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HEME ENZYMES IN A CHLORINA HYBRID OF GROUND NUT (*ARACHIS HYPOGAEA*) AND ITS PARENTS

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Abstract—Chlorophyll biosynthesis in the Chlorina hybrid was affected due to the lower levels of the enzyme δ-amino levulinate dehydratase responsible for the synthesis of porphobilinogen. A comparison of the amounts of different heme containing enzymes from the etiolated and green seedlings of the Chlorina and its parents suggested that the chlorophyll and heme moiety of catalase share the same pool of porphobilinogen and that this pool is different to the one shared by peroxidase and indole acetic acid oxidase. The enzyme δ-amino levulinate dehydratase possesses two isoenzyme bands. These isoenzymes may be spatially separated and responsible for the synthesis of two pools of porphobilinogen.

INTRODUCTION

In plants protoporphyrin IX is a common precursor of chlorophyll and the heme moiety of several important enzymes such as catalase, peroxidase, IAA oxidase, cytochrome, etc. It is not yet known whether the protoporphyrin IX used for the synthesis of chlorophyll and heme containing enzymes is coming from the same pool of its precursors α-amino levulinic acid (δ-ALA) and porphobilinogen. In recent years there has been a great deal of interest in understanding the relation existing between the synthesis of heme containing enzymes and chlorophylls and in the regulatory aspects of their biosynthesis. The studies of Castelfranco and Jones [1] and a recent report from Hendry and Stobart [2] suggest that both heme and chlorophyll may share the same pool of δ-ALA.

Boyce and Jones [3] using levulinic acid—an inhibitor of δ-ALA dehydratase, demonstrated that this inhibitor suppressed the synthesis of chlorophyll and nitrate reductase but it did not affect the activities of 'control enzymes' such as phosphoglycerate kinase, catalase and peroxidase. In order to understand which of the heme containing enzymes share the common pool of precursors with the chlorophyll, we have studied the activities of different heme containing enzymes in a Chlorina hybrid in which the chlorophyll biosynthesis is low due to a low activity of δ-ALA dehydratase (δ-ALAd) but not δ-ALA synthesizing (δ-ALAs) enzymes. The data suggest that catalase and chlorophyll may be sharing the same pool of porphobilinogen which is independent of the one shared by peroxidase and IAA oxidase.

RESULTS AND DISCUSSION

The amounts of δ-ALAs, δ-ALAd, peroxidase, IAA oxidase and catalase in etiolated plants are shown in Table 1. The chlorophyll content per g fr. wt routinely observed in the green leaves of these varieties is also shown at the bottom of the table. The data for enzyme activities are expressed on a per g fr. wt basis but the trend observed is similar if compared on a per mg soluble protein basis. The level of δ-ALAs in the Chlorina is more or less the same as in the parents. The lower concentration of chlorophyll in the Chlorina (routinely observed to be 15–20% of parents) therefore could not be due to lower rate of ALA synthesis. The activity of δALAd is quite low in the Chlorina and more or less parallels that of chlorophyll. This low activity can explain the lower content of chlorophyll in the Chlorina. In fact by studying the protochlorophyllide accumulation in the presence of excess δ-ALA, incorporation of radioactivity from labelled δ-ALA into chlorophyll and accumulation of δ-ALA in the presence of levulinic acid in the 3 varieties, we have observed that the chlorophyll deficiency in the Chlorina is indeed due to the limiting levels of δ-ALAd.

Since in the Chlorina δ-ALAd is limiting the synthesis of chlorophyll, this we thought would help us in distinguishing those enzymes that share the common pool of porphobilinogen with chlorophyll and those that do not. The activity of those heme enzymes sharing a common pool of porphobilinogen with chlorphyll would be reduced in the Chlorina whereas the activity of other heme enzymes may not be altered. The data presented in Table 1 show that in the seedlings of Chlorina the activity of catalase was considerably reduced whereas the activities of the enzymes peroxidase and IAA oxidase were, if anything, present in somewhat higher amounts than in either of the parents. In fully green leaves of the seedlings grown under field conditions the trend observed was the same for the activities of the 3 enzymes (Table 2). Catalase activity was considerably lower in the

Table 1. Amounts of chlorophyll, δ-ALA synthesizing enzyme, δ-ALA dehydratase and other heme containing enzymes per g fr. wt in etiolated seedlings of Chlorina and its parents—Spanish and Krinkle varieties of ground nut

Variety	ALAs*	ALAd†	Peroxidase‡	IAA§ oxidase	Catalase‖
Chlorina	87	0.33	7290	547	475
Spanish	98	2.20	6280	481	1420
Krinkle	92	2.07	5600	387	1420

The chlorophyll levels routinely observed in the green leaves of Chlorina, Spanish and Krinkle are 0.3 mg, 2.0 mg and 1.8 mg per g fresh weight respectively. *nmol of δ-ALA synthesized per hr; † μmol per hr; ‡Δ A at 470 nm per hr; § μmol O_2 taken up per hr; ‖ Δ A at 240 μm per hr.

Chlorina than in either of the parents whereas the activity of the enzymes peroxidase and IAA oxidase was somewhat higher in the Chlorina. Nitrate reductase which can only be detected in green leaves was present in much smaller amount in the Chlorina than it is in Spanish or Krinkle.

