On the Action of Chlorosis-Inducing Herbicides in Leaves

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The chlorosis induced by several different herbicides (group 1: aminotriazole, haloxidine; group 2: Sandoz 6706, difunon) in developing leaves of rye seedlings *(Secale cereale* L.) growing in light of 5,000 lx was, in addition to the absence of chlorophyll and carotenoids, accompanied by specific deficiencies of the 70 S ribosomes and of both chloroplastic and peroxisomal enzymes as previously described (Plant Physiol. **61**, 1017–1022 (1978)) while growth and mitochondrial activities were little or not impaired.

In dim light (10 lx) chlorophyll was formed and the chloroplastic and peroxisomal enzymes reached activities comparable to those in untreated leaves at 10 lx. Upon exposure to 30,000 lx a rapid bleaching of existing chlorophyll and a rapid inactivation of the plastidic enzyme NADP-glyceraldehydephosphate dehydrogenase occured after treatment with herbicides of group 2 but not in the presence of herbicides of group 1. After all treatments catalase was rapidly inactivated after transfer to high light intensity. The bleaching of chlorophyll and the enzyme inactivations occurred also at 0 °C. In the herbicide-bleached leaves grown at 5,000 lx the in vivo synthesis of δ-aminolevulinic acid was low and in the presence of Sandoz 6706 and difunon the capacity for protochlorophyll(ide) synthesis was largely inactivated.

Introduction

Several chemically unrelated herbicides are known to cause chlorosis of treated plants [1, 2]. All chlorosis-inducing herbicides so far investigated have in common that they prevent or strongly diminish the accumulation of colored carotenoids which is widely regarded as their basic mechanism of action [1–4]. The synthesis and phototransformation of Pchl(ide) was not blocked in treated leaves and the chlorosis is assumed to arise from an photooxidative bleaching of chlorophyll resulting from the carotenoid-deficiency [1, 3, 4]. Carotenoids are thought to protect against photooxidation because they are able to quench the reactive triplet states of excited chlorophyll or its potential reaction product $^1O_2$ and thus prevent the generation of activated oxygen species which are capable of causing oxidative degradation of chlorophyll as well as of other chloroplast constituents [5–8].

Unexpected and not easily understood as a consequence of photodestructions in the plastids were, however, the observations that after treatment with all chlorosis-inducing herbicides that we have investigated also the catalase activity and, in some instances (Sandoz 6706, difunon), even additional other peroxisomal enzymes were strongly and specifically inactivated [2]. If these peroxisomal defects were parallel or completely separate herbicide effects the possibility has to be considered that they might by themselves essentially contribute to the generation of photooxidative damage and to bleaching because catalase is no longer available for the detoxification of H_2O_2 and, after the complete elimination of the peroxisomal reactions, the whole photorespiratory pathway which is also regarded as a means protecting against photooxidation [9] would no longer be functional. Therefore, we have tried to obtain further information about the site of origin of the photooxidative events – whether they arise at a purely photochemical level [5, 7] or are initiated by deviations of the photosynthetic electron transport chain [8] and would then depend on enzyme-catalyzed reactions – and about their relationship to the inactivation of peroxisomal functions.

Abbreviations: ALA, δ-aminolevulinic acid; Pchl(ide), protochlorophyll(ide); A, aminotriazole; H, haloxidine; S, Sandoz 6706; D, difunon; F, fluometuron; SD, Bayer SDR 5175.

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Materials and Methods

Experiments were performed with winter rye (*Secale cereale* L.) cv. Petkus "Kustro". Growing conditions and herbicide-treatments and concentra-

tions were as previously described [2], except where otherwise indicated.

For the demonstration of photodestructions seedlings were grown at 10 lx. On day 6 the upper and lower quarters of 20 first leaves were excised and the remaining middle sections were floated either on the herbicide solutions used during growth (for enzyme assays) or on 0.1 M K-phosphate buffer, pH 6.0 (for pigment determinations) in petri dishes and kept at either 0 °C or 30 °C. Under these conditions the leaf segments were illuminated at 30,000 lx.

Measurements of the respiration rates of leaf segments, pigments, *in vivo* activity of δ-aminolevulinic acid synthesis and the estimation of other enzyme activities have been described elsewhere [2, 10, 11].

