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BRIEF COMMUNICATION

Triazine inhibits electron transfer in photosystem 2 and induces lipid peroxidation in thylakoid membrane of maize

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Abstract

Triazine treatment of thylakoid membranes isolated from the primary leaves of *Zea mays* L. showed an 80 % inhibition of photosystem (PS) 2 activity. No detectable change of PS 1 activity was found. The inhibited membranes showed a selective reduction of the most unsaturated linolenic acid ($C_{18:3}$) biosynthesis by about 15 % coupled with a corresponding increase in stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids. Thus the inhibition of electron transfer of PS 2 induced by triazine treatment was followed by lipid peroxidation and changes in the thylakoid membrane fluidity.

Key words: herbicide, linolenic acid, linoleic acid, membrane fluidity, myristic acid, oleic acid, palmitic acid, photosystem 1, stearic acid, *Zea mays*

The site of action of many herbicides, including triazine, is in the photosynthetic apparatus (Bairamov *et al.* 1992). Triazine inhibits the electron transfer at various sites of the photosynthetic electron transport chain, apparently as a result of the interaction with the quinones of PS 2 (Garadner 1981, Ducruet 1984). There are two possible mechanisms of the effect of herbicides: (*1*) The herbicide induces a conformational change in the protein-bound membrane as it binds to the Q_B protein in PS 2 complex with a molecular mass of 32 kDa (Pfister *et al.* 1981, Sigematsu *et al.* 1989); (*2*) The herbicide facilitates lipid peroxidation of thylakoid membrane. Gronwald (1991) reported that herbicides impair the synthesis of lipids and decrease the degree of unsaturation of plastid galactolipids due to their ability to inhibit fatty

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acid desaturases located in chloroplasts of higher plants. Experiments on isolated thylakoid membrane of various plants with a low ratio of unsaturated fatty acids showed a large inhibition of electron transport indicating the relationship between fluidity of the membrane and the efficiency of photosynthetic process (Vigh *et al.* 1979, Gombos *et al.* 1988, Covello *et al.* 1989). Moreover, the degree of unsaturation may control the association of the peripheral chlorophyll (Chl *a/b*) light harvesting complex 2 with PS 2α, thus determining the PS 2 heterogeneity in PS 2α and PS 2β (Horvath *et al.* 1979). Increase in thylakoid membrane fluidity is an indication of the extent of adaptive modification of the lipid matrix, accomplished either by changing fatty acid saturation, lipid class composition, or the ratio of protein to lipid (Barber 1985, Vigh *et al.* 1985, Vigh 1987). We studied the relationship between fluidity of the thylakoid membrane isolated from triazine-treated plants and the electron transport capacity in order to clarify the mechanism of herbicide mediated inhibition of photosynthesis.

Zea mays L. seeds (from the Saka Agriculture Center) were soaked overnight in distilled water. Imbibed grains germinated at 37 °C on filter paper moistened with 10 cm^3 of water in glass Petri dishes. Germination were carried out under a irradiance of 26 W m^{-2} at 15/9 h day/night photoperiod. 0.6 mM 2-chloro-5-ethyl-amino-6-isopropyl-amine-5-triazine was applied to 3 d-old seedlings. After 12 d, the primary leaves were collected for measurements.

Thylakoids were isolated as described by Osman and El-Shintinawy (1988). Leaves (10 g) were homogenized for 5 s in 60 cm^3 of isolation buffer (50 mM Tricine, pH 7.8, 50 mM NaCl, 3 mM $MgCl_2 \cdot 6\,H_2O$, and 0.5 mM EDTA) using a mixer. The homogenate was filtered through eight layers of cheesecloth and centrifuged for 2 min at 2 000 *g*. The resulting chloroplast pellet was resuspended in 20 cm^3 of suspension medium containing 400 mM sorbitol, 50 mM KH_2PO_4 (pH 7.5), 2 mM $MgCl_2 \cdot 6\,H_2O$, and 0.1 % bovine serum albumin. The suspension was centrifuged at 3 000 *g* for 90 s. The pellet was suspended in 2 cm^3 of suspension buffer. Thylakoids containing 30 μg(Chl) cm^{-3}(suspension) were used for different measurements. Chl *a* concentration was determined according to Mackinney (1941).

Electron transport measurements were carried out essentially as described by Osman and El-Shintinawy (1988). Clark type oxygen electrode (*Yellow Springs Instruments*, England) and a slide projector providing an irradiance of 300 W m^{-2} at the cuvette surface were used. The basic reaction mixture (pH 7.8) used for all electron transport assays contained 40 mM sorbitol, 50 mM KH_2PO_4, 10 μM DCMU (for PS 1) and 4 mM $MgCl_2 \cdot 6\,H_2O$. Steady state electron transport measurements of water → 2,5-dimethyl-*p*-benzoquinone (BQ; 1 mM) in the presence of 0.5 mM $K_3Fe(CN)_6$ gave PS 2 activity, of water → methyl viologen (MV; 0.5 mM) showed both PS 2 and PS 1 activities, and ascorbate → MV (0.5 mM MV, 20 μM DCPIP, 1 mM Na-ascorbate) showed PS 1 activity only.

