Ethane Formation and Chlorophyll Bleaching in DCMU-Treated *Euglena gracilis* Cells and Isolated Spinach Chloroplast Lamellae

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Light dependent chlorophyll bleaching and ethane formation in *Euglena gracilis* cells at slightly acidic pH values above 5.2 are enhanced by DCMU and other photosystem II blockers. Excited chlorophyll under conditions of limited energy dissipation seems to be the oxidant for ω-3 unsaturated fatty acids, yielding ethane and other (ethylene, "malondialdehyde") oxidation products, cooxidizing chlorophyll.

Introduction

Loss of photosynthetic pigments is induced by certain herbicides and may generally be caused by either 1) inhibition of biosynthesis, and 2) stimulation of degradation of these pigments [1 – 6]. In the case of electron transport blockers of the "DCMU" type, photobleaching with concomitant unsaturated fatty acid oxidation ("malondialdehyde"-formation) as a consequence of limited energy dissipation has been suggested as the responsible mechanism [5, 7 – 9].

We recently reported on ethane determination as a simple and non-destructive method for following unsaturated fatty acid oxidation in plant tissue after mechanical wounding (decompartmentalization) or paraquat treatment [9, 10]. In context with several chemical models presented by others (see below) we wish to report on the mechanism of ethane formation and its stimulation by DCMU (see also ref. [11]).

Materials and Methods

All methods as well as materials used in the present publication have been described recently (Elstner, E. F., Z. Naturforsch. 1979, manuscript submitted). Spinach chloroplasts were "aged" by incubation at pH 7.6 at 4 °C for 48 h. These chloroplast particles show no photosynthetic electron transport at the expense of water as electron donator.

Results and Discussion

Illuminated *Euglena gracilis* cells evolve both ethane and ethylene in a ratio of ca. 7 – 12 to one, besides other, minor hydrocarbon compounds. Since ethane is by far the major component in the gaseous headspace of illuminated *E. gracilis* cells, we shall only report on the characteristics of the production of ethane. As shown in the figure, light dependent ethane production is stimulated at decreasing pH-values from 6.0 to 5.2 and by DCMU at pH-values above 5.2. Certain N-substituted maleimide derivatives (generously donated by Drs. H. P. Fischer and E. Ebert, Ciba-Geigy, Basel) which act as electron transport blockers at a similar site as DCMU (see Brugnoni *et al.*, this issue), also

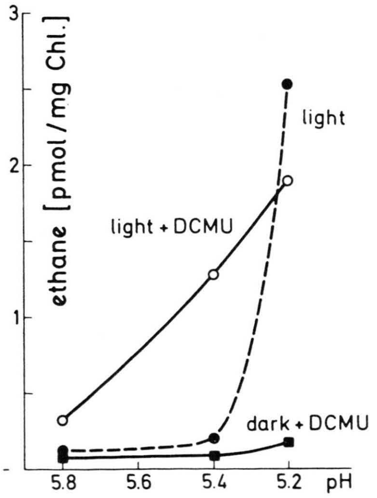

Fig. Ethane formation by *Euglena gracilis* cells: dependence on pH and effect of DCMU. For reaction conditions see Table I.

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; Chl, chlorophyll.

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Table I. Ethane formation and chlorophyll bleaching in *Euglena gracilis:* Effect of electron transport blockers.

Conditions: *Euglena gracilis* cells were incubated for 5 h at pH 5.6 in a photothermostate (35 Klux, 22 °C) with illumination from the bottom. Ethane was withdrawn from the gaseous headspace of the rubber-sealed incubation vessels. Electron transport rates were determined with isolated spinach chloroplast lamellae (methylviologen reduction).

Additions [M]	Electron transport % MV-reduction	Chlorophyll bleaching [%]	Ethane production [pmol/mg Chl]
control (dark)	—	0	50
control (light)	100	3	130
2×10^{-5} M DCMU	0	98	700
10^{-5} M 3,4-dimethyl-2-hydroxy-5-oxo-2.5 dihydropyrrolones: N-substituent:			
3',4'-dichlorophenyl-...	4	97	710
3'-chloro-4'trifluoromethyl-...	4	99	880
2'-trifluoromethyl-1,3,4-thiodiazole-...	9	98	950

stimulate light – dependent chlorophyll bleaching and ethane formation (Table I).

