NITRATE AND NITRITE REDUCTASE ACTIVITIES IN INDUCED CHLOROPHYLL MUTANTS OF BARLEY

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1. Introduction

Assimilation of nitrate in higher plants is stimulated by light. It was shown earlier [1] that in rice seedlings the lower rate of nitrate utilization in the dark could not be attributed to the production of inhibitors of nitrate reductase or to depressed uptake of the inducer. A direct effect of light in the synthesis of nitrate reductase was indicated in rice seedlings by Shibata et al. [2], but the exact role of radiation in this respect is not known. Travis et al. [3] found that light grown seedlings had a higher proportion of polyribosomes as compared with etiolated plants and this was responsible for higher rate of synthesis of nitrate reductase in light. As against this, it was demonstrated that this specific requirement for light could be eliminated in tobacco leaves if gibberellic acid and kinetin are supplied in the dark [4,5]. We have investigated the synthesis of nitrate and nitrite reductases in ethyl methane sulphonate (EMS) induced albino mutants of barley. The main advantage of using chlorophyll-less leaves over etiolated seedlings is that the effect of light can be investigated independently of the photosynthetic reactions. If etiolated seedlings are exposed to light, chlorophyll synthesis also commences and photosynthetic reactions cannot be eliminated. We find that the synthesis of nitrate reductase is intimately linked with the photosystems involving chlorophyll while that of nitrite reductase seems to be independent of these reactions.

2. Materials and methods

The albino mutants of barley (*Hordeum vulgare*) were obtained by chemical mutagenesis of the seeds with EMS. The M_1 seeds were selected and sown again to raise M_2 generation. The chlorina mutants had pale green colour while in some of the albino mutants a few leaves were normal green and others were complete albinos. The chlorophyll mutants were removed from the field, provided with complete Hoagland solution containing 15 mM nitrate and kept in an illuminated chamber at 30° for 24 hr. In one of the sets the Hoagland solution was supplemented with 2% sucrose. During day time the seedlings were exposed to natural sunlight. Leaf extracts were prepared and nitrate and nitrite reductase activities were assayed by established methods [6,7]. Protein was determined by the method of Lowry et al. [8].

3. Results and discussion

Nitrate and nitrite reductase activities were assayed in normal plants and chlorina mutants. In albino mutants the green and the albino leaves were separately examined.

Table 1 shows that in albino leaves nitrate reductase activity was negligible but the green leaves of the same plant had substantial activity, comparable to that in normal seedlings. At the same time, very slight difference was observed in the total protein

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Table 1
Nitrate and nitrite reductase activities in chlorophyll mutants of barley seedlings.

Seedlings	mg protein per 500 mg leaf tissue	Nitrate reductase (nmoles NO_2^- formed per hr)		Nitrite reductase (μmoles NO_2^- reduced per hr)	
		per g tissue	per mg protein	per g tissue	per mg protein
Normal	9.10	216.0	11.80	72.0	3.96
Chlorina	9.10	131.4	7.23	73.8	4.05
Albino					
a) green leaves	5.85	227.7	19.46	72.0	6.15
b) albino leaves	4.10	not detected	–	15.3	1.86

in the extracts of green and white leaves of the albino plants, thus showing a specific effect on inhibition of synthesis of nitrate reductase in the albino leaves. The chlorina mutants showed less activity of this enzyme than the normal plants but nitrite reductase activity was about the same in both. It is interesting to note that small but significant nitrite reductase activity could be detected in the albino leaves.

Table 2 shows that addition of sucrose is not effective in enhancing the synthesis of nitrate reductase in albino leaves. On the contrary, it slightly depressed the activities in these as well as in normal leaves. Supply of carbon compounds thus may not be a limiting factor in diminished synthesis of nitrate reductase in albino leaves. Ritenour et al. [9] suggested that in leaves nitrite reductase is localised in the chloroplast while nitrate reductase is not. Schrader et al. [10] also arrived at the same conclusion by using chloramphenicol, which inhibited the synthesis of the former but not of the latter. As against this, Grant et al. [11] by using differential centrifugation of leaf homogenates concluded that both enzymes are largely located outside the chloroplasts. Albino leaves are completely devoid of chloroplasts but contain undifferentiated proplastids [12] and hence the presence of significant nitrite reductase activity in them indicates that the enzyme may not be necessarily associated with complete chloroplast structure.

Table 2
Effect of sucrose on nitrate and nitrite reductase activities in normal and albino seedlings of barley.

Treatment	mg protein per 500 mg leaf tissue	Nitrate reductase (nmoles NO_2^- formed per hr)		Nitrite reductase (μmoles NO_2^- reduced per hr)	
		per g tissue	per mg protein	per g tissue	per mg protein
A. Normal Seedlings					
i) Control	8.69	672.0	38.71	73.9	4.26
ii) +Sucrose	9.33	483.0	25.90	70.5	3.79
B. Albino Seedlings					
i) Control	6.26	39.2	3.58	20.3	1.63
ii) +Sucrose	6.15	31.9	2.27	17.9	1.45

Reduced ferredoxin generated during photoreactions has been suggested as an electron donor for chloroplast nitrite reductase [13,14], but since the proplastids of albino leaves are photosynthetically inactive and presumably lack ferredoxin also, the source of reductant has to be sought elsewhere. Moreover, since the albinos have negligible nitrate reductase activity, the physiological significance, if any, of the presence of nitrite reductase is obscure, unless nitrite is translocated to them from other plant parts.

High nitrate reductase activity in the green leaves of albino plants indicates that photosynthetic reactions are in some way required for the synthesis of the enzyme, since the only apparent difference between these and the albino leaves is that the former possess functional chloroplasts. Nothing is known about the integrity of the polyribosome structure [3] in albino leaves, but the protein content of albino and normal leaves did not differ markedly. The suggestion regarding the role of gibberellic acid and kinetin [4,5] in the synthesis of nitrate reductase cannot be completely ruled out, but it appears inconceivable that hormones from the green parts of the same plant might not be available to the albino leaves exposed to light. Kannangara and Woolhouse [15] showed that in addition to nitrate and light, presence of carbon dioxide is also essential for the synthesis of nitrate reductase in the leaves of *Perilla frutescens*. Absence of enzyme synthesis in carbon dioxide free atmosphere could not be attributed to lack of carbohydrate reserves nor could supplementation with glucose improve the rate of enzyme formation. It has been shown by Warburg and Krippahl [16] and Good [17] that presence of carbon dioxide is essential for the Hill reaction and hence it is likely that the effect of carbon dioxide deprivation might be acting through the inhibition of Hill reaction. Results reported here considered in conjunction with the earlier finding [1] that inhibitors of photoreactions such as DCMU (3,(3,4-dichlorophenyl)-1,1-dimethyl urea) and simazine also inhibit the synthesis of nitrate reductase suggest that the Hill reaction somehow creates conditions favourable for the synthesis of nitrate reductase. It is pertinent to point out here that in the facultative anaerobes induced synthesis of nitrate reductase is facilitated by reduced oxygen tension [18]. Thus by analogy, generation of Hill reductant in the green tissues may possibly play an important role by altering the internal redox state which might be necessary for induction of nitrate reductase in photosynthetic tissues.

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