

Regulation of Chloroplast Differentiation

*Proceedings of an International Meeting on the Regulation
of Chloroplast Differentiation, held in Rhodes, Greece
July 14–20, 1985*

Editors

George Akoyunoglou

Department of Biology
Nuclear Research Center "Demokritos"
Athens, Greece

Horst Senger

Fachbereich Biologie/Botanik
Philipps Universität
Marburg, Federal Republic of Germany

Alan R. Liss, Inc., New York

Contents

Contributors xiii

Preface

G. Akoyunoglou and H. Senger xxv

Section I. Biosynthesis, Compartmentation, and Transport of Chloroplast Components: Pigments—Lipids

Regulation of Chlorophyll Biosynthesis

Wolfhart Rüdiger 3

Chloroplast Biogenesis 52: Demonstration in Toto of Monovinyl and Divinyl Monocarboxylic Chlorophyll Biosynthetic Routes in Higher Plants

Constantin A. Rebeiz, Baishnab C. Tripathy, Shi-Ming Wu, Ahmad Montazer-Zouhoor, and Edward E. Carey 13

Selected Topics in Chlorophyll Biosynthesis

Dieter Dörnemann 25

Formation of 5-Aminolevulinic Acid Via the C5-Pathway *In Vitro*

Astrid Kah, Dieter Dörnemann, and Horst Senger 35

RNA Is Required for Enzymatic Conversion of Glutamate to δ -Aminolevulinic Acid by Algal Extracts

Jon D. Weinstein, Sandra M. Mayer, and Samuel I. Beale 43

Enzymes for Chlorophyll Synthesis in Developing Peas

Alison G. Smith 49

Properties of the Mg-Protoporphyrin IX Monomethyl Ester (Oxidative) Cyclase System

P.A. Castelfranco, Y.-S. Wong, and J.D. Olechno 55

Affinity Chromatographic Purification of Two Enzymes of the Latter Stages of Chlorophyll Synthesis

Leslie Y.-M. Kwan, Deborah L. Darling, and William R. Richards 57

Chloroplast Culture XI. Involvement of Phytohormones in the Greening of Higher Plants

Henry Daniell and Constantin A. Rebeiz 63

vi / Contents

Photoreduction of Protochlorophyllide and Its Relationship to 5-Amino-laevulinic Acid Synthesis in Leaves of Dark-Grown Barley (<i>Hordeum vulgare</i>)	
Keith Stobart and Ibraheem Ameen-Buckari	71
Formation of Protochlorophyllide Aggregates; Absorbing at 650 nm, Induced by Potassium Chloride	
Anna Widell and Eva Selstam	81
Localization of NADPH-Protochlorophyllide Oxidoreductase in Dark-Grown and Greening Leaves by On-Section Immuno-Marking With Protein A-Gold	
Margareta Ryberg and Katayoon Dehesh	87
Photoactive Protochlorophyllide Oxidoreductase in Prolamellar Bodies of Dark-Grown Pine Seedlings	
Eva Selstam and Anna Widell	93
Light Independent Proteolysis of Protochlorophyllide Reductase	
C.J. Walker and W.T. Griffiths	99
Chlorophyllide <i>b</i> Synthesis in Phenanthroline-Treated <i>Chlamydomonas reinhardtii</i> <i>y</i>-1 in the Dark	
Daniel P. Bednarik and J.Kenneth Hooper.	105
Relation Between Enzymatic Destruction of Magnesium Porphyrins and Chloroplast Development	
Merrill Gassman and Ponnusamy Ramanujam	115
Biosynthesis of Chloroplast Carotenoids	
George Britton	125
Phosphatidylcholine Distribution in Chloroplast Membranes	
Albert-Jean Dorne, Maryse A. Block, Jacques Joyard, and Roland Douce . .	135
Cytochemical Localization of a Lipid-Synthesizing Enzyme in Developing Tobacco Cells	
Judy Brangeon and Arlette Forchioni	141
Lipid Metabolism in Immature Cotyledons of Safflower (<i>Carthamus tinctorius</i>, L.) Exposed to Light	
Gareth Griffiths, Sten Stymne, Alan Beckett, and Keith Stobart	147
Use of HPLC and Partial Sequence Determination for Analysis of the P700 Chl <i>a</i> Apoprotein of PSI. PSI-A1 and PSI-A2 Are Both Present in Maize CPI	
Leonard E. Fish and Lawrence Bogorad	157
Effect of Dichloroacetamide Antidote of Sulfolipid Biosynthesis in Corn	
Elizabeth Blee	163
Section II. Biosynthesis, Compartmentation, and Transport of Chloroplast Components: Proteins	
Compartmentation of Protein Synthesis Within the Chloroplast	
Maurice M. Margulies	171

