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The isolation and composition of chloroplasts and etiolated plastids from corn seedlings

Differential centrifugation procedures for the isolation of chloroplasts have been used extensively in studies on their composition and metabolism[1,2]. Several investigators have recently incorporated density-gradient techniques in procedures for the isolation of chloroplasts[3–6] and etiolated plastids[6,7]. Enhanced purification was demonstrated through enzyme-distribution measurements. Chemical analyses were generally limited to relative total nitrogen and chlorophyll content. In this study, the percentage composition of chloroplasts isolated by a differential density-gradient scheme is compared with chloroplasts isolated by differential centrifugation alone. Purified chloroplasts are then compared with etiolated plastids isolated by an identical procedure. Corn seedlings (*Zea mays*, Seneca Chief) were chosen as the plant source since their composition has not been reported even though they are used frequently in structural studies[8].

In differential centrifugation[9], seedlings 14–19 days old were first placed in a dark room for 36–72 h to reduce the starch content. Leaves were rinsed with distilled water, blotted dry, and 100 g homogenized with sand in a prechilled mortar using 200 ml of 0.35 M NaCl. The homogenate was strained through a double thickness of cheesecloth and the sediment obtained by centrifugation at 200 × *g* for 1 min discarded. Chloroplasts were sedimented by centrifugation at 1000 × *g* for 15–20 min. They were suspended in 0.35 M NaCl, re-centrifuged, and finally suspended in 30 ml of 0.35 M NaCl.

Calibrated[10] sucrose gradients (density range 1.09–1.26) were used in density-gradient purification[11]. 10-ml aliquots of the final chloroplast suspension were layered over 22-ml gradients and centrifuged in a Spinco Model L ultracentrifuge (SW 25.1 rotor) at 35000 × *g* (average) for 40 min. The dark-green chloroplast layer centered in the lower third of the gradient was separated with a tube-cutter, diluted with an equal volume of 0.35 M NaCl, sedimented, refractionated in a gradient, and finally suspended in 0.35 M NaCl. Plastids, isolated by a similar procedure from etiolated seedlings grown 23 days in a dark-room, formed an orange-brown layer in the same gradient region as chloroplasts.

Total phosphorus[12], nitrogen[13], and chlorophyll[14] were determined by standard procedures. Lipid phosphorus and nitrogen were determined on alcohol–ether (3:1, v/v) extracts. After standing for 30 min, the sample–solvent mixture was boiled 5 min, filtered under slight nitrogen pressure, and diluted to volume. Since the OGUR AND ROSEN[15] procedure for plant nucleic acids has been questioned[16–18], nucleic acids were extracted by the CHIBA AND SUGAHARA[16] method and their phosphorus content determined[12]. Samples were dialyzed with gentle shaking for several days against 20–40 vol. of 0.35 M NaCl and dry weights estimated by subtracting dialysate from retentate after drying, *in vacuo*, at 70° to constant weight.

Chloroplast sediments obtained by differential centrifugation were examined by phase-contrast microscopy and were found to contain intact chloroplasts together with broken fragments, smaller particles presumably mitochondria and immature plastids, and starch granules. The gradient fraction contained thin circular and ellipsoidal discs and was largely uncontaminated with other material. Chlorophyll and total nitrogen were increased significantly in gradient purification while total

TABLE I

COMPOSITION OF CHLOROPLAST AND PURIFIED CHLOROPLAST FRACTIONS

Component	Chloroplasts*	Purified chloroplasts**
	μg/mg dry wt.***	
Chlorophyll	58.6 ± 13.3 (5)	105 ± 36 (5)
Total N	65.4 ± 19.7 (5)	97.0 ± 15.4 (5)
Lipid N	8.53 ± 0.41 (3)	11.8 ± 2.8 (4)
Total P	1.8 ± 0.77 (6)	1.5 ± 0.22 (5)
Lipid P	1.2 ± 0.55 (6)	0.98 ± 0.20 (5)
RNA P	0.20 (2)	0.12 (2)
DNA P	0.22 (1)	0.17 (1)

* Differential centrifugation.
** Chloroplast layer from gradient.
*** Mean ± standard deviation of observation. Figures in parenthesis indicate number of preparations analyzed.

phosphorus, lipid phosphorus, and the two nucleic acid phosphorus fractions were unchanged or slightly lower (Table I). The increment in chlorophyll nitrogen accounted for the increment in lipid nitrogen. Increments in chlorophyll and total nitrogen and the relative decrease in total, lipid, and nucleic acid phosphorus when compared to chlorophyll or total nitrogen suggest that chloroplasts isolated by differential centrifugation alone are contaminated with starch, nucleic acid-rich nuclear, and phospholipid-rich mitochondrial fractions. These significant differences in composition were not reflected in chlorophyll: total nitrogen ratios, 0.90 and 1.08. An additional comparison such as the total phosphorus: total nitrogen ratio should be included in the evaluation of isolation procedures (Table II). Similar chlorophyll: total nitrogen ratios have been obtained from other plant sources by aqueous isolation procedures[1,3,19,20], while a non-aqueous procedure yields lower ratios[19]. A higher total phosphorus and nucleic acid phosphorus content was obtained in other studies[20]. The lipid phosphorus content was similar to the value reported here[20].

TABLE II

COMPOSITION OF CHLOROPLAST AND ETIOLATED PLASTID FRACTIONS

Fraction	Total P* / Total N	Lipid N* / Total N	Lipid P* / Total P	Lipid P* / Lipid N
Chloroplasts** (mean)	0.028	0.13	0.67	0.14
Purified chloroplasts** (mean)	0.015	0.12	0.65	0.083
Etiolated plastid – A***				
Plastid layer	0.034			
Sediment	0.028			
Etiolated plastid – B***				
Plastid layer	0.044	0.052	0.31	0.26
Sediment	0.034	0.037	0.32	0.29

* Ratios expressed as μg/μg.
** Calculated from data in Table I.
*** A and B are different plastid preparations.

Gradient centrifugation of etiolated plastids yielded approximately equal quantities of two fractions, one sedimenting in the same region as chloroplasts, and a second sedimenting to the bottom of the tube. This heavier fraction was subsequently found to have a density greater than 1.35. These fractions could not be distinguished from each other by phase-contrast microscopy, nor did they differ appreciably in relative chemical composition (Table II). They were composed of small irregularly shaped bodies presumably plastids together with a few larger circular particles. When chloroplasts and etiolated plastids are compared (Table II), a significant increment in total phosphorus relative to both total nitrogen and lipid phosphorus is observed. A low relative lipid nitrogen reflects the absence of chlorophyll. The relative nitrogen increment in chloroplasts may reflect protein synthesis which occurs at a rapid rate during plastid development[21,22]. The relative lipid phosphorus increment suggests that phospholipid synthesis also occurs during plastid development. For example, non-green Euglena cells have higher concentrations of acid-soluble phosphorus and polyphosphate and lower concentrations of lipid phosphorus than green cells[23].

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