The Effect of PS II Herbicides, Amitrol and SAN 6706 on the Activity of 3-Hydroxy-3-methylglutaryl-coenzyme-A-reductase and the Incorporation of [2-^{14}C]Acetate and [2-^{3}H]Mevalonate into Chloroplast Pigments of Radish Seedlings

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The influence of photosystem II herbicides (DCMU, bentazone) and herbicides that block chloroplast-pigment formation (amitrol, SAN 6706) on the incorporation of [^{14}C]acetate and [^{3}H]-mevalonate in chlorophylls and carotenoids was studied in radish seedlings *(Raphanus sativus* L.).

1. Though the pigment content in SAN 6706-treated plants is very low, they exhibit the highest specific incorporation rate as compared to controls and to the other herbicide-treated plants. This high metabolic activity coincides with a high specific activity of HMG-CoA-reductase in the plastidic and cytoplasmic fraction.

2. [2-^{3}H]mevalonic acid was incorporated in chlorophylls and carotenoids of controls and all herbicide-treated plants.

3. [2-^{14}C]acetate was only incorporated in β-carotene and lycopene, but not in α-carotene or xanthophylls. From this it is concluded that β-carotene is synthesized by two different biosynthetic pathways.

4. The incorporation of [^{14}C]acetate in DCMU- and bentazone-treated plants proceeded at a much lower rate (chlorophyll a) — or not at all (chlorophyll b) — than in controls of plants treated with amitrol or SAN 6706.

Introduction

Different functions of the mode of action of herbicides have been proposed. They inhibit photosynthesis [1] or interfere with the formation of chloroplasts by blocking carotenoid and chlorophyll accumulation [2 – 6]. The present investigation was performed to study whether four different herbicides (DCMU, bentazone, amitrol, San 6706) affect the incorporation of [2-^{14}C]acetate together with [2-^{3}H]mevalonate in chlorophylls and carotenoids, and was also compared with the activity of HMG-CoA-reductase [7], the key enzyme of the terpenoid pathway, in the organell and in the microsomal fraction.

Material and Methods

Radish seedlings were grown in bentazone, DCMU, amitrol (10^{-4} M each) and SAN 6706 (10^{-5} M) in white light (0.8 mW/cm^2). After 6 days [2-^{14}C]acetate and [2-^{3}H]mevalonate were applied for 24 h, chlorophylls and carotenoids were extracted, purified [8, 9], and assayed for radioactivity. The organell ($16\,000 \times g$) and microsomal fractions ($105\,000 \times g$) were assayed for HMG-CoA-reductase activity using a modified radioactive test method [10] with increasing amounts of HMG-CoA (radio isotope dilution). V_{max} values were calculated by the linear plot method [11].

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Results and Discussion

Photosystem II herbicide-treated plants are green, as are control plants, while amitrol and SAN 6706 [5] induce a strong chlorosis resulting in yellow or white leaves. The accumulation of chlorophyll a and b is suppressed by amitrol and SAN 6706, while PS II herbicides mainly affect the formation of chlorophyll a.

Amitrol specifically inhibits the cyclization of lycopene to α- and β-carotene, resulting in the accumulation of lycopene. Besides lycopene, phytoene, phytofluene, neurosporene and γ-carotene were identified. SAN 6706 is thought to inhibit the desaturation reaction in the carotenoid biosynthesis as seen by high amounts of phytoene. DCMU and bentazone induce a shade adaption [12].

Table I. Molspecific radioactivity of *Raphanus* chlorophylls after 24 h incorporation with [2-^{14}C]acetate and DL-[2-^{3}H]mevalonic acid as precursors.

	Control	10^{-4} M DCMU	10^{-4} M Bentazon	10^{-4} M Amitrol	10^{-5} M SAN 6706	
Chlorophyll a	7.22×10^4	8.78×10^4	6.91×10^4	5.39×10^4	62.27×10^4	[^{3}H]DPM/μmol
	1.23×10^4	0.28×10^4	0.23×10^4	1.09×10^4	16.64×10^4	[^{14}C]DPM/μmol
	5.88	31.96	30.11	4.93	3.74	[^{3}H]/^{14}C
Chlorophyll b	6.56×10^4	5.9×10^4	5.84×10^4	6.31×10^4	78.13×10^4	[^{3}H]DPM/μmol
	0.66×10^4	0	0	1.44×10^4	18.21×10^4	[^{14}C]DPM/μmol
	9.90	—	—	4.38		[^{3}H]/^{14}C

Table II. Molspecific radioactivity of *Raphanus* carotenoids after 24 h incorporation with [2-^{14}C]acetate and DL-[2-^{3}H] mevalonic acid as precursors.