Biosynthesis of the Chlorophyll-a P₇₀₀ Polypeptide(s) M.M. Margulies, H.L. Tiffany, and T. Hattori	181
Synthesis and Degradation of Thylakoid Polypeptides Petra Gerloff and Michael Wettern	187
Thylakoid Proteins in Seeds and Etiolated Plants of Spinach Brigitte Paproth and Günter Hauska	193
Comparative Serological Studies on Some Proteins of Etioplasts and Chloroplasts of <i>Phaseolus vulgaris</i> var. <i>Commodore</i> L. A. Radunz, G.H. Schmid, M. Bertrand, and E. Dujardin	197
Purification and Properties of Chloroplast and Cytoplasmic Phosphoglycerate Kinase From Barley Eileen M. McMorro and J. William Bradbeer	205
Processing of Precursors of the Chlorophyll a/b Binding Proteins and of the Small Subunit of Ribulose Bisphosphate Carboxylase <i>In Vitro</i> Dawn B. Marks, Barbara J. Keller, and J. Kenneth Hooper.	211
The Chloroplast Envelope Membranes: A Key Structure in Chloroplast Biogenesis Jacques Joyard, Maryse A. Block, Albert-Jean Dorne, Jacques Covès, Bernard Pineau, and Roland Douce	217
Isolation of a Fraction Enriched in Envelope Membranes From Purified Pea (<i>Pisum sativum</i>) Etioplasts Jürgen Soll, Gerhard Wanner, Günter Henkelmann, Ursula Röper, and Michaela Schulze	229
Crosslinking of Envelope Proteins Presumably Involved in the Binding of Nuclear Coded Chloroplast Precursor Proteins Klaus Kloppstech and Annette Bitsch	235
Reconstitution of the Solubilized Envelope Receptors for Nuclear-Coded Precursor Proteins Annette Bitsch and Klaus Kloppstech	241
 Section III. Assembly and Organization of the Photosynthetic Membrane—Development of Function	
Electrophoretic Separation of Chlorophyll-Protein Complexes and Their Apoproteins J. Philip Thornber, Gary F. Peter, Rachel Nechushtai, Parag R. Chitnis, Fiona A. Hunter, and Elaine M. Tobin.	249
Organization of the Light-Harvesting Apparatus During Chloroplast Biogenesis in Wheat Guy J. Bredenkamp, Michael P. Percival, Andrew N. Webber, and Neil R. Baker	259
Pigment and Polypeptide Composition of the Light-Harvesting Complex of the Photosystem I Unit Poulcheria Antonopoulou and George Akoyunoglou	267

The Conditions for the Assembly of Functional PS II Units in Etiolated Leaves F. Franck, C. Sironval, R. Gysembergh, and G.H. Schmid	273
Characterization of Etiochloroplast Membrane Fractions Isolated From Dark-Grown Pine (<i>Pinus jeffreyi</i>) Colette Cahay, Marie-Rose Michel-Wolwertz, and Michel Brouers	283
Photosynthetic Energy Transduction in Protoplasts From Developing Light-Grown Wheat Candida D. Paige and Michael F. Hipkins	291
Changes in the Molecular Organization of Thylakoid Membranes During Ontogenetic Development of <i>Scenedesmus obliquus</i> Karin Krupinska	297
Changes in Activity and Unit-Size of Photosystem II During the Cell Cycle of <i>Scenedesmus obliquus</i> Karin Krupinska and Horst Senger	305
Synthesis and Assembly of Chlorophyll-Protein Complexes and Cytochromes in Isolated Developing Chloroplasts Devaki Bhaya and Paul A. Castelfranco	313
Apoproteins of the Iron-Sulfur Centers A and B in the Photosystem I Primary Electron Acceptor Complex William Ortiz, Julian Bonnerjea, and Richard Malkin	315
The Supramolecular Structure of ATP-Synthases Erhard Mörschel and Martin Bokranz	323
Is Lateral Movement of Pigment-Protein Complexes Required for Grana Unstacking? Joan Argyroudi-Akoyunoglou and George Akoyunoglou	329
Thylakoid Protein Phosphorylation in the Red Algae <i>Porphyridium cruentum</i> Jochen Kirschner and Horst Senger	339
Thylakoid Protein Phosphorylation During the Life Cycle of Synchronized <i>Scenedesmus obliquus</i> Wolfgang Heil and Horst Senger	345
Reversible Inactivation of the LHC II Thylakoid Protein Kinase During Photoinhibition of <i>Chlamydomonas reinhardtii</i> Gadi Schuster, Donna Oksenberg, Andrew L. Staehelin, and Itzhak Ohad	351
Development and Differentiation of the Photosynthetic Prokaryotes: Role of Membrane Growth Initiation Sites in the Development of Photosynthetic Membranes in <i>Rhodospseudomonas sphaeroides</i> Patricia A. Reilly, Joseph Van Houten, and Robert A. Niederman	359