These results can be explained on the basis of the following proposal. As suggested by earlier workers [1, 2] both chlorophyll and heme moiety of heme containing enzymes may arise in a common pool of δ-ALA. However, the pool of porphobilinogen used for the synthesis of chlorophyll may be different from the pool of porphobilinogen used for the synthesis of some of the heme containing enzymes. Thus the synthesis of porphobilinogen may take place at two sites in the cell, one of which may be in the plastids and responsible for providing porphobilinogen for the synthesis of chlorophyll and catalase. The other site of porphobilinogen synthesis may be in the cytoplasm and this may provide porphobilinogen for the synthesis of heme moiety of such enzymes as peroxidase and IAA oxidase. Previous studies [4, 5] have demonstrated that the plastids when supplied with δ-ALA can synthesize protoporphyrin IX and chlorophyll indicating that all the enzyme systems responsible for synthesizing chlorophyll from δ-ALA are present in the plastids. Thus the enzyme δ-ALAd may be present in two different sites in the cell.

In the Chlorina under study, the amount of plastid δ-ALAd may be low resulting in the lower levels of chlorophyll and catalase. If the pool of δ-ALA is common for chlorophyll and heme biosynthesis, then in the Chlorina relatively larger amounts of δ-ALA will be available for the synthesis of heme moiety of those enzymes which originate in the cytoplasmic pool of porphobilinogen. This can explain the somewhat higher activities of IAA oxidase and peroxidase in the Chlorina. We, therefore, believe that the enzyme δ-ALAd is present in two spatially different locales. If this is so it is logical to expect two isoenzyme bands of this enzyme. The isoenzyme pattern of δ-ALAd shows that in all the 3 varieties there were two isoenzyme bands of δ-ALAd. Although the traces can be considered to show the activity of the two isoenzyme bands only semi-quantitatively, it appears that the band II relative to band I is much higher in the parents than in Chlorina. One of these bands may be located in the chloroplasts and the other in the cytoplasm.

Earlier studies on δ-ALAd [6, 7] have shown the presence of only one isoenzyme band. It is possible that during the purification procedure employed by the earlier workers one of the isoenzyme bands may have been lost. During the course of the present studies we observed that the enzyme extract stored overnight shows only one band. Further we have confirmed the presence of two isoenzyme bands of δ-ALAd in the pea and barley seedlings. The proposal that porphyrin synthesis may take place in more than one compartment, as brought out by the present studies, has previously been considered (see [8]).

Are there two enzyme systems responsible for the synthesis of δ-ALA to be utilized by the two isoenzymes of δ-ALA dehydratase? Such a possibility cannot be ruled out in view of the demonstration of the existence of two pathways of δ-ALA synthesis [2, 9].

The activity of nitrate reductase was less than one-third in the Chlorina (Table 2). This is not unexpected as Sawhney *et al.* [10] have previously observed lower

Table 2. Amounts of porphyrin containing enzymes per g fr. wt in 6-week-old green leaves of Chlorina and its parents—Spanish and Krinkle varieties of ground nut

Variety	Peroxidase	IAA oxidase	Catalase	Nitrate reductase
Chlorina	6240	483	430	5
Spanish	4200	264	1150	19
Krinkle	5760	305	1150	17

The nitrate reductase activity has been expressed as μg of NO_2^- produced per hr. Other enzyme activities are expressed as in Table 1.

nitrate reductase activity in the chlorophyll deficient mutants. Since for the induction of nitrate reductase activity reducing power generated during the photosynthetic electron transport is essential [10, 11], it is possible that the lower nitrate reductase activity in the Chlorina may be due to lower chlorophyll content and hence lower rates of photosynthetic electron transport in it. However, the possibility that lower nitrate reductase activity in the Chlorina could be due to the inhibition of the synthesis of heme protein (cytochrome *b*-557) which is a component of higher plant nitrate reductase [12] as a result of lower δ-ALAd activity cannot be ruled out. Boyce and Jones [3] have, in fact, shown that inhibition of δ-ALAd by levulinic acid inhibits nitrate reductase activity although it does not affect partial electron transport reactions of photosynthesis. In this connection it is interesting to note that Konis *et al.* [13] observed inhibition of photosystem II activity (as also chlorophyll–protein complex related to this system) by levulinic acid in greening maize leaves. We are not sure whether lower nitrate reductase activity in the Chlorina is due to lower activity of δ-ALAd or due indirectly to the lower chlorophyll content and hence lower rates of electron transport.

EXPERIMENTAL

The Chlorina hybrid was from the cross between the wild types Spanish and Krinkle varieties of ground nut (*Arachis hypogaea*). Seeds of the 3 varieties (2 parents and the Chlorina) after surface sterilization with Na hypochlorite (0.05%) were sown in Vermiculite and grown in the field for 6 weeks. These served as the source of the enzymes for studies involving green leaves. The enzymes peroxidase, IAA oxidase and catalase were estimated in etiolated as well as green leaves. Nitrate reductase was estimated in the green leaves only, as in etiolated plants it was not detectable. The activity of δ-ALAs was estimated *in vivo* by the method of ref. [14]. The δ-ALAd was measured by using the procedure of ref. [15]. Peroxidase and IAA oxidase were extracted by using the extraction procedure of ref. [16] and assayed according to ref. [17] for peroxidase, and ref. [18] for IAA oxidase. Catalase levels were measured by the procedure in ref. [19]. Nitrate reductase was assayed as in ref. [20] and protein was estimated by Lowry's method [21]. Electrophoresis was carried out according to ref. [22]. The pH of the buffer used during electrophoresis was 8.3. The gels were stained for δ-ALAd isoenzyme bands by first incubating them with 4 mM δ-ALA in 0.2 M Tris, pH 8.2, for 1 hr and then developing the colour by modified Ehrlich's reagent. The gels were scanned immediately since the bands are unstable. All expts were repeated at least 3 times and the trends obtained were always the same. The data presented are from one set of experiments and represent the trends observed.

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