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Effect of 3-formylchromones substituted with 4-aminosalicylic acid and some other aniline derivatives on photosynthesis inhibition in spinach chloroplasts

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Abstract

The inhibitory effect was investigated of 16 different 3-formylchromone derivatives, the condensation products of 6-R^1-3-formylchromone with 4-aminosalicylic acid and of the adducts of 6-R^1-3-formylchromone with *n*-alcohols and aminosalicylic acids or some other derivatives of aniline, on the photochemical activity of spinach chloroplasts. The inhibitory activity of the compounds studied correlated with the lipophilicity of the R^1 and R^2 (alkoxy) substituents. Using fluorescence study it was found that the site of action of the studied effectors is photosystem (PS) 2. By EPR spectroscopy it was confirmed that the studied effectors interact with the intermediates Z^+/Y^+ which are situated in D 1 and D 2 proteins on the donor side of PS 2 which is reflected in a partial decrease in the photosynthetic electron flow through PS 2 to PS 1. It was found that the core of PS 2 is not damaged.

Additional key words: EPR spectra, fluorescence emission, 3-formylchromone, *Spinacia oleracea*.

Introduction

Natural and synthetic chromone derivatives exhibit a wide spectrum of biological activity, *e.g.* antianaphylactic (Klutchko *et al.* 1979), antiasthmatic (Nohara *et al.* 1978), antialergic (Klutchko *et al.* 1977, Nohara *et al.* 1978), and antimicrobial (Abdelhafez *et al.* 1993, Lácová *et al.* 1995). According to Houghton *et al.* (1994), some natural and semi-synthetic chromone alkaloids show activity against human

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Abbreviations: Chl - chlorophyll; DPC - diphenylcarbazide; DPIP - dichlorophenol-indophenol; EPR - electron paramagnetic resonance; IC_{50} - molar effector concentration causing 50 % inhibition of oxygen evolution rate in spinach chloroplasts; OER - oxygen evolution rate; PAS - 4-aminosalicylic acid; PS - photosystem.

immunodeficiency virus (HIV) and herpes simplex virus. The presence of a piperidine ring and unsaturated hydroxy groups on the molecules enhanced anti-HIV activity which was considered to be due to irreversible binding to glycoprotein 120. Luthria *et al.* (1993) investigated insect antifeedant activity of chromone derivatives and found that maximum activity exhibited furochromones with an alkoxy substituent at C-4 or C-9. Substitution of C-7 methyl of the γ-pyrone ring and dealkylation of C-4 or C-9 methoxyl caused considerable reduction in feeding deterrent activity.

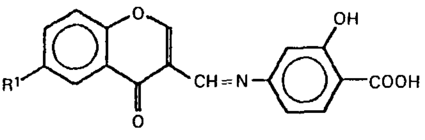
One of the most well-known biologically active aniline derivatives is the antituberculotic drug 4-aminosalicylic acid (Lehmann 1946, Korolkovas and Burckhalter 1976). From the viewpoint of the synthesis of potential biologically active effectors exhibiting a wide spectrum of effects, molecules consisting of two or more biologically active skeletons present a subject of great interest.

The aim of this paper is to investigate the effect of selected 3-formylchromones substituted with 4-aminosalicylic acid and some other aniline derivatives on photochemical activity of spinach chloroplasts.

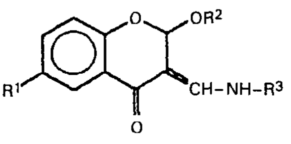
Materials and methods

The compounds studied (formulas A and B) were synthesized according to Lácová *et al.* (1995). They were condensation products of 4-aminosalicylic acid (PAS) with 6-R^1-3-formylchromone [R^1 = H (1), Cl(2), Br (3)] (formula A), adducts of 6-R^1-3-formylchromone with PAS and ethanol [R^1 = H (4), CH_3 (5), Cl (6), Br (7); R^2 = C_2H_5; R^3 = 3-OH,4-(COOH)-Ph] and adducts of 6-chloro-3-formylchromone (R^1 = Cl) with PAS [R^3 = 3-OH,4-(COOH)-Ph] and propanol [R^2 = C_3H_7 (8)], butanol [R^2 = C_4H_9 (9)], hexanol [R^2 = C_6H_{13} (10)] and heptanol [R^2 = C_7H_{15} (11)] (formula B). The above set of effectors was completed with the adducts of 6-chloro-3-formylchromone (R^1 = Cl) with ethanol (R^2 = C_2H_5) and 3-aminosalicylic acid [R^3 = 2-OH,4-(COOH)-Ph) (12)], 2-hydroxyaniline [R^3 = 2-OH-Ph (13)], 4-chloro-2-hydroxyaniline [R^3 = 2-OH,4-Cl-Ph (14)], 4-aminobenzoic acid [R^3 = 4-(COOH)-Ph (15)] and methylamine [R^3 = CH_3 (16)] (formula B).

