Heterogeneity in Chloroplasts of Siphonacious Algae as Compared with Higher Plant Chloroplasts

Angela Lüttke, Ursula Rahmsdorf, and Rainer Schmid

Institut für Pflanzenphysiologie und Zellbiologie der Freien Universität Berlin

(Z. Naturforsch. **31 c**, 108–110 [1976]; received September 29, 1975)

Acetabularia Chloroplasts, Buoyant Densities, Isopycnic Gradient Centrifugation

The chloroplast population of *Acetabularia* contains four subpopulations of intact plastids with the buoyant densities at 1.21 g/cm³–1.18 g/cm³–1.14 g/cm³–1.10 g/cm³ on the discontinuous sucrose gradient. The starch content varies with the buoyant densities. The subpopulations probably represent entities of different biochemical function.

Acetabularia chloroplasts display broad morphological heterogeneity [1, 2]. The plastids differ largely in form, size, reserve material content [1, 2], and quantity of DNA [3]. A first indication for disparate biochemical properties is given by the CO_2 fixation rates in the apical and basal part of the cell [4]. Hence, separation methods of functionally different chloroplasts are needed in order to study their properties in detail. The results presented here demonstrate that isopycnic gradient centrifugation is a useful technique to resolve the plastids into discrete fractions for biochemical and metabolic studies.

In addition to three *Acetabularia* species, *A. mediterranea*, *A. cliftonii*, *A. major*, the investigations were extended to another siphonacious alga, *Bryopsis plumosa*. Since Spinach chloroplasts have been defined by density gradient centrifugation [5], these plastids were run for comparison. The *Acetabularia* species and *Bryopsis plumosa* were grown as described by Hämmerling [6]. Spinach *(Spinacea oleracea)* was purchased from a local market.

The chloroplast suspensions for sucrose density centrifugation were obtained by gently homogenizing the algae in isolation buffer (6.7 mM phosphate buffer, pH 7.1, containing 0.33 M sucrose, 2 mM EDTA **, 50 mM MES *, 0.05% mercaptoethanol) using a glass homogenizer. The homogenate was filtered through miller gauze, and the filtrate centrifuged at 200 × *g* for 90 sec. The resulting supernatant was centrifuged at 1000 × *g* for 10 min, and the sediment washed twice with isolation buffer. Spinach leaves were ground in a chilled mortar after addition of isolation buffer and of seasand. The homogenate was filtered through nylon gauze and centrifuged at 800 – 1200 × *g* several times for 10 min. All steps were carried out at 4 °C.

The conditions for the sucrose density gradients are described in legends to figures. After the centrifugation, fractions were collected through a hole in the bottom of the tube, and their sucrose concentrations were determined by refractometry, their chlorophyll content by the method of Arnon [7].

The *Acetabularia* patterns of the discontinuous gradients (Fig. 1) display four distinct bands containing chloroplasts, irrespective of the duration of centrifugation. A similar pattern was obtained on the linear gradient (Fig. 1). The sedimentation profiles of the chloroplasts from the three *Acetabularia* species were remarkably similar in that the chloroplasts migrated to identical densities (Fig. 2 a). The *Bryopsis* plastids resolved into three density types only, one type being predominant (Fig. 2 b). Compared to *Acetabularia*, the densities of the *Bryopsis* chloroplasts were lighter. Again in contrast to *Acetabularia* plastids, spinach chloroplasts resolved into two bands (Fig. 3) under identical centrifugation conditions. This result agrees with earlier reports on

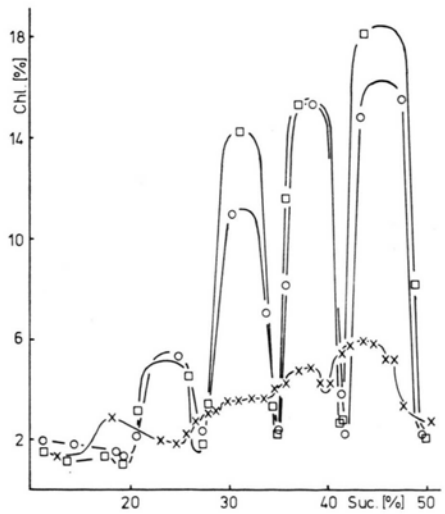

Fig. 1. Distribution of *Acetabularia mediterranea* chloroplasts. The chloroplast sediment was resuspended and layered on top of either a continuous or a discontinuous sucrose gradient. The continuous gradient ranged from 1.8 to 0.6 M sucrose in isolation buffer and was centrifuged for 1 hour (×–×). The discontinuous gradient consisted of 2 ml cushions of 1.8 M, 1.45 M, 1.2 M, 0.9 M, and 0.6 M sucrose. The gradients were centrifuged for 1 hour (□–□) or 3 hours (○–○). The centrifugation was carried out at 41000 × *g* (SW 41) and 4 °C.

