Prolamellar Body Development in Etioplasts of *Pisum sativum* L. var. 'Alaska'

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The term “prolamellar body” is generally used to describe the membrane system of leaf etioplasts in which “vesicles” or “tubules” aggregate together. Menke (1962) and Gibbs (1970) described “thylakoids” as being fundamental units of an internal system of membranes within the developing plastid and have the appearance of “flattened membrane-limited sacs”, Ben-Shaul, et al. (1964) referred to similar structures as “discs”. Kirk and Tilney-Basset (1967) defined “etioplast” as being plastids formed in the leaf cells of dark-grown angiosperms.

The rate of chloroplast maturation and its ability to form prolamellar bodies depends upon cell age (Rascio 1976). Also, in barley the recrystallization of the prolamellar body with the resynthesis of protochlorophyllide in darkness, subsequent to photoconversion upon illumination, is dependent upon seedling age (Henningson and Boynton 1969).

There have been many investigations in the ultrastructural development and arrangement of prolamellar bodies within etioplasts of dark-grown seedlings (Klein and Poljakoff-Mayber 1961, Kahn 1966, Kahn 1968a, 1968b, Kahn, Bordman and Thorne 1970).

Prolamellar bodies have been reported in very young maize leaves of plants that had been exposed to 25,000 lux (Leech, Rumsby, Thomson, Crosby, and Wood 1972).


Several workers have proposed explanations or models for the interpretation of the sequential development of prolamellar bodies in plant cell etioplasts. Gunning and Jagoe (1967) suggest that the prolamellar body consists of a framework of tubules positioned in the three major axes of a common cuboidal lattice. A different view of prolamellar body development has been presented by Wehrmeyer, (1965a, b, c, and Wehrmeyer and Robbelien 1965), wherein the main portion of the crystalline part of the prolamellar body is conceived as being arranged in a six-sided crystal lattice structure. In studies of prolamellar body development in *Phaseolus*, Ikeda (1968) agrees with the Wehrmeyer concept of multiple
hexagonal units undergoing aggregation to make up the prolamellar body proper.

Another view concerning prolamellar body formation has been proposed by von Wettstein (1958, 1967, Engelbrecht and Weier 1967), wherein the inner plastid envelope produces discrete vesicles and these specific vesicles accumulate within the stroma, undergoing subsequent fusion to form the prolamellar body.

Schnepf (1964) and Menke (1962) envision the prolamellar body as consisting of an array of helically coiled tubules devoid of an interconnecting tubular network. Other studies by Gunning and Jagoe (1967) suggest that porous membranes comprise the makeup of the peripheral lamallae, with ribosomes partaking in the spatial arrangement of the tubules within the prolamellar body (Brown and Gunning 1966). Etioplasts, upon illumination, reveal that the tubule-type of structure is reformed and becomes transformed into vesicles (Virgin et al, 1963). In essence, these tubules—formed either during dark exposure or upon exposure of the etioplasts to illumination—are quite similar, with respect to ultrastructural appearance.

An alternate view of prolamellar body development is offered by Weier and Brown (1970), in studies of Phaseolus, wherein they suggest that rather than the plastid envelope-derived vesicles undergoing fusion to form the prolamellar body (von Wettstein 1958, 1967), there is instead a process of gradual contraction of the sheets of porous membranes into a more ordered tubular arrangement.

When KMnO₄ is used as a fixative, the developmental process was interpreted as being the formation and coalescence of discrete vesicles (Virgin, Kahn, and von Wettstein 1963). Also, when the prolamellar body undergoes transformation by light, the paracrystalline structure separates into discrete vesicles which upon dispersal, become primary thylakoid layers (Virgin, Kahn, and von Wettstein 1963). However, when similar studies were repeated with glutaraldehyde as the primary fixative, followed by osmium tetroxide, the discrete vesicles appeared to be interconnected into tubular membranes, in both the formation and transformation of the prolamellar body (Henningston and Boynton 1969).

