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Possible Nature of an Electron Dense Substance in the Thylakoid Lumen of Chloroplasts

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Abstract

The fixation of leaves of *Tanacetum vulgare* L. in glutaraldehyde makes it possible to isolate chloroplasts without sacrificing an electron dense substance in the thylakoid lumen. The extraction of lipids from the chloroplasts isolated from the leaves preliminarily fixed in glutaraldehyde has demonstrated that glycerolipids (galactolipids and phospholipids) are not manifested in TLC, whereas isoprenoid lipids (chlorophyll, carotenoids) are manifested. Presumably, isoprenoid lipids are not fixed with glutaraldehyde and are extracted from the thylakoid membrane. The ultrastructural control demonstrates that the electron dense substance from the thylakoid lumen is also extracted. It is possible that this substance is of isoprenoid nature.

Keywords

Tanacetum vulgare L., Intrathylakoid Electron Dense Substance, Glutar Chloroplasts, Ultrastructure, TLC of Lipids

1. Introduction

Thylakoids, photosynthesizing membranes of chloroplasts, are flattened closed membrane structures with an inner space, the lumen. The thylakoid lumen can contain an electron dense substance (ED substance), which has been recorded for numerous species of cultivated and wild plants [1]-[8].

Personal observations of various types of wild plants have demonstrated that ED inclusions in the thylakoid lumen with varying degrees of occupancy are typical for the chloroplasts of plants under field conditions. One cell can contain the chloroplasts both with impacted thylakoids with no inclusions and with the thylakoids filled with an ED substance to a very large extent [9]. The ED substance is formed in the thylakoid lumen, then enters the chloroplast matrix and after that, the cytoplasm [6] [10].

Such a prevalence, probably total, of a substance in the inner space of thylakoids in wild flora requires its nature to be determined. The purpose of the work is to discover the nature of a yet unidentified ED substance localized in the thylakoid lumen.

2. Materials and Methods

The work was conducted in quadruplicate using upper leaves and isolated chloroplasts of a wild *Tanacetum vulgare* L. (Asteraceae) plant at the stage of flower bud formation.

The pieces of leaves were fixed in 2% glutaraldehyde dissolved in 30 mM phosphate buffer (pH 7.2) with postfixation in 1% OsO_4 solution or without it.

Chloroplasts were isolated both from fresh leaves (fresh chloroplasts) and from the leaves preliminarily fixed in 2% glutaraldehyde dissolved in phosphate buffer during 2 - 24 h (glutar chloroplasts), as described in the work of [6].

Fresh chloroplast sediment was fixed in 2% glutaraldehyde solution. The fresh and glutar chloroplasts were additionally fixed in 1% of OsO_4 or left without osmium plating. The fixed material was dehydrated in alcohols and acetone and then embedded in the Epon 812 epoxy resin (Fluka, Germany).

Ultrathin sections were contrasted in saturated aqueous solution of uranyl acetate (Sewa, Czech Republic) and 0.25% solution of lead citrate (British Drug Houses, England) and were examined with an electron microscope (Jeol, Japan).

Lipids were extracted using a chloroform/methanol (1:2, v/v) solution [11] from the isolated chloroplasts, which were obtained both from fresh leaves and from those preliminary fixed in glutaraldehyde.

Qualitative analysis of lipids was carried out using thin layer chromatography (TLC) on silica gel (Merk, Germany) in a chloroform-methanol-water system (65:25:4). The chromatogram was developed in 50% sulfuric acid with subsequent heating to 120˚C.

Classes of lipids were determined using the standards [12].

3. Results

In the leaves of tansy at the stage of flower bud formation, an electron dense substance in the thylakoid lumen was always present in mesophyll chloroplasts (**Figure 1(a)** and **Figure 1(c)**).

Figure 1 presents regions of mesophyll cells and demonstrates that the thylakoid lumen is filled with the ED substance that is retained upon both glutar-osmic fixation (**Figure 1(a)** and **Figure 1(c)**) and glutar fixation without osmium plating (**Figure 1(b)** and **Figure 1(d)**).

Numerous plastoglobules that are electron dense upon glutar-osmic fixation appear electron transparent upon purely glutar fixation, presumably due to extraction in a series of alcohols and acetone.

Upon the isolation of chloroplasts from fresh leaves, they lose not only their membranes and stromas, but also ED inclusions in the thylakoid lumen (**Figure 2(a)** and **Figure 2(c)**).