As reported elsewhere (Elstner, Z. Naturforsch. manuscript submitted) *E. gracilis* homogenates exhibit light dependent α-linolenic acid oxidation with concomitant ethane formation and chlorophyll bleaching. This ethane formation is not inhibited by:

a) electron transport blockers, b) superoxide dismutase or catalase and c) several $OH^{\cdot}$ scavengers, but is inhibited by the carotenoid crocin and strongly stimulated by reducing agents as ascorbate and bisulfite. A model reaction for α-linolenic acid dependent chlorophyll photobleaching is presented in Table II: It consists of isolated potato mitochondria and isolated spinach chloroplasts. Potato mitochondria have been reported to produce ethane from α-linolenic acid [12]; as shown in Table II, α-linolenic acid strongly stimulated ethane formation in isolated potato mitochondria as expected [12] but shows only little or no effect in isolated, aged chloroplasts, which lack photosynthetic electron transport in the absence of artificial electron donors. In the presence of both α-linolenic acid and isolated mitochondria, isolated chloroplast lamellae exhibit a stimulated chlorophyll bleaching as compared to controls incubated at the same pH (5.5).

Illumination of chloroplasts slightly stimulates ethane formation and strongly enhances chlorophyll bleaching. Both ethane formation and chlorophyll bleaching are enhanced in the presence of either mitochondria, α-linolenic acid or both. Thus, isolated mitochondria in the presence of α-linolenic acid can partially substitute for the light. This effect is probably due to mitochondrial lipoxygenase, since plant lipoxygenases are known to catalyze cooxidations of dyes and pigments like chlorophyll or carotenoids [13, 14]. It is remarkable, however, that in isolated,

Table II. Chlorophyll bleaching and ethane formation by isolated spinach chloroplast lamellae: modelreaction with isolated potato mitochondria and linolenate.

Conditions: Aged (24 h at 4 °C in pH 7.6 Tris HCl-buffer), isolated spinach chloroplast lamellae with 1 mg Chl/ml were incubated with isolated potato mitochondria with 100 mg dry weight (12) or with 30 μmol α-linolenic acid for 2 h at pH 5.5. Illumination with 35 Klux, where indicated.

Incubations	Dark		Light	
	ethane [nmol/mg Chl/h]	% Chl bleached	ethane [nmol/mg Chl/h]	% Chl bleached
mitochondria	1.2	—	0.9	—
mitochondria + α-lin.	41.6	—	41.2	—
chloroplasts	0	34	1.2	73
chloroplasts + α-lin.	0.1	38	0.9	100
mitoch. + chloropl.	2.8	39	7.2	100
mitoch. + chloropl. + α-lin.	33.7	73	43	100

aged chloroplasts addition of α-linolenic acid has no effect on light – dependent ethane formation; in freshly prepared chloroplasts, α-linolenic acid stimulates ethane formation, further enhanced by addition of cytochromes, reducing agents or paraquat (data not shown). Ethane formation by aged chloroplasts from an endogeneous substrate is stimulated by light. These findings are indicative for different mechanisms of ethane formation from α-linolenic acid as far as the coupling to the light reactions is concerned: one reaction may involve the reducing side of photosystem I and another one may be driven solely by excited chlorophyll, independent of an intact electron transport system.

Inhibition by chloroplasts of ethane formation by potato mitochondria after addition of α-linolenic acid (Table II) may partially be due to the α-linolenic acid binding capacity of chloroplasts [15]. The relatively high rates of chlorophyll bleaching (ca. 35 – 40%) in the dark at pH 5.5 may be connected with the release of unsaturated fatty acid (see citations in ref. [15]) from the thylakoid structure, since aged chloroplasts (in contrast to freshly prepared chloroplasts) produce considerable amounts of ethane in the light without any addition of substrate.

From the presented results we can conclude that in isolated chloroplasts, as in intact *E. gracilis* cells, chlorophyll is photobleached independently of intact electron transport but dependent on oxidation products of α-linolenic acid, as represented by alkoxy – or hydroperoxy radicals as proposed for carotene cooxidations [14, 16].

