Fine structure of lipotubuloids (elaioplasts) in *Malva neglecta* and *Althaea rosea*

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(Received: November 16, 1970.)

Abstract:

The elaioplasts in the genus *Malva*, like those of *Ornithogalum umbellatum* are lipotubuloids, that means that they are aggregations of osmiophilic granules surrounded by a network of microtubules which in the lipotubuloids of *Malva*, are more labile than those of *Ornithogalum*.

INTRODUCTION

Observations in the light microscope (Wąlek-Czernecka, Kwiatkowska, 1961) demonstrated that the elaioplasts in *Malva*, like the lipotubuloids of *Ornithogalum umbellatum* (Kwiatkowska 1971 a), are agglomerations of osmiophilic granules. Also as regards chemical composition, the elaioplasts in *Malva* resemble *Ornithogalum* lipotubuloids. They contain neutral lipids (triglycerides), phospholipids and protein substances which may be revealed both without lipid extraction or after their removal by means of pyridine (Wąlek-Czernecka, Kwiatkowska 1961). In the elaioplasts of *Malva neglecta*, hydrolytic enzyme activity characteristic of spherosomes (cf. Wąlek-Czernecka 1965) has also been demonstrated (Kwiatkowska, Stęplewski 1966).

The here described results of observation in the electron microscope confirm the preceding conclusion that the elaioplasts in *Malva* are not plastides, neither are they genetically related to the latter.

The osmiophilic granules aggregated in elaioplasts in the root cells of *Malva* are surrounded by microtubules similarly as are the osmiophilic granules in the lipotubuloids of *Ornithogalum*. One may, therefore assume that the agglomerations of granules in *Malva* and *Ornithogalum* represent the same category of bodies for which the term lipotubuloids was introduced (Kwiatkowska, 1971 a, b).

MATERIAL AND METHODS

Cells of the apical zone of roots of *Althaea rosea* and *Malva neglecta* served as material for the investigations. There were no noticeable cytological differences between the two species, therefore they will be further discussed jointly. The material was fixed in OsO_4 after Palade, in glutaraldehyde with secondary fixation in OsO_4 after Sabatini et al. and also in 2 per cent potassium permanganate. The sections were dehydrated in a series of alcohols and embedded in methacrylate or in epon. The sections were prepared on a Freiburger Präzisionsmechanik or on a Russian microtome. They were stained with uranyl acetate and lead nitrate. The observations were made under Tesla BS 242, UMW 100 and JEM-5 microscopes.

RESULTS OF OBSERVATION

In the cells of *M. neglecta* and *A. rosea* roots, there occur various stages of lipotubuloid formation, from small irregular accumulations of osmiophilic granules up to large almost spherical bodies characteristic for hypocotyl cells and flower organs (Wałek-Czernecka, Kwiatkowska, 1961). The size of the osmiophilic granules forming the aggregations varies from 0.04 to 1.0 μ . Single osmiophilic granules of identical appearance and size may also be seen in the cell (Figs. 3, 5, 6). The granule agglomerates are rather compact, sometimes looser. They are generally localised near the nucleus, and sometimes in its deep concavity (Figs. 1, 2, 3) as are the juvenile stages of *Ornithogalum* lipotubuloids. In the highly vacuolised cells, the aggregates occur at a certain distance from the nucleus (Fig. 4) between the vacuoles.

The osmiophilic granules of the root cortex cells with a light cytoplasm (Figs. 5 and 6) and those of the central cylinder with very dark cytoplasm (Figs 1 and 2) are similar in appearance. In the material fixed with glutaraldehyde they are homogeneous, electron-dense and spherical. After the use of other fixatives, e.g. Palade's fixative, the osmiophilic granules behave somewhat differently. Their contents are also dark and homogeneous, and their shape on the cross section is irregularly spherical or polygonal (Fig. 7). The contour of some of them is sharply delineated, and of others completely obliterated.