Section IV. Changes in Structure of the Photosynthetic Apparatus During Development

Development of Bioenergetic Function in Light-Grown Seedlings

Alan R. Wellburn, Ioannis Gounaris, John H. Owen, Johanna E.M. Laybourn-Parry, and Florence A.M. Wellburn 371

NADPH-Dependent Prolamellar Body Transformations *In Vitro*

Margareta Ryberg and Christer Sundqvist. 383

Chloroplast Biogenesis 53: Ultrastructural Study of Chloroplast Development During Photoperiodic Greening

Carole C. Rebeiz and Constantin A. Rebeiz 389

General Features of Changes in Ultrastructure and Composition of Chloroplasts During Their Development

Zdeněk Šesták 397

Differentiation of Structure and Function of the Plastid During Greening of the G-2 Mutant of *Chlorella fusca*

Elisabeth Przibilla and Gottfried Galling 407

Interactions Between the Nucleus and Cytoplasmic Organelles During the Cell Cycle of *Euglena gracilis* in Synchronized Cultures. III

Tomoko Ehara, Shuji Sumida, Tetsuaki Osafune, and Eiji Hase 413

Behavior of Proplastids and Their Nucleoids in Dark-Dividing Cells of *Euglena gracilis*, With Special Reference to Their Temporary Association With the Nucleus

Tetsuaki Osafune, Shinya Tsukada, and Eiji Hase 419

Section V. Regulation of Chloroplast Development: Transcriptional, Translational, and Post-Translational Regulation

Chloroplast Development

Dennis E. Buetow 427

Chloroplast Development in Algae and Higher Plants: A General Survey

Gottfried Galling and Allan Michaels 433

Euglena Plastid Constituents: Their Source and Biosynthetic Regulation in Light and Darkness

Anthony J. Spano and Jerome A. Schiff 443

A Special Type of Nucleus-Plastid-Interactions: Nuclear Gene Induced Plastome Mutations

Rudolf Hagemann 455

x / Contents

Conservative and Variable Features of the Chloroplast Genome of <i>Acetabularia</i> Hans-Georg Schweiger, Egon J. de Groot, Michael B. Leible, and Martin J. Tymms	467
DNA Synthesis in Suspension Cultures of <i>Nicotiana tabacum</i> and <i>Glycine max</i> Sabine Heinhorst, Gordon C. Cannon, and Arthur Weissbach	477
Organization and Structure of Chloroplast tRNA Genes André Steinmetz and Jacques H. Weil	489
Identification, Organization and Photoregulated Expression of the Maize Plastid Genes for the Alpha Subunit of CF₁ and Subunits I and III of CF₀ Steven R. Rodermel and Lawrence Bogorad	499
Regulation of Expression of Nuclear Genes Coding Plastid Proteins in Cultured Soybean Cells Geza Erdős, Kenji Shinohara, and Dennis E. Buetow	505
Expression and DNA Sequence of the Chlamydomonas Chloroplast Gene Homologous to α Subunit of RNA Polymerase of <i>E. coli</i> Stefan J. Surzycki, T-H. Hong, and Judith A. Surzycki	511
Gene Transfer as a Tool to Study the Synthesis of the Small Subunit of Ribulose Bisphosphate Carboxylase Robert B. Simpson and Thomas D. McKnight	517
Changes in the Amount of Different Nucleic Acids in Cotyledons of Ageing Mustard (<i>Sinapis alba</i>, L.) Seedlings Detlef Rosemann, Gabriele Dietrich, Gerhard Link, and Helga Kasemir	523
Early Light Inducible Proteins of Barley: Evidence for the Existence of a Multigene Family Bernhard Grimm and Klaus Kloppstech	531
Characterization of Light-Induced Chloroplast DNases From <i>Euglena gracilis</i> B. Boyer, E. Brownell, J. Tornabene, R. Grzesik, and H. Lyman	537
Response of RuBP Carboxylase mRNA and Protein in Adult Tobacco Leaves Transferred From Low to High Light Regime Jean-Louis Prioul, Agnès Reyss, and Mireille Tenaud	543
Gene Expression in Blue Light-Dependent Chloroplast Differentiation of Cultured Plant Cells Gerhard Richter, Ralf Einspanier, Wolfgang Hüsemann, Anja Dudel, and Klaus Wessel	549
Age and Phytochrome-Induced Changes at the Level of the Translatable mRNA Coding for the LHC-II Apoprotein of <i>Phaseolus vulgaris</i> Leaves Paraskevi Tavladoraki, George Akoyunoglou, Annette Bitsch, Gabriele Meyer, and Klaus Kloppstech.	559