We therefore might outline chlorophyll bleaching and ethane formation by illuminated *E. gracilis* cells in the presence of DCMU (or other conditions where energy dissipation is strongly reduced) as follows:

I) Production of excited (triplet?) chlorophyll and activated oxygen

a) $\mathrm{Chl} \xrightarrow{\mathrm{hxv}} {}^{\mathrm{T}}(\mathrm{Chl}^*) \xrightarrow{\mathrm{DCMU}} \mathrm{plastoquinone}$

b) ${}^{\mathrm{T}}(\mathrm{Chl}^*) + \mathrm{O}_2 \rightarrow (\mathrm{Chl}^+ - \mathrm{O}_2^-)$, or
${}^{\mathrm{T}}(\mathrm{Chl}^*) + \mathrm{O}_2 \rightarrow \mathrm{Chl} + {}^1\mathrm{O}_2$

c) $(\mathrm{Chl}^+ - \mathrm{O}_2^-) + \mathrm{OH}^- \rightarrow (\mathrm{Chl} - \mathrm{O}_2^-) + \mathrm{OH}^{\cdot}$, or
$(\mathrm{Chl}^+ - \mathrm{O}_2^-) + \mathrm{RH} \rightarrow (\mathrm{Chl} - \mathrm{O}_2^-) + \mathrm{H}^+ + \mathrm{R}^{\cdot}$.

We have no indication, however, for the production of the very reactive OH-radical ($\mathrm{OH}^{\cdot}$) as outlined in I c) and as proposed by Harbour and Bolton [19]. The inhibition of both chlorophyll bleaching and ethane formation in *E. gracilis* homogenates by the water soluble carotenoid crocin (E. F. Elstner, Z. Naturforsch., manuscript submitted) may indicate a participation of singlet oxygen (${}^1\mathrm{O}_2$) in the above process as outlined in reaction I b).

II) Activation of ω-unsaturated fatty acids (α-lin) and ethane formation

In agreement with references [5, 13, 17 – 20] we may write:

a) $\mathrm{R}^{\cdot} + \alpha\text{-lin} \rightarrow \mathrm{RH} + \alpha\text{-lin}^{\cdot}$

b) $\alpha\text{-lin}^{\cdot} + \mathrm{O}_2 \rightarrow \alpha\text{lin OO}^{\cdot}$
(ω-3 peroxy radical)

c) $\alpha\text{-lin OO}^{\cdot} + \mathrm{RH} \rightarrow \mathrm{R}^{\cdot} + \alpha\text{-lin OOH}$
(ω-3-alkyl peroxide)

d) $\alpha\text{-lin OOH} + \mathrm{Me}^{n+} \rightarrow \mathrm{Me}^{(n+1)} + \mathrm{OH}^- + \alpha\text{-lin O}^{\cdot}$
(ω-3-alkoxy radical)

e) $\alpha\text{-lin O}^{\cdot} \xrightarrow{\text{ß- scission}} \mathrm{CH}_3 - \mathrm{CH}_2^{\cdot} + \mathrm{CHO} - - \mathrm{CH} = \mathrm{CH} - \mathrm{R}$

f) $\mathrm{CH}_3 - \mathrm{CH}_2^{\cdot} \xrightarrow{\text{reduction}} \text{ethane}$

g) α-lin OOH may also be formed directly from α-lin by reaction with ${}^1\mathrm{O}_2$:
$\alpha\text{-lin} + {}^1\mathrm{O}_2 \rightarrow \alpha\text{-lin OOH}$.

$\mathrm{R}^{\cdot}$ may be represented by Chl^+ and/or other alkyl radicals.

III) Chlorophyll bleaching

Oxidized Chlorophyll (Chl^+) may decay into bleached oxidation products. Chl^+ is formed either from excited chlorophyll, Chl^*, by charge separation or by reaction with $\mathrm{R}^{\cdot}$ (α-lin$^{\cdot}$).

Measurement of ethane production, in addition to the measurement of other parameters of lipid degradation, may prove as a valuable tool for the identification and classification of certain activities of herbicides.

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