Effect of Substituted Pyridazinone Herbicides and of Difunone (EMD-IT 5914) on Carotenoid Biosynthesis in Green Algae

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The carotenoid biosynthesis of the green alga *Ankistrodesmus braunii* is blocked if these cells are cultured in presence of sublethal doses of pyridazinone herbicides (San 9789, San 6706, BASF 44521) or of the herbicide difunone (EMD-IT 5914). The amount of colored carotenoids normally found in these algae is reduced drastically and the precursors phytoene and phytofluene are accumulated. Furthermore a decrease in the chlorophyll level occurs in the treated cells, but there is a stronger loss of chlorophyll a, resulting in a lowering of the chlorophyll a/b ratio with time. Concerning the activity of substituted pyridazinones leading to inhibition of carotenoid biosynthesis this effect can be related to the chemical structure of these compounds: a trifluoromethyl substitution of the phenyl ring *and* a mono- or dimethyl substitution of the amine (San 9789, San 6706) or a methoxy group instead of the substituted amine (BASF 44521) are required both for this effect. Other pyridazinone derivatives with *either* a trifluoromethyl substitution of the phenyl ring (San 9774) *or* a dimethyl substitution of the amine (San 9785) *or* a methoxy group (BASF 13761) are without any effect on the pigment pattern of these algae.

Introduction

Pyridazinone herbicides are active inhibitors of the photosynthetic electron transport chain at photosystem II [1]. Like NH_2-substituted compounds (pyrazon, San 9774) also N-methylated (San 9789) and N-dimethylated derivatives (San 6706) and even pyridazinone derivatives with no nitrogen in the side chain but with a methoxy group (BASF 13761, BASF 44521) (see Fig. 1) cause inhibition of photosynthetic oxygen evolution [2, 3]. Concerning the effect of the N-dimethylated pyridazinone derivative San 6706, Hilton *et al.* [1] pointed out that it might inhibit more than one reaction in plants. In the meantime at least three sites of action of San 6706 have been found in plant cells: inhibition of Hill reaction, interference with the formation of polar lipids of chloroplast membranes [4] and with carotenoid biosynthesis [5]. It is well established that San 6706 inhibits carotenoid formation in higher plants [5, 6], algae [7] and bacteria [8] causing an accumulation of colorless polyenes and a diminishing of colored carotenoids followed by bleaching of chlorophyll.

Studying the effect of several substituted pyridazinones on oxygen evolution of photosynthesis, growth rate and pigments of green algae we observed that not only the N-dimethylated compound (San 6706) but also the N-methylated (Son 9789) as well as the methoxylated derivative (BASF 44521) cause a bleaching of algal cells. Therefore

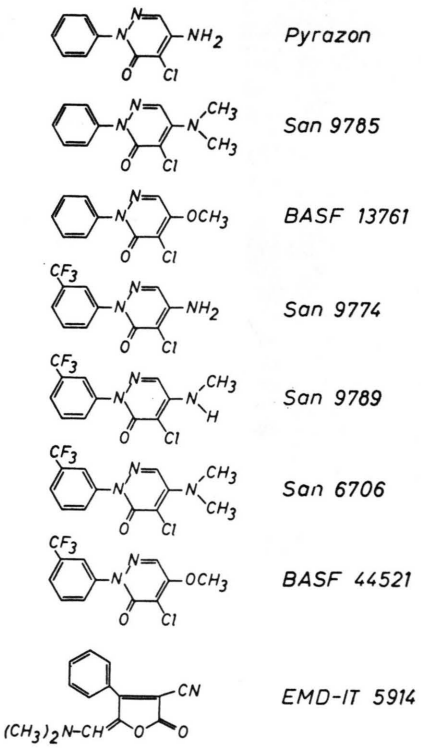

Fig. 1. Structure of substituted pyridazinones and of difunone tested in the experiments.

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we studied the effects of diverse substituted pyridazinones on carotenoid formation and on the chlorophyll content in the green alga *Ankistrodesmus braunii* to find out, whether special substituents are responsible for this kind of action of pyridazinone derivatives. In comparison to the pyridazinone herbicides also the herbicide difunone (EMD-IT 5914) has been investigated in order to test its effect on carotenoid pattern. This herbicide is known to affect development and differentiation of plastids [9], and the chlorophyll synthesis of seedlings [10], while the photosynthetic oxygen evolution of green alga is influenced only to a small degree if tested in short term experiments [11].