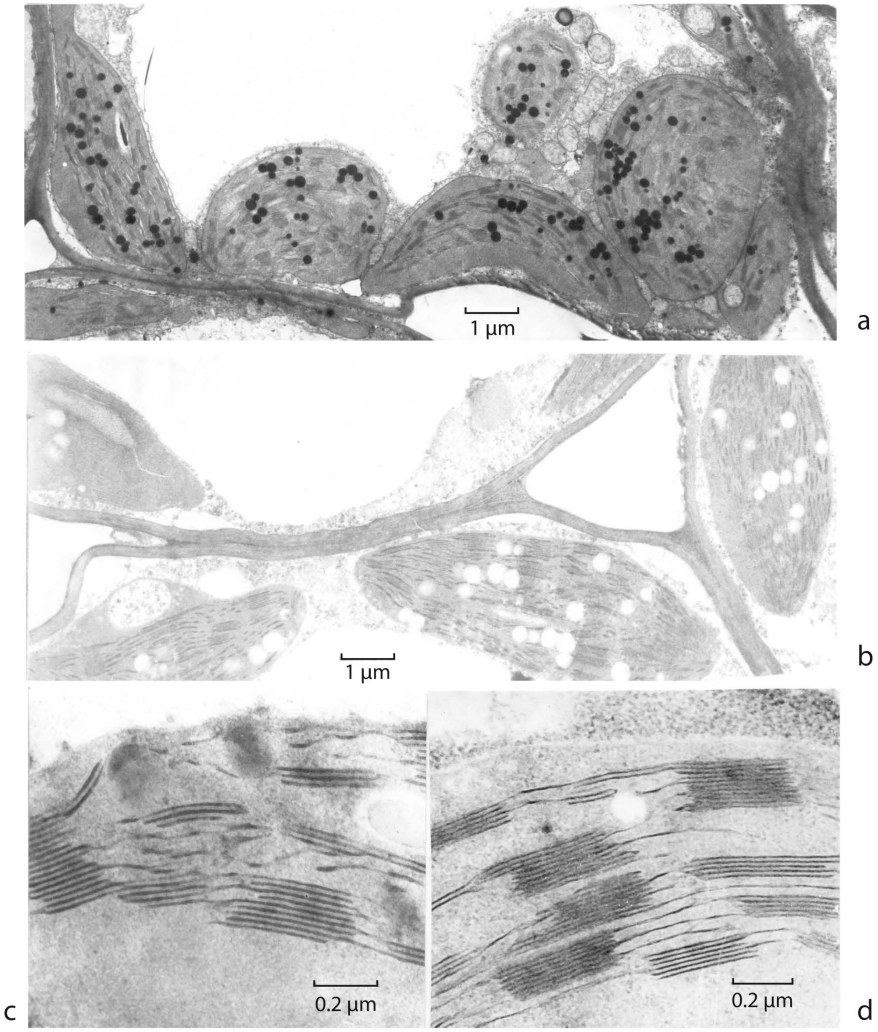

Figure 1. The ED substance retains in the thylakoid lumen. (a) Chloroplasts in the tansy leaves fixed with glutaraldehyde with subsequent osmium plating (small zoom); (b) Chloroplasts in the tansy leaves fixed with glutaraldehyde without osmium plating (small zoom); (c) Chloroplasts in the tansy leaves fixed with glutaraldehyde with subsequent osmium plating (large zoom); (d) Chloroplasts in the tansy leaves fixed with glutaraldehyde without osmium plating (large zoom).

Upon the isolation of chloroplasts from the leaves preliminarily fixed in glutaraldehyde (glutar chloroplasts), the ED substance retains in the thylakoid lumen along with the chloroplast matrix (**Figure 2(b)** and **Figure 2(d)**).

Extraction of lipids from fresh chloroplasts and from glutar ones has shown different pictures.

From fresh chloroplasts, all membrane lipids are extracted, both glycerolipids and isoprenoids (**Figure 3**).

From glutar chloroplasts, glycerolipids are not extracted. Presumably, they bind tightly with membrane proteins. But both isoprenoids (**Figure 3**) and the ED substance from thylakoids are extracted (**Figure 4**). Thylakoids in grana are impacted and have no ED substance.