On preparations fixed with potassium permanganate (embedded in methacrylate or epon), the lipotubuloids of *Malva* undergo complete destruction. Of the osmiophilic granular aggregations, there remain only fragments of the torn highly contrasting, covers, forming a reticulation against the light background (cf. Fig. 8 with Figs. 7 and 3). Similar fragments of these covers of high electron density are visible in the cytoplasm beside the lipotubuloids (Fig. 8, bodies denoted by L). In the author's opinion, they are remains of destroyed (single or several) osmiophilic granules. The bodies surrounded with a contrasting cover sometimes adjoin to the endoplasmic reticulum forming its prolongation. The cell organelles beyond the lipotubuloid range, and the endoplasmic reticulum membranes are relatively well preserved in the way typical for this fixative.

Figs. 1 and 2. *A. rosea* — cells of root fixed in glutaraldehyde and OsO_4 (embedded in epon). Aggregations of osmophilic granules localised close to the nucleus.

Notations: Lt — lipotubuloid (aggregations of osmiophilic granules), OG — osmiophilic granules, M — mitochondria, P — plastids with starch grains, V — vacuoles, mn — nuclear envelope (in fig. 2 partly cut tangentially) ER — endoplasmic reticulum. Micrographs taken with JEM-5

Figs. 3 and 4. *A. rosea* — lipotubuloids surrounded by a number of rough reticulum membranes. Ground cytoplasm within lipotubuloid is lighter. In fig. 3 reticulum with enlarged canals (ERI). Fixation and notations as in figs 1 and 2 (JEM-5)

Figs. 5 and 6. *A. rosea* — osmiophilic granules from root cortex zone. Notations as above (JEM-5).

Figs. 7 and 8. *A. rosea*—cell fragments with lipotubuloids close to nucleus. 7—fixed after Palade, 8—fixed in 2% KMnO₄. The sections were embedded in methacrylate.

Photographed in UMW 100 and Tesla BS 242 (fig. 8). L—deformed bodies of the type of lipid droplets, S—bodies resembling spherosomes, other notations as in preceding captions)

Figs. 9 and 10. *A. rosea*. Two fragments of root cells fixed in KMnO_4 (methacrylate). Spherosome close to lipotubuloid (fig. 9) and within it (fig. 10). Mitochondria contacting lipotubuloid (Tesla BS 242)

Figs. 11, 12 and 13. *A. rosea* — in fig. 11 dictyosome close to osmiophilic granules (glutaraldehyde and OsO_4 , epon). JEM-5. Figs 12 and 13 — dictyosomes arranged parallelly to lipotubuloid surface. Fixed in 2% KMnO_4 (methacrylate). UMW 100. In fig. 12 — mitochondria inside lipotubuloid

Figs. 14 and 15. *A. rosea* — osmiophilic granules from root cells surrounded with microtubules (mt). Fixed in glutaraldehyde and OsO₄ (methacrylate). JEM-5

Sometimes on the periphery of the deformed lipotubuloids or in their vicinity, and sometimes within them (Figs. 9 and 10), bodies surrounded by a weakly contrasting membrane may be seen, filled with fine-grained contents. Similar spherical or oval bodies occur in various places within the cell (e.g. Fig. 8). They frequently adhere to the endoplasmic reticulum membrane (Fig. 9). These bodies are somewhat smaller than the mitochondria (ca. $0.2\text{--}0.4\ \mu$) and less numerous than the latter. No intermediate forms between the bodies undergoing destruction in potassium permanganate and the spherical structures described could be found. In OsO_4 -fixed preparations, it is also difficult, as in *Ornithogalum*, to find the counterpart of these spherical bodies distinguishable after KMnO_4 fixation. The somewhat oval or elliptic bodies filled with contrasting granular contents resemble somewhat the spherical bodies, their number, however, does not correspond to that of the bodies surrounded with a single membrane and preserved in potassium permanganate. They were found very rarely after using Palade's fixative (fig. 7 S), and were completely absent on preparations fixed with glutaraldehyde.

