Interference of Dimethazone with Formation of Terpenoid Compounds

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Dimethazone is a bleaching herbicide without any peroxidative activity. In addition to the inhibition of chlorophylls, carotenes, and xanthophylls, decreased formation of other prenyl lipids (phytol and α-tocopherol) can be observed in the presence of dimethazone. Application of this herbicide to pea plants results in the inhibition of longitudinal growth of the newly formed internodes which can be reversed by gibberellic acid. Apparently, dimethazone also decreases the endogenous gibberellin levels.

As the formation of all the compounds assayed is inhibited to the same extent and as I_{50}-values for chlorophyll and carotenoid biosynthesis are identical (about 10 μM dimethazone), the various effects of dimethazone are explained by a single enzyme target in the terpenoid biosynthetic pathway between acetate and geranylgeranyl pyrophosphate.

Introduction

Dimethazone, 2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone (= FMC 57020) is a newly developed preemergence herbicide for weed control in soybeans [1, 2]. Multiple effects of dimethazone on plant metabolism have been reported [3, 4]. This compound exhibits bleaching activities, by affecting the content of plastidic pigments [5]. In contrast to many other bleaching herbicides, however, an accumulation of phytoene was not observed [3]. In addition to low pigment concentrations, chloroplasts develop with ultrastructural disorganization. Higher sucrose and glucose levels in dimethazone-treated seedlings were interpreted in terms of a blocked utilization of these sugars by dimethazone. A decreased Shibata shift was taken as indication for low phytylation activity [3, 5].

In this publication we report on the inhibitory effect of dimethazone on the formation of several terpenoid compounds and determined I_{50}-values for inhibition of both chlorophyll and carotenoid formation. All data presented suggest a common inhibitory site for dimethazone in the terpenoid pathway before or at the prenyl-pyrophosphate step.

Materials and Methods

Scenedesmus acutus, strain 276-3a, was cultivated for 3 days in an autotrophic medium in the light (45 W/m^2) or in a heterotrophic medium supplemented with glucose and yeast extract [6] in the dark. Peas (*Pisum sativum* L., var. Kromboeck) were germinated and grown in vermiculite for 2 weeks. Then 20 μl of a solution of dimethazone or gibberellic acid in 0.1% Tween 20 was applied to the terminal bud of groups of 6 plants. After 3 days the length of the newly-formed internode was measured.

Carotenes and xanthophylls were extracted as described [7]. Separation was performed by TLC on silica plates using 15% toluene (v/v) in petrol (b.p. 100–140 °C). Chlorophylls *a* and *b* were determined after hot methanol extraction (65 °C, 15 min) according to Mackinney [8]. Extraction of α-tocopherol and phytol from freeze-dried algal cells was done with 80% acetone (v/v) containing 0.1% Na-ascorbate and heating at 50 °C for 15 min. The extract was partitioned against hexane. After washing with 80% ethanol, the hexane phase was used for α-tocopherol determination by HPLC [9]. Aliquots of the hexane extract were evaporated and redissolved in methanol. Then phytol was determined by HPLC on a Spherosorb 5 ODS column with 5% H_2O (v/v) in methanol as mobile phase. The UV-absorption was recorded at 230 nm, the flow was 1 ml/min, the retention time for phytol 8.5 min.

Ethane evolution from *Scenedesmus* cells was taken as an indicator of peroxidative action of dimethazone and oxyfluorfen. The hydrocarbon was accumulated in sealed head-space vials and the gas phase automatically injected into the gas chromatograph. Details of this procedure were given previous-

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ly [10]. Packed cell volume (pcv) was determined in graduated microcentrifuge tubes of 80 µl capacity.

Results and Discussion

The effect of dimethazone on prenyl lipids is shown in Table I. In addition to autotrophic cultures of *Scenedesmus, heterotrophically* grown cells were assayed for pigment content to avoid and exclude possible peroxidative degradation in the light. In the presence of dimethazone the formation of total carotenoids and total chlorophylls was inhibited to the same extent (63% of control). This simultaneous decrease of both types of plastidic pigments in heterotrophic cultures resembles the effect of nitrodiphenyl-ether herbicides and may be indicative of peroxidative activities of dimethazone [7]. However, peroxidative ethane evolution could not be found with *Scenedesmus* cells treated with high concentrations of dimethazone (Table II). When the nitrodiphenyl-ether herbicide oxyfluorfen was added as a reference compound it caused substantial peroxidative ethane evolution already at a 0.1 µM concentration. The data obtained with autotrophic *Scenedesmus* cells show that the formation of chlorophylls *a* and *b* was inhibited by dimethazone and that chlorophyll *a*, the precursor of chlorophyll *b*, is even more strongly affected (Table I). The same tendency can be observed for carotenes and xanthophylls. The levels of both carotenoids were also decreased by dimethazone, but the levels of the xanthophyll precursors α- and β-carotene were decreased more strongly than those of the end products of this biosynthetic pathway. Many bleaching herbicides interfering with carotenoid biosynthesis cause the accumulation of phytoene [11]. In contrast to the action of those herbicides, inhibition of carotenoid formation by dimethazone was not accompanied by phytoene accumulation in *Scenedesmus*, nor was phytoene detected when higher plants were treated with dimethazone [3].

Free phytol and α-tocopherol could be detected in autotrophic *Scenedesmus* cells. The α-tocopherol content in *Scenedesmus* is about 10- to 100-fold lower than in leaves of various species of higher plants [9].

Table II. Peroxidative formation of ethane in autotrophic *Scenedesmus* cells.

	Ethane evolution* [nmol/ml packed cell volume]
Control	0
100 µM Dimethazone	0
0.1 µM Oxyfluorfen	5.4
1 µM Oxyfluorfen	13.8

* Ethane was accumulated over 16 h.

