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CONTENTS

Dedication V

Preface VII

LIPID METABOLISM IN CHLOROPLASTS AND LEAVES

Oleic acid, the central substrate
P.K. Stumpf, D.N. Kuhn, D.J. Murphy, M.R. Pollard, T. McKeon and J. MacCarthy 3

The role of chloroplasts in leaf lipid metabolism and polyunsaturated fatty acid synthesis
G. Roughan and R. Slack 11

Use of mesophyll protoplasts to study lipid metabolism in cell organelles
R. Haas and E. Heinz 19

The structural organization of chloroplast lipids *in vivo* and in model systems: Some aspects
C. Liljenberg 29

Lysogalactolipids as intermediates in galactolipid synthesis in chloroplasts
K.-P. Heiŝe and A. Sauer 39

Galactolipid formation in chloroplast envelopes. A discussion on differences between experiments *in vivo* and *in vitro*
J.F.G.M. Wintermans, A. van Besouw, G. Bögemann and J. Aerts 49

Studies on the biosynthesis of sulfoquinovosyl diacylglycerol in higher plants
J.B. Mudd, R. Dezacks and J. Smith 57

Long chain acyl-coenzyme A thioesters as substrates in glycerolipid biosynthesis of chloroplasts
M. Bertrams and E. Heinz 67

Synthesis of long-chain acyl-CoA in chloroplast envelope membranes
J. Joyard and P.K. Stumpf 73

Cooperation between chloroplasts and the extra-plastidial compartment for the biosynthesis of leaf lipids
A. Trémolières, J.-P. Dubacq, D. Drapier and P. Mazliak 77

Phospholipid exchange proteins from photosynthetic tissues
M. Julienne, D. Douady, J.-P. Dubacq, A. Trémolières, D. Drapier, M. Grosbois, P. Mazliak and J.-C. Kader 81

Changes in lipid composition and synthesis and in chloroplast structure observed in greening barley leaves
A.O. Davies, A.T. James, R. Jeffcoat and J.L. Harwood 85

A hypothetic role for phosphatidylglycerol and 3-*trans*-hexadecenoic acid in the light reactions of the photosynthetic process
J.-C. Duval, J.-P. Dubacq and A. Trémolières 91

Trans-3-hexadecenoic acid and grana stacking
T. Guillot-Salomon, A. Trémolières, C. Tuquet and J.-P. Dubacq 95

Lipid changes in plastids isolated from alfalfa seedlings grown under salt-stress
F. Harzallah-Skhiri, T. Guillot-Salomon and M. Signol 99

Influence of growth temperature on acyl lipids of leaves
D.J. Chapman and J. Barber 103

Fatty acid synthesis in isolated chromoplasts and chromoplast homogenates. ACP stimulation, substrate utilisation, and cerulenin inhibition
B. Liedvogel and H. Kleinig 107

Partial purification and properties of a soluble fatty acid synthetase from olive leaves
L.M. Daza, M. Garrido and J.P. Donaire 111

Function and distribution of phospholipids in spinach thylakoid membranes as revealed by phospholipase A_2 treatment
A. Rawyler and P.-A. Siegenthaler 117

Characterization and meaning of chloroplast lipoxygenase activities
R. Douillard 121

Galactolipid biosynthesis in *Euglena gracilis*
E. Blee and R. Schantz 127

Phosphatidylglycerol biosynthesis in *Euglena gracilis*
A. Chammai and R. Schantz 131

Discovery of a new glyceroglycolipid in blue-green algae and its role in galactolipid biosynthesis
G.B. Feige, E. Heinz, K. Wrage, N. Cochems and E. Ponzelar 135

LIPID METABOLISM IN NON-PHOTOSYNTHETIC TISSUES

Fatty acid synthesis
J.L. Harwood 143

Phospholipid localization in biological membranes
J.A.F. Op den Kamp, T. Kauerz and G. van Meer 153

Phospholipid exchange protein from higher plants
M. Yamada, T. Tanaka and J.-I. Ohnishi 161

Lipid metabolism as a factor in environmental adaptation
P.J.C. Kuiper 169

Biogenesis of lipids in oilseed plants
L.-Å. Appelqvist 177

The biochemistry of lipids in cereal crops
T. Galliard and P.J. Barnes 191

Lipid composition of sycamore cells cultivated at various temperatures
M. Gawer, F. Trapy, J. Guern and P. Mazliak 199

Oxygen and temperature effects on the fatty acid composition of sycamore cells (*Acer pseudoplatanus* L.)
F. Rebeille, R. Bligny and R. Douce 203

Desaturation of fatty acids in lipids in response to the growth temperature in the blue-green alga, *Anabaena variabilis*
N. Sato and N. Murata 207

Subcellular localisation of fatty acid synthetases in cell cultures of higher plants
F. Spener 211

