BBA 43246

Density of pea chloroplasts determined by four different methods

The determination of the density of cells or organelles is generally based on the attainment of an equilibrium position upon centrifugation in a density gradient. Accurate measurements necessitate using isotonic suspending media and intact particles, conditions which have usually been disregarded when determining chloroplast densities. Using pea chloroplasts isolated nonaqueously, an upper limit for the density is here estimated to be 1.18 g/cm^3. Techniques involving centrifugation in a density gradient, a pycnometer, and Stokes' law produced results indicating that the density of intact chloroplasts isosmotic to the plant cell is 1.10–1.11 g/cm^3.

Density gradient. Chloroplasts were isolated from leaves of 14-day-old *Pisum sativum* in the light[1]. Isolation required 2 min and yielded 95 % Class I (intact) chloroplasts[1]. The isolation medium was 0.26 M sucrose, 5 mM *N*-tris-(hydroxymethyl)-methyl-2-aminoethane sulfonate (TES)–NaOH (pH 7.5). This solution has approximately the same osmolality as sap expressed from the pea leaves[2], and hence the isolated chloroplasts presumably had the same volume and density as *in vivo*. Since sucrose does not readily enter these intact chloroplasts[2], it is a suitable osmoticum for density studies. A linear density gradient was prepared by varying the amount of Ficoll (approximate molecular weight, 400000; Pharmacia Fine Chemicals, Piscataway, N.J.) from 7 % at the top to 40 % at the bottom (all solutions had 0.26 M sucrose, 5 mM TES–NaOH, pH 7.5). Chloroplasts containing 0.5 mg chlorophyll were layered on top. Centrifugation was for 30 min at 40000 × *g* at 0° in a Spinco Model Ti-14 zonal rotor. A band of Class I chloroplasts was centered at a density of 1.103 g/cm^3 (Table I). When the centrifugation time was extended to 60 min, the peak of the Class I band was at 1.109 g/cm^3, but the percentage of Class I chloroplasts decreased to about half of that in Table I. Since the Class I band did not appreciably shift when the centrifugation time was doubled, the position in the gradient could well be close to the density of pea chloroplasts. However, only a small fraction (3.7 % in Table I) of the chloroplasts remain intact. Also using a zonal centrifuge, PRICE

TABLE I

SEDIMENTATION PATTERN OF INTACT PEA CHLOROPLASTS IN A DENSITY GRADIENT MADE WITH FICOLL

After centrifugation (see text), thirteen 50-ml aliquots were collected in 90 min, only those tubes containing intact chloroplasts being indicated. Each sample was examined in a counting chamber for Class I chloroplasts using a Zeiss phase contrast research microscope. The density was obtained at 20.00° with a 25-ml pycnometer.

Tube No.	*Class I chloroplasts (% of original)*	*Density (g/cm^3)*
6	0.2	1.088
7	0.9	1.096
8	1.8	1.103
9	0.6	1.110
10	0.2	1.117

Abbreviation: TES, *N*-tris-(hydroxymethyl)methyl-2-aminoethane sulfonate.

AND HIRVONEN[3] have reported an equilibrium density for bean chloroplasts of 1.27 g/cm³, the high value occurring probably because either the plastids contracted osmotically in the sucrose gradient employed or were actually broken.

Nonaqueous fractionation. After lyophilization of pea leaves, chloroplasts can be isolated nonaqueously from the dry powdered material using differential centrifugation in organic reagents[4]. When the density of a hexane–carbon tetrachloride mixture was 1.33 g/cm³, such chloroplasts did not move up or down in the tube when centrifuged for 60 min at 40000 × *g*. Thus, the nonaqueous components of pea chloroplasts may have a density near 1.33 g/cm³. The volume of pea chloroplasts from plants in the light is 29.0 μ^3 at the same osmolality as the cell sap[2]. The nonaqueous volume (*b* in the Boyle–Van't Hoff relation) is 16.2 μ^3 per chloroplast[2]. Using a water density of 1.00 g/cm³, the density of chloroplasts is thus $[(16.2)(1.33) + (29.0-16.2)(1.00)]/29.0$ or 1.18 g/cm³. This estimate assumes that the chlorophyll-containing lyophilized material was solely chloroplasts. However, many cytoplasmic ribosomes and presumably a considerable amount of cytoplasmic protein, both having relatively high densities, adhere to chloroplasts isolated nonaqueously[4]. Thus 1.18 g/cm³ is only an upper estimate of chloroplast density.