Table I. Prenyl-lipid levels in autotrophic *Scenedesmus* treated with dimethazone.

	Control	+20 µM Dimethazone	% Control	
	[µg/mg dry weight]			
A) Heterotrophic cells				
Chlorophyll *a*	19.5	11.4	63	
Chlorophyll *b*	6.9	5.4		
Colored carotenoids*	3.3	2.1	63	
B) Autotrophic cells				
Chlorophyll *a*	37.8	13.8	37	**
Chlorophyll *b*	14.7	7.8	53	
α- + β-Carotene*	1.3	0.5	39	
Xanthophylls	4.4	2.8	64	
Phytol	111.6×10^{-3}	74.2×10^{-3}	66	
α-Tocopherol	7.6×10^{-3}	4.9×10^{-3}	64	

* Accumulation of phytoene could not be observed in dimethazone-treated cells. Growth inhibition of autotrophic or heterotrophic cultures was about 30 to 40%.

** In autotrophic cultures an additional decrease of the pigment levels is observed due to photoxidative degradation especially when pigment biosynthesis is impaired.

The pool of free phytol in *Scenedesmus* represents about 0.2% of the phytol bound to chlorophylls. Dimethazone inhibited the formation of both prenyl lipids and we found the same degree of inhibition as for carotenoids and chlorophylls in the dark-grown cultures. Thus, there is strong evidence that impaired chlorophyll formation, seen in the presence of dimethazone, is the result of inhibition of phytol biosynthesis. Consequently, the decrease of the Shibata shift in the presence of dimethazone [3] can be explained by low phytylation activities due to poor phytol supply. This agrees with the finding of Duke and Kenyon [5] that this herbicide decreased chlorophyllide conversion.

I_{50}-values for the inhibition of chlorophyll as well as carotenoid formation have been determined in heterotrophic (dark) cultures (Fig. 1). In an adapted Dixon plot [12] of dimethazone concentration *versus* reciprocal pigment concentration straight lines were obtained and the intersection with the abscissa gave identical I_{50}-values of 10 to 11 μM dimethazone for inhibition of both chlorophyll and carotenoid formation, respectively. These identical I_{50}-values and similar degrees of inhibition of the formation of prenyl lipids as shown in Table I can be taken as evidence of only *one* site being affected in the terpenoid pathway.

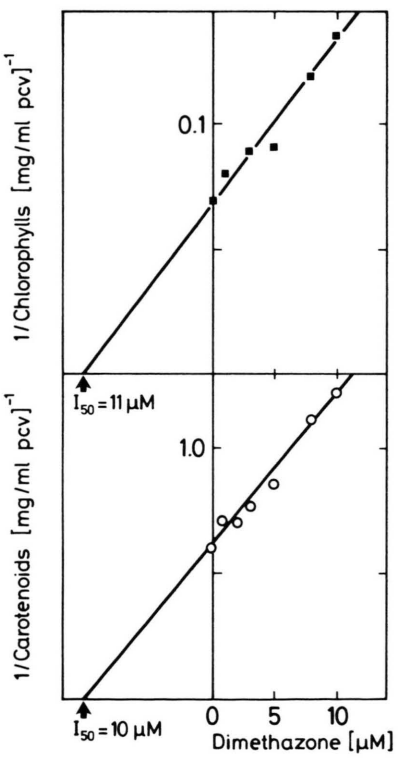

Fig. 1. Determination of I_{50}-values for inhibition of chlorophyll (upper part) and carotenoid formation (lower part) by dimethazone in heterotrophically-grown *Scenedesmus* cells. Reciprocal concentrations of total chlorophyll and total carotenoids (mg/ml packed cell volume) were plotted against dimethazone concentration.

Table III. Internode length of *Pisum sativum* plants treated with dimethazone and gibberellic acid (GA_3).*

Treatment	Length [cm] ± standard error
1) Control	3.8 ± 0.3
2) 100 μM Dimethazone	1.8 ± 0.3
3) 100 μM Dimethazone + 100 μM GA_3	3.1 ± 0.1
4) 100 μM GA_3	4.1 ± 0.4

* 20 μl, suspended in 0.1% Tween 20, were applied to the terminal bud of 2-week old seedlings.

Inhibition of seedling growth by dimethazone has been reported [3]. We have tried to reverse the dimethazone-induced decrease of internode length in *Pisum* plants by gibberellic acid (Table III). After application of dimethazone, longitudinal growth of the terminal internode was found only half of the control plants. Simultaneous treatment with identical amounts of gibberellic acid and dimethazone prevented most of the inhibition caused by the latter. Variance analysis including Fisher's LSD-Test (1% level, two-tailed; degrees of freedom were 20) showed that internode lengths of dimethazone-treated and control plants as well as of plants treated with dimethazone and dimethazone + gibberellic acid were significantly different from each other. This result is taken as evidence that the biosynthesis of gibberellins, another group of terpenoid compounds, is inhibited by dimethazone.

Conclusion

Dimethazone inhibits the formation of several terpenoids in *Scenedesmus*. Furthermore, there is indirect evidence that gibberellin biosynthesis is affected in *Pisum*. The common biosynthetic route for all these terpenoids is the pathway from acetate *via* mevalonate to geranylgeranyl pyrophosphate. The latter compound is the precursor from which all the compounds of Table I branch off, including gibberel-

lins. The results presented suggest firstly, that the inhibition site of dimethazone is located either within the formation of prenyl pyrophosphates from acetate and mevalonate or within their interconversion, and, secondly, that only *one* enzyme target is sufficient to account for the decreased formation of all the prenyl lipids.

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