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SHORT COMMUNICATION

LIGHT MEDIATED INCREASE IN ACTIVITY OF PORPHOBILINOGEN DEAMINASE/UROPORPHYRINOGEN III COSYNTHETASE AND δ-AMINOLEVULINATE DEHYDRATASE IN TISSUE CULTURES OF TOBACCO

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Abstract—Purified enzyme extracts from tissue cultures of tobacco which have been submitted to continuous white illumination show an increase in activity of porphobilinogen deaminase/uroporphyrinogen III cosynthetase and δ-aminolevulinate dehydratase. The specific activities increase linear over a range of about 4 days. Following a lag phase, the chlorophyll content increase is also linear. The time courses show that multiplication of enzymatic activities of the porphyrin biosynthesis chain is involved in chlorophyll synthesis.

INTRODUCTION

IT HAS been suggested that light regulates chlorophyll synthesis by mediating the formation of the enzymes of δ-aminolevulinate synthesis[1, 2] or by mediating the formation of the protein part of protochlorophyllide holochrome.[3] These two proteins mark the beginning and the end of a chain of enzymes which are only partly characterized in higher plants.[4–7] Furthermore, the potential activities of the enzymes involved are unknown. Recently, we have shown that chlorophyll synthesis is paralleled by an increase in specific activity of δ-aminolevulinate dehydratase in tissue cultures of tobacco.[8] Similar results are reported for bean leaves[9] and tissue cultures of kalanchoe.[10] In continuation of the earlier experiments, this paper presents data on the synthesis of uroporphyrinogen in relation to the formation of porphobilinogen and chlorophyll.

RESULTS

The experiments were performed with greening and well growing tissue cultures of *Nicotiana tabacum* var. 'Samsun' precultured in darkness for 5–6 days. The conditions for culture are described elsewhere.[8,11]

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Enzyme extracts were prepared by grinding 20 g of freshly harvested tissue in a precooled mortar with additions of 0·9–1·0 ml of a 1 M solution of tris, to give a final pH of 8·2, 0·025 ml of mercaptoethanol and quartz sand. The homogenate was filtered through a cloth and was centrifuged for 1 hr at 18,000 *g*. The supernatant was submitted to purification by means of gelfiltration (Sephadex G 25) using the centrifuge technique.[12] This procedure removes impurities which block the enzyme activity of ALAD in crude extracts. The rate of uroporphyrinogen synthesis from porphobilinogen is about the same in purified and crude extracts, when low quantities of enzyme solutions are used.

The incubation mixtures contained the following solutions: Porphobilinogen deaminase/uroporphyrinogen III cosynthetase: 0·5 ml of tris-HCl buffer pH 8·2, 0·2 M; 0·1 ml of EDTA, 0·025 M, adjusted to pH 8·2; 0·05 ml of porphobilinogen, 1 mg/ml; 0·05–0·1 ml of enzyme and H_2O to a final volume of 1 ml.

δ-Aminolevulinate dehydratase: 0·5 ml of tris-HCl buffer pH 8·2, 0·1 M; 0·05 ml of $MgCl_2$, 0·1 M; 0·05 ml of mercaptoethanol, 0·1 M; 0·1 ml of δ-aminolevulinate, 1 mg/ml; 0·05–0·2 ml of enzyme and H_2O to a final volume of 1 ml.

The incubation at 32° was terminated after 4 hr by addition of 0·2 ml of a 3 M TCA solution per ml of sample. 0·1 ml of a 0·1 M solution of $HgCl_2$ removed mercaptoethanol from porphobilinogen assays. Following centrifugation the supernatants were diluted (1:1) with 5 N HCl and Ehrlich's reagent respectively.

Porphobilinogen values were read at 555 nm (E_M: $62 \cdot 10^3$ 1/M cm)[13] 10 min after mixing and uroporphyrinogen values 2–3 hr later after autoxidation to uroporphyrin at 406 nm (E_M: $530 \cdot 10^3$ 1/M cm).[6] Porphobilinogen values were corrected for losses of uroporphyrinogen.