In cells fixed with glutaraldehyde and osmium tetroxide there occur around the osmiophilic granules minute contrasting amorphous bodies adhering to the outer membrane of the granules. These bodies are not found in material fixed after Palade and in 2 per cent KMnO_4 . These bodies may be found around both single osmiophilic granules and those aggregated in a lipotubuloid (Figs 1-6). On some photographs one can see that the osmiophilic granules are surrounded with rings or fragments of short tubules with highly contrasting walls, ca. $200\ \text{\AA}$ in diameter (figs 14 and 15). By analogy to the picture found in the lipotubuloids of *Ornithogalum*, the conclusion may be advanced that we are dealing here with microtubules preserved to a various extent, which are more labile than those of *Ornithogalum*.

In the close vicinity of the lipotubuloids, parallel to their periphery profiles of the endoplasmic reticulum are seen in all fixed preparations (cf. Figs. 3, 4, 7 and 8). They are most numerous after fixing in glutaraldehyde. The osmium fixative and glutaraldehyde visualise the ribosomes on their surface. The reticular membranes frequently surrounded the lipotubuloid on one or all sides. If the lipotubuloid is close to the nucleus, it is surrounded on one side by the nuclear membrane caving into the nucleus, and on the other by parallel reticulum membranes (Fig. 3). In this way the lipotubuloid is partly separated from the remaining area of the cell. This isolation is not, however, complete, in many places there are gaps between the membranes, and sometimes the latter are absent around the granular aggregations.

In highly vacuolised cells in which the lipotubuloids are distant from the nucleus and situated in the vicinity of the vacuoles they are surrounded at least on one side with granular reticulum (Fig. 4).

It is characteristic that, after fixation in glutaraldehyde, which preserves well the ground cytoplasm, the cytoplasm within the aggregate of osmiophilic granules is lighter and less granular than in the remaining regions (Figs. 3 and 4). This is observed both within the large and minute agglomerations (Fig. 1). Between some of the agglomerations of osmiophilic granules, reticulum membranes are visible

with greatly enlarged canals (Fig. 3). The lumen of these canals seems lighter than the remaining part of the cells.

Close to the lipotubuloids, and sometimes inside them also, dictyosomes and mitochondria may be found. The latter adhere sometimes to the osmiophilic granules (Fig. 12). The mitochondria are oval in shape or elongated, and characterised by numerous cristae and a dense matrix in the material fixed with glutaraldehyde or OsO_4 , whereas in KMnO_4 the matrix is not preserved and the cristae are less developed.

The Golgi apparatus is generally visible on the periphery of the lipotubuloids. They are situated either with cisterns parallel to their surface or with one of their edges parallel, from which vesicles are seen to detach (Figs 12 and 13). On the lipotubuloid periphery there are sometimes several (3 to 8) dictyosomes which may lie very close to one another. They also occur in the close vicinity of single osmiophilic granules (Fig. 11).

DISCUSSION

Malva lipotubuloids exhibit a number of essential features common with those of the *Ornithogalum* lipotubuloids, they have, however, certain specific traits.

The osmiophilic granules forming lipotubuloids in *Malva* represent — as shown by the cell pattern obtained after fixing in KMnO_4 — two categories. One, of bodies surrounded by a single membrane which preserve their spherical shape and partly their finegrained contents, the other in which they undergo destruction leaving only the contrasting cover. Of these two categories, the first bodies seem rather to correspond to spherosomes, whereas the others have the appearance of lipid droplets which are poorly fixed in KMnO_4 . On the OsO_4 preparations the former cannot be distinguished from the latter. Therefore I have reserved the name osmiophilic granules for designating the bodies filled with homogeneous electron-dense contents and surrounded with a single membrane seen in the OsO_4 -fixed cells similarly as in the *Ornithogalum* cells.

