The Development of Daffodil Chromoplasts in the Presence of Herbicides SAN 9789 and SAN 9785

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Pyridazinone Herbicides, Chromoplast Ultrastructures, Carotenoids

The effects of two pyridazinone herbicides (SAN 9789 and SAN 9785) were studied on the fine structure and carotenoid composition during the transformation of chloroplasts into chromoplasts in daffodil (*Narcissus poëticus* L.) flowers. SAN 9789 caused the absence of big characteristic carotene crystals and the appearance of numerous nonosmiophilic plastoglobuli in chromoplasts. The accumulation of all carotenoids was drastically reduced and the content of carotenes was 40-fold lower than in the control. SAN 9785 caused no gross abnormalities in ultrastructure of chromoplasts. The synthesis of carotenes was partially reduced to about 75%. In spite of this the accumulation of xanthophylls is two times higher than in the control. The interaction between chromoplast ultrastructure and carotenoid composition is discussed.

Introduction

The chromoplasts from red and yellow coronae of daffodil flowers contain big crystals of β-carotene [1] and chromoplast internal membranes with an extremely high lipid content with lutein as the prominent component followed by violaxanthin and neoxanthin [2, 3].

In plastids, carotenoids are always associated with lipid molecules either in membranes or in plastoglobuli and it is therefore of great interest to investigate the relationship between the structure of the plastids and the synthesis of carotenoids. The characterization and the biosynthesis of carotenoids in higher plants are well investigated [4]. However, the relation between biosynthesis of carotenoids and structure of plastids is poorly understood.

There are many substances which have been reported to interfere with carotenoid synthesis in higher plants. CTPA has been shown to cause the accumulation of the red pigment lycopene [5], while the herbicides amitrole, dichlormate and pyriclor [6] have been found to cause the accumulation of the yellow pigment ζ-carotene and of two colourless polyenen. Pyridazinone herbicides are the potent inhibitors of carotenoid synthesis [7–13]. The pyridazinone herbicide SAN 9789 has been reported to be a strong and direct inhibitor of carotenoid biosynthesis, which causes, more or less, to a complete absence of coloured carotenoids. SAN 9785 has been shown to be a specific inhibitor of the desaturation reaction between linoleic and linolenic acid [14–16] and causes indirectly minor effects on pigment accumulation.

The action of pyridazinone on ultrastructure and photosynthetic activity [17–20] and lipid composition [21–22] of chloroplasts has been investigated. However, the action of pyridazinone herbicides on the ultrastructure and pigment composition of chromoplasts is still inadequately investigated. In order to obtain this information the effects of two pyridazinone derivates, SAN 9789 and SAN 9785 on chromoplast development during the transformation of chloroplasts into chromoplasts were studied in red coronae of daffodil flowers.

Materials and Methods

The daffodil flowers (*Narcissus poëticus* L.) were grown in the Botanical garden under natural conditions. All experiments were performed in March and April during two seasons. Young undetached buds (about 15 cm long) of daffodil flowers with pale green coronae were carefully opened and whole petals and coronae were imersed for 20 h in 0.2 mM solution of herbicides in tap water (control). Herbicide SAN 9789 was dissolved directly in tap water, but SAN 9785 was first dissolved in a few drops of methanol before tap water was added. After the

Abbreviations: SAN 9789, 4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3-(2H)-pyridazinone; SAN 9785, 4-chloro-5-(dimethylamino)-2-phenyl-3-(2H)-pyridazinone.

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treatment the buds were carefully closed so that coronae and petals were protected from withering until the natural opening of the flowers. The samples for macroscopic observation, microscopic investigation and pigment analysis were taken 5, 9 and 14 days after the treatment. In all experiments only the intensively coloured 1–2 mm wide distal border of corona was used.

For electron microscopy the material was fixed in 1% glutaraldehyde and postfixed in 1% OsO_4 (at 1 °C) and embedded in Araldit after dehydration. Sections were cut on the Reichert Om U2 ultramicrotome, stained with uranyl acetate and lead citrate and examined in the Siemens Elmiskop I.