Material and Methods

Ankistrodesmus braunii (Naegeli) was grown in an inorganic medium [12] with a light-dark rhythm of 14 to 10 hours (3500 lx white light, 30 °C, pH 6.2, air + 1.5% CO_2). The pyridazinone herbicides dissolved in methanol and EMD-IT-5914 dissolved in acetone-methanol (1 : 9) were added to the culture medium in a final concentration of 10^{-6} M (the concentration of methanol or acetone in the nutrient solution was 0.1% methanol or in the case of EMD-IT-5914 0.09% methanol + 0.01% acetone). The algae were grown in the herbicide containing medium for about 5 – 7 days.

The following compounds were used in our studies: pyrazon (5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone), San 9785 (4-chloro-5-(dimethylamino)-2-phenyl-3(2H)-pyridazinone), San 9774 (5-amino-4-chloro-2(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone), San 9789 (4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone), San 6706 (4-chloro-5-(dimethylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone) supplied from Sandoz AG Basel; BASF 13761 (4-chloro-5-methoxy-2-phenyl-3(2H)-pyridazinone), BASF 44521 (4-chloro-5-methoxy-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone) supplied from BASF, Ludwigshafen and EMD-IT-5914 (5-dimethylamino-methylene-2-oxo-4-phenyl-2,5-dihydrofurane-carbonitrile-(3)) supplied from Celamerck, Ingelheim.

For extraction of pigments the cells were harvested by centrifugation. The extraction of pigments and the thin layer chromatography was carried out by the methods of Hager and Meyer-Bertenrath [13] and Britton and Goodwin [14]. Extinction coefficients ($E^{1\%}_{1cm}$) obtained from Hager and Meyer-Bertenrath [13] and Davis [15] were used to calculate the quantity of the pigments.

Results and Discussion

The carotenoid pattern of the green alga *Ankistrodesmus braunii* is changed if algae are grown in a medium containing sublethal doses of the herbicides San 9789, San 6706, BASF 44521 or EMD-IT-5914. Absorption spectra of the α-carotene fraction from cells treated with these herbicides reveal besides the absorption maxima of α-carotene those of phytofluene and in the UV part of the spectrum those of phytoene (Fig. 2). The level of colored carotenoids normaly found in these algae (mainly α-carotene, β-carotene, violaxanthin, lutein, neoxanthin, zeaxanthin) is reduced after treatment with

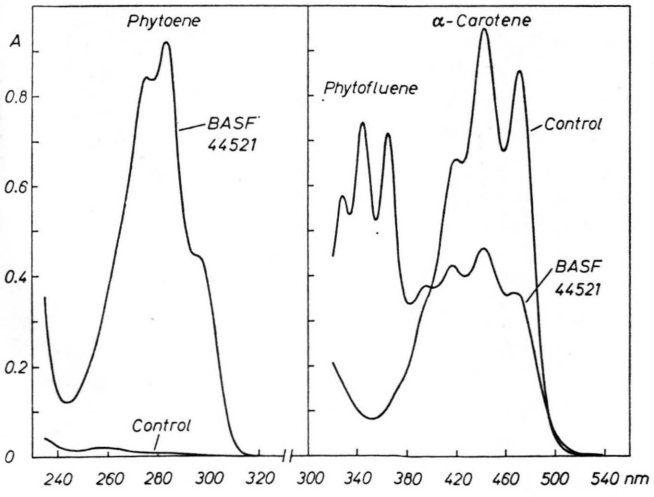

Fig. 2. Absorption spectra of the α-carotene fraction (light petroleum) from control algae and from algae treated with BASF 44521 (treatment with San 9789, San 6706 or EMD-IT-5914 causes corresponding changes of the spectrum).

Table I. Effect of substituted pyridazinones and difunone (EMD-IT-5914) on the content of pigments in the green alga *Ankistrodesmus braunii*.

	mg Pigment/g dry weight					
	Phytoene	Phytofluene	Colored carotenoids	Chl a	Chl b	Chl a/b
Control	0	0	9.3	31.1	13.4	2.3
Pyrazon	0	0	10.0	35.6	16.1	2.2
San 9785	0	0	9.8	33.2	15.5	2.1
BASF 13761	0	0	9.5	35.6	17.1	2.0
San 9774	0	0	8.6	28.8	13.4	2.1
San 9789	5.2	1.1	2.8	10.8	7.5	1.4
San 6706	9.9	2.6	1.6	3.4	2.6	1.4
BASF 44521	4.8	0.9	2.1	11.6	7.6	1.5
EMD-IT-5914	2.1	0.13	1.2	4.0	2.2	1.8

Table II. Distribution of precursors, carotenes and xanthophylls in green algae after treatment with substituted pyridazinones and difunone (EMD-IT-5914).