Kinetin-Induced Accumulation of mRNA Encoding the Apoprotein of the Light-Harvesting Chlorophyll a/b-Protein Complex in Tobacco Cell Suspensions

Bernard Teyssendier de la Serve, Michèle Axelos, and Claude Péaud-Lenoël 565

Post-Translational Regulation of Chloroplast Differentiation

George Akoyunoglou and Joan Argyroudi-Akoyunoglou 571

Turnover of Ribulose 1,5 Bisphosphate Carboxylase in *Chlorella fusca* and in Its Light Dependent Mutant g-2

Jürgen Bullmann and Gottfried Galling 583

Regulation of Protein Synthesis During Plastid Development in *Euglena gracilis*

Catherine Bouet, Rodolphe Schantz, Bernard Pineau, Guy Dubertret, and Gérard Ledoigt 587

A Variety of Chloroplast-Located Proteases

Xiang-Qin Liu and André T. Jagendorf 597

Control of 32kDa Thylakoid Protein Degradation as a Consequence of Herbicide Binding to Its Receptor

Autar K. Mattoo, Jonathan B. Marder, Victor Gaba, and Marvin Edelman. . 607

The Effect of Cross-Linking on the Native and Denatured 32 kDa-Q_B Protein of *Chlamydomonas reinhardtii* Thylakoids

Noam Adir, Achim Trebst, and Itzhak Ohad 615

Section VI. Regulation of Chloroplast Development: Photoregulation

Control by Light of Plastidogenesis as Part of a Control System

Hans Mohr 623

Twofold Action of Phytochrome on Development of the Capacity for Photophosphorylation in Mustard Cotyledons (*Sinapis alba* L.)

Heidemarie Oelze-Karow and Hans Mohr. 635

Is the Antenna to Reaction Center Ratio in Pea Chloroplasts Regulated for Optimum Energy Conversion?

J. Whitmarsh and W.-J. Lee 643

Light-Quality Regulates Photosystem Stoichiometry in Cyanobacteria

Annamaria Manodori and Anastasios Melis 653

Efficiency of Low Irradiance Red and Blue Light in Development of Corn Mesophyll and Bundle Sheath Chloroplasts