For determination of protein the biuret method was used. Chlorophyll was extracted from lyophylized cells[14] by 80% acetone and was estimated by means of a nomogram.[3]

Porphobilinogen was enzymatically synthesized using δ-aminolevulinate dehydratase from spinach, purified about 15 fold by acetone precipitation.[5] The reaction mixture was composed of 100 mg of δ-aminolevulinate, neutralized with tris, about 125 mg of protein, 0·5 mM of mercaptoethanol, 0·5 mM of $MgCl_2$, 5 mM of tris-HCl buffer pH 8·3 and sodium dodecyl sulphate to give minimal porphyrin synthesis in a final volume of 100 ml. After incubation, porphobilinogen was isolated following the procedure of Shemin[15] (yield based on δ-aminolevulinate: about 45 per cent).

Both the specific activity of porphobilinogen deaminase/uroporphyrinogen III cosynthetase and the chlorophyll content of the cultures increase in a similar manner, when colourless cultures are exposed to continuous white light (3500 lx). Furthermore, there is a synchronous increase in specific activity of δ-aminolevulinate dehydratase. In dark grown cultures the enzymatic activities remain constant. The increasing specific activities exhibit a much shorter or even failing lag phase than chlorophyll synthesis. The rapid increase in enzymatic activity probably indicates a dependency of chlorophyll synthesis on new formation of enzymes, although these enzymes are already present in dark grown cells. It is not possible, however, at present, to distinguish clearly between causative reactions and parallel effects. Samples taken from green tissues, cultured for several passages in light show higher

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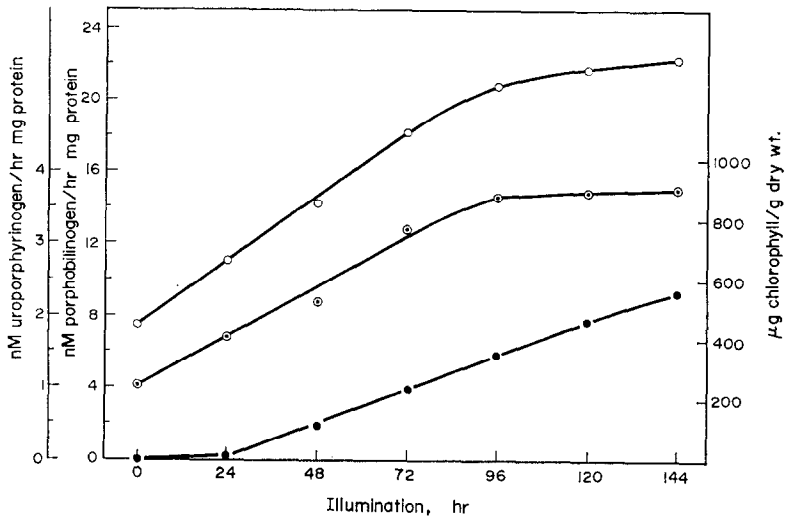

FIG. 1. PORPHOBILINOGEN DEAMINASE/UROPORPHYRINOGEN III COSYNTHETASE ACTIVITY. (◉), 5-Aminolevulinate dehydratase activity (○) and chlorophyll content (●) of dark grown tissue cultures of tobacco during continuous white, fluorescent light (3500 lx). The figure combines two experiments performed under similar conditions. Specific enzyme activity and chlorophyll content respectively versus time of illumination.

specific activities than samples from day 5 or day 6, but the increase in specific activities and chlorophyll content are no longer proportional. Possibly the level of enzymes has reached a steady state after a certain time.

It has been suggested that the parallel increase in chlorophyll content and δ-aminolevulinate dehydratase activity is due to a common intracellular site[8,9] and a common control mechanism.[9] This suggestion is supported by our results of uroporphyrinogen synthesis in extracts of greening tissue cultures of tobacco. During development the increments of activity of δ-aminolevulinate dehydratase and porphobilinogen deaminase/uroporphyrinogen III cosynthetase exhibit parallel time courses.

The findings indicate that multiplication of several enzymes of the porphyrin biosynthesis chain is involved in chlorophyll synthesis. An inhibition of chlorophyll synthesis by antibiotics, blocking protein synthesis, may therefore not only be due to a deficiency in δ-aminolevulinate synthetase[1,2] and the protein moiety of protochlorophyll holochrome,[3] but also to a deficiency in other enzymes of this biosynthesis chain.