	% of total carotenoids							
	Phytoene	Phytofluene	α-Carotene	β-Carotene	Violaxanthin	Lutein	Neoxanthin	Zeaxanthin
Control	0	0	11.4	16.0	11.2	30.7	12.8	16.7
Pyrazon	0	0	10.4	12.9	11.8	28.8	15.0	19.8
San 9785	0	0	12.1	16.8	10.8	29.6	12.3	14.4
BASF 13761	0	0	11.0	15.4	12.6	29.2	14.3	16.6
San 9774	0	0	10.0	15.4	11.7	31.0	13.2	17.3
San 9789	60.5	11.0	1.0	1.4	1.3	10.2	5.6	7.3
San 6706	70.7	18.5	0.3	0.5	0.2	4.4	2.0	2.5
BASF 44521	60.1	11.0	0.3	1.3	2.0	11.0	5.3	6.2
EMD-IT-5914	58.9	3.6	0.6	2.3	2.1	17.1	6.7	8.7

these herbicides and saturated precursors, the colorless compounds phytoene and phytofluene are accumulated (Table I).

The carotenoid precursors phytoene and phytofluene are not present in detectable amounts in algae of the control culture (grown under the same conditions containing 0.1% methanol in the nutrient solution). No change in quality and quantity of the pigments has also been observed, if algae were cultured in presence of the pyridazinone derivatives pyrazon, San 9785, San 9774 or BASF 13761 (Table I). The carotenoid pattern is like that of algae grown under normal conditions (Table II).

The three substituted pyridazinones which interfere with the carotenoid biosynthesis even cause a decrease of the content of the chlorophyll in algal cells (Table I) like observed by Böger and Schlue [16]. The chlorophyll content is drastically reduced also by the herbicide EMD-IT-5914. In algae treated with the other pyridazinones in sublethal doses the chlorophyll level remains like that of control algae.

Accumulation of intermediates of carotenoid biosynthesis has been observed in *Chlorella* mutants [17] and in many other organisms in the presence of diphenylamine and other compounds [18, 19]. The herbicides amitrole, dichlormate and pyriclor cause an accumulation of saturated precursors of carotenoid biosynthesis resulting in a loss of colored carotenoids. Wheat seedlings treated with dichlormate show an accumulation of ζ-carotene and in pyriclor and amitrole treated seedlings phytoene, phytofluene and ζ-carotene have been detected [1]. Since after treatment with San 6706, San 9789 or BASF 44521 and even with the herbicide difunone high amounts of the colorless phytoene and in a lower degree of phytofluene have been found, it may be concluded that these herbicides interfere with the mechanism of desaturation by blocking the step wise dehydrogenation of the prepursors of carotenes normally obtained in these algae. If cyclization reactions would be blocked by these herbicides an accumulation of colored acyclic carotenes should occur like observed after treatment with pyriclor, amitrole and dichlormate.

It has been postulated that carotenoids are universally associated with photosynthetic tissues be-

cause they can protect cells against harmfull effects of visible light [21]. The ability to provide photoprotection *in vivo* is related to the lenght of the conjugated double bond chain of the carotenoid molecule [22, 23]. A minimum of 9 conjugated double bonds seems to be necessary to confer protection from light damage. Concerning the decrease of the chlorophyll level observed in algal cells treated with San 9789, San 6706, BASF 44521 or EMD-IT-5914 it may be suggested that these cells lost the normal protective mechanism by lacking most of the colored carotenoids and in consequence the chlorophyll is destroyed by photooxydation. This conclusion is supported by the observation that in algal cells treated with these compounds the decrease of chlorophyll a is stronger than that of chlorophyll b, resulting in a lowering of the chlorophyll a/b ratio (Table I). With regard to the effect of EMD-IT-5914 it has been shown by Hampp *et al.* [10] that the activity of the enzyme porphobilinogenase is inhibited by this herbicide. Therefore it may be suggested that the inhibitory action of this compound is not restricted to carotenoid synthesis accompanied by photodestruction of chlorophyll but that chlorophyll synthesis is affected directly.

From our results it can be concluded that the pyridazinone derivatives with both substitutions, a trifluoromethyl substitution of the phenyl ring *and* a methyl- or dimethyl substitution of the amine (or a methoxy group) are active as inhibitors of carotenoid biosynthesis. This process is not affected by compounds carrying *either* a trifluoromethyl substitution of the phenyl ring *or* a dimethyl substitution of the amine (or a methoxy group). Comparing the molecular structure of the pyridazinone derivatives it may be concluded that special substituents are responsible for the effect on carotenoid biosynthesis. Until now, however, correlation between activity and structure of the different herbicides (amitrole, pyriclor, dichlormate, difunone, pyridazinones) known to interfere with carotenoid biosynthesis has not been found.

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