Kenneth Eskins and Susan McCarthy 663

Blue Light-Induced Starch Breakdown in *Chlorella* Cells

Akio Kamiya and Wolfgang Kowallik 671

Regulation of Blue Light-Enhanced Carbohydrate Breakdown During Chloroplast Development of <i>Scenedesmus</i> Mutant C-2A': A ³¹P NMR Study Günter Ruyters	677
Differentiation of Chloroplasts in Leaves of Aurea Plants Mercedes Wrischer, Alenka Hloušek-Radojčić, Ljerka Kunst, and Nikola Ljubešić	685
Changes in Shape of Chloroplast Spectra During Ontogeny of Primary Leaves of French Bean Correlate With Rate of Chlorophyll Accumulation Pavel Šiffel, Nikolai N. Lebedev, and Zdeněk Šesták	691
Effect of the Rate of Chlorophyll <i>a</i> Formation on Thylakoid Development in Higher Plants George Tzinias, George Akoyunoglou, and Agapios Akoyunoglou	697
Studies of the Physiology and Molecular Genetics of Phycobilisome Development in <i>Anacystis nidulans</i> After a Light Shift From White to Red Light Anders Lönneborg, S. Roger Kalla, Jonas Lidholm, Lisbet K. Lind, Petter Gustafsson, and Gunnar Öquist.	703
Developmental and Functional Relations Between the Thylakoids and Stroma in the Regulation of Phosphoribulokinase J. William Bradbeer, Marianne E. Rüffer Turner, and Kevin M. Fallon	707
 Section VII. Regulation of Chloroplast Development: Environmental Regulation	
CO₂ and HCO₃⁻ Accumulation by Microalgae James V. Moroney, H. David Husic, and N.E. Tolbert	715
Regulatory and Developmental Factors Affecting Photoinhibitory Damage and Recovery in Chilling Sensitive Rice Katherine E. Steinback and Benjamin A. Moll	725
Nuclear-Coded Chloroplast Heat-Shock Proteins in Pea Gabriele Meyer and Klaus Kloppstech	731
The Effect of Sugars on the Development of Fern Gametophytes Zoltán Kristóf, Gábor Tóth, Gyula Paless, Abd El-Hamid Ali, and Anna H. Nagy	737
Influence of Glucose on the Oxygen-Evolving Capacity of Heterotrophically Grown <i>Chlorella</i> Klaus P. Bader and Günter Ruyters.	745
Chloroplast Gene Organization in Pea, Common Bean and Broad Bean Gerhard Bookjans, Christine Michalowski, Mfika Mubumbila, Bjarn M. Stummann, Knud W. Henningsen, Jacques-Henry Weil, Hans J. Bohnert, and Edwin J. Crouse	751
Index	759

ISOLATION OF A FRACTION ENRICHED IN ENVELOPE MEMBRANES
FROM PURIFIED PEA (*PISUM SATIVUM*) ETIOPLASTS

Jürgen Soll, Gerhard Wanner, Günter Henkelmann,
Ursula Röper and Michaela Schulze
Botanisches Institut der Universität München
Menzinger Str. 67, D-8000 München 19

INTRODUCTION

The envelope membrane is a feature common to all members of the plastid family. It is conservatively retained as a two membrane barrier, consisting of inner and outer envelope, at all levels of plastid development, e.g. proplastids, etioplasts, chromoplasts, chloroplasts (Douce et al., 1984). Studies on chloroplast envelopes from pea and spinach have revealed its essential role in the proper function of the organelle (Douce et al., 1984, Soll et al., 1980, Cline et al., 1982, Pfisterer et al., 1982). No data are available so far on the function of the plastid envelope in chloroplast development, though some initial reports have been published from etioplasts envelope of cereals (Sandelius and Selstam, 1984, Hönighaus and Feierabend, 1985). These results are difficult to compare with data obtained from dicotyledons. We have therefore developed a method to purify etioplasts from etiolated pea plants and to isolate envelope membranes of these plastids thus enabling us to compare directly data from different developmental stages.

MATERIAL AND METHODS

Pea plants (*Pisum sativum*, var. Miranda) were soaked for 12 hours in running tap water and grown in vermiculith in the dark for 11 days at 23°C. About 200 gr. of primary leaves were normally used for the etioplast preparation. Leaves were homogenized in a blender equipped with razor blades (Kanangara et al., 1977) using the following buffer;

Grind 200 gm of etiolated pea leaves, age 10-11 days;
 0.5 M sucrose; tricine-KOH 30 mM, pH 7.2; 1 mM MgCl₂,
 1 mM EdTA

