosynthetic processes taking place in OEC. An electron donor-acceptor complex formation between the compounds studied and OEC can be assumed as the probable mechanism of action of these effectors characterized by good electron donor properties. A more precise determination of their site as well as mechanism of action requires further and more detailed studies.

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