Results and Discussion

1. Specificity of the herbicide action

a) Growth and mitochondrial activity. When seedlings of rye or wheat are grown from the onset of germination on solutions of the herbicides aminotriazole, the aminotriazole derivative SDR 5175, haloxidine, Sandoz 6706, fluometuron, or difunon in light of 5,000 lx the leaves grow and expand normally, however, they become completely chlorotic. At appropriate concentrations the total amino-nitrogen content of the leaves was not or only little affected [2]. A high degree of specificity in the interference of the herbicides with chloroplasts was further emphasized by the findings that mitochondrial enzyme activities [2] and respiration rates (Fig. 1a) were either only slightly influenced or even higher than for untreated controls. A comparison of the influence of cyanide and salicylhydroxamic acid, an inhibitor of the cyanide-insensitive respiration, shows that the herbicide-bleached leaves had as a particular modification, as compared to untreated leaves, acquired the cyanide-insensitive pathway in addition to the cyanide-sensitive respiration (Fig. 1 b). This seems to be characteristic for chlorotic leaf tissues and not particularly related to the herbicide action since it was similarly found in heat-bleached rye leaves [10].

b) Effects on chloroplasts and peroxisomes. In addition to the absence of chlorophyll and carotenoids all herbicide treatments prevented the accumulation of chloroplastic 70 S ribosomes, as indicated by the absence of plastidic rRNA at 5,000 lx [2], and, consequently, all proteins which are totally or partially synthesized on chloroplastic ribosomes, such as ribulosebisphosphate carboxylase [2], were absent from the chlorotic leaves. The elimination of the 70 S ribosomes would by itself be sufficient to cause chlorosis of the leaves [2, 10, 11]. For a chloroplast-specific enzyme which is synthesized on cytoplasmic ribosomes, NADP-glyceraldehydephosphate dehydrogenase, two types of behavior were observed. After treatment with aminotriazole or haloxidine (group 1) the NADP-glyceraldehydephosphate dehydrogenase reached high activity in light-grown leaves, whereas after treatment with Sandoz 6706 and difunon (group 2) its activity remained extremely low [2]. In fluometuron-treated leaves the behavior was intermediate. In darkgrown leaves the accumulation of plastidic rRNA as well as that of chloroplast-specific enzymes was unaffected

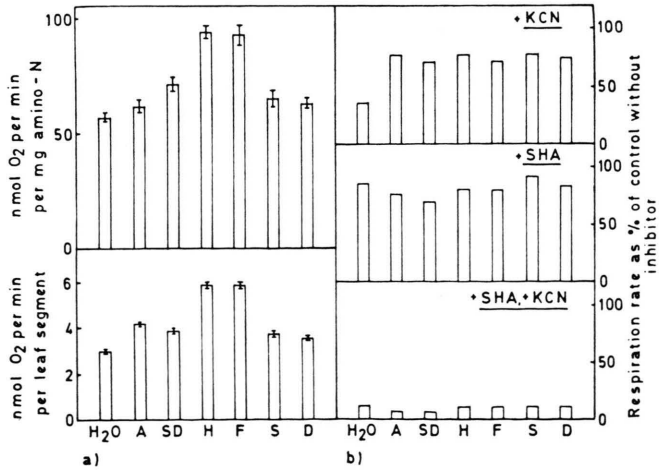

Fig. 1. Comparison of respiration in leaf tissue from 6-day-old rye seedlings grown in light on H_2O or in the presence of herbicides. a) Respiration rates. b) Influence of 15 min preincubation with 1.7 mM KCN, 20 min preincubation with 2.5 mM salicylhydroxamic acid (SHA), or 20 min preincubation with SHA plus 10 min KCN. The acetone used as solvent for SHA had no effect at the concentration applied.

by all herbicide treatments indicating that, directly or indirectly secondary photodestructions were responsible for their elimination in light [2].

Peroxisomal enzymes were also quite strikingly affected. In all light-grown leaves catalase activity remained very low and, after treatment with herbicides of group 2, also the activities of other peroxisomal enzymes, such as glycolate oxidase and hydroxypyruvate reductase were hardly detectable [2]. That most of these peroxisomal defects also occurred only in light suggests that photodestructions seem to be involved which are, however, difficult to understand as consequence of photooxidative events in the chloroplasts.