The nonhomogeneous character of the osmiophilic granules was revealed under the influence of KMnO_4 also in the epidermal cells of the *Ornithogalum* ovary. The degree of deformation of the granules of lipid droplets type was not the same in the genera discussed. In *Ornithogalum* they are shapeless, irregularly stellate, they have a highly contrasting edge and a light, sometimes grey interior. On the other hand, the granules of lipid droplets type are much more injured in *Malva* when fixed in KMnO_4 . The only thing that remains of them are fragments of the contrasting cover which is thicker than the plasmatic membrane. The site once occupied by the lipotubuloid is filled with a tangle of these elements. It is supposed that the differences in the behaviour of the osmiophilic granules of *Ornithogalum* and *Malva* lipotubuloids are due to the different chemical properties of the lipids they contain. Large quantities of phospholipids are characteristic for *Ornithogalum*, they occur in smaller amounts in *Malva* (there are distinct differences in the staining of lipotubuloids in Barker's hematein test). In the lipotubuloids of *Malva* neutral lipids

prevail. They are characterised by an affinity towards OsO_4 , in the vapours of which the lipotubuloids and the entire fixed part of the root turn black within 1–2 seconds, this indicating a large number of unsaturated bonds. The lipotubuloids of *Ornithogalum*, on the contrary, take on a brown hue in OsO_4 vapours.

The lability of the osmiophilic granules in *Malva*, their tendency to coalescence and deformation, were noticeable also in *intra vitam* observations of the cells. Labile proved also to be the microtubules in *Malva* which were preserved only in few ultrathin sections of cells fixed in glutaraldehyde, whereas in *Ornithogalum*, the microtubules were well visible even when Palade's fixative was used.

The arrangement of a strongly developed system of membranes of the endoplasmic reticulum around the lipotubuloids is rather characteristic for the root cells in *Malva*, perhaps this is due to a different development stage of these cells. In *Ornithogalum* this kind of protection was very exceptional.

The presence of Golgi apparatus and mitochondria on the periphery of the lipotubuloids, and sometimes within them is characteristic both for the lipotubuloids in *Malva* and in *Ornithogalum* as well as for the elaioplasts of *Haemanthus albiflos* described by Tourte (1964, 1966). The existence of a topographical relation between the Golgi apparatus and the lipotubuloids in the root cells of *Malva* was indicated by the results of earlier cytochemical investigations (Kwiatkowska and Stęplewski 1966). It was, namely, found that at the point where the lipotubuloids are localised, there occur elements exhibiting thiamine pyrophosphatase activity, an enzyme known as marker of the Golgi apparatus (Poux 1967; Dauwalder et al. 1969). Structures similar to those which gave a positive reaction in the test to TPP-ase also revealed the presence of alkaline phosphatase which is also found in the Golgi apparatus, (to quote Novikoff et al. 1961; Dominas et al. 1963). Similar bodies could also be revealed by the method of silver impregnation of Da Fano (Kwiatkowska and Stęplewski 1966).

Confrontation of the results of electron microscope observations with the picture of TPP-ase localisation in the light microscope seems at first glance to indicate that they do not strictly correspond to one another. On many electron micrographs no Golgi structures can be seen close to the lipotubuloids or within them. If they do appear, their dimensions are so small that they could not give a reaction as strong as that obtained in the test for TPP-ase. The difference in special distribution of the structures seen as a whole in an uncut cell and in the appearance of the same lipotubuloids on ultrathin sections, should, however, be borne in mind. In the intact cell, the dictyosomes arranged parallelly to the lipotubuloid surface will occupy a much wider space than on the ultrathin section of the same structure.

It should also be remembered that the results of the reaction for TPP-ase varied widely (Kwiatkowska and Stęplewski 1966). In many cells TPP-ase occurred in minute elongated or granular elements dispersed within the cell, and sometimes aggregated around the nucleus. In some cells the localisation of this enzyme was identical with that of the osmiophilic aggregations. It is, therefore, probable that a periodical translocation of the Golgi apparatus closer to the lipotubuloid takes place here. There is more chance of visualizing this phenomenon in the light microscope