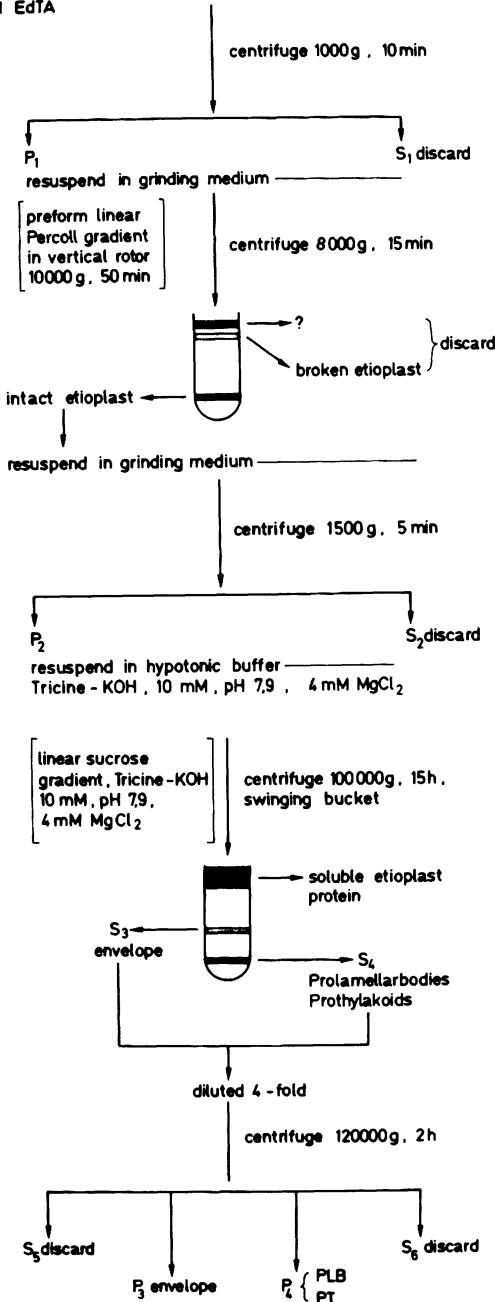


Figure 1. Purification scheme of pea etioplast subfractions

0.5 M sucrose, 1 mM MgCl₂, 1 mM EdTA, 30 mM N-(tris-(hydroxymethyl) methyl) glycine-KOH, pH 7.2. The slurry was filtered through a nylon-net (30 μm aperture) and a crude etioplast pellet was obtained after centrifugation at 1000 g for 10 min. The plastids were further purified on a linear silica-sol gradient. The gradient was made of 5 ml 80% percoll cushion and 20 ml 30% percoll solution. A linear gradient was formed by centrifugation at 10.000 g for 50 min. in a vertical rotor (see also Fig. 1). The purified, intact, etioplasts were recovered from the interface 80% percoll/linear gradient after centrifugation for 15 min. at 8000 g. The etioplast suspension was diluted in excess isolation medium and washed free of percoll. They were then lysed in hypotonic buffer (see Fig. 1) and the plastid components separated on a linear sucrose gradient

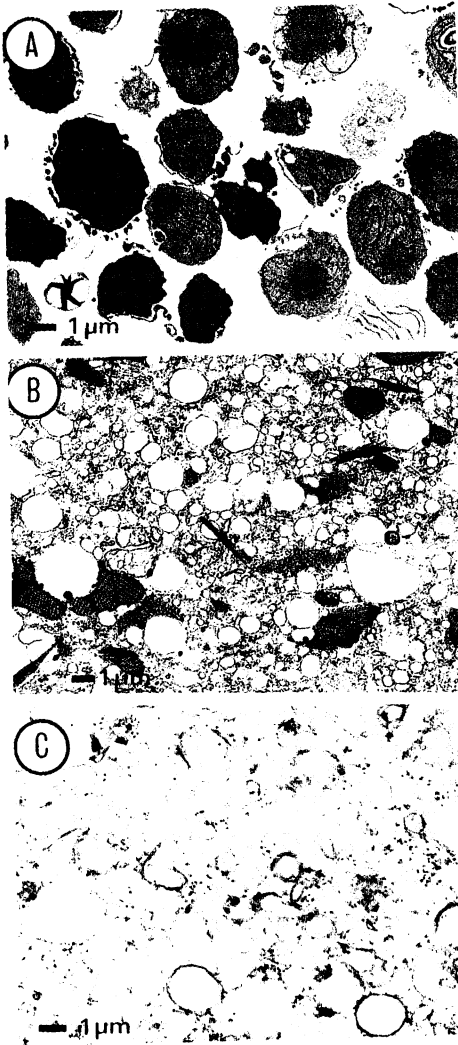


Figure 2. Electronmicroscopic studies; A) isolated pea etioplasts after purification on a linear percoll gradient; B) mixture of prolamellar bodies and prothylakoids, obtained from fraction 10 of the linear sucrose gradient (see Fig. 1, Fig. 5); C) envelope membranes, fraction 6 (Fig. 5)

(0.6-1.2 M sucrose, underlaid with a 55% sucrose cushion) buffered as in Fig. 1. Envelope membranes and a mixture of prolamellar bodies and prothylakoids were recovered from the gradient and pelleted by centrifugation (Fig. 5).