The present study concerns the development of prolamellar bodies within etioplasts in apical leaf tissue of dark-grown pea seedlings (Pisum sativum L. var. ‘Alaska’).

Materials and methods

Pea seedlings (Pisum sativum L. var. ‘Alaska’) were grown in the dark from 2 to 8 days at a temperature of 23±2°C during the day and 20°C at night. The seedlings were grown in vermiculite saturated with half-strength Hoaland’s nutrient medium.

Tissue from the apical leaves of 2, 4, 6, and 8-day-old dark-grown seedlings was excised and fixed in darkness to prevent protochlorophyllide conversion. Immediately after excision, the respective tissue samples were fixed in 3% glutaraldehyde buffered with 0.1 M phosphate (pH 7.2) for 1 hr at 4°C. Following several rinses in buffer, the tissue was cut into smaller pieces (2 mm sp.) and post-fixed in 1% OsO₄, in 0.1 M phosphate at a pH of 7.2 for 1 hr at 4°C. The
Fig. 1. Etioplast of 2-day-old, dark-grown seedling. Tubules (T) are present in the stroma (S). Some of the tubules are linked together to form chain-like organelles (arrows). × 95,000.
material was dehydrated in a graded ethanol series, treated with propylene oxide, and then embedded in Epon 812 (Luft 1961). This sections were cut with a glass knife on a Porter-Blum MT-2 ultramicrotome, and collected on cleaned 200 or 400 mesh copper grids. The sections were then stained with uranyl salts only and examined with a Phillips 200 electron microscope.

Fig. 2. The etioplast tubules (T) of 4-day-old, dark-grown seedlings show the congregation of some tubules near the central portion of the stroma (S). With many tubules now linked up to form individual lamellar strands (L), all emanating out from the “future core” portion of the forming prolamellar body (PB). ×81,000.
Fig. 3. An oblique section showing an etioplast of 6-day-old, dark-grown seedling. The tubular nature of the stroma lamellar (L) is evident (arrows). Many of the lamellae assume an ordered orientation and thereby give a hexagonal, crystalline lattice formation to the crystalline core portion of the prolamellar body (PB). Some of the tubules of the more peripheral lamellae appear to be in very close proximity to the inner envelope membrane of the etioplast (IN). ×109,000.
Fig. 4. An 8-day-old etioplast showing a very dense core area (arrows) of the prolamellar body (PB). Several thylakoids (TH) emanate outward from the core portion into the surrounding stroma (S). Osmiophilic globules (OG) are also present near the core portion of the prolamellar body (PB). Some lamellae (L) are in close proximity to the inner envelope membrane (IN) along a portion of its length (arrows). ×112,000.
Fig. 5–6. A fully formed crystalline portion of an 8-day-old etioplast of a dark-grown seedling. The crystalline portion of the prolamellar body (PB) has a hexagonal, crystal lattice type of orientation with an interconnecting tubular arrangement. Several thylakoids (TH) emanate out from the crystalline portion of the prolamellar body into the stroma. Individual tubules (T) are also present. ×53,000.
Results

The stroma of the etioplast (Fig. 1) is thickly populated with tubules which appear to be aggregating (arrows). Some of the tubules have started to coalesce into tube-like strands.

The etioplast in Fig. 2 shows a more extensive mobilization of the tubules within the stroma prior to the formation of a “core area” towards the middle portion of the stroma. Emanating outward from the “core area” are individual tube-like strands formed from the linking up of individual tubules.

An oblique tissue section (Fig. 3) shows the tubular nature of the aggregate vesicles that partake in the formation of the core portion of the prolamellar body (arrows). The lengthy tubules appear to be of considerable length and assume tube-like interconnections as the formation of the hexagonal crystalline core progresses.

The etioplast (Fig. 4) shows a prolamellar body (PB) with a very dense core, with thylakoid membranes (TH) branching out into the periphery of the stroma. Osmiophilic globules (OG) are also present within the stroma near the thylakoid membranes. A portion of the thylakoid membrane is in close proximity to the inner etioplast membrane (arrows).