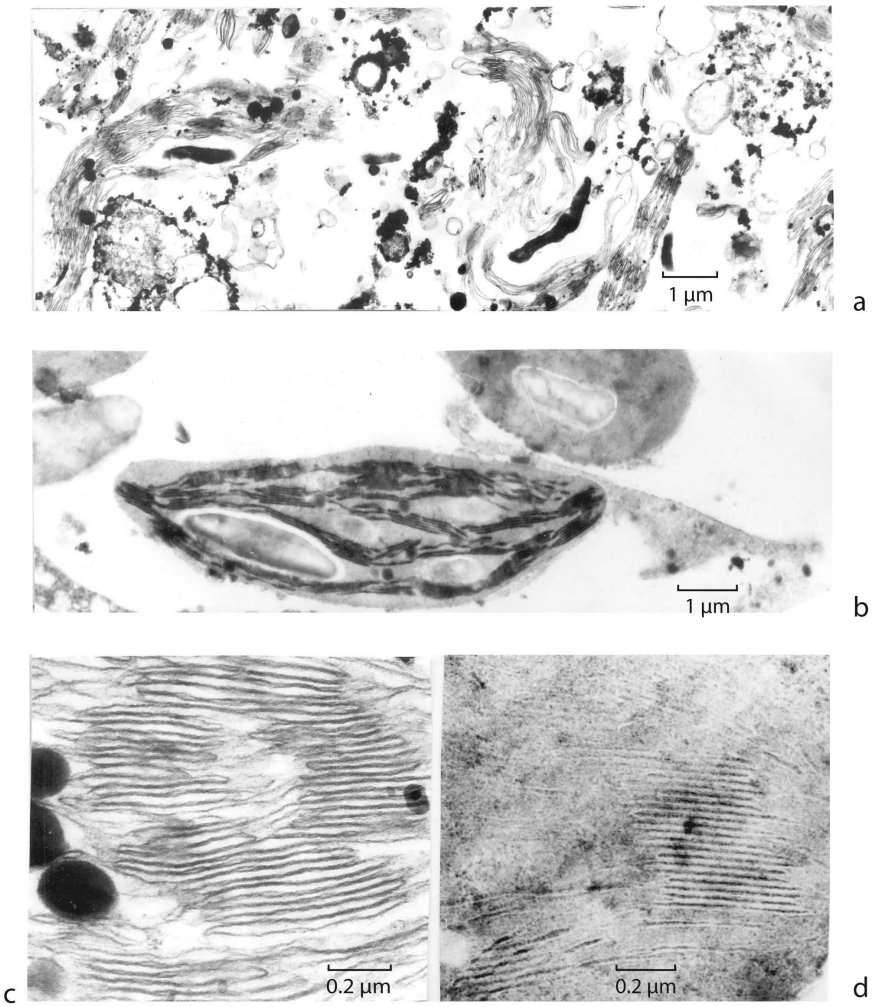

Figure 2. Contents ED substance in the thylakoid lumen. (a) Chloroplasts isolated from fresh tansy leaves (small zoom); (b) Chloroplasts isolated from the leaves previously fixed with glutaraldehyde (small zoom); (c) Chloroplasts isolated from fresh tansy leaves (large zoom); (d) Chloroplasts isolated from the leaves previously fixed with glutaraldehyde (large zoom). Thylakoid lumens are electron transparent and do not contain the ED substance upon isolation from fresh leaves ((a) and (c)). Glutar chloroplasts retain their form and the ED substance in the thylakoid lumen ((b) and (d)).

4. Discussion

The plants that are grown under conditions of stable temperature and lighting have an electron transparent thylakoid lumen. And the plants that grow in the wild with temperature and lighting fluctuations very often have the ED substance in the thylakoid lumen, which was proved for the members of a wide variety of plant families by personal observations.

Additionally, the presence of the ED substance in the thylakoid lumen of the plants growing under field conditions can depend both on the age of a plant and on the time of day. The ED substance comes out of the lumen and enters the chloroplast matrix in the form of ED globules and then the cytoplasm in the form of ED aggregates [6] [10] [13].

