Influence of Aminotriazol on the Biosynthesis of Chlorophyll and Phytol

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Treatment of wheat caryops with 3-amino-1,2,4-triazol followed by dark germination leads to phytol deficiency (based on the chlorophyll content) during the subsequent greening process in the light. The analysis of the pigment fraction reveales the presence of chlorophyll esterified with geranylgeraniol and dihydrogeranylgeraniol in agreement with earlier results [5].

Chlorophyll esterified with geranylgeraniol is shown *in vivo* and *in vitro* to be the precursor of phytylated chlorophyll. Aminotriazol presumably inhibits the hydrogenation of geranylgeraniol to phytol in the pigment.

Introduction

Aminotriazol (3-amino-1,2,4-triazol) belongs to the group of "bleaching" herbicides, *i. e.* it leads to the lack of pigments like chlorophyll in light-grown plants. Burns *et al.* [1] presumed a primary inhibition of carotenoid biosynthesis followed by photodestruction of chlorophyll because protecting carotenoids are not present. Such a mechanism has also been proposed for the pyridazinon herbicides SAN 6706 and SAN 9789 [2–4]. Whereas the latter two herbicides inhibit carotenoid accumulation in the dark as well as in the light [5], no inhibition was found with aminotriazol in the dark [5–7]. Because manyfold effects of aminotriazol have been described, *e. g.* accumulation of nucleotides, amino acids and ammonium ion [8–10], inhibition of protein and lipid biosynthesis [6, 7], chlorophyll accumulation must not necessarily be impaired via inhibition of carotenoid synthesis. The accumulation of geranylgeraniol-containing chlorophyll (Chl_{GG}) in aminotriazol-treated wheat seedlings [5] suggests that the last step of phytol biosynthesis (*i. e.* the hydrogenation step) might be inhibited by the herbicide. However, it was not clear at the time of that investigation whether Chl_{GG} is a normal intermediate of the biosynthesis of Chl_P. A reinvestigation of the aminotriazol effect on chlorophyll biosynthesis and a careful comparison with normal conditions seemed therefore necessary. The first preliminary results of this research are described in the present paper.

Results and Discussion

The kind of application of aminotriazol is essential for the observed results. We soaked the wheat caryops for 24 hours in 10^{-3} M aminotriazol and germinated the treated caryops immediately on vermiculite moistened with water [5]. This treatment provided a good comparison for the kinetics of greening in various light periods whereas a permanent treatment with the herbicide could lead to different amounts of herbicide in the plants after various incubation periods because of the possibility of permanent uptake. The treated caryops must not be washed before germination because the herbicide effect is eliminated by washing. The duration of the dark germination (in our experiments 96 hours at 25 °C) period is as well essential because the plants escape the herbicide action during longer dark germination (120 hours or more).

The accumulation of total phytol in aminotriazol-treated seedlings during the light period after 96 hours dark germination is somewhat slower than the accumulation of total chlorophyll (Fig. 1) whereas the water control plants accumulate more phytol than chlorophyll. This is consistent with earlier observations [5]. The accumulation of chlorophyll and phytol stops in the herbicide-treated plants after 24 hours illumination whereas the control plants continue the accumulation. The phytol deficiency (as

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Abbreviations: Chl_{GG}, chlorophyll esterified with geranylgeraniol; Chl_{DHGG}, chlorophyll esterified with dihydrogeranylgeraniol; Chl_{THGG}, chlorophyll esterified with tetrahydrogeranylgeraniol; Chl_P, chlorophyll esterified with phytol.

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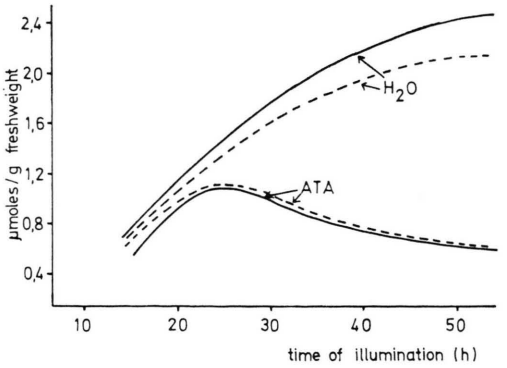

Fig. 1. Accumulation of total phytol (—) and total chlorophyll (- - -) in 96 hours old etiolated wheat seedlings in continuous white light (2500 lux). H_2O: controls, ATA: caryops soaked in 10^{-3} aminotriazol for 24 hours before germination.

compared to total chlorophyll) is largest at the beginning of the light period (pigment and alcohols can at first be determined after 10 hours illumination). Phytol deficiency in herbicide-treated plants and phytol surplus (related to chlorophyll) in water controls (Fig. 2) corresponds to earlier results [5]. Analysis of the phytol deficient pigment fractions (up to 48 hours illumination) reveales the presence of chlorophyll esterified with geranylgeraniol and dihydrogeranylgeraniol as described earlier [5].