Requests for reprints should be sent to Dr. Rainer Schmid, Freie Universität Berlin, Fachbereich 23, Institut für Pflanzenphysiologie und Zellbiologie, Königin-Luise-Str. 12–16a, *D-1000 Berlin 33*.

*, ** *Abbreviations:* MES, Morpholinoethanesulfonic acid; EDTA, Ethylenediaminetetraacetic acid.

the sedimentation properties of higher plant chloroplasts [8–11].

It is generally recognized that the two bands contain opaque and dark organelles in the phase contrast [8] characterizing intact and broken plastids,

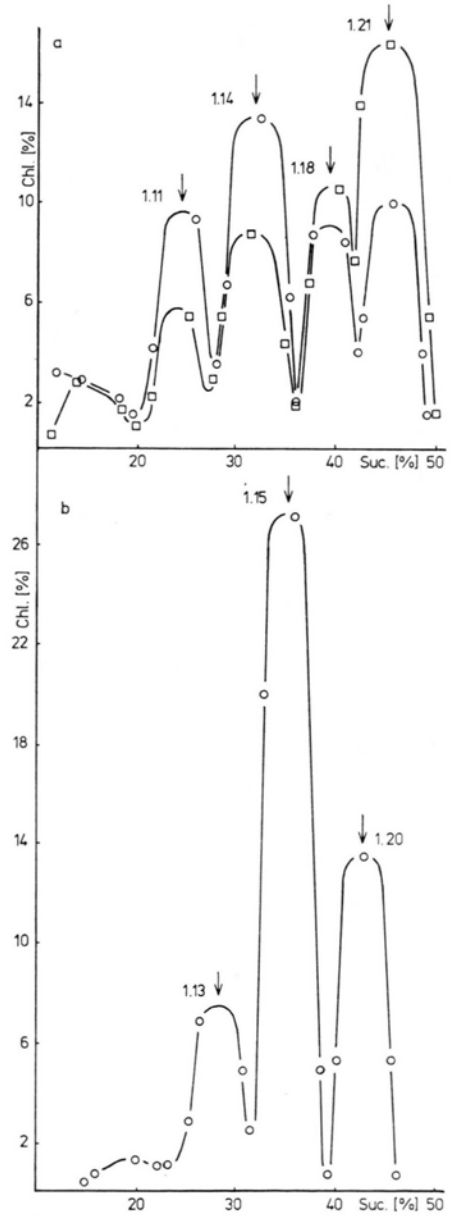

Fig. 2. Density distribution profiles of chloroplasts on discontinuous sucrose gradients. For gradient composition see legend for Fig. 1. The centrifugation was carried out for 2 hours. The arrows denote equilibrium densities of chloroplasts from *A. cliftonii* (□—□) and *A. major* (○—○) in Fig. 2 a, and of chloroplasts from *Bryopsis plumosa* (○—○) in Fig. 2 b.