where we dispose of a higher number of cells visible as a whole than in the ultrathin sections which allow us to see only a small fragment of the cell. What are the relations between the Golgi structures and the lipotubuloids is an open question. Investigations on the incorporation of labelled palmitate and glycerol in animal cells (Stein and Stein 1966, 1967) suggest that the Golgi apparatus takes part in lipid synthesis and is one of the earliest sites of incorporation of the lipid precursors, before they become localised in lipid droplets. Other studies on animal material indicate, on the contrary, that the Golgi apparatus contributes to lipid break-down. Numerous Golgi structures appear, namely, in the process of absorption and digestion of fat in food (Adamstone 1959; Palley and Karlin et al. 1959). A similar role might be ascribed to the Golgi structures in connection with the appearance in them of the enzyme capable of hydrolysing lipids, i.e. lipase (Seligman et al. 1966 — in animal material, Olszewska et al., 1966 — in plant cells).

SUMMARY

The initial stages of elaioplasts in *Malva*, occurring in the apical part of the root were investigated. It was found that elaioplasts are not plastides, but structures of the same kind as the lipotubuloids of *Ornithogalum umbellatum*. Therefore, the elaioplasts of *Malva* are also considered as lipotubuloids.

When seen in the electron microscope these bodies look like an aggregation of osmiophilic granules. The aggregations are usually surrounded partly by a number of parallel membranes of rough endoplasmic reticulum.

The osmiophilic granules after fixation of the section in OsO_4 , are surrounded by a membrane and filled with electron-dense homogeneous contents. After fixing the material in KMnO_4 , most of the osmiophilic granules of the lipotubuloid are destroyed, there only remain the torn contrasting covers. Some of the osmiophilic granules are preserved in the form of bodies with morphological characters corresponding to spherosomes. They are surrounded by a single membrane and filled with fine-granular contents. These structures occur on the periphery of the lipotubuloids or within them, and also in the cytoplasm beyond the lipotubuloids.

The microtubules around the osmiophilic granules in *Malva*, aggregated in lipotubuloids, or dispersed in the cytoplasm are only visible in some of the preparations fixed in glutaraldehyde and OsO_4 . On most of the electron micrographs of cells the microtubules are destroyed or deformed.

Mitochondria and Golgi apparatus occur most frequently around the lipotubuloids, and seldom within them.

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Lipotubuloidy (elajoplasty) Malva neglecta i Althea rosea

Streszczenie

Zbadano inicjalne stadia elajoplastów malw występujące w wierzchołkowej części korzeni. Stwierdzono, że elajoplasty te nie są plastydami, lecz strukturami tego samego rodzaju, co lipotubuloidy *Ornithogalum umbellatum*. W związku z tym elajoplasty malw uznano również za lipotubuloidy.

Lipotubuloidy malw oglądane w mikroskopie elektronowym przedstawiają się jako skupienia osmoofilnych granul. Skupienia te zwykle objęte są częściowo szeregiem równoległych membran szorstkiego retikulum endoplazmatycznego.

Osmofilne granule po utrwaleniu obiektu OsO_4 otoczone są membraną i wypełnione gęstą dla elektronów, homogenną treścią. Po utrwaleniu materiału KMnO_4 większość osmoofilnych granul lipotubuloidu niszczy się, zostają z nich jedynie porożrywane, kontrastowe powłoki. Niektóre osmoofilne granule zachowują się w postaci ciał, których cechy morfologiczne odpowiadają sferosomom. Otacza je pojedyncza membrana i wypełnia drobnoziarnista treść. Struktury te występują na peryferiach lipotubuloidów lub w ich obrębie, a także w cytoplazmie poza lipotubuloidem.

Mikrotubule, widoczne wokół osmoofilnych granul malwy skupionych w lipotubuloidy bądź rozproszonych w cytoplazmie, widać tylko w niektórych preparatach. Na większości fotografii z komórek utrwalanych aldehydem glutarowym i OsO_4 mikrotubule są zniszczone lub zdeformowane.

Mitochondria i struktury Golgiego występują najczęściej wokół lipotubuloidów, rzadko zaś na ich terenie.