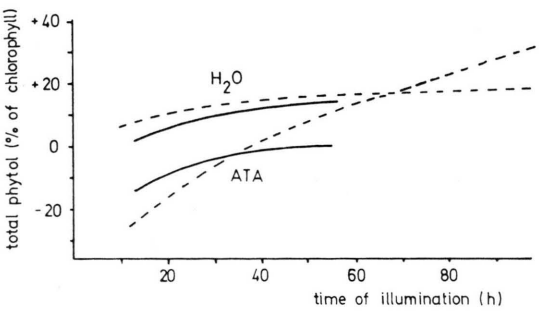

Fig. 2. Deficiency and surplus of phytol based on the chlorophyll content during greening of etiolated wheat seedlings, (—) values of present investigation, (- - -) values of Steffens [17].

The same pigments Chl_{GG}, Chl_{DHGG} and in addition Chl_{THGG} have been detected as immediate precursors of Chl_P during the normal greening process of etiolated plants. The sequence given in Fig. 3 has been shown for etiolated oat [11] and bean [12] seedlings. These findings are important in connection with the results obtained with aminotriazol-treated plants because some of the earlier discussed possibilities of herbicide action seem to be unlikely now. One of the unlikely possibilities is the photooxidation of Chl_P to Chl_{GG} or Chl_{DHGG}. Such a preferential photooxidation of phytol (over the chlorophyllide residue of chlorophyll) has never been found; photooxidation on the contrary leads to preferential destruction of the chlorophyllide residue (over the phytol residue) of chlorophyll ([13], Benz and Rüdiger unpublished results). The assumption that chlorophyllide could be esterified with geranylgeraniol (and dihydrogeranylgeraniol) because of lack of phytol under herbicide action [5] is as well unlikely if Chl_{GG} is the natural precursor of Chl_P. The kinetics do not yet exclude the possibility, however, that an equilibrium exists between more or less hydrogenated diterpene alcohols in the pigment, *i. e.* $Chl_{GG} \rightleftharpoons Chl_{DHGG} \rightleftharpoons Chl_{THGG} \rightleftharpoons Chl_P$. Such an equilibrium could then be shifted to the left or right side according to the conditions within the plant cell.

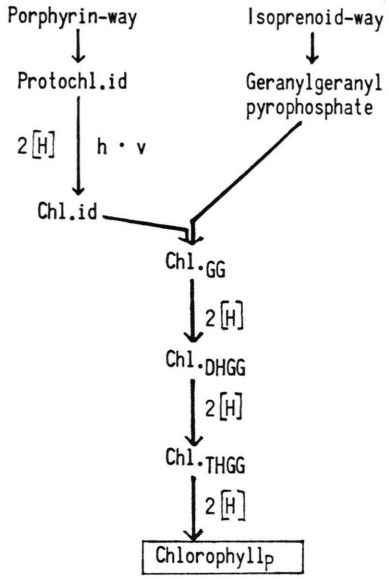

Fig. 3. Pathway of the last step of chlorophyll biosynthesis derived from kinetics determined in oat [11] and bean [12] seedlings.

To check this possibility, we used an *in vitro* system for the esterification of chlorophyllide. The system described at first for maize shoots [14] was modified and applied to oat seedlings [15]. It consists of purified etioplasts which are broken by an

osmotic shock and – after phototransformation of protochlorophyllide to chlorophyllide – incubated with geranylgeranyl pyrophosphate or phytyl pyrophosphate. This cell-free system incorporated either geranylgeraniol or phytol into chlorophyll [15]. Although the specificity is somewhat better for geranylgeranyl pyrophosphate than for phytol pyrophosphate we do not consider this as a convincing evidence for the presumed sequence $Chl_{GG} \rightarrow Chl_P$. However, incubation with NADPH leads to a transformation of Chl_{GG} to Chl_P via the intermediates (Fig. 4). This transformation has also been confirmed with labelled geranylgeranyl pyrophosphate whereas the label of phytylpyrophosphate is not found in any other pigment than Chl_P [15]. These experiments contradict the possibility of the above mentioned equilibrium. They further demonstrate that the hydrogenation of Chl_{GG} to Chl_P needs NADPH whereas NADH is not effective [15].

Taking together all available evidence, the most likely effect of aminotriazol seems to be the inhibition of the hydrogenation of Chl_{GG} to Chl_P. This step of the chlorophyll biosynthesis seems to be sensitive not only to aminotriazol but also to other unfavorable conditions: it is also inhibited by anaerobiosis of the seedlings [16]. It is not yet clear whether aminotriazol acts via decrease of the available NADPH pool or via decrease of the hydrogenase system (either by inhibition of enzyme activity or of enzyme biosynthesis). The *in vitro* system used in this study will be valuable also for the investigation of this question.

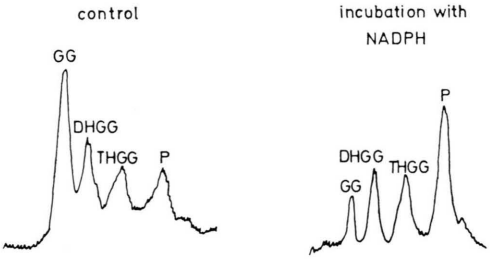

Fig. 4. HPLC analysis of chlorophylls esterified with various alcohols after *in vitro* esterification of chlorophyllide. Etioplast fraction from oat seedlings were broken by osmotic shock, irradiated for 1 min with white light and incubated for *in vitro* esterification of chlorophyllide [14]. Incubation with NADPH (ratio NADPH/chlorophyll = 1 mol/mmol). leads to preferential formation of Chl_P. Conditions of HPLC analysis as in [12].

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