EFFECT OF 2-ALKYLTHIO-4-PYRIDINECARBOTHIAMIDES ON PHOTOSYNTHETIC ELECTRON TRANSPORT IN SPINACH CHLOROPLASTS

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2-Alkylthio-4-pyridinecarbothiamides (**1**) inhibit photosynthetic electron transport in spinach chloroplasts. The dependence of photosynthesis-inhibiting activity upon the lipophilicity of 2-alkylthio substituent shows quasi-parabolic course with maximum effectiveness at 2-octyl derivative. The site of action of **1** is the intermediate D^+, corresponding to tyrosine radical, which is situated in D_2 protein on the donor side of photosystem **2**.

Key words: 2-Alkylthio-4-pyridinecarbothiamides; Photosynthesis inhibition; Photosynthetic centres.

Several heterocyclic compounds with alkylthio substituent are known to be biologically active agents. 2-Alkylthio-3-alkylbenzothiazolium salts show concentration-dependent plant growth-regulating activity, *i.e.* stimulating at low and inhibitory at relatively high effector concentrations[1]. The 6-(*R*)-substituted 2-alkylthio- and 2-alkylcarbonylmethylthiobenzothiazoles exhibit antifungal[2,3], antiyeast[3] and antimycobacterial[4] activity and they inhibit also photosynthetic processes in *Chlorella vulgaris* and spinach chloroplasts[3,5–8]. The 2-alkylthio-4-pyridinecarbothiamides (**1**), similarly to the above mentioned effectors, show also antifungal[9,10] and antimycobacterial[11] activity. The dependence of biological activities of the above mentioned alkylthio substituted heterocyclic effectors upon the alkyl chain length of alkylthio substituent shows a quasi-parabolic course[9,10]. However, the dependence of antimycobacterial activity of **1** against *Mycobacterium tuberculosis* upon the alkyl chain length of the 2-alkylthio substituent shows two maxima of the highest activity what can be connected with the presence of two pharmacophore groups – the alkylthio group and thioamide group – in the molecules of these compounds[11].

A great variety of experimental methods, *e.g.* UV-VIS, fluorescence, electron paramagnetic resonance (EPR) and other spectroscopic techniques, measurements of oxygen evolution rate in photosynthesizing organisms as well as the use of various

artificial electron donors and acceptors with known site of action can be applied for the study of photosynthetic apparatus and photochemical processes.

The EPR spectroscopy is an experimental method suitable for studying the photosynthetic apparatus because the chloroplasts of higher green plants exhibit EPR signals belonging to both photosystems (PS) (so called signal I and signal II) in the region of free radicals[12] ($g \approx 2.00$). Signal I belongs to the chlorophyll dimer in the core of PS 1 (P 700) (ref.[12]). Signal II consists from two constituents belonging to the intermediates Z^+/D^+, which are situated on the donor side of PS 2 and secure the electron transfer from the oxygen evolving complex to the core of PS 2 (P 680). It was found that the signal II_{slow} which is stable in the dark during several hours belongs to the intermediate D^+ corresponding to tyrosine 160 (Y_D^+) which is situated in D_2 protein[13–16]. The second part of signal II, so called signal $II_{very\ fast}$, which is present in EPR spectra of illuminated chloroplasts, belongs to the intermediate Z^+. This intermediate corresponds to tyrosine 161 (Y_Z^+) which is located in D_1 protein[13–15].

The aim of this work is the study of the effect of **1** on photochemical activity of spinach chloroplasts and the determination of the site and mechanism of their action in photosynthetic electron transport.

EXPERIMENTAL

The compounds studied were prepared according to ref.[9]. The chemicals used for phosphate buffer preparation $Na_2HPO_4 . 12\ H_2O$, KH_2PO_4, $MgCl_2 . 6\ H_2O$, NaCl as well as saccharose, sodium salt of 2,6-dichlorophenolindophenol (DCPIP), and dimethyl sulfoxide (DMSO) were obtained from Lachema, Brno, 1,5-diphenylcarbazide (DPC) was a product of Sigma. All applied chemicals were of analytical grade.