Lipids were extracted from isolated thylakoid membranes as described by Bligh and Dyer (1959) and fatty acids were determined using gas chromatography according to Flood (1981). Approximately 150 mg of isolated thylakoid membranes were mixed with 4 cm^3 of 0.66 M KOH in methanol, then placed in a 20 cm^3 screw-capped tube. The tube was flushed with N_2, capped and heated at 100 °C for 5 min.

The mixture was cooled and 4 cm^3 of boron trifluoride (BF_3) in methanol (14 % m/v - BDH) was added and the tube was flushed with N_2, capped and heated at 100 °C for another 5 min. The mixture was cooled and 4 cm^3 of each of saturated NaCl, water and petroleum ether (b.p. 40 - 60 °C) were added. The supernatant was removed, washed with water, dried over anhydrous Na_2SO_4, and concentrated by a stream of N_2 gas at room temperature to about 1 cm^3 (2 mm^3 of methylester of tridecanoic acid was added as internal standard). A sample of 5 mm^3 of the dried supernatant was injected into a *GLC-Variant 6000*. Flame ionization detector, 2 m column, 3.2 mm internal diameter, packed with 15 % *OV-275*, *Chrom P/AW/80-100* mesh operating at 180 °C, injection temperature 230 °C, detector temperature 250 °C with carried gas of He at a flow rate of 0.42 $cm^3\ s^{-1}$. Fatty acid methyl esters were identified by comparison with standards (*Polscience Corporation*, kit no. *61CX*), and the quantities were calculated from the areas obtained by the *KLB-2220* Recording Integrator. The fluidity was determined as the ratio of unsaturated fatty acids of the thylakoid membranes.

Triazine treatment (0.6 mM) resulted in 80 ± 2 % reduction in PS 2 activity [control rate 2.8 $mmol(O_2)\ kg^{-1}\ s^{-1}$], whole electron transport rate [control rate 8.3 $mmol(O_2)\ kg^{-1}\ s^{-1}$] was reduced by 35 ± 5 %, PS 1 activity [control rate 12.0 $mmol(O_2)\ kg^{-1}\ s^{-1}$] was not significantly affected by triazine treatment. The results are in agreement with those of Ort *et al.* (1983) using triazine resistant pigweed. According to Shimazaki *et al.* (1984), the decrease in the binding constant for the added artificial electron acceptor is not accompanied by a change in binding site of triazine is at PS 2 quinones which is in agreement with the report of Dekker (1993).

Table 1. Fatty acid composition [%, m/m] of triazine (0.6 mM) treated maize chloroplasts from 12-d-old leaves.

Fatty acids	Control	Triazine
$C_{14:0}$ (myristic acid)	0.74	<0.10
$C_{16:0}$ (palmitic acid)	9.60	12.42
$C_{16:1}$ (palmitoleic acid)	6.11	11.44
$C_{18:0}$ (stearic acid)	2.16	4.00
$C_{18:1}$ (oleic acid)	7.25	18.30
$C_{18:2}$ (linoleic acid)	49.34	53.74
$C_{18:3}$ (linolenic acid)	14.80	<0.10

The possible relationship between the observed inhibition of electron flow of PS 2 and the fluidity of thylakoid membrane was examined by monitoring the fatty acid composition in herbicide-inhibited thylakoid membranes (Table 1). A most significant decline was recorded in the relatively unsaturated linolenic acid. The ratio of $(C_{18:0}+C_{18:1}+C_{18:2})/C_{18:3}$ was greatly increased in triazine treated membranes which reflected the selective decline in linolenic acid, a highly unsaturated fatty acid. This increased ratio is an indication of lipid peroxidation induced by the herbicide treatment. The inhibition in linolenic acid content was accompanied with a

stimulation in linoleic, oleic and stearic acids content. Stearic, oleic and linolenic acids are the major unsaturated fatty acids in higher plant tissue. Athough linolenic acid represents about 17 % of the C_{18} fatty acids in maize chloroplasts, the observed inhibition of its biosynthesis caused a dramatic decrease in PS 2 activity. The results are in agreement with those of Horvath *et al.* (1987) and suggest that even low lipid peroxidation causes pronounced changes in membrane structure and semipermeability. An optimum fatty acid unsaturation maintains an efficient electron transfer from Q_A to the plastoquione pool. Thompson *et al.* (1987) mention that lipid peroxidation leads to the profilation of free radicals capable of damaging membranes and proteins and deesterifying membrane fatty acids (Peiser *et al.* 1982).

Thus triazine affected the electron transport via PS 2 quinones while the PS 1 activity was not significantly changed. Valadon and Kates (1984) and Sigematsu *et al.* (1989) report that triazine binds the plastoquinone acceptor of PS 2 (Q_B). The inhibition of electron flow of PS 2 is accompanied by an inhibition of the most unsaturated fatty acid $C_{18:3}$ suggesting its regulatory role in membrane fluidity. Apparently, there is a linkage between the level of saturation of thylakoid lipids and of the linear electron transport. Thus, lipid biosynthesis may be added to plant metabolic process such as photosynthesis, carotenoid biosynthesis and essential amino acid biosynthesis (Argenta 1991) that are inhibited by herbicides.

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