CHLOROPHYLL SYNTHESIS BY ISOLATED INTACT ETIOPLASTS

F.A.M. Wellburn and A.R. Wellburn

Department of Biological Sciences,
University of Lancaster,
Lancaster,
United Kingdom.

Received August 25, 1971

Summary

The light stimulated incorporation of label from ^{14}C-labelled glycine and succinic acid into chlorophyll by isolated intact etioplasts indicates the presence of δ-amino-levulinic acid (δ-ALA) synthetase within the higher plant plastid.

There is evidence that all the enzymes that convert δ-amino-levulinic acid (δ-ALA) to chlorophyll together with succinyl thiokinase are present in the higher plant plastid[1,2] in non-limiting amounts[2,3]. However, the localization of δ-ALA synthetase within the higher plant is not known. Nadler and Granick have proposed a model for control of chlorophyll synthesis based on a light induced activation at the translational level of the synthesis of δ-ALA synthetase and other short half-life proteins limiting δ-ALA formation[3]. Recently, Rebeiz and Castelfranco have shown etioplasts to be capable of synthesising labelled protochlorophylls from ^{14}C-δ-ALA[4].

We have described a new method for the preparation of suspensions of intact etioplasts which have small amounts of mitochondrial (0.2%) and bacterial (0.1%) contamination[5]. These plastids have both inner and outer plastid envelopes undamaged and show normal light dependent development characteristics. In this communication we would like to report the incorporation of ^{14}C-labelled glycine, succinic acid or δ-ALA into chlorophyll by nine similar preparations of etioplasts over a period of 3 hours in the light or the dark.

The incubation medium used in these experiments was the isolation medium of our method[5] plus 0.1% bovine serum albumin and pyridoxal phosphate (3 μ-moles) together with all the additives of Rebeiz and Castelfranco[4], at similar concentrations, but with double the concentration of co-enzyme A. The only other additions are shown in Table 1, all at a concentration of 30μ moles in a total of 7 ml of each plastid suspension. Immediately prior to extraction of pigments the dark incubation mixture was briefly illuminated in an attempt to photoconvert any labelled protochlorophyllide formed. Extraction of pigments followed the procedure of Rebeiz and Castelfranco[6], carrier chlorophylls being added and purification to constant specific activity was carried out using the paper chromatographic procedures described by the same authors[4,6] and the cellulose TLC method of Schneider[7,8].

The results in Table 1 show a light stimulated incorporation of label from ^{14}C-labelled glycine and succinic acid into chlorophyll$_a$ indicating δ-ALA synthetase activity is present within illuminated etioplasts. It is unlikely that the low level of mitochondria present in these etioplast preparations would be capable of releasing sufficient δ-ALA for biosynthesis on this scale and any bacterial metabolism may be eliminated from suspicion by the light-stimulated nature of the biosynthetic activity. Approximately double the activity was incorporated from ^{14}C-δ-ALA but the incorporation of label from δ-ALA in the dark was not as high as in the light though significantly higher that the amount of radioactivity found in chlorophyll$_a$ from etioplasts incubated with labelled glycine or succinic acid. This may reflect the quiescent nature of metabolic activity within undifferentiating etioplasts.

No significant ^{14}C-chlorophyll$_b$ biosynthesis was detected. This is in agreement with the earlier observations of Rebeiz who was unable to show chlorophyll$_b$ biosynthesis in 2.5 hours but only after 4.5 hours in cucumber cotyledons implying an *in vivo* lag period[9] involving an unknown control mechanism.

Incorporation of radioactivity from labelled glycine, succinic acid and δ-amino-levulinic acid into chlorophyll$_a$ and chlorophyll$_b$ by isolated intact etioplasts from Avena

Additional components of basic incubation medium	Incorporation of ^{14}C label into:	Disintegrations min^{-1}. 100 mg $protein^{-1}$		
		3 hrs light (500 lux)	3 hrs light (500 lux)	3 hrs dark followed by 1 min. light
Glycine-2-^{14}C (33μc) + succinic acid	Chlorophyll$_a$	49572	37246	2772
Succinic acid-2,3-^{14}C (33μc) + glycine	"	47799	36689	2559
δ-Amino-levulinic acid-4-^{14}C (33μc)	"	76183	62055	14001
Glycine-2-^{14}C (33μc) + succinic acid	Chlorophyll$_b$	846	771	683
Succinic acid-2,3-^{14}C (33μc) + glycine	"	678	781	1041
δ-Amino-levulinic acid-4-^{14}C (33μc)	"	780	922	733

The amounts of radioactivity incorporated from ^{14}C-δ-ALA by these etioplasts are not as high as those found by Rebeiz and Castelfranco who used lower activities of precursors (2μc) but also significantly longer incubation periods (16 hrs). Three hours has been found to be the permissible period of satisfactory development of etioplasts from Avena using incubation media presently available. As described elsewhere[10], etioplasts tend to lose their outer envelope upon prolonged incubation and thereafter normal chloroplast development is not observed. Until the problem of the maintainance of the etioplast outer envelope during incubation is solved, amongst many other possible factors, the

complete differentiation in vitro of etioplasts into chloroplasts will remain unachieved. Nevertheless, short term incubations can yield useful information and we hope to report more fully on the biosynthetic capabilities of isolated intact etioplasts over this short period of normal morphogenesis.

References

1. Granick, S. Plant Physiol. Supp. 36, xlviii (1961).
2. Nandi, D.L., and Waygood, E.R. Can. J. Biochem. 43, 1605 (1965).
3. Nadler, K., and Granick, S. Plant Physiol. 46, 240 (1970).
4. Rebeiz, C.A., and Castelfranco, P.A. Plant Physiol. 47, 24 (1971).
5. Wellburn, A.R., and Wellburn, F.A.M. J. Exptl. Bot. in the press (Nov. 1971).
6. Rebeiz, C.A., and Castelfranco, P.A. Plant Physiol. 47, 33 (1971).
7. Schneider, H.J.A.W. J. Chrom. 21, 448 (1966).
8. Schneider, H.J.A.W. Phytochem. 7, 885 (1968).
9. Rebeiz, C.A. Magon, Série Scientifique 13, 1 (1967).
10. Wellburn, A.R. and Wellburn, F.A.M. Proc. II Int. Cong. Photosyn. Res., Stresa (1971).