Stereometrical analysis of number and size of prolamellar bodies during pea chloroplast development

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Abstract

The plastid prolamellar bodies in dark-grown pea seedlings undergo gradual transformation and decay after illumination with low intensity light. Random micrographs do not give direct information concerning the sizes and average numbers of prolamellar bodies in a plastid. These values were obtained after evaluation by a stereometrical method from the ratio of prolamellar bodies sizes to the plastid size and from the frequency of prolamellar body sections of a given diameter. Plastids of dark-grown seedlings contained on the average at least one prolamellar body. After illumination the size of the bodies decreased rapidly owing to dispersion into primary thylakoids and split into much smaller numerous prolamellar bodies.

Key words: chloroplast development, prolamellar body, Pisum sativum, stereology

INTRODUCTION

In plants grown in darkness proplastids differentiate into etioplasts with prolamellar bodies which after exposure to light convert into grana and stroma thylakoids. Quantitative data on the size and number of prolamellar bodies are necessary for analysis of chloroplast differentiation, especially conversion of prolamellar bodies into primary thylakoids.

Gunning and Jagoe (1967) developed a method of calculating the area of membranes stored in the prolamellar body. The total area of the membranes can be calculated from the volume of the prolamellar body. It is possible, therefore, to measure the amount of the thylakoid membranes originating from prolamellar bodies.

Radom ultrathin sections give no direct information on the size and number of prolamellar bodies in a plastid. Serial sections can be obtained, but usually their number is too small for quantitative analysis. Thus, stereometry is required to evaluate the real number and size of structures in plastids. The volume of the structures having arbitrary shapes can be calculated from analysis of sections (Weibel and Gomez 1962, Weibel et al. 1966). This method does not allow determination of the total number of structures of different size.

There has been no data concerning the number and sizes of prolamellar bodies. Only the total volume of prolamellar bodies in a plastid is given, without taking into account the dispersion of their sizes (Bradbeer et al. 1974a, b, c). It was essential, therefore, to find the total number and actual dimensions of prolamellar bodies. Straightforward application of the existing stereometrical methods (Komender 1973) was not possible.

The stereometric method presented here allows, although with some crude approximations, to estimate the average number and real size of prolamellar bodies in a plastid. It seems more accurate in the assessment of the results than simple comparison of cross sections areas.

MATERIAL AND METHODS

Seeds of *Pisum sativum* L. var. Bördi sown in pots with humid sand were kept in darkness at 25°C. Eight days after germination the seedlings were illuminated by continuous white light ($0.98 \cdot 10^3 \text{ erg} \cdot \text{cm}^{-2}$) for 2, 4, 8, 12, 24, 48 and 72 hours. Pieces of about 1 mm² cut near the main vein from the broadest part of the second leaf above the cotyledons were fixed in 2.5% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.4 for 2 hours, washed in buffer and placed overnight in cold 2% OsO₄ in the same buffer. The specimens were dehydrated in an ethanol-propylene oxide series and embedded in epon (Luft 1961). Sections cut on an LKB ultramicrotome were stained with uranyl acetate and lead citrate (Reynolds 1963) and examined under a Tesla BS 500 electron microscope.

The major and minor axes of plastid sections and diameters of prolamellar body sections were measured in the mesophyll cells. Measurements of 50 to 140 plastid sections from each experiment were carried out. Results were analysed by standart statistical methods.

STEREOMETRICAL METHOD OF DETERMINING THE NUMBER AND SIZE OF PROLAMELLAR BODIES

Sections of prolamellar bodies visible on micrographs are of different size. There are two possible reasons for this: the actual size of prolamellar

Fig. 1. Etioplast of dark-grown, eight-day-old seedling of *Pisum sativum*. x 36 000
Figs. 2, 3. *Pisum sativum* chloroplasts with prolamellar bodies after illumination with white light: Fig. 2 — 2 hours, Fig. 3 — 4 hours. x 48 000

Figs. 4, 5. *Pisum sativum* chloroplasts with prolamellar bodies after illumination with white light: Fig. 4 — 12 hours, Fig. 5 — 48 hours. x 48 000

bodies in a plastid are different or the plane of section may lie at a different distance from the centre of the prolamellar body.