For pigment analysis the samples were cut into small pieces, mixed with a small amount of $BaCO_3$ and quartz sand and ground in 100% acetone in a mortar to extract the pigments. For quantitative analysis the obtained carotenoids were separated by thin-layer chromatography on silica gel G plates with the mixture of petrol ether (60–80 °C): ethyl acetate: diethylamine (58:30:12) as solvent. The bands which contained carotenoids were eluted with 80% acetone. The absorbance for chlorophylls at 663 nm and 645 nm and for carotenoids at 450 nm [4], were measured with the spectrophotometer Specol 10 (Zeiss, Jena).

Results

Macroscopic observation

The corona of the young daffodil flower bud is pale greenish in colour. Untreated coronae become intensively red in less than two weeks, whereas the treatment with SAN 9789 results in flowers with only slightly coloured coronae. They are uniformly yellow and without any distinctly coloured border. However, the herbicide SAN 9785 did not cause any macroscopically visible effects regarding colour and size of coronae.

Ultrastructure of plastids

Control. The pale greenish corona in the flower bud contained chloroplasts which are not yet fully differentiated (Fig. 1A). These chloroplasts were small and contained less thylakoids and much less grana, any with only few thylakoids. In the course of further development the colour of the coronae turned red, the grana disappeared and long single thylakoids and chromoplast internal membranes gradually assembled. Within this membrane system, part of which persisted until the final stages of chromoplast development, big carotene crystals (more than 10 μm long) appeared (Fig. 1B). Plastoglobuli (about 0.1 μm in diameter) were very often arranged in small groups.

SAN 9789. The process of transformation of chloroplasts into chromoplasts in treated daffodil flowers was very similar to some processes of the control during the first five days. The grana disappeared and long chromoplast internal membranes gradually formed. But in the coronae of fully developed flowers the cross sectioned chromoplasts were irregular in shape and contained a large membranous system, forming very long bundles of partially stuck membranes. Carotene crystals were very small (less than 1 μm long) and rare. Plastoglobuli were numerous having very little osmiophilic nature and greater than 0.2 μm in diameter (Fig. 1C).

SAN 9785. Electron micrographs of plastids treated with this herbicide revealed no gross abnormalities in chromoplast morphology. Carotene crystals were formed, but their dimension was smaller (1–5 μm long) and the shape was less regular than in control flowers. Single thylakoids and chromoplast internal membranes were present but poorly developed compared with untreated flowers. Plastoglobuli were small and rare (Fig. 1D).

Pigment analysis

Control. In greenish coronae the majority of pigments were chlorophylls (1.70 mg/g fr.wt.). The ratio carotenoids/chlorophylls was 1.3 as in normal photosynthetic tissue. The carotenes (0.58 mg/g fr.wt.) and xanthophylls (1.66 mg/g fr.wt.) seemed unaffected with a ratio (carotenes/xanthophylls) of 0.34. Thin-layer chromatography showed that lutein represented the majority of xanthophylls. Violaxanthin, zeaxanthin and neoxanthin were considerably less present (Table I).

Red coronae in fully developed flowers were completely without chlorophylls. At the same time the concentration of carotenes was very high (8.14 mg/g fr.wt.) In spite of this the amount of xanthophylls diminished permanently with the age of the flowers to about 0.44 mg/g fr.wt. after 14 days. In consequence the ratio carotenes/xanthophylls increased to 13.5 (Table I).