2. On the mechanism of bleaching

a) Capacity for chlorophyll synthesis. The series of enzymes converting ALA to Pchl(ide) is made on cytoplasmic ribosomes [11] and all herbicide-bleached leaves were at least to some degree able to form Pchl(ide), when they were supplied with ALA in darkness (Fig. 2 a). The capacity for Pchl(ide) formation was, however, much smaller after treatment with herbicides of group 2 than in the presence of group 1 herbicides (Fig. 2 a). The capacity for Pchl(ide) formation is typically much greater in the growing basal than in the more mature tip parts of the leaves (Fig. 2 a, [11]). Similarly also the *in vivo* activity of the ALA-synthesizing reactions was preferentially restricted to the young basal leaf region [11] and, therefore, only the basal leaf parts were compared in Fig. 2 b. In all herbicide-bleached leaves the *in vivo* synthesis of ALA was rather low, particularly in the presence of group 2 herbicides and thus the efficiency of chlorophyll synthesis seemed to be strongly restricted.

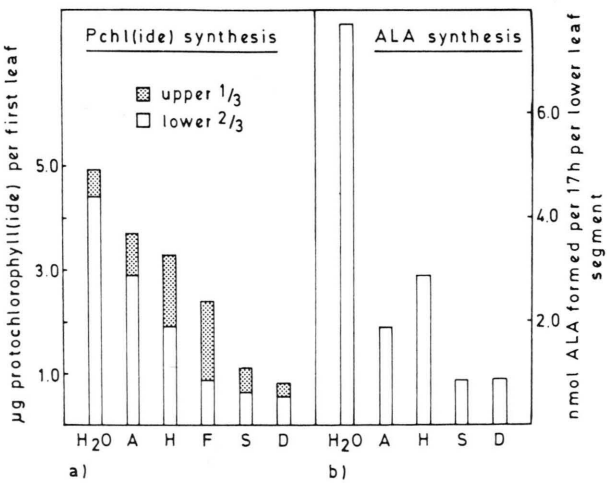

Fig. 2. a) Pchl(ide) formation within 17 h of dark-incubation on 20 mM ALA in sections of the first leaves of 6-days-old rye seedlings grown in light on H_2O or in the presence of herbicides. b) *in vivo* synthesis of ALA in the basal parts (lower 2/3) of the first leaves of 6-days-old rye seedlings grown in light on H_2O or in the presence of herbicides during a 17 h incubation on 80 mM levulinic acid in light.

b) Photooxidation of chlorophyll. Photooxidation of chlorophyll is usually weakened when plants are grown in dim light. Accordingly, rye leaves grown at only 10 lx clearly accumulated chlorophyll in the presence of all herbicides. The amounts reached 40 – 100% of the chlorophyll content of untreated leaves grown at 10 lx. However, at 10 lx the carotenoid contents of the treated leaves were also much higher than at 5,000 lx and only in the presence of Sandoz 6706 and difunon the ratio of carotenoid to chlorophyll was considerably lower than in untreated leaves. When segments of leaves grown at 10 lx in the presence of Sandoz 6706 or difunon were exposed to a high light intensity of 30,000 lx the chlorophyll was bleached within a few hours both at 30 °C and 0 °C (Fig. 3). However, after treatments with herbicides of group 1 the photobleaching of chlorophyll was at both temperatures much slower than for group 2 herbicides and hardly greater than in untreated leaves, even when much higher and already growth-inhibiting concentrations were applied than were sufficient for the induction of chlorosis at 5,000 lx (Fig. 3).

c) Photoinactivation of chloroplastic and peroxisomal enzymes. At 10 lx the activities of the NADP-glyceraldehydephosphate dehydrogenase and of all peroxisomal enzymes of herbicide-treated leaves were equal to those of untreated leaves, with the exception of catalase which is inactivated by aminotriazole. After exposure to 30,000 lx NADP-glyceraldehydephosphate dehydrogenase of leaves treated with Sandoz 6706 or difunon and the catalase of all treated leaves were strikingly inactivated within 6 h at 30 °C as well as at 0 °C (Fig. 3). Within this time the activities of glycolate oxidase and hydroxypyruvate reductase were little affected.

d) Conclusions. For Sandoz 6706 and difunon the results confirm the hypothesis that the chlorosis is generated through a rapid photooxidative destruc-