The supposition, that prolamellar bodies in plastids at a given developmental stage are of the same size cannot be excluded. If so, the dispersion of section sizes would originate from the randomness of the section plane position. We will assume, however, and that will be confirmed by further analysis, that there are prolamellar bodies of different sizes in plastids. A very simplified model is proposed which allows the determination of the actual dimensions from random sections dimensions. The model assumes that the prolamellar bodies are spherical, since the great majority of their sections are of circular shape (Figs. 1-5). The same assumption was made by Gunning and Jagoe (1967) and Bradbeer et al. (1974a, b, c) in their studies of prolamellar body structure. The present model also assumes that the planes of the sections and the position of prolamellar bodies within the plastid are random, although this is a rather crude approximation since the positions may often be correlated, for example, when a large prolamellar body splits, the fragments will be most probably close to each other.

The probability that a section of a plastid includes a prolamellar body is $\frac{b_1}{a}$, where b_1 represents the diameter of the prolamellar body, a is the average of the major and minor axes of the plastid (Fig. 6). The probability is averaged over various positions of the prolamellar body in the plastid. The diameter of the prolamellar body section depends on the plane of the section and is smaller than, or equal to the actual diameter — b_1 . Let us introduce classes of prolamellar body diameters: from b_1 to b_2 , from b_2 to b_3 etc. The probability $P_{b_1 b_2}$ that the diameter of the prolamellar body section between b_2 and b_1 is equal to the ratio

Fig. 6. Model of plastid with prolamellar body. b_1 — diameter of prolamellar body.

$$a_1 \text{ — major plastid axis, } a_2 \text{ — minor plastid axis, } a = \frac{a_1 + a_2}{2}$$

of the length $A_1 A_2$ (see Fig. 7) to one half of the averaged axis length:

$$P_{b_1 b_2} = \frac{A_1 A_2}{\left(\frac{a}{2}\right)} = \frac{\sqrt{b_1^2 - b_2^2}}{a}.$$

Similarly, the probability that the section diameter is between b_2 and b_3 is equal to the ratio of the length $A_2 A_3$ to the average axis a divided by 2, hence:

$$P_{b_2 b_3} = \frac{A_2 A_3}{\frac{a}{2}} = \frac{\sqrt{b_1^2 - b_3^2} - \sqrt{b_1^2 - b_2^2}}{a}.$$

N plastid sections were analysed after each illumination time. As in the case of prolamellar bodies, the plastid sections have different diameters. The maximal section diameter was chosen as the real one. It was obtained from the central section of a plastid and should be the best approximation to its real size. After each illumination time plastid diameters were similar on each section. One can conclude, therefore, that plastids have similar diameters not only on each section but in a whole cell as well.

It was determined that among N plastid sections there are n_1 sections of prolamellar bodies with diameters between b_1 and b_2 , n_2 sections with diameters between b_2 and b_3 etc. The rest of plastid sections did not contain prolamellar body sections at all. The following values were chosen: $b_1 = 1.6 \mu\text{m}$, $b_2 = 1.3 \mu\text{m}$, $b_3 = 1.0 \mu\text{m}$, $b_4 = 0.8 \mu\text{m}$, $b_5 = 0.6 \mu\text{m}$, $b_6 = 0.4 \mu\text{m}$, $b_7 = 0.2 \mu\text{m}$. Sections with the largest diameters ($b_1 - b_2$) originated from prolamellar bodies with the largest actual diameters. Such sections will be called central sections. Some of the sections with smaller diameters originate also from the largest prolamellar bodies, but these are side sections.

With the help of the prolamellar body model (Fig. 7) and analysis of micrographs, the actual average number of a given size class of prolamellar

Fig. 7. Model of prolamellar body with diameter b_1 , b_2 , b_3 , b_4 — diameters of side sections

bodies in a plastid can be determined. Since there are n_1 central sections of prolamellar bodies with diameters between b_1 and b_2 , it follows from the model that: $\frac{n_1 P_{b_2 b_3}}{P_{b_1 b_2}}$ sections with diameters between b_2 and b_3 are side sections of the largest prolamellar bodies ($b_1 - b_2$). Other side sections are in subsequent classes of diameters ($b_3 - b_4$, etc.). The total number of sections of the largest prolamellar bodies is equal to:

$$n_1 + \frac{n_1 P_{b_2 b_3}}{P_{b_1 b_2}} + \frac{n_1 P_{b_3 b_4}}{P_{b_1 b_2}} + \dots$$

If there was one prolamellar body with central section diameter between b_1 and b_2 in a plastid, we should find $\frac{Nb_1}{a}$ prolamellar body sections in N plastid sections. Therefore, we have on the average in a plastid:

$$m_1 = \frac{n_1 + \frac{n_1 P_{b_2 b_3}}{P_{b_1 b_2}} + \frac{n_1 P_{b_3 b_4}}{P_{b_1 b_2}} + \dots}{\frac{Nb_1}{a}}$$

prolamellar bodies with actual diameter between b_1 and b_2 . Quantities $n_1, N, \frac{b_1}{a}, m_1$ for each illumination period are given in Tables 1, 2 and 3.