Oxygen evolution rate in spinach chloroplasts in the presence of electron acceptor DCPIP was determined according to the method described in ref.[17]. The compounds were dissolved in DMSO because of their too low aqueous solubility. The applied DMSO concentration did not affect oxygen evolution rate.

The emission fluorescence spectra were recorded by a fluorescence spectrophotometer F-2000 (Hitachi, Tokyo) at room temperature (24 °C). The samples of chloroplast suspension (10 μg chlorophyll dm^{-3}) were excited at 436 nm using s slit width of 10 nm and they were kept in the dark 10 min prior to the measurements.

The electron paramagnetic resonance spectra were registered by an ERS 230 apparatus (ZWG AdW, Berlin) which operates in the X-band at 24 °C. The chlorophyll content in the chloroplast suspension was about 4 g dm^{-3}, the used microwave power 5 mW, the modulation amplitude $5 . 10^{-4}$ T. The samples were illuminated directly in the resonator cavity with a 250 W halogen lamp and they were protected against warming by a water filter.

RESULTS AND DISCUSSION

The IC_{50} values, *i.e.* molar concentrations of the effectors causing a 50% decrease of the oxygen evolution rate in the suspension of spinach chloroplasts, vary for the investigated set of **1** in the range from 72 μmol dm^{-3} (for octyl) to 6.24 mmol dm^{-3} (for

methyl derivative). The logarithms of $1/IC_{50}$ values and the corresponding values of hydrophobic fragment constants f[18] for the alkyl substituents are summarized in Table I. It is evident that the dependence of the photosynthesis-inhibiting activity of **1** upon the lipophilicity of the alkyl substituent shows quasi-parabolic course. The corresponding correlation can be expressed by the following equation:

$$\log (1/IC_{50}) = -0.1190\ (\pm 0.0111) f^2 + 1.1064\ (\pm 0.0852) f + 1.5039\ (\pm 0.1450)$$

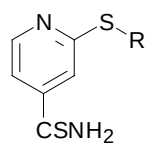
$$r = 0.9807;\ s = 0.1202;\ F = 113.1;\ n = 12;\ f_{opt} = 4.6485\ . \qquad (1)$$

The results of statistical analysis confirm that the Hansch's parabolic model is suitable for description of the correlation between photosynthesis-inhibiting activity and lipophilicity of **1**. Similar results have been obtained also for the dependence of the biological activity of 2-alkylthio- and 2-alkylcarbonylmethylthiobenzothiazoles[2–8] and antifungal activity of **1** (refs[9,10]) upon lipophilicity of the effectors.

The effects of **1** on the photosynthetic centres of chloroplasts were investigated by studying chlorophyll *a* fluorescence. The decreased intensity of the emission band at 686 nm, belonging to the pigment–protein complexes in photosystem 2 (ref.[19]) (Fig. 1) suggested PS 2 as the site of action of the studied effectors.

TABLE I

Logarithms of $1/IC_{50}$ values of 2-alkylthio-4-pyridinecarbothiamides and corresponding values of hydrophobic fragment constants (f) of the alkyl substituent. (IC_{50} values correspond to the molar concentrations of effectors causing a 50% decrease of oxygen evolution rate in spinach chloroplasts.) The values of f were taken from ref.[18]



R	log ($1/IC_{50}$)	f	R	log ($1/IC_{50}$)	f
Methyl	2.2045	0.77	Cyclohexyl	3.9854	3.63
Ethyl	3.1060	1.43	Hexyl	3.9326	3.57
Propyl	3.1698	1.97	Heptyl	4.0016	4.10
Butyl	3.3497	2.51	Octyl	4.1456	4.63
Isobutyl	3.3930	2.38	Decyl	3.9908	5.63
Pentyl	3.8540	3.10	Dodecyl	3.5156	6.75