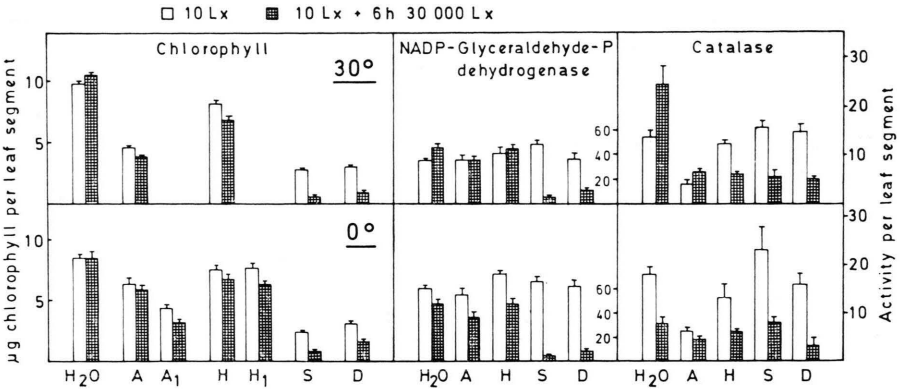

Fig. 3. Chlorophyll contents and activities of NADP-glyceraldehydephosphate dehydrogenase (nmol substrate reacted per min and segment) and catalase (µmol per min and segment) in segments from the leaves of 6-days-old rye seedlings grown at 10 lx on H_2O or in the presence of herbicides, before and after a 6 h illumination at 30,000 lx at either 30 °C or 0 °C. A: 0.25 mM, A_1: 0.3 mM aminotriazole, H: 0.05 mM, H_1: 0.15 mM haloxidine.

tion of chlorophyll and other chloroplast constituents, presumably including enzymes of chlorophyll synthesis, in treated leaves. It is also evident that after all herbicide treatments catalase was affected by a rapid secondary inactivation in bright light. Since all these photodestructions were also able to proceed at 0 °C they seem to be predominantly initiated by purely photochemical reactions which, in the case of group 2 herbicides, are probably caused by the carotene-deficiency. The inactivation of catalase conceivably indicated increased H_2O_2 accumulation leading to the formation of the inactive complex II of catalase [12]. The reasons for the inactivation of other peroxisomal enzymes during continuous growth at 5,000 lx are still unclear.

The results for aminotriazole and haloxidine are less unequivocal. Treated leaves grown in dim light contained also appreciable amounts of carotenoids and, whereas new synthesis of chlorophyll was effectively blocked upon exposure to bright light, the bleaching of existing chlorophyll was so slow, as compared to treatments with Sandoz 6706 or difunon, that it can hardly serve as a convincing sole explanation for the genesis of the chlorosis. Conceivably, photooxidations, though much more attenuated than in the presence of group 2 herbicides, were, nevertheless, sufficient to cause the destruction of the plastidic ribosomes. The 70 S ribosome-deficiency is known to prevent further chlorophyll accumulation and induce chlorosis of leaves, presumably because chlorophyll is not sufficiently stabilized in the resulting defective plastid membranes and its synthesis is strongly diminished by regulatory feedback mechanisms suppressing *e. g.* the activity of ALA synthesis [11], as observed for all herbicide-bleached leaves (Fig. 2 b).

Acknowledgements

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[1] J. R. Corbett, The biochemical mode of action of pesticides. Academic Press, London, New York 1974.
[2] J. Feierabend and B. Schubert, Plant Physiol. **61,** 1017–1022 (1978).
[3] P. G. Barthels and C. McCullough, Biochem. Biophys. Res. Commun. **48,** 16–22 (1972).
[4] E. R. Burns, G. A. Buchanan, and M. C. Carter, Plant Physiol. **47,** 144–148 (1971).
[5] G. Renger and C. Wolff, Biochim. Biophys. Acta **460,** 47–57 (1977).
[6] B. Halliwell, Cell Biol. Int. Reports **2,** 113–128 (1978).
[7] J. R. Harbour and J. R. Bolton, Photochem. Photobiol. **28,** 231–234 (1978).
[8] E. F. Elstner and R. Youngman, Ber. Deutsch. Bot. Ges. **91,** 569–577 (1978).
[9] M. R. Kirk and U. Heber, Planta **132,** 131–141 (1976.)
[10] J. Feierabend and U. Schrader-Reichhardt, Planta **129,** 133–145 (1976).
[11] J. Feierabend, Planta **135,** 83–88 (1977).
[12] B. Chance, Biochem. J. **46,** 387–402 (1950).