The average number of prolamellar bodies with actual diameter between b_2 and b_3 per plastid can be now calculated. Sections of these prolamellar bodies have diameters between b_2 and b_3 and smaller (Fig. 8). There are n_2 sections with diameters between b_2 and b_3 . Part of them, namely, $\frac{n_1 P_{b_2 b_3}}{P_{b_1 b_2}}$ are the side sections of the largest prolamellar bodies ($b_1 - b_2$). The remaining part:

$$\bar{n}_2 = n_2 - \frac{n_1 P_{b_2 b_3}}{P_{b_1 b_2}}$$

are the central sections of smaller prolamellar bodies ($b_2 - b_3$). It has been determined that $\bar{n}_2$ is positive, this supporting above formula.

The numbers of side sections in the successive classes of prolamellar bodies are:

$$\frac{\bar{n}_2 P_{b_3 b_4}}{P_{b_2 b_3}}, \frac{\bar{n}_2 P_{b_4 b_5}}{P_{b_2 b_3}}, \text{ etc.}$$

Fig. 8. Model of prolamellar body with diameter $b_2 \cdot b_3 - b_4$ — diameters of side sections

Table 1

Plastids and prolamellar bodies (pbs) in dark-grown pea seedlings and after illumination with weak white light

Hours of illumination	Number of			b_1/a
	plastid sections (N)	plastid sections with pbs	pbs per section	
0	90	51	0.57	0.7
2	70	56	1.06	0.44
4	80	50	0.86	0.35
8	114	90	1.26	0.34
12	120	104	1.53	0.32
24	128	94	1.39	0.25
48	111	89	1.49	0.20
72	135	70	1.29	0.19

a — average minor and major axes of the largest plastid section, b_1 — the largest diameter of the prolamellar body.

Table 2

Number of prolamellar body sections of different dimensions of dark-grown pea seedlings and after illumination with weak white light

Hours of illumination	Diameter (μm) and number of sections						
	0-0.20 n_7	0.21-0.40 n_6	0.41-0.60 n_5	0.61-0.80 n_4	0.81-1.00 n_3	1.01-1.30 n_2	1.31-1.60 n_1
0				14	17	9	11
2	5	24	31	14			
4	19	20	25	6			
8	42	65	34	1			
12	69	82	32				
24	67	65	42	3			
48	60	61	38	6			
72	35	104	29	6			

Table 3

Average number of prolamellar bodies in a plastid of dark-grown and illuminated with weak white light pea seedlings

Hours of illumination	Diameter (μm) and average number of prolamellar bodies							
	0-0.20 m_7	0.21-0.40 m_6	0.41-0.60 m_5	0.61-0.80 m_4	0.81-1.00 m_3	1.01-1.3 m_2	1.31-1.60 m_1	Total
0				0.25 $\pm$ 0.11	0.55 $\pm$ 0.10	0.08 $\pm$ 0.06	0.25 $\pm$ 0.05	1.13 $\pm$ 0.17
2	0.39 $\pm$ 0.29*	1.35 $\pm$ 0.28	1.43 $\pm$ 0.20	0.62 $\pm$ 0.11				3.79 $\pm$ 0.46
4	2.36 $\pm$ 0.53	1.11 $\pm$ 0.28	1.35 $\pm$ 0.20	0.29 $\pm$ 0.09				5.11 $\pm$ 0.64
8	2.70 $\pm$ 0.42	2.57 $\pm$ 0.22	1.15 $\pm$ 0.14	0.03 $\pm$ 0.02				6.45 $\pm$ 0.49
12	4.61 $\pm$ 0.41	3.36 $\pm$ 0.21	1.04 $\pm$ 0.13					9.01 $\pm$ 0.48
24	6.62 $\pm$ 0.64	3.65 $\pm$ 0.33	1.94 $\pm$ 0.20	0.13 $\pm$ 0.06				12.34 $\pm$ 0.75
48	9.55 $\pm$ 0.96	5.32 $\pm$ 0.49	2.70 $\pm$ 0.31	0.36 $\pm$ 0.11				17.93 $\pm$ 1.12
72	2.71 $\pm$ 0.63	6.79 $\pm$ 0.31	1.44 $\pm$ 0.22	0.35 $\pm$ 0.10				11.29 $\pm$ 0.75

* Average mean $\pm$ SE.

