THE DIFFERENCE IN TURNOVER RATE BETWEEN THE CHLOROPHYLL a IN THE P700-CHLOROPHYLL a-PROTEIN AND IN THE TOTAL CHLOROPLAST MEMBRANES†

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Green, 10-day old corn plants were excised at the first node and labeled with δ-[^{3}H]-aminolevulinic acid, a precursor of chlorophyll. From 6 to 72 hrs after incorporation of the radioactive label, chloroplast membrane fragments were prepared and dissolved in 1% Triton X-100. From this extract CPI, the P700-chlorophyll a-protein complex, was isolated by hydroxylapatite chromatography. The specific activity of the chlorophyll a in CPI was found to be about half as great as that in the total extract suggesting a slower turnover of this complex and probable isolation from other chlorophyll in vivo.

A P700-chl a-protein complex (CPI) has been isolated from a wide variety of plants (1). This complex which appears to be the heart of photosystem I has a molecular weight of 110,000 daltons and contains 12-15 moles of chl a per mole of protein (2). It has approx. 40 molecules of chl a per P700 in leaf and green algal preparations; this represents a ten-fold enrichment for P700. The complex can be isolated either by hydroxylapatite chromatography following solubilization with Triton or, in a slightly altered form, by sodium dodecyl sulfate - gel electrophoresis. It usually comprises about 10% of the total chl in the plant.

In the work reported here we labeled the chl in green corn leaves with tritiated δ-aminolevulinic acid and subsequently measured the specific activity (SA) of the chl in the total Triton-solubilized chloroplast membranes and in the isolated CPI. We found that the SA of the chl a in CPI was about half of that in the total extract suggesting a slower rate of turnover of this specialized complex.

Abbreviations: ALA = δ-aminolevulinic acid, chl = chlorophyll, CPI = P700-chl a-protein, SA = specific activity

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MATERIALS AND METHODS

Plant Material; Incorporation of Label. Seeds of Zea mays L. line INRA 260 were grown at 25°C in continuous light on vermiculite wetted with distilled water. The plants were used after 10-12 days at which time the third leaf was not fully unfurled and still yellowish in color.

For incorporation of radioactive label the aerial parts of each plant were separated from the roots at the first node with a razor blade. The excised zone was then immersed in a tube containing 40 µl of a solution of [^{3}H] ALA with a SA of 33 Ci/mM. A total of 1 mCi of radioactivity (or 3×10^{-5} mmoles of ALA) was used in each experiment. After about 2 hrs in the light there was complete absorption of radioactivity (an amount of ALA that does not modify the pool of pigment precursors (3)), and the plants were placed in distilled water.

In the first two experiments the plants were next equilibrated for about 22 hrs under continuous illumination. In Exp. 3, 1 mCi of [^{3}H] ALA was incorporated into 80 plants. These were then incubated in a chamber of the phytotron of the CNRS at Gif-sur-Yvette under 18,000 lux at 22°C and 70% relative humidity. Groups of 20 of these plants were analysed after 6, 24, 48 and 72 hrs, respectively.

Preparation of Triton Extract and CPI. The plants were chilled in darkness for 30 min and then ground in a mortar with sand and 50 mM Tris buffer, pH 7.5 containing 10 mM sodium ascorbate. The homogenate was filtered through cotton gauze, centrifuged lightly to remove sand and large pieces, and then centrifuged at 10,000 g for 5 min to sediment the chloroplasts. These were resuspended in more buffer and washed twice. The amount of chl in the washed pellet was determined (see below) and an amount of 1% Triton X-100 added to provide a ratio of Triton to chl of 75 (w/w). All of the chl-containing membranes were dissolved by this procedure. The Triton extract was passed through a column of hydroxylapatite (Hypatite C, Clarkson Chemical Co. Inc., Williamsport, Pa., USA), and CPI was finally

eluted by 0.2 M phosphate buffer, pH 7.5 according to Shiowaza et al (2).

Measurements of Chl, P700 and Radioactivity. The chl determinations were made in 80% acetone and water using the equations of Mackinney (4). P700 was estimated according to Dietrich and Thornber (5).

For the radioactivity measurements the pigments were extracted from CPI and the Triton extract with 80% acetone and transferred to petroleum ether. This extract was chromatographed on Whatman paper No 3 in a solvent of petroleum ether-benzene-acetone (34:10:4, v/v/v). Chls a and b were eluted separately in 80% acetone. Their concentration was measured spectroscopically and radioactivity was determined with a liquid scintillation counter. Care was taken to avoid error due to fluorescence or concentration quenching.