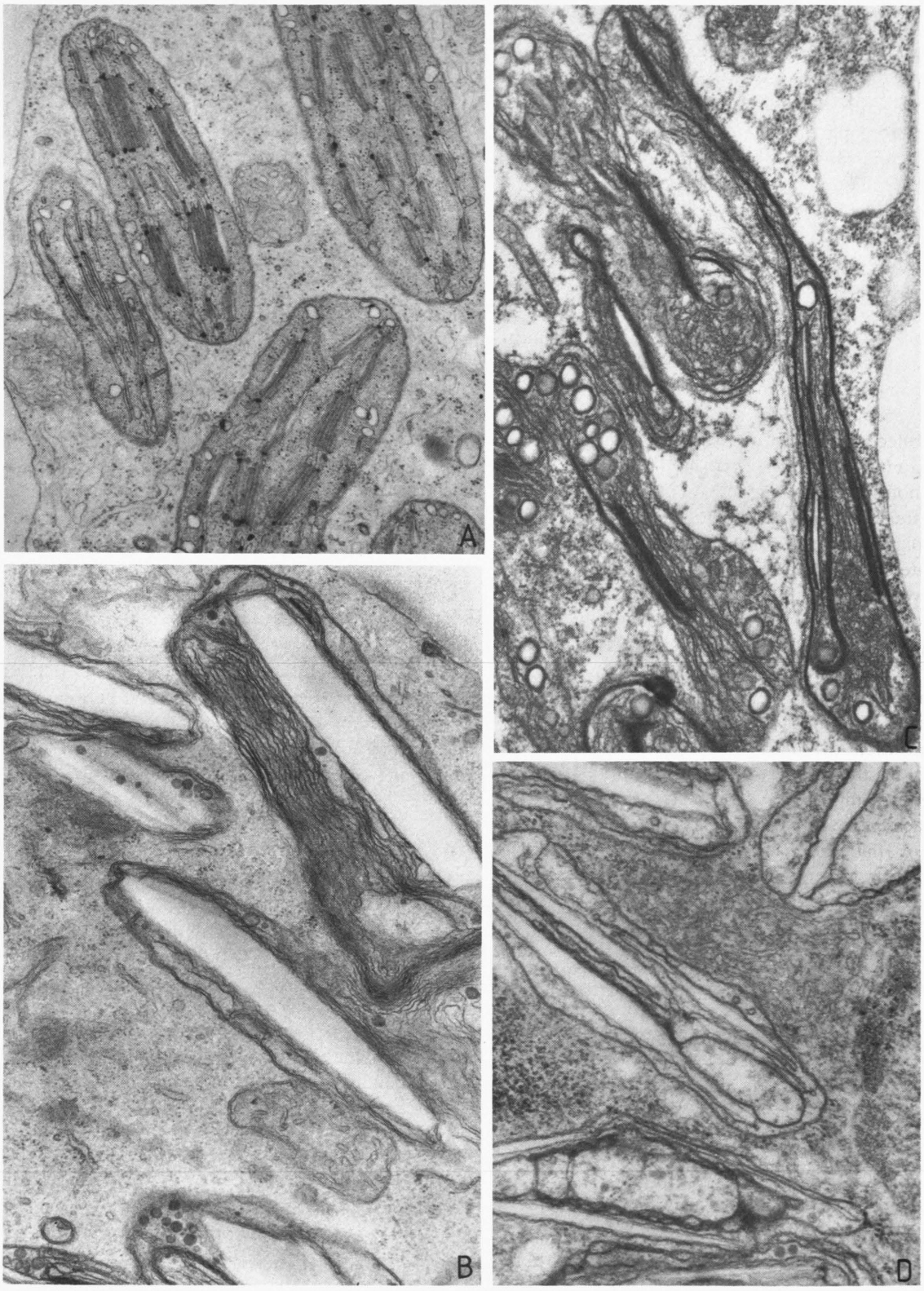

Fig. 1. Plastids from coronae of daffodil flowers:
A. Chloroplasts from flower bud. ×23,000.
B. Chromoplasts from fully developed flowers. ×28,000.
C. Chromoplasts treated with SAN 9789. ×25,000.
D. Chromoplasts treated with SAN 9785. ×33,000.

Table I. Content of carotenoids and chlorophylls in coronae during the development of untreated and treated daffodil flowers. Each value is the mean ± standard deviation of six independent experiments during two seasons.