Pycnometer. A pycnometer can be precisely filled to some volume at a given temperature, and thus densities of fluids can be deduced from suitable weighings. The densities of the medium (0.26 M sucrose, 5 mM TES–NaOH, pH 7.5) and of chloroplast suspensions isolated aqueously as described above were determined using a 10-ml Weld pycnometer (Table II). The results in the fifth column indicate an average chloroplast density of 1.097 g/cm³. Uncertainties include contributions from a few broken chloroplasts, whole cells and mitochondria occurring with the isolated chloroplasts.

TABLE II

CHLOROPLAST DENSITY DETERMINED WITH A PYCNOMETER

The volume of pea chloroplasts in 0.26 M sucrose, 5 mM TES–NaOH (pH 7.5) is 0.02697 ml/mg chlorophyll[2]. This number times the chlorophyll concentration (column 3) gives the chloroplast volume fraction (column 4). The volume fraction × ($\rho_{chloroplasts}-\rho_{medium}$) equals the increment of the suspension density over that of the medium (column 2 − column 1), and so the chloroplast density can be calculated.

ρ_{medium} at 20.00° (g/cm³)	$\rho_{suspension}$ at 20.00° (g/cm³)	Chlorophyll (mg/ml)	Chloroplast volume fraction (ml/ml)	$\rho_{chloroplast}$ (g/cm³)
1.02625	1.02816	1.04	0.0281	1.094
1.02726	1.02964	1.09	0.0294	1.108
1.02694	1.02879	1.04	0.0281	1.093
1.02676	1.02898	1.11	0.0300	1.101
1.02681	1.02859	1.08	0.0291	1.088

Stokes' law. A spherical particle of radius *r* moving under the influence of gravity in a fluid achieves a terminal velocity given by Stokes' law:

$$\text{Velocity} = 2/9 \cdot r^2 g (\rho_{particle}-\rho_{medium})/\eta$$

where *g* is 980 cm/sec². The viscosity, η, of the isolation and suspension media at 20° was measured as 0.0131 g/cm·sec using an Ostwald viscometer. The time for a Class I

chloroplast to fall vertically a known distance was determined using a graduated eyepiece on a horizontally positioned phase contrast microscope. From the distribution of velocities (Fig. 1), the mean velocity of fall was $4.84 \cdot 10^{-5}$ cm/sec. If chloroplasts were spheres of volume 29.0 μ^3, r^2 would equal $3.64 \cdot 10^{-8}$ cm^2. Using these values and ρ_{medium} from Table II, the chloroplast density is 1.107 g/cm^3. For oblate ellipsoids having a ratio of major to minor axes of 2.3 (similar to illuminated pea chloroplasts *in vivo*[4]), the frictional drag is about 6 % greater than for spheres of the same volume[5], which would lead to an underestimate of the chloroplast density by about 0.005 g/cm^3 in the present case.

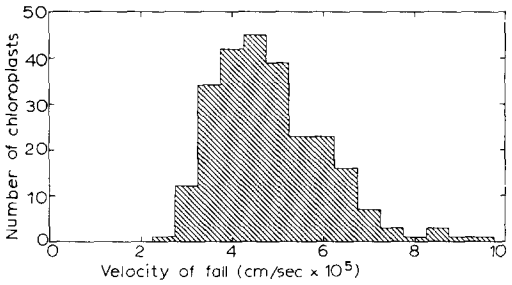

Fig. 1. Velocities of pea chloroplasts falling under the influence of gravity in 0.26 M sucrose, 5 mM TES–NaOH (pH 7.5).

In summary, the four techniques yield results which are consistent with 1.10–1.11 g/cm^3 for the density of intact pea chloroplasts at an osmolality approximating that *in vivo*. Such values are considerably greater than the density of water, which reflects the high protein content of chloroplasts.

This research was supported by Public Health Service Research Grant GM 15183 from the National Institute of General Medical Sciences.

Department of Botanical Sciences and Molecular Biology Institute, University of California, Los Angeles, Calif. 90024 (U.S.A.)

PARK S. NOBEL

1 P. S. NOBEL, *Plant Physiol.*, 43 (1968) 781.
2 P. S. NOBEL, *Biochim. Biophys. Acta*, 172 (1969) 134.
3 C. A. PRICE AND A. P. HIRVONEN, *Biochim. Biophys. Acta*, 148 (1967) 531.
4 P. S. NOBEL, D. T. CHANG, C-T. WANG, S. S. SMITH AND D. E. BARCUS, *Plant Physiol.*, 44 (1969) 655.
5 H. B. BULL, *An Introduction to Physical Biochemistry*, F. A. Davis Co., Philadelphia, 1964, pp. 264, 330.

Received August 1st, 1969

Biochim. Biophys. Acta, 189 (1969) 452–454