RESULTS AND DISCUSSION

Three separate experiments were performed. In the first experiment 22 plants were used, and 13 mg of chl were recovered in the 1% Triton extract. The chl a/b was 3.40. About 4% of the total chl chromatographed on hydroxylapatite was recovered in CPI in each experiment. In Exp. 2 forty plants were tritiated. After incubation the two expanded leaves of each plant were cut in half. The apical halves were ground separately from the basal halves combined with the young third leaves where new chl synthesis occurs (6). In this way those regions of active chl synthesis were separated from the more mature regions where metabolic turnover predominates. We recovered 13 and 8 mg total chl from the upper and lower parts of the plants respectively in the detergent extracts, both of which had a chl a/b of 3.00.

In the third experiment the SA of the chls was measured with 20-plant samples 6, 24, 48 and 72 hrs after incorporation of the label. Approx. 11 mg of chl was recovered from each of the first 3 time periods with chl a to b ratios of 3.40, 3.35 and 3.38, whereas only about 4 mg was recovered

Table I. The specific activities of chls *a* and *b* in the Triton extracts and of chl *a* in CPI.

Experiment	Triton Extract chl *a*	Triton Extract chl *b*	CPI chl *a*
	(c.p.m. · 10^6 · mg^{-1}chl)		
1 (22 hrs)	16.0	4.52	6.30
2 (22 hrs Basal	20.8	5.88	11.0
Apical	5.08	1.08	2.80
3 (6 hrs)	4.77	0.77	2.33
(24 hrs)	4.99	1.80	2.60
(48 hrs)	4.70	1.87	3.23
(72 hrs)	4.30	1.87	3.40

from the 72 hr sample, the chl *a*/*b* of which had dropped to 3.15. The leaves were obviously senescent after 72 hrs.

The average ratios of chl to P700 in the Triton extracts and chl-protein complexes were 435 and 44 respectively showing a 10-fold enrichment in the reaction center except for the 72-hr plants which had about 100 fewer chls per P700 in total but nearly the same ratio (49) in CPI. This difference indicates that the bulk chl breaks down faster than the reaction center chl during senescence.

Table I shows the SAs of the chls in the 7 samples from 3 experiments. In Table II are the calculated ratios of SA of chl *a* to chl *b* in the Triton extracts and of chl *a* in CPI to the chl *a* in the Triton extracts. Because different numbers of plants were labeled with the same total amount of readioactivity in each experiment, the SA values are not comparable between experiments.

Since a fraction of chl *a* is a precursor of chl *b* (7,8), the finding that chl *b* was labeled at a slower rate than chl *a* (higher ratio in Table II) in the apical as opposed to the basal parts of the leaves and in the 6-hr

Table II. The ratios of specific activities of chl a to b and of chl a in CPI to chl a in the Triton extract.

Experiment	Triton Extract chl a/b	Chl a CPI/Triton Extract
1 (22 hrs)	3.5	0.39
2 (22 hrs)		
Basal	3.5	0.53
Apical	4.7	0.55
3 (6 hrs)	6.2	0.49
(24 hrs)	3.0	0.52
(48 hrs)	2.5	0.69
(72 hrs)	2.3	0.79

compared to later samples was to be anticipated. After 24 hrs the SA of chl a decreased while the SA of chl b increased up to 48 hrs and remained the same at 72 hrs. This resulted in a decrease in the ratio of SA of chl a to b during senescence. The ratio of the amounts of chls a and b also decreased during this period indicating a more rapid break down of chl a than chl b under the conditions of our experiment. Since the rootless stems and leaves were exposed to continuous light, this may reflect the more rapid bleaching of chl a than chl b previously reported for chloroplasts (9) and broken Chlorella (10).

The SA of the chl a in CPI was found to be about one-half that of the total chl a in all experiments except for the 48 and 72 hr samples in Exp. 3 (i.e. during senescence) when the SA of chl a in CPI still increased slightly while the total chl a decreased both in amount and SA.

These results all suggest that CPI in vivo differs physically and metabolically from the rest of the chl-containing membrane. All the chls must of course be close enough for efficient energy transfer to occur during photosynthesis, but at the same time isolated biosynthetically such

that the radioactive label does not become incorporated or lost at the same rate as in or from the bulk chl. The very fact that Triton extracts much of the chl in the membrane and forms micelles with it, but does not dissociate the chl from CPI indicates a very special organization of this chl-protein. For other discussions of the effects of Triton on chloroplast membranes see Refs. 11, 12 and 13.

One must not overextend interpretation of these results. Little should be inferred about relative rates of chl synthesis versus turnover because we were labeling less than one in every 10,000 chl molecules. We studied CPI because we are able to isolate it, but there may well be other chl-protein complexes which also have a different labeling pattern from the bulk chl.

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