The total number of smaller prolamellar bodies ($b_2 - b_3$) is:

$$\bar{n}_2 + \frac{\bar{n}_2 P_{b_3 b_4}}{P_{b_2 b_3}} + \frac{\bar{n}_2 P_{b_4 b_5}}{P_{b_2 b_3}} + \dots$$

If in a plastid there was one prolamellar body ($b_2 - b_3$), we should see $\frac{Nb_2}{a}$ prolamellar body sections in N plastid sections. Hence, in a plastid there are on the average:

$$m_2 = \frac{\bar{n}_2 + \frac{\bar{n}_2 P_{b_3 b_4}}{P_{b_2 b_3}} + \frac{\bar{n}_2 P_{b_4 b_5}}{P_{b_2 b_3}} + \dots}{\frac{Nb_2}{a}}$$

smaller prolamellar bodies ($b_2 - b_3$). The value of $\frac{b_2}{a}$ can be found from the formula:

$$\frac{b_2}{a} = \frac{\frac{b_1}{a}}{\frac{b_2}{b_1}}$$

Similar analysis was carried out for the subsequent classes. It has been determined that $\bar{n}_3, \bar{n}_4$ etc. are positive, just as $\bar{n}_2$. Thus, prolamellar bodies of all size classes are present in plastids.

The total average volume of prolamellar bodies in a plastid was determined from the average number of prolamellar bodies of a given size in a plastid (Table 3). Assuming that all prolamellar bodies are spherical, their total volume is given by:

$$V = \sum_i m_i \frac{4}{3} \left(\frac{b_i}{2} \right)^3,$$

where m_i denotes average numbers of prolamellar bodies of a given diameter (Table 4), b_i denotes diameters.

Table 4

Total average volume of prolamellar bodies in a plastid

Hours of illumination	Volume, μm^3
0	0.72
2	0.19
4	0.16
8	0.18
12	0.12
24	0.31
48	0.32
72	0.30

RESULTS

Prolamellar bodies formed in etioplasts during their development from proplastids in darkness. On each of 51 etioplast sections out of 90 analysed, one prolamellar body was seen, hence, on the average there were 0.57 prolamellar bodies per etioplast section. With the help of the method presented above the number of prolamellar bodies per etioplast was determined. Sections of prolamellar bodies in etioplasts had a diameter between $0.6\text{ }\mu\text{m}$ and $1.6\text{ }\mu\text{m}$ (Table 2). The numbers of prolamellar body sections of consecutive size classes as found from micrographs are denoted by n_1, n_2, n_3, n_4 and are given in Table 2. The actual average number of prolamellar bodies per etioplast was calculated to be 1.13. Average numbers of prolamellar bodies per etioplast of consecutive size classes are denoted by m_1, m_2, m_3, m_4 and are given in Table 3.

Similar analysis was carried out for plastids developing in white light after several illumination times (Table 3). It was found that the number of prolamellar bodies per plastid section after initial growth remained almost constant for 3 days (Table 1). However, the absolute number of prolamellar bodies per plastid increased continuously for 2 days and decreased later (Table 3). Probably most of the prolamellar bodies which existed in illuminated seedlings originated from decaying etioplast prolamellar bodies. During the process of prolamellar body decay the following regularity was observed: after 8, 12, 24, 48 hours the bigger the prolamellar bodies the less abundant they were.

The decrease of the total average volume of prolamellar bodies in a plastid (in the first 2 hours) was due to the dispersion of prolamellar bodies into primary thylakoids (Table 4). This quantity increased after 24 hours of illumination and was accompanied by an increase of the number of prolamellar bodies in a plastid. Probably new small prolamellar bodies were formed and possibly fused with the existing ones (Table 4).

After 72 hours of illumination numerous (11.29 per plastid) and relatively big (up to $0.8\text{ }\mu\text{m}$ in diameter) prolamellar bodies still existed in the plastids (Table 3).