	Control	10^{-4} m DCMU	10^{-4} m Bentazon	10^{-4} m Amitrol	10^{-5} SAN 6706	
Lycopene	208.5×10^5	125.2×10^5	504.7×10^5	213.3×10^5	608.7×10^5	[^{3}H]DPM/μmol
	310.5×10^5	160.2×10^5	252.4×10^5	29.3×10^5	247.5×10^5	[^{14}C]DPM/μmol
	0.67	0.78	2.00	7.30	2.50	^{3}H/^{14}C
α-carotene	154.0×10^5	308.9×10^5	620.0×10^5	308.9×10^5	4356.0×10^5	[^{3}H]DPM/μmol
	0	0	0	0	0	[^{14}C]DPM/μmol
	—	—	—	—	—	^{3}H/^{14}C
β-carotene	23.6×10^5	89.1×10^5	128.3×10^5	29.1×10^5	140.8×10^5	[^{3}H]DPM/μmol
	9.4×10^4	6.3×10^4	13.0×10^4	17.0×10^4	19.0×10^4	[^{14}C]DPM/μmol
	25.1	140.5	97.4	16.7	72.1	^{3}H/^{14}C
Lutein	4.3×10^5	4.7×10^5	27.2×10^5	4.4×10^5	45.38×10^5	[^{3}H]DPM/μmol
	0	0	0	0	0	[^{14}C]DPM/μmol
	—	—	—	—	—	^{3}H/^{14}C
Violaxanthin	5.6×10^5	2.2×10^5	$6.9\times10.^5$	2.0×10^5	51.30×10^5	[^{3}H]DPM/μmol
	0	0	0	0		[^{14}C]DPM/μmol
	—	—	—	—	—	^{3}H/^{14}C

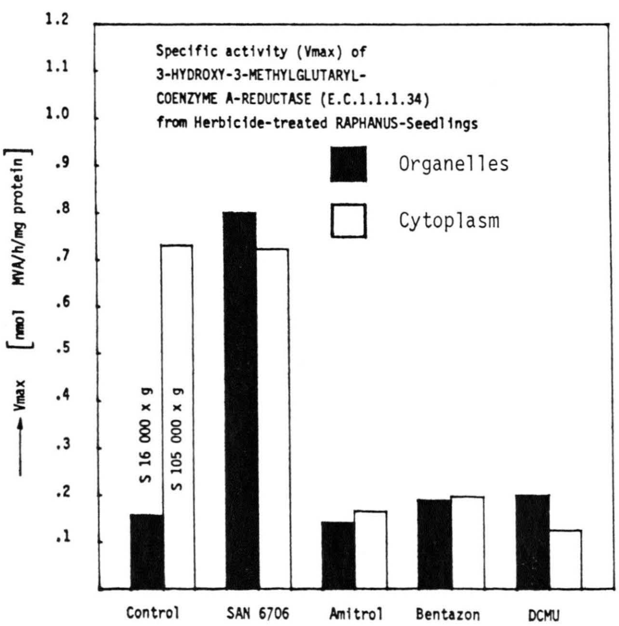

Fig. 1: Specific activity of 3-Hydroxy-3-methylglutaryl-coenzyme A-reductase in the microsomal and plastid fraction of radish seedlings treated with different herbicides.

The very high incorporation of [^{14}C]acetate and [^{3}H]mevalonate in SAN 6706-treated plants Tables I, II) coincides with a high activity of HMG-CoA-reductase in the plastid and microsomal fraction (Fig. 1). In plants treated with DCMU, bentazone and amitrol, the enzyme activity of the microsomal fraction is strongly decreased, but that of the plastid fraction is nearly the same as in untreated plants.

Incorporation experiments with [2-^{14}C]acetate and [2-^{3}H]mevalonate reveal that DCMU and bentazone-treated plants incorporate acetate at a much lower rate than amitrol, SAN 6706 and control-plants. Mevalonate was incorporated in chlorophyll a and b in the herbicide-treated plants to the same extent as in control plants, except for SAN 6706-

treated plants. The latter show the highest incorporation rate into chlorophylls and carotenoids, indicating a very high biosynthetic capacity. In spite of this, their pigment content is very low. This indicates that SAN 6706 treated plants can synthesize but not accumulate chlorophylls and carotenoids, possibly since other structural components of the thylakoid are missing.

The observation that ^{14}C-label from the acetate was only found in lycopene and β-carotene but not in α-carotene and xanthophylls (Table II) suggests that β-carotene is present in the chloroplast in two different pools of different biosynthetic pathways.

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