Contamination of the etioplast fraction by mitochondria and peroxisomes were tested using cytochrome-c-oxidase and OH-pyruvate-reductase, respectively, as marker enzymes (Jackson and Moore, 1979) (Table 1).

Lipid analysis was done by thin layer chromatography on HPTLC-plates (silica-gel 60, Merck)

using acetone benzene/water (91/30/8) as a solvent system. Lipids were visualized with the following stain (made from 2 gr $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 180 mg KMnO_4 , 6 ml H_2SO_4 , 200 ml H_2O). The plates were heated for 10 min. at 120°C and then scanned at 583 nm. Calibration experiments had shown, that the different lipid species stain with the same intensity ($\pm 5\%$).

Function	Volume (ml)	cytochrome-c-oxidase		HO-Pyruvat-reductase	
		total activity (mol/min)	mol/mg-min.	total activity (nmol/min)	μmol/mg-min.
crude pellet, P ₁	10	2745	25.3	2.42	0.022
top layer Percoll gradient, O ₁	2	1220	36	0.66	0.0197
broken etioplasts O ₂	4	1028	33.2	0.51	0.0166
purified, intact etioplast	1	6.4	0.74	0.005	0.0006

TABLE 1.

Contaminations of percoll purified, intact etioplasts were determined as in Jackson and Moore 1979.

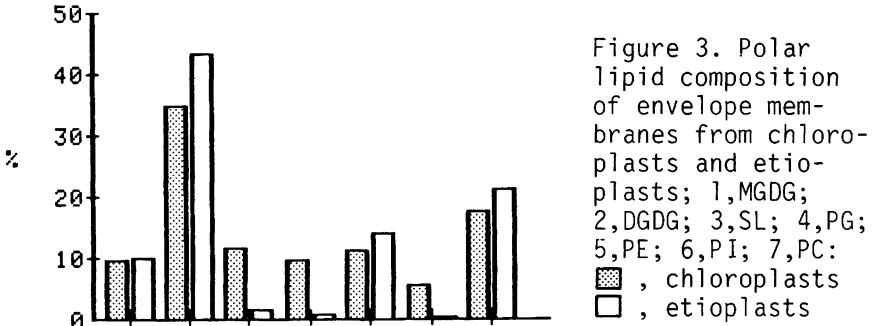


Figure 3. Polar lipid composition of envelope membranes from chloroplasts and etioplasts; 1, MGDG; 2, DGDG; 3, SL; 4, PG; 5, PE; 6, PI; 7, PC; , chloroplasts , etioplasts

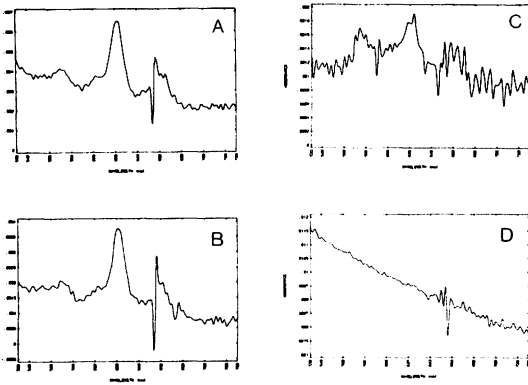


Figure 4. Distribution of protochlorophyllide (λ_{max} 620 nm) in different fractions. Similar amounts of protein were extracted and analysed spectrophotometrically. A) intact etioplasts, B, C, D) fraction 10, 8, 6 respectively of linear sucrose gradient.

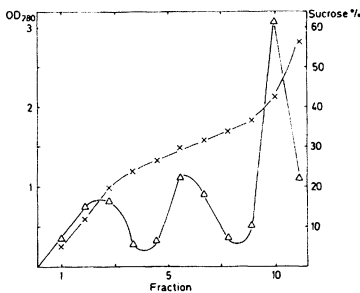


Figure 5. Separation of etioplast components on a linear sucrose gradient. Envelope fractions 6,7; Prothylakoids and prolamellar bodies, fraction 10.