DISCUSSION

Prolamellar bodies disperse and desintegrate when an etioplast transforms into a chloroplast. To assess the dynamics of this process it seems necessary to have quantitative data on prolamellar bodies. Their number seen in ultrathin sections bears no simple relation with the absolute number of prolamellar bodies inside plastids. It is possible, however, to work out the absolute number, because it is related to the relative number and size

of prolamellar bodies and plastids. We show that in weakly illuminated pea seedlings the number of prolamellar bodies per plastid section after an initial increase remains almost stable for three days (Table 1), while their absolute number in plastids increases continuously for two days (Table 3). A decrease of prolamellar bodies diameters reduces the probability of finding them in random sections. This is the cause of the discrepancy between the number of prolamellar body sections and the number of prolamellar bodies in plastids.

Papers where quantitative relations are given often restrict their analysis to the number of prolamellar bodies in plastid sections, but do not assess their total number in a plastid (for example Henningsen and Boynton 1969). The decrease of prolamellar body volume is attributed only to dispersion into thylakoids (Bradbeer et al. 1974b, c). The dispersion induced by weak white light, however, is correlated with the disappearance of large prolamellar bodies and the occurrence of much smaller but numerous ones. They presumably originate from the large bodies (Figs. 1-5). Therefore, the decrease in size should be caused by both dispersion and splitting of the existing prolamellar bodies.

Dispersion alone is a cause of the total volume decrease; its magnitude may be found by the assessment of the total number of prolamellar bodies and their size. The total volume of prolamellar bodies in our experiment decreased at first rapidly, then stabilized and finally began to rise slightly (Table 4). Without the application of the stereometrical method the result of observations would have been somewhat different; the volume would have decreased constantly throughout the experiment.

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REFERENCES

- Bradbeer J. W., Ireland H. M. M., Rest J., Edge H. J. W., 1974a. Plastid development in primary leaves of *Phaseolus vulgaris*. VII. Development during growth in continuous darkness. *New Phytol.* 73: 263-270.
- Bradbeer J. W., Gyldenholm A. O., Ireland H. M. M., Smith J. W., Rest J., Edge W. J. W., 1974b. VIII. The effects of the transfer of dark-grown plants to continuous illumination. *New Phytol.* 73: 271-279.
- Bradbeer J. W., Gyldenholm A. O., Smith J. W., Rest J., Edge H. J. W., 1974c. IX. The effects of short light treatment on plastid development. *New Phytol.* 73: 281-290.
- Gunning B. E. S., Jagoe M. P., 1967. The prolamellar body. In: *Biochemistry of chloroplasts*. T. W. Goodwin (ed.). Acad. Press, London. pp. 656-676.

- Henningsen K. W., Boynton J. E., 1969. Macromolecular physiology of plastids. VII. The effect of brief illumination on plastids of dark-grown barley leaves. *J. Cell Sci.* 5: 757-793.
- Komender J., 1973. Wybrane metody cytologiczne. In: *Ultrastruktura i funkcja komórki*. J. Kawiak, Z. Osuchowska, A. Przełęcka (eds.). PWN, Warszawa. pp. 41-46.
- Luft J. H., 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* 9: 409-416.
- Reynolds E. S., 1963. The use of lead citrate at high pH as an electronopaque stain in electron microscopy. *J. Cell Biol.* 17: 208-214.
- Weibel E. R., Gomez D. M., 1962. A principle of counting tissue structures on random sections. *J. Appl. Physiol.* 17: 343-348.
- Weibel E. R., Kistler G. S., Scherle W. F., 1966. Practical stereological methods for morphometric cytology. *J. Cell Biol.* 30: 23-38.

*Stereometryczna analiza liczby i wielkości ciał prolamellarnych
w rozwijających się chloroplastach grochu*

Streszczenie

Ciała prolamellarne utworzone w plastydach siewek grochu, rosnących w ciemności ulegają transformacji i rozpadowi po oświetleniu siewek słabym, białym światłem. Fotografie z mikroskopu elektronowego nie dają bezpośredniej informacji o wielkości i przeciętnej liczbie ciał prolamellarnych w plastydzie. Te wartości zostały znalezione po obliczeniach metodą stereometryczną na podstawie stosunku wielkości ciał prolamellarnych i plastydów oraz częstości występowania przekrojów ciał prolamellarnych danej wielkości. Plastydy siewek rosnących w ciemności zawierały przeciętnie przynajmniej po jednym ciele prolamellarnym. Po oświetleniu, wielkość ciał prolamellarnych szybko się zmniejszyła skutkiem ich rozpraszania w pierwotne tylakoidy i rozpadu na mniejsze ciała.