Formula A (compounds 1 - 3)



Formula B (compounds 4 - 16)



Spinach chloroplasts used in the study of photosynthetic activity were prepared by a partly modified procedure of current preparation methods described by Walker

(1980) using TRIS buffer (20 mM, pH 7.0) containing 0.4 M saccharose and 0.2 mM $MgCl_2$ (for details see Šeršeň *et al.* 1990). The oxygen evolution rate (OER) in spinach chloroplasts (C_{chl} = 30 mg dm^{-3}) was determined spectrophotometrically (*Specord UV-VIS, Zeiss*, Jena, Germany) using 2,6-dichlorophenol-indophenol (DPIP) (30 µM) as an electron acceptor and phosphate buffer (20 mM, pH = 7.2) containing saccharose (0.4 M), $MgCl_2$ (5 mM) and NaCl (15 mM) according to the method described by Kráľová *et al.* (1992). The effect of the studied effectors on OER was expressed by inhibitory concentration values of IC_{50} [mol dm^{-3}] corresponding to concentrations causing 50 % inhibition with respect to the untreated control samples, or by their negative logarithms (pI_{50}). Because of low solubility of the studied compounds in water, these were dissolved in dimethyl sulphoxide. The applied solvent content (up to 3 %) did not affect DPIP photoreduction or oxygen evolution rate in spinach chloroplasts.

The effect of the compounds studied on photosynthetic centres of chloroplasts was investigated by studying chlorophyll *a* (Chl *a*) fluorescence (*F-2000 Hitachi*, Tokyo, Japan; excitation wavelength 436 nm; slit 10 nm; accomodation of samples in the dark 10 min prior to the measurements; Chl content in the samples 10 mg dm^{-3}) at room temperature.

EPR measurements were carried out with an instrument *ERS 230* (*WG, Akademie der Wissenschaften*, Berlin, Germany) operating in the 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 the compounds studied (0.05 mol dm^{-3}) were recorded in the dark and in the light at room temperature. Chl content in the samples was 4 kg m^{-3}. The samples were irradiated with a 250 W halogen lamp through a water filter. Because of low solubility of the studied compounds these were dissolved in dimethyl sulphoxide, however the applied solvent content in the samples (10 %) affected the investigated EPR signals in the range of experimental error of this method, *i.e.* ± 10 %.

Results

From the adducts of 6-chloro-3-formylchromone with ethanol and some substituted aniline derivatives (6, 12 - 15) the maximum OER inhibition in spinach chloroplasts was shown by the adduct with PAS (6) (IC_{50} = 63 µmol dm^{-3}) (Table 1).

The dependence of pI_{50} values of the effectors studied upon the hydrophobic π parameters of the 6-R^1-substituent of compounds 1 - 3 and 4 - 9, as well as upon the corresponding π parameters of 2-alkoxy substituent for compounds 6 and 8 - 11, are illustrated in Fig. 1. The applied π-values were taken from Hansch *et al.* (1973) and from Pešák *et al.* (1980). The inhibitory activity concerning OER in spinach chloroplasts increases with increasing lipophilicity of the 6-R^1-substituent for the condensation products of PAS with 6-R^1-3-formylchromone (Fig. 1, *circles*), and for the adducts of 6-R^1-3-formylchromone with PAS and ethanol

Table 1. IC_{50}-values concerning OER inhibition in spinach chloroplasts by the studied condensation products of 4-aminosalicylic acid with 6-R^1-3-formylchromone and by the adducts of 6-R^1-3-formylchromone with *n*-alcohols and aniline derivatives (these compounds correspond to formula A or formula B).