	Young bud	Control			SAN 9789			SAN 9785		
Days	0	5	9	14	5	9	14	5	9	14
Carotenes mg/g fresh weight	0.58 ±0.15	0.55 ±0.12	0.44 ±0.11	8.14 ±1.42	0.68 ±0.15	0.30 ±0.05	0.20 ±0.05	0.65 ±0.13	0.20 ±0.05	6.10 ±1.10
Xanthophylls mg/g fresh weight	1.66 ±0.34	1.58 ±0.31	1.09 ±0.21	0.44 ±0.12	1.49 ±0.35	0.68 ±0.13	0.28 ±0.05	0.90 ±0.19	0.15 ±0.03	1.00 ±0.28
Chlorophyll $a+b$ mg/g fresh weight	1.70 ±0.24	2.85 ±0.48	0.83 ±0.16	– –	2.07 ±0.42	0.99 ±0.19	– –	2.61 ±0.45	0.84 ±0.20	– –

SAN 9789. In the first 5 days this herbicide had some effects on the quantitative or qualitative pigment composition. After 9 days the concentration of carotenoids began to decrease, opposite to that of the untreated plants. In the next few days the content of all pigments rapidly dropped, so that the coronae of treated flowers accumulated 40-fold less carotenes and two-fold less xanthophylls than control plants (carotenes/xanthophylls ratio: 0.7). The chlorophylls were present only in traces (Table I).

SAN 9785. Pigment composition in treated flowers was similar during the first 5 days to that in the untreated flowers (Table I), but after 9 days the concentration of carotenes and xanthophylls was lower. In the next 6 days the amount of all carotenoids rapidly raised, although carotenes did not reach the level of carotenes in control flowers. In spite of this the concentration of xanthophylls (1.0 mg/g fr. wt.) was two times higher than in untreated flowers (carotenes/xanthophylls ratio was 6.1). The chlorophylls were not present even in traces (Table I).

Discussion

The results show that treatment with 0.2 mM SAN 9785 induces only subtle changes in daffodil chromoplast structures, although it is known as a potent inhibitor of lipid synthesis. The observations indicate that the inhibition of some component of plastids may not cause the stop of synthesis of other components such as carotenoids. By contrast SAN 9789 inhibits carotene synthesis [10] but has no particular effect on the biosynthesis of chromoplast membrane structures. This means that the inhibition of one part of the plastid membrane components (lipids) does not prevent the synthesis of other components such as carotenoids and *vice versa*. It appears that some of the processes in plastids during the transformations of chloroplasts into chromoplasts are mutual independent.

The process of disappearance of chlorophylls during the development of daffodil flowers treated with SAN 9789 and SAN 9785 is very similar to that in control, and we cannot find any remarkable differences. Photodestruction of chlorophylls [23] is not present as a consequence of the lack of the protective role of carotenoids in material treated with both herbicides, because the corona is protected in closed buds from strong light during the process of flower development.

Our results indicate that the sublethal concentration of pyridazinone herbicides applied do not drastically affect the membrane structures of chromoplasts. It seems that only SAN 9785 slightly reduces the amount of membranes. According to some earlier investigations, we, however, conclude that their chemical composition is remarkably changed. The membranes of treated barley chromoplasts contain higher proportions of lipids and a different fatty acid composition than under normal conditions [16, 19]. We expect that treatment with SAN 9785 causes big changes in lipid composition in chromoplast internal membranes which mostly consist of lipids (more than 70%) [3].

The appearance of big non-osmiophilic plastoglobuli in chromoplasts treated with SAN 9789 is not yet clear. Probably the increase in total fatty acid content (3-fold in the SAN 9789-treated plants) [16] may be due to the appearance of such structures. It is still unknown whether the plastoglobuli contain some pigments or not but the amount of carotenoids is

quite small in comparison with their possible carriers (membranes, plastoglobuli and small crystals).

It has previously been found that SAN 9789 induces the complete inhibition of coloured carotenoids [10]. Our observation supports this finding. On the one hand we believe that small amounts of carotenoids, present in treated daffodil coronae, were synthesized already before treatment. On the other hand, diminishing of the amount of some carotenoid pigments is not the result of their destruction under the influence of herbicides. During the treatment (15 days) the coronae grew rapidly, their fresh weight multiplied several times, thereby leading to a relative dilution of the carotenoid concentration. Only the destruction of chlorophylls was obvious, but this is not induced by herbicides.

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