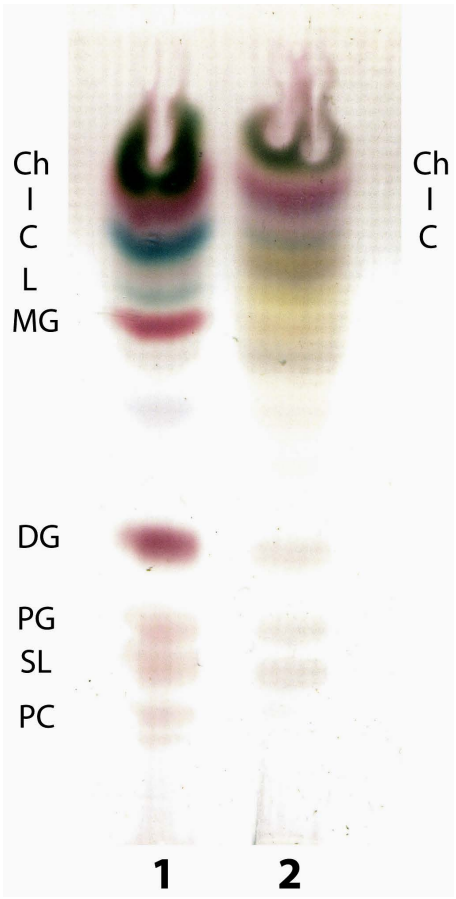

Figure 3. TLC of tansy lipids extracted from fresh chloroplasts (1) and from glutar chloroplasts (2). (Ch) chlorophyll; (C) carotene; (L) lutein; (MG) monogalactosyldiglyceride; (DG) digalactosyldiglyceride; (PG) phosphoglycerol; (SL) sulfolipid; (PC) phosphatidylcholine; (I) possible isoprene.

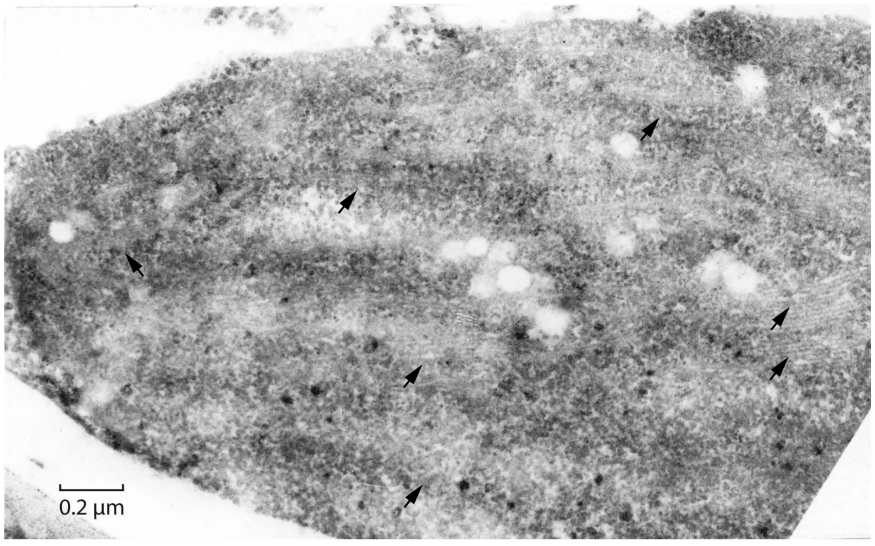

Figure 4. Chloroplasts isolated from the leaves previously fixed in glutaraldehyde and then extracted with chloroform-methanol. Thylakoids in grana are impacted and do not contain the ED substance (arrows).

In the plants growing under strong lighting, isoprenoid lipids (carotenoids, α-tocopherol, and plastoquinone-9) accumulate in chloroplasts in significant numbers in the form of ED globules [14].

In the cells of *Stevia rebaudiana*, steviol glycoside, which is isoprenoid, is synthesized; the chloroplast thylakoids contain ED inclusions, and the chloroplast matrix contains ED globules [8].

A series of cytochemical reactions performed in order to determine the nature of the ED substance in the thylakoid lumen demonstrated that it is not protein, not lipid, not polysaccharide [15]. An attempt to identify the nature of the ED substance in the thylakoids of *Stevia rebaudiana* was made in the work of [16] using the method of gas chromatography-mass spectrometry. The authors claimed that the ED substance is triacylglycerol. The authors isolated chloroplasts from fresh leaves, while in the work of [6] it was demonstrated that upon the extraction from fresh leaves, the ED substance leaves the thylakoid lumen for the isolation medium. As far as no structural control was presented in the work of [16], it is doubtful that this substance is triacylglycerol. As shown above in this work, extraction of lipids from fresh and glutar chloroplasts results in different pictures.

From glutar chloroplasts, only pigments (chlorophyll and carotenoids) are extracted, whereas glycerolipids remain binded and are not manifested in TLC. Simultaneously, the ED substance from the thylakoid lumen is extracted, which was demonstrated by the structural control. Both chlorophyll and carotenoids are derivatives of isoprene.

It can be assumed that the ED substance from the thylakoid lumen is of isoprenoid nature.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Srivastava, L.M. (1966) On the Fine Structure of the Cambium of *Fraxinus americana* L. *Journal of Cell Biology*, **31**, 79-93. https://doi.org/10.1083/jcb.31.1.79

[2] Casadoro, G. and Rascio, V. (1979) Plastid Ultrastructural Features in the Various Tissues of Sunflower Leaves. *Cytobios*, **24**, 157-166.

