DETERMINATION OF THE SITES OF SYNTHESIS OF CHLOROPHYLL SYNTHESIZING ENZYMES IN CELL CULTURES OF NICOTIANA TABACUM

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SUMMARY

The activity of a sequence of enzymes involved in chlorophyll biosynthesis (δ-aminolevulinic acid synthetase (ALAS), δ-aminolevulinic acid dehydratase, porphobilinogenase and chlorophyllase) was followed during greening of tobacco cell cultures under the influence of chloramphenicol (CAP). The photosynthetic enzymes ribulose diphosphate carboxylase (RuDPCO) and NADP linked glyceraldehyde dehydrogenase (NADP-GDH) were used as markers for penetration and action of the inhibitor. RuCPCO was inhibited at concentrations of CAP which still allowed good chlorophyll accumulation. The enzymes of chlorophyll biosynthesis, the activity of which increased during illumination and CAP treatment, behaved like NADP-GDH which is known to be synthesized in the cytoplasm. The results suggest that synthesis of enzymes of chlorophyll biosynthesis takes place in the cytoplasm. Decreasing light induced increment of ALAS activity caused by CAP may possibly be taken as an indication that things are more complicated with this enzyme.

INTRODUCTION

Chlorophyll biosynthesis is blocked by various inhibitors of nucleic acid and protein synthesis, including chloramphenicol (for literature see 1,2). However, the role of this substance, concerning inhibition of chlorophyll biosynthesis, is doubtful.

Abbreviations: ALAD: δ-aminolevulinic acid dehydratase, ALAS: δ-aminolevulinic acid synthetase, CHLase: chlorophyllase, CAP: chloramphenicol, NADP-GDH: NADP linked glyceraldehyde dehydrogenase, PBGase: porphobilinogenase, RuDPCO: ribulose diphosphate carboxylase.

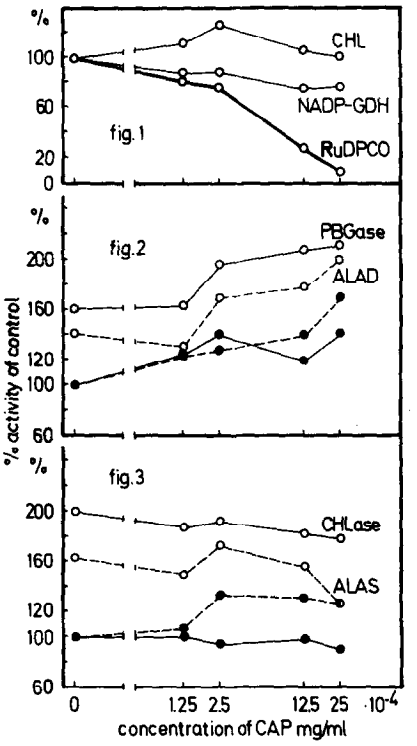
There are also reports demonstrating no inhibition or inhibition only under high concentrations of CAP (e.g. 3-6). Since CAP is known to inhibit translation in chloroplasts (cf. 2), these findings would implicate either that CAP has not penetrated through the plastid envelope or that the enzymes of chlorophyll biosynthesis, although located in the plastids (7-10), are synthesized in the cytoplasm.

To test these possibilities the activities of a squence of enzymes in chlorophyll biosynthesis (ALAS, ALAD, PBGase, and CHLase) were followed in addition to chlorophyll accumulation and activities of photosynthetic enzymes under the influence of CAP during greening of cell cultures of tobacco. NADP-GDH, which is known to be synthesized in the cytoplasm, and RuDPCO, the proteins of which are synthesized in the plastid as well as in the cytoplasm (for literature see 11), were used as markers for penetration and action of CAP.

RESULTS AND DISCUSSION

The experiments were performed with mixotrophic cell cultures of tobacco, the greening of which is preceded and accompanied by an increase in enzyme activities of chlorophyll and porphyrin biosynthesis (12,13). This increase takes place during the transformation of proplastids and amyloplasts to chloroplasts (14). Concentrations of CAP as used in our experiments scarcely affected growth and protein content of the tobacco cells (15).

