THE PROMPT EFFECT OF PYRIDAZINONE HERBICIDES ON THE PRIMARY PROCESSES OF PHOTOSYNTHESIS

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1. Introduction

Substituted pyridazinone herbicides San 6706, San 9785 and San 9789 applied for long times (e.g., for several days) inhibit the development of chloroplast structure and alter its pigment composition [1–3]. After pyridazinone treatment the photosynthetic activity of plants (Hill reaction, CO_2 fixation) is strongly diminished [4,5]. However, it was found [5] that a prompt inhibition of ferricyanide reduction in the Hill reaction occurs in isolated chloroplasts. The purpose of our investigation was to find the mechanism of this action. We present data indicating that San 6706, San 9785 and San 9789 pyridazinone herbicides directly block the O_2-evolving activity of *Chlorella*. Furthermore, using thermoluminescence and delayed luminescence techniques, it is shown that the site of action of pyridazinone herbicides is at the location of the water-splitting enzyme of photosystem II.

2. Experimental

Chlorella pyrenoidosa (strain Emerson) was cultured in Tamiya medium [6] at 25°C and synchronized by 16 h light/8 h dark regime. All experiments were carried out with algal cultures in which cells were in D_a developing state [7].

Thermoluminescence measurements were made from –80 to +90°C using equipment similar to that in [8]. The samples were illuminated with white light at 10 W . m^{-2} for 5 min during continuous cooling from +20 to –80°C, then heated at a constant rate of 10°C/min.

Delayed luminescence was measured by the phosphoroscope method [9]. The luminescence of the samples was generated by broad-band blue light at 10 W . m^{-2} (Corning 4-96 filter). Delayed luminescence was detected through a Corning 2-64 filter by an EMI 9558 photomultiplier. The amplified output of the photocurrent was stored in a ICA70 multichannel analyzer and finally displayed on a chart recorder.

The oxygen-evolving activities were measured polarographically with a Clark electrode at 30°C.

3. Results and discussion

Table 1 shows that pyridazinone herbicides are fairly strong inhibitors of the O_2-evolving activity. However, even at 10^{-4} M herbicides do not influence delayed luminescence (fig.1, curves a). The sensitivity to DCMU, a specific inhibitor of electron transport chain after the Q primary electron acceptor of photosystem II, is unaffected (fig.1, curves b). Since delayed luminescence results from charge recombination between Q and the electron donor side of photosystem II [10], and the delayed luminescence remains

Abbreviations: San 6706, 4-chloro-5-(dimethylamino)-2-(α,α,α,-trifluoro-*m*-tolyl)-3(2H)-pyridazinone; San 9785, 4-chloro-5-(dimethylamino)-2-phenyl-3(2H)-pyridazinone; San 9789, 4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone; Q, the primary electron acceptor of photosystem II; PQ, plastoquinone; Z, the primary electron donor of photosystem II; M, water-splitting enzyme; DCMU, 3-(3,4-dichlorophenyl)-1,1 dimethylurea

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Table 1
Inhibition of O_2-evolving activity of *Chlorella pyrenoidosa* by herbicides

Herbicide	Concentration for 50% inhibition (μmol)
San 6706	70
San 9785	52
San 9789	85

Cells were suspended in the growth medium and adjusted to 10 μg chl./ml suspension

intact after herbicide treatment we can conclude that the pyridazinone herbicides do not act either at Q or Z. Therefore the electron transport chain components after Q and the water-splitting enzyme complex remain as possible targets of the herbicide action.

Comparing the glow curves of the untreated and herbicide-treated algae (fig.2), the most apparent alteration after herbicide addition is the reduction or disappearance of the maximum at +25°C. This maximum is ascribed either to the positively charged water-splitting enzyme [11,12], or to the PQ-pool [13]. If the herbicides act on PQ, their application after DCMU-treatment should not affect the glow curves. The thermoluminescence curves ought to appear similar to that shown by a broken line in fig.2. However, if DCMU was added to *Chlorella* a few minutes before or after the addition of the herbicides,