The prolamellar body has assumed a hexagonal, crystal lattice appearance in the core area (Figs. 5–7). Several thylakoid membranes (TH) are connected to the membranes of the prolamellar body and emanate out into the periphery of the stroma.

Discussion

Structural models for the crystalline portion of the prolamellar body have been proposed by several investigators (von Wettstein 1958, Menke 1962, Gunning 1965, Wehrmeyer 1965a, b, c, Brown and Gunning 1966, Gunning and Jagoe 1967, Lang and Rae 1967, Ikeda 1968, Weier and Brown 1970). Gunning (1965) proposed a cubic model of lattice structure, whereas Ikeda (1968) suggested a hexagonal model for the crystalline lattice structure of the prolamellar body. Weier and Brown (1970), in a study of Phaseolus etioplasts, proposed a model structure that is in essential agreement with that of Wehrmeyer (1965a, b, c) and virtually identical to that of Ikeda (1968) in that they both suggest the following: a) the involvement of tubules of equal length comprising the core of the crystalline prolamellar body; and b) the basic crystalline structural unit has hexagonal (six-side star) character, thereby allowing for four separate tubules to unite at each of the respective nodes.

It is important to acknowledge that some of the ideas of prolamellar body development heretofore mentioned have recently become modified in terms of developmental biogenesis (Weier and Brown 1970, Wellburn and Wellburn 1971, Wellburn and Wellburn 1973, Robertson and Laetsch 1974, Rascio 1976, and Leech, Rumsby, Thomson, Crosby and Wood 1972).

In terms of the ultimate derivation of the fundamental structural elements
Fig. 7. An 8-day-old etioplast showing two crystalline areas of prolamellar body (PB) development, with thylakoids (TH) lying in the stroma (S) between them. Individual tubules (T) and tubular lamellae (L) are in close proximity to the inner envelope membrane (IN). $\times 61,000.$
that give rise to the prolamellar body, (von Wettstein 1958, 1967), the present study does not implicate the inner envelope membrane as being the site of origin of discrete vesicles which later aggregate to form the tubular network that subsequently becomes the prolamellar body. Other workers have proposed an alternative model wherein the peripheral membranes of the prolamellar body are derived from lamellar sheets rather than originating as discrete vesicles from the inner envelope membrane (Weier and Brown 1970). The present investigation is more in agreement with this latter concept (Figs. 1–7).

The etioplast stroma has a number of tubules prior to the formation of a “core” or crystalline portion of the prolamellar body (Figs. 1–2).

Oblique sections of etioplasts indicate that the tubular network comprising the crystalline portion of the prolamellar body core appears to be formed by the aggregation of tubular membranes (Fig. 3).

The crystalline portion of the prolamellar bodies in this study show an ordered hexagonal arrangement of a lattice-type interconnecting tubular structure (Figs. 3–7).

To summarize, dark-grown apical pea leaf etioplasts undergo several, discrete, ultrastructural alterations within the stroma of the etioplast during an 8 hr dark-exposure period (Figs. 1–7). The developing prolamellar body tubules aggregate to form the paracrystalline prolamellar body (Figs. 1–7). Thus, the prolamellar paracrystalline body appears to be composed of perforated sheets which assume a more reticulate appearance when sectioned.

Summary

The development of the prolamellar body in young (2–8-day-old) apical leaf etioplasts of dark-grown pea seedlings (Pisum sativum L. var. ‘Alaska’) has been examined at the ultrastructural level. The early stages of prolamellar body biogenesis involves the formation of discrete stroma tubules. Subsequently (8 days), these tubules aggregate into tubular strands which move centrally, serving as orientation points for the later development of the lattice-tubule-framework that ultimately develops in the stroma. The developing crystalline portion slowly assumes a hexagonal arrangement and ultimately becomes the “crystalline core” of the prolamellar body. Thylakoid membranes emanate out from the crystalline portion of the prolamellar body into the peripheral areas of the etioplast stroma. The relationship of prolamellar body development in pea tissue is discussed with respect to similar organelles described at the fine structural level in etioplasts of other plant tissues.

References