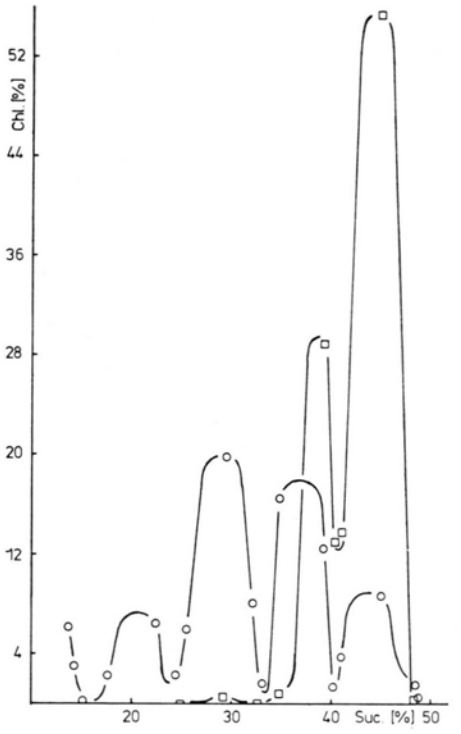

Fig. 3. Distribution of chloroplasts on discontinuous gradients from *A. mediterranea* (○—○) and *Spinacia oleracea* (□—□). For gradient composition see legend for Fig. 1. The centrifugation was carried out for 2 hours.

respectively. Monitoring our fractions by light and phase microscopy, only intact chloroplasts with an opaque appearance were detected. Hence, we conclude that the four bands in fact represent chloroplast subpopulations from *Acetabularia*.

A particularly striking criterion observed by electron microscopal studies [1, 12, 13], is the variable starch content of the plastids in the different parts of the *Acetabularia* cell. The number of polysaccharide granules follows an apicobasal gradient, the highest number found in chloroplasts from the base of the cell. An apicobasal gradient was also reported for the division of the plastids [2]. The plastids resolved on the basis of density in our gradients, also varied in the starch content. The ratio of starch/chlorophyll of the subpopulations increased with increasing density.

Apparently the varying starch content of the *Acetabularia* chloroplasts unlike the more uniform starch content in the chloroplasts of higher plants, is the main factor for the different buoyant densities of the organelles. Taking into account that the aging of the plastids is visualized by their polysaccharide content, it is quite probable that the bands consist

of chloroplasts with different biochemical capacities. We may even speculate that the separation pattern on the sucrose gradient corresponds to the gradient observed for the chloroplast fine structure [2, 12] and their photosynthetic capacities [4] within the living cell.

[1] M. Boloukhère, J. Microsc. **13**, 401 [1972].
[2] S. Puiseux-Dao and A.-C. Dazy, Biology of Acetabularia, 111 (J. Brachet and S. Bonotto, eds.), Academic Press, New York—London 1970.
[3] C. L. F. Woodcock and L. Bogorad, J. Cell Biol. **44**, 361 [1969].
[4] O. Issinger, I. Maass, and H. Clauss, Planta **101**, 360 [1971].
[5] R. McC Lilley, M. P. Fitzgerald, K. G. Rienits, and A. D. Walker, New Phytol. **75**, 1 [1975].
[6] J. Hämmerling, Ann. Rev. Plant Physiol. **14**, 65 [1963].
[7] D. J. Arnon, Plant Physiol. **24**, 1 [1949].
[8] M. V. Laycock, D. Boulter, and D. A. Thurman, Biochemistry of Chloroplasts, **Vol. I**, p. 293, (T. W. Goodwin, ed.), Academic Press, New York—London 1966
[9] J. W. Lyttleton, J. E. M. Ballantine, and B. J. Forde, Autonomy and Biogenesis of Mitochondria and Chloroplasts, p. 447, (N. K. Boardman, A. W. Linnane, and R. M. Smillie, eds.), North Holland, Amsterdam—London 1971.
[10] R. K. Yamazaki and N. E. Tolbert, Biochim. Biophys. Acta **178**, 11 [1969].
[11] V. Rocha and I. P. Ting, Arch. Biochem. Biophys. **140**, 398 [1970].
[12] S. Puiseux-Dao, A.-C. Dazy, D. Hoursiangou, and E. Matthys, Biology and Radiobiology of Anucleate Systems, II. Plant Cells, p. 101, (S. Bonotto, R. Goutier, R. Kirchmann, and J.-R. Maisin, eds.), Academic Press, New York—London 1972.
[13] D. Hoursiangou-Neubrun and S. Puiseux-Dao, Plant Sci. Lett. **2**, 209 [1974].

Erratum

H. J. Rurainski, Antagonistic Relationships between Electron Transport and P_{700} in Chloroplasts and Intact Algae, Z. Naturforsch. **30 c**, 761 [1975]. Page 763, Eqn (4) should be read:

$$p^*(t) = p_0^* + p_\omega^* \sin(\omega t - \varphi)$$

Satz und Druck: Konrad Triltsch, Würzburg