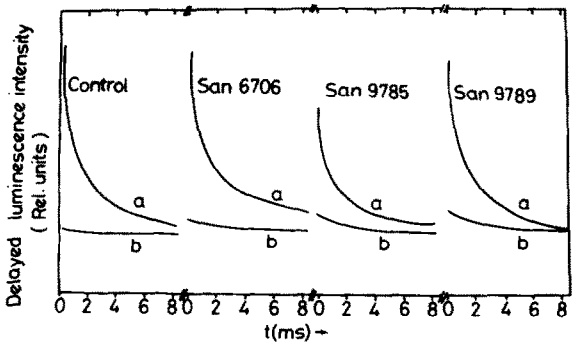

Fig.1. Delayed luminescence of *Chlorella* in the presence of 0.1 mM pyridazinone herbicides (curves a) and in the presence of 10 μM DCMU (curves b). The effect of DCMU + herbicide does not depend on the order of addition of the chemicals. Chlorophyll was 10 μg/ml suspension.

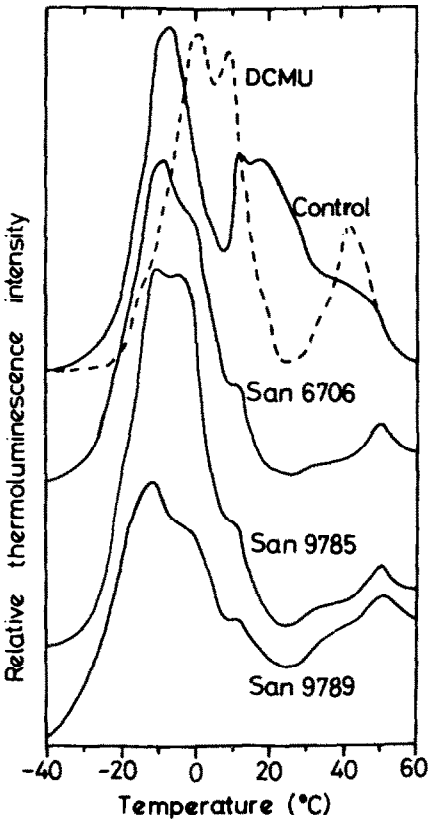

Fig.2. Glow curves of *Chlorella* in the presence of 0.1 mM pyridazinone herbicides and 10 μM DCMU (broken line) taken after a few minutes incubation. The chemicals were added to the cell suspensions before illumination. Chlorophyll was 160 μg/ml suspension.

the glow curves became less structured and the overall intensities of thermoluminescence (not seen in the relative presentation) became very low (fig.3). We conclude that the 3 types of substituted pyridazinone herbicides are not DCMU-type inhibitors of photosynthesis, but act directly on the water-splitting enzyme.

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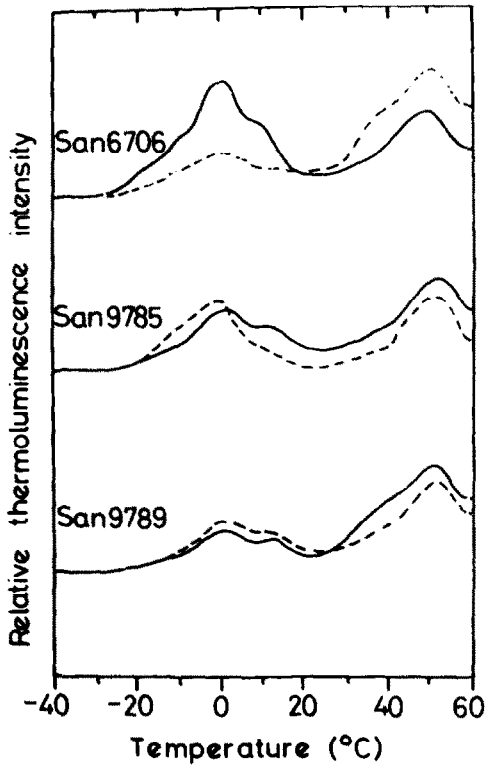

Fig.3. The simultaneous effect of 0.1 mM pyridazinone herbicides and 10 μM DCMU on the glow curve of *Chlorella*. There is no significant difference when DCMU was added before (solid line) or after (broken line) the herbicide addition. Chlorophyll was 160 μg/ml suspension.

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