Compound	Formula	R^1	R^2	R^3	IC_{50} [μmol dm^{-3}]
1	A	H	-	-	1466.8
2	A	Cl	-	-	259.4
3	A	Br	-	-	144.7
4	B	H	C_2H_5	3-OH,4-(COOH)-Ph	452.3
5	B	CH_3	C_2H_5	3-OH,4-(COOH)-Ph	117.9
6	B	Cl	C_2H_5	3-OH,4-(COOH)-Ph	63.0
7	B	Br	C_2H_5	3-OH,4-(COOH)-Ph	36.5
8	B	Cl	C_3H_7	3-OH,4-(COOH)-Ph	13.2
9	B	Cl	C_4H_9	3-OH,4-(COOH)-Ph	4.2
10	B	Cl	C_6H_{13}	3-OH,4-(COOH)-Ph	4.3
11	B	Cl	C_7H_{15}	3-OH,4-(COOH)-Ph	11.2
12	B	Cl	C_2H_5	2-OH,4-(COOH)-Ph	174.8
13	B	Cl	C_2H_5	2-OH-Ph	171.1
14	B	Cl	C_2H_5	2-OH,4-Cl-Ph	351.4
15	B	Cl	C_2H_5	4-(COOH)-Ph	14028.0
16	B	Cl	C_2H_5	CH_3	732.0

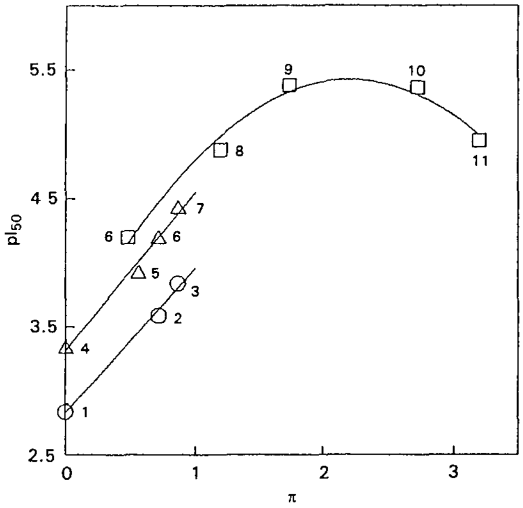


Fig. 1. Dependence of pI_{50}-values upon the hydrophobic parameters π of the R^1-substituent for the condensation products of PAS with 6-R^1-3-formylchromone [*circles*; R^1 = H (1), Cl (2), Br (3)] and for the adducts of 6-R^1-3-formylchromone with PAS and ethanol [*triangles*; R^1 = H (4), CH_3 (5), Cl (6), Br (7)], and upon the hydrophobic parameters π of the alkoxy substituent of the adducts of 6-chloro-3-formylchromone with PAS and *n*-alcohols [*squares*; alcohol = ethanol (6), *n*-propanol (8), *n*-butanol (9), *n*-hexanol (10) and *n*-heptanol (11)]. The π values are taken from Hansch *et al.* (1973) and Pešák *et al.* (1980).

(Fig. 1, *triangles*). However, the dependence of pI_{50}-values upon the π parameters of the 2-alkoxy substituent of the adducts of 6-chloro-3-formylchromone with PAS and *n*-alcohols exhibits a quasi-parabolic course, the so-called "cut- off" effect (Fig. 1, *squares*).

With respect to the intensity of the Chl fluorescence emission band at 686 nm obtained with untreated control chloroplast suspensions, this showed decreases in the presence of the studied compounds, reflecting interaction of these effectors with the photosynthetic apparatus of spinach chloroplasts (Fig. 2).

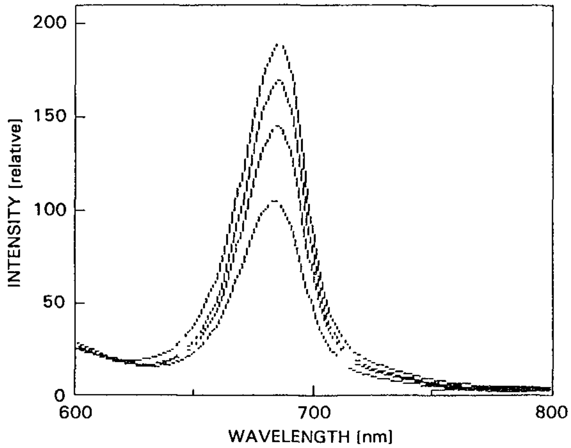


Fig. 2. Fluorescence emission spectra of untreated spinach chloroplasts and in the presence of 5.5, 16.4 and 32.2 μmol dm^{-3} of compound 9 (*curves from top to bottom*).

In the presence of compound 8, the intensity of the EPR signal II was decreased to approximately one half and that of the signal I showed an approximately twofold increase (Fig. 3B) with respect to the corresponding signal intensities of the untreated sample (Fig. 3A).