Figure 1 shows that there is also no apparent inhibition of chlorophyll biosynthesis by CAP at concentrations which are strongly inhibitory for RuDPCO. At lower concentrations of CAP



Figures 1,2, and 3: The influence of light and various concentrations of chloramphenicol (CAP) on the activity of the enzymes δ-aminolevulinic acid dehydratase (ALAD), δ-aminolevulinic acid synthetase (ALAS), chlorophyllase (CHLase), NADP linked glyceraldehyd dehydrogenase (NADP-GDH), porphobilinogenase (PBGase), and ribulose diphosphate carboxylase (RuDPCO), and on chlorophyll accumulation (CHL) in tobacco cell cultures. 100% correspond to enzyme activities without CAP, and without illumination, if the enzymes are active in the dark. O = activities after light treatment, ● = activities in the dark. CAP concentrations are given as mg/ml of culture medium. The graphs represent the average of 4 experiments.

chlorophyll accumulation is even slightly higher than in the control. As demonstrated by the inhibition of RuDPCO, CAP has penetrated through plastid envelopes, but no harmful influence is exerted on the synthesis of proteins of cytoplasmic origin, which are represented by NADP-GDH.

If RuDPCO inhibition is taken as a measure for the inability of plastid ribosomes to synthesize proteins and enzymes, we may

infer that enzymes responsible for chlorophyll biosynthesis in cells treated with CAP cannot be synthesized within these organelles.

However, since the enzymes of chlorophyll biosynthesis, including ALAS, are already present in dark-grown cells (12,13) and before CAP treatment, outlasting enzymes might be the origin of chlorophyll biosynthesis under the influence of CAP. In the present experiments this possibility was excluded by assays of enzyme activities and the demonstration of a light induced increase in activities of ALAS, ALAD, PBGase, and CHLase after CAP treatment (fig. 2,3). Previously it had been already shown that chlorophyll accumulation in tobacco cells was tightly bound to actual enzyme activities (12). This finding also argues against the assumption of chlorophyll biosynthesis without concomitant enzyme synthesis.

After 6 days of illumination, the activities of the enzymes ALAS, ALAD, PBGase and CHLase were significantly higher than in the control in the dark (cf. also 12,13). ALAD and PBGase showed even higher activities under the influence of increasing concentrations of CAP, but this increase in activity was paralleled by an increase of dark activities of the two enzymes. CHLase activity decreased slightly under illumination and darkness, subject to the concentration of CAP. The light induced increment of ALAS activity decreased with increasing amounts of CAP.

The results demonstrate that the enzymes of chlorophyll biosynthesis behave like NADP-GDH, which is synthesized in the cytoplasm, although NADP-GDH is more subject to inhibition by CAP. This observation corroborates the finding that the same enzymes are inhibited by the inhibitor of cytoplasmic

translation, cycloheximide, and that they are scarcely affected by rifamycin SV (16) which is known to inhibit transcription in the plastid (17). Decreasing activities of ALAS and CHLase under the influence of CAP may be ascribed to an inhibition of the synthesis of subsidiary proteins necessary for transport and localisation of these enzymes according to an assumption made previously (5,18). But in light of results obtained with Rhodopseudomonas spheroides (19) the decreasing increment of light induced ALAS activity caused by CAP might be interpreted as an indication for two species of ALAS, different in sensibility to CAP and light. In addition our experiments indicate that the sites of primary light response are most likely located in the cytoplasm (cf. 20).

EXPERIMENTAL ANNOTATIONS

The experiments were performed with a cell clone of Nicotiana tabacum var. Samsun isolated by Bergmann (21) and cultivated as described by Bergmann and Berger (14). After 5 days of cultivation in the dark the cell cultures were illuminated for 6 days (3500 lx). CAP was applied at the beginning of the light period. The cells were harvested and broken in Tris-HCl buffer, pH 7,8, with 2-mercaptoethanol by means of a Potter-Elvehjem homogenizer. After centrifugation and purification by gel centrifugation the homogenate was used for enzyme assays. RuDPCO and NADP-GDH were assayed according to Rabin and Trown (22) and Ziegler and Ziegler (23), respectively. ALAS, ALAD, and PBGase from tobacco cells were measured as already described elsewhere (13,24). CHLase was tested with reference to Böger (25).

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