For more precise determination of the site of action of **1** in the photosynthetic apparatus of spinach chloroplasts EPR spectroscopy has been used. Figure 2 presents EPR spectra of the untreated chloroplast suspension as well as that in the presence of **1** in the dark and in the light. Practically the whole EPR signal at $g = 2.0046$ and line width $\Delta B \approx 2$ mT belongs to signal II_{slow} (Fig. 2a, full line). EPR signal induced by light practically corresponds to signal $II_{very\ fast}$ (Fig. 2a, the difference of the signal intensity in the light and in the dark). From Fig. 2b it is evident that the intensity of EPR signal II, mainly the intensity of its constituent signal II_{slow}, has been decreased by the studied compounds. That means that the studied compounds interact with D^+ intermediate. Due to the interaction of **1** with this part of PS 2, the photosynthetic electron transport between PS 2 and PS 1 is impaired and consequently a pronounced increase of signal I intensity in the light

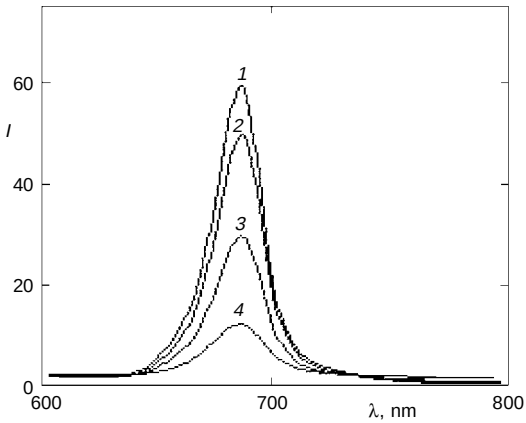


FIG. 1
Fluorescence spectra of untreated spinach chloroplasts (*1*) and of chloroplasts treated with 8.5 (*2*), 42.0 (*3*), and 85.0 (*4*) μmol dm^{-3} of 2-hexylthio-4-pyridinecarbothiamide

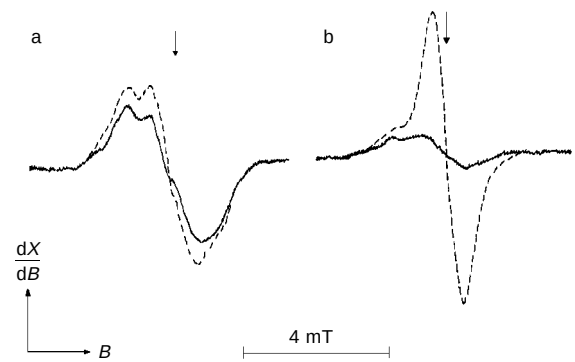


FIG. 2
EPR spectra of untreated spinach chloroplasts (a) and of chloroplasts treated with 0.05 mol dm^{-3} of 2-butylthio-4-pyridinecarbothiamide (b). The full lines correspond to chloroplasts kept in the dark, the dashed lines to the illuminated chloroplasts

(Fig. 2b, dashed line; g = 2.0026, ΔB = 0.7 mT) belonging to the cation-radical of the chlorophyll dimer in the core of PS 1 can be observed.

The DPC is an artificial electron donor of PS 2 with known site of action in the intermediate Z^+/D^+ on the donor side of PS 2. Consequently, it can restore the photosynthetic electron transport inhibited by such effectors which do not impair the own core of PS 2. Upon addition of DPC (0.5 mmol dm^{-3}) to chloroplasts inhibited by the studied compounds the oxygen evolution rate was practically completely restored. Since the site of action of this artificial donor of PS 2 is the intermediate Z^+/D^+ (ref.[20]), it can be assumed that in the presence of the studied effectors the own core of PS 2 (P 680) and a part of the electron transport chain – at least up to plastoquinone – remain intact.

The site of action of the studied 2-alkylthio-4-pyridinecarbothiamides, *i.e.* the intermediate D^+, differs from that of 2-alkylthio and 2-alkylcarbonylmethylthio substituted benzothiazole derivatives which interact with the oxygen evolving complex on the donor side of PS 2 (refs[6,7]).

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