[3] Rascio, N., Casadoro, C. and Di Chio, L. (1979) Ettoplast-Chloroplast Transformation in Sunflower Cotyledons. *Protoplasma*, **100**, 45-52. https://doi.org/10.1007/BF01276300

[4] Nurmi, A. (1985) Comparison between Thylakoid Composition and Chloroplast Ultrastructure in Developing Plants of *Brassica*, *Helianthus*, *Sisymbrium* and *Tanacetum*. *Journal of Ultrastructure Research*, **92**, 190-200. https://doi.org/10.1016/0889-1605(85)90046-1

[5] Danilova, M.F. and Kashina, T.K. (1999) Strukturnye osnovy aktinoritmicheskoy regulyatsii tsveteniya (Structural Bases of Actinorhythmic Regulation of Flowering). Nauka, Moscow.

[6] Semenova, G.A. (2005) Vnutritilakodnoe electronno-plotnoe veshchestvo v khloroplastakh pizhmy *Tanacetum vulgare* L. (Intrathylakoid Electron Dense Substance in Chloroplasts of Tansy *Tanacetum vulgare* L.). *Cytologia*, **47**, 510-517.

[7] Lee, K.B. (2007) Ultrastructure of Crystalline Inclusions in the Thylakoids of Dodder (*Cuskuta japonica*) Plastids. *Journal of Plant Biology*, **50**, 325-330. https://doi.org/10.1007/BF03030662

[8] Ladygin, V.G., Bondarev, N.I., Semenova, G.A., Smolov, A.A., Reshettnyak, O.V. and Nosov, A.M. (2008) Chloroplast Ultrastructure, Photosynthetic Apparatus Activities and Production of Steviol Glycosides in *Stevia rebaudiana in vivo* and *in vitro*. *Biologia Plantarum*, **52**, 9-16. https://doi.org/10.1007/s10535-008-0002-y

[9] Rozentsvet, O., Nesterov, V., Bogdanova, E., Kosobryukhov, A., Subova, S. and Semenova, G. (2018) Structural and Molecular Strategy of Photosynthetic Apparatus Organisation of Wild Flora Halophytes. *Plant Physiology and Biochemistry*, **129**, 213-220. https://doi.org/10.1016/j.plaphy.2018.06.006

[10] Bogdanova, E., Ivanova, L., Yudina, P., Semenova, G., Nesterov, V. and Rozentsvet, O. (2022) Seasonal Dynamics of Functional Parameters of Winter-Green Steppe Relict *Globularia punctata* Lapeyr. *Flora*, **289**, Article ID: 152037. https://doi.org/10.1016/j.flora.2022.152037

[11] Bligh, E.G. and Dyer, W.J. (1959) A Rapid Method for Total Lipid Extraction and Purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911-917.

[12] Semenova, G. (1999) The Relationship between the Transformation of Thylakoid Acyl Lipids and the Formation of Tubular Lipid Aggregates Visible on Fracture Faces. *Journal of Plant Physiology*, **155**, 669-677. https://doi.org/10.1016/S0176-1617(99)80081-9

[13] Semenova, G.A. (1985) Elektronno-plotnoe veshchestvo v kletkakh mezofilla lista kartofelya (Electron Dense Substance in the Cells of Potato Leaf Mesophyll). *Fiziologya Rastenij*, **32**, 461-464.

[14] Lichtenthaler, H.K. (2013) Plastoglobuli, Thylakoids, Chloroplast Structure and Development of Plastids. In: Biswal, B., Krupinska, K. and Biswal, U.C., Eds., *Plastid Development in Leaves during Growth and Senescence*, Springer, Dordrecht, 337-361. https://doi.org/10.1007/978-94-007-5724-0_15

[15] Van Steveninck, M.E. and Van Steveninck, R.F.M. (1980) Plastids with Densely Staining Thylakoid Contents in *Nymphoides indica*. II. Characterization of Stainable Substance. *Protoplasma*, **103**, 343-360. https://doi.org/10.1007/BF01276961

[16] Bondarev, N.I., Kurilov, D.V., Bondareva, T.A. and Nosov, A.M. (2018) Gliceriltrikaprat v tilakoidakh *Stevia rebaudiana in Vitro* i ego fiziologicheskaya rol' (Glyceryl Tricaprate in Thylakoids of *Stevia rebaudiana in Vitro* and Its Physiological Role). *Izvestiya RAN. Seriya Biologicheskaya*, **1**, 37-41.