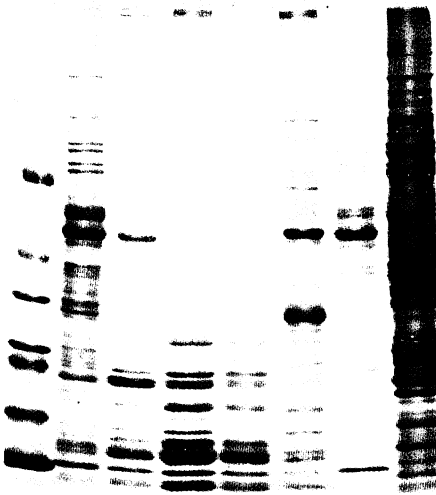


Figure 6. SDS-Polyacrylamide gelelectrophoresis of etioplasts subfractions obtained from the linear sucrose gradient from left to right lane;

1, Molecular weight standards
12,20,24,29,36,45,68 kDa;
2, etioplasts; 3, fract. 6;
4, fract. 7; 5, fract. 8;
6, fract. 10; 7, fract. 3;
8, fraction 2.

CONCLUSIONS

We have developed a large scale method for the purification of etioplasts from

dark grown pea. As outlined in Fig. 1 and shown in Table 1 for the marker enzyme distribution a very pure etioplast fraction is obtained in respect of mitochondrial and peroxisomal contaminations. Electronmicroscopy excludes also the contamination by membranes of other cell compartments (Fig. 2A). To have highly purified plastids is a prerequisite for the isolation of envelope membranes (Douce et al., 1984). Our separation procedure, as summarized in Fig. 1 has yielded a discrete membran fraction different from the mixture of prothylakoid and prolamellar bodies. Initial attempts to characterize this fraction are outlined in Fig. 3-6 and support our view that we have obtained envelope membranes from pea etioplasts.

Our data on the polar lipid composition of envelope membranes show major differences in the content of sulfur lipid (SL), phosphatidylglycerol (PG) and phosphatidylinositol (PI) which are much less in envelope membranes from etioplasts. The content of phosphoethanolamine (PE), a lipid which is normally not found in envelope (Douce et al., 1984) is probably due to contamination with rough endoplasmic reticulum and currently under further investigation. No differences are observed in the galactolipid content (MGDG, DGDG) As shown in Fig. 4, no protochlorophyllide is detectable in the envelope membrane fraction, indicating that this fraction is devoid of prolamellar bodies. These results are further supported by polyacrylamide gel electro-

phoresis with subsequent Western-blot analysis stained for coupling-factor using specific antibodies. In fraction 6 (envelope fraction) coupling factor was at the limit of detection while fraction 9 and 10 (prolamellarbodies, prothylakoids) showed high coupling factor content (compare also Fig. 6, data not shown).

REFERENCES

- Douce R, Block MA, Dorne AJ, Joyard J (1984). The plastid envelope membranes. *Subcell Biochemistry* 10, 1-84.
- Soll J, Kemmerling M, Schultz G, (1980). Tocopherol and plastoquinone synthesis in chloroplast subfractions. *Arch Biochem Biophys* 204, 544-550.
- Cline K, Werner-Washburne M, Andrews J, Keegstra K (1981). Separation and characterization of inner and outer envelope membranes of pea chloroplast. *Proc Natl Acad Sci USA* 78, 3595-3599
- Pfisterer J, Lachmann P, Kloppstech K (1982). Transport of proteins into chloroplasts. *Eur J Biochem* 126, 143-148.
- Sandelius AS, Selstam E (1984). Localization of galactolipid biosynthesis in etioplasts isolated from dark grown wheat. *Plant Physiol* 76, 1041-1046.
- Hönighaus R, Feierabend J (1985). Origin and developmental changes of plastid envelope proteins and translocator activities. *Planta* in press.
- Kannangara CG, Gough SP, Hansen B, Rasmussen JN, Simpson DJ (1977). A homogenizer with replacable razor blades for bulk isolation of active barley plastids. *Carlsberg Res Commun* 42, 431-439.
- Jackson C, Moore AL (1979). Isolation of higher plant mitochondria. In *Plant Organelles*, Reid E (ed) Ellis Horwood publisher, pp 1-13.

This work was supported in part by the Deutsche Forschungsgemeinschaft and Freunden und Förderern der Universität München.