Discussion

The decreased intensity of the Chl fluorescence emission band at 686 nm (Fig. 2) belonging to the pigment-protein complex in PS 2 (Atal *et al.* 1991) obtained in the presence of the compounds studied with respect to that of the untreated control suggested PS 2 as the site of action of the effectors studied.

The decrease of the EPR signal II (Fig. 3 B, full line) confirmed that the effector interacts with the intermediates Z^+/Y^+ which are situated in D 1 and D 2 proteins taking their place on the donor side of PS 2. These intermediates supply the electrons into the core of PS 2. Since due to the interaction of the effector with Z^+/Y^+ intermediates an approximately 50 % loss of their function can be observed, it is evident that the photosynthetic electron flow through PS 2 to PS 1 will be also proportionally limited. Consequently the limited reduction of PS 1 is reflected in approximately twofold increase in the light of the signal I belonging to oxidized PS 1 (Fig. 3). The use of the artificial electron donor diphenylcarbazide (DPC) acting in the region of Z^+/Y^+ (Jagerschöld and Styring 1991) can be helpful for determinating whether the studied effectors interact with the core of PS 2 (P_{680}). It is evident that the core of PS 2 is not impaired by the investigated compounds, while the reduction of DPIP in chloroplasts inhibited up to 90 % by the effectors studied was practically completely restored after the addition of 0.2 mmol dm^{-3} DPC.

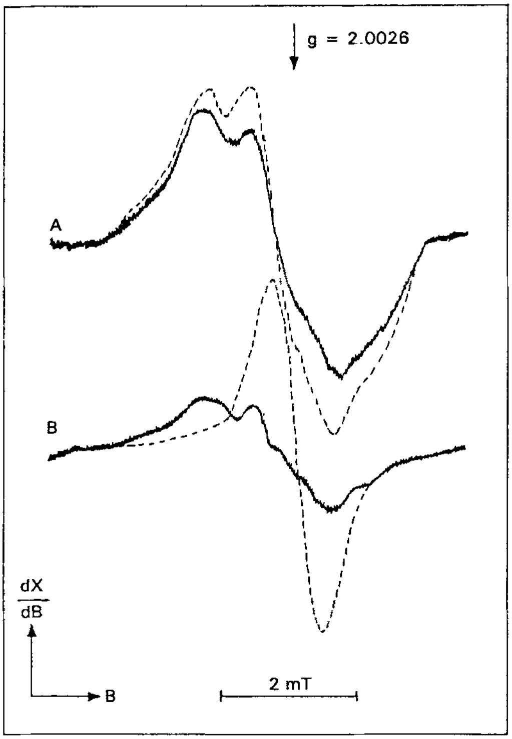


Fig. 3. EPR spectra of untreated spinach chloroplasts (*A*) and of chloroplasts treated with 0.05 mol dm^{-3} of compound 8 (*B*). *Full lines* correspond to chloroplasts kept in the dark, *dashed lines* to the illuminated chloroplasts. The *dashed* spectrum in B was recorded at a sensitivity two-fold lower than the recordings of the other spectra.

Since the site of action of the adducts of 6-chloro-3-formylchromone with PAS and *n*-alcohols are the intermediates Z^+/Y^+, the quasi-parabolic course of the dependence of pI_{50} values upon the π parameters of 2-alkoxy substituent (Fig. 1) can be connected with the fact that the highest inhibitory activity will be exhibited by effectors having sufficiently high lipophilicity for securing their passage through the lipidic parts of biological membranes, but simultaneously enabling sufficiently high effector concentration in the aqueous phase which is indispensable for the interactions with proteins. The lower activity of the effectors with shorter alkoxy substituents is caused by their limited transition through the lipidic part of the thylakoid membranes (due to their low lipophilicity) and consequently by their lower final concentration inside thylakoids, *i.e.* in the neighbourhood of the interacting PS 2 proteins. On the other hand, the number of effector molecules with long alkoxy substituents reaching the above-mentioned site of action will also be limited since these lipophilic effectors remain predominantly incorporated in the lipidic part of the membrane. The enhancement of the inhibitory activity of the condensation products of PAS with 6-R^1-3-formylchromone (1 - 3) and of the adducts of 6-R^1-3-formylchromone with PAS and ethanol (4 - 7) with the increasing lipophilicity of the R^1-substituent is connected with the improvement of effector transition through the lipidic part of thylakoid membranes. The comparison of the inhibitory activity of 6-chloro-3-formylchromone with ethanol and some aniline derivatives confirmed the most pronounced efficiency for the adduct with the biologically active PAS molecule.

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