Biosynthesis of parinaric acid (9,11,13,15-octadecatetraenoic acid)
M. Noda, K. Ohga, Y. Nakagawa and K. Ichihara 215

A comparison of the polypeptide and phospholipid composition of oil body and microsomal preparations from safflower and linseed cotyledons
R. Slack and G. Roughan 219

Lipid metabolism in developing wheat seeds
D.N. Stokes, T. Galliard and J.L. Harwood 223

α-ketodicarboxylic acids in lipogenic substrates in Flacourtiaceae
I. Tober and F. Spener 227

Root phospholipid composition as a factor of the differential Ca-sensitivity of plants
M. Rossignol and C. Grignon 231

Calcium binding, phosphatidic acid formation and fatty acid breakdown in plant mitochondria
J. Dupont and C. Lance 235

The availability of palmitoyl-CoA influences the activity of palmitoyl-CoA hydrolase in carrot homogenate
P. Baardseth and E. Slinde 239

Effect of sodium chloride and calcium sulfate on the lipid composition of sunflower leaves (*Helianthus annuus L.*)
L. Bettaieb, M. Gharsalli and A. Cherif 243

Relationships between temperature, microviscosity and desaturases activities in the microsomal membranes of two fungi with different behaviour
L. Chavant, C. Montant and C. Wolf 249

Scopulariopsis brevicaulis (Bainier) : Study of the lipid content in relation to growth
J.-L. Fonvieille and M. Sancholle 253

Fatty acid biosynthesis in cell free preparations of *Anabaena cylindrica*
Z.T. Al'Araji and T.J. Walton 259

The effect of biotrophic fungal infection on the lipid metabolism of green plants
D.M. Lösel 263

WAX METABOLISM

Light promotes synthesis of the very long fatty acid acyl chains in maize wax
P. von Wettstein-Knowles, P. Avato and J. D. Mikkelsen 271

Alkanes and alkenes in the epicuticular waxes from Cistus plants
P.-G. Gülz 275

Stearoyl-CoA metabolism in the microsomes from leek epidermal cells: Thioesterase, acyltransferase and elongase activities
T. Abdul-Karim, R. Lessire and C. Cassagne 281

Synthesis of lipids by epidermal and mesophyll protoplasts isolated from barley leaf sheaths
J.D. Mikkelsen 285

Long chain fatty acid activation: Relations with very long chain fatty acids biosynthesis
R. Lessire and C. Cassagne 291

PIGMENTS

Prenylquinones in plant leaves
H.K. Lichtenthaler 299

Current concept of organization of chlorophyll biosynthesis
A.A. Shlyk 311

Carotenoid biosynthesis in plants
J.W. Porter, S.L. Spurgeon and D. Pan 321

The role of carotenoids in chloroplasts of higher plants
D. Siefermann-Harms 331

Biosynthesis of α-tocopherol and plastoquinone-9 in spinach chloroplasts
J. Soll and G. Schultz 341

Carotenoid biosynthesis in *Scenedesmus obliquus* (Chlorophyta): Experiments with deuterium oxide
G. Britton and A.P. Mundy 345

The influence of kinetin on the chlorophyll biosynthesis in radish cotyledons
C. Buschmann 349

Properties of membrane-bound 3-hydroxy-3-methylglutaryl-coenzyme A reductase (EC 1.1.1.34) from radish seedlings and some aspects of its regulation
T.J. Bach, H.K. Lichtenthaler and J. Rétey 355

Carotenoid biosynthesis: Biogenesis of capsanthin and capsorubin in pepper fruits (*Capsicum annuum*)
B. Camara and R. Monéger 363

Level of chlorophyll b and the light harvesting chlorophyll-protein complex in Raphanus seedlings grown at different light quanta fluence rates
U. Prenzel, H.K. Lichtenthaler and D. Meier 369

Circular dichroism studies of the spontaneous organization of xantophyll compounds in water-alcohol solutions. Correlations with the role played by the polyene substances in the structural organization of biological systems
J. Lematre, B. Maudinas and C. Ernst 373

Light influence on zeaxanthin epoxidation inhibition of S-ethyl dipropylthiocarbamate
R.E. Wilkinson 375

Lipids, pigments, light-harvesting chlorophyll protein complex and structure of a virescent mutant of maize
E. Selstam 379

STEROLS AND TERPENES

Function as an evolutionary determinant of biosynthesis
W.R. Nes 387

The use of mutants and azasterols in studies of yeast sterol biosynthesis
A.C. Oehlschlager, H.D. Pierce, Jr., A.M. Pierce, R.H. Angus,
E. Quantin-Martenot, A.M. Unrau and R. Srinivasan 395

Biosynthesis of sterol conjugates in plants
Z.A. Wojciechowski 405

A monolayer study of lipid: Protein interactions in the chloroplast membrane
D.G. Bishop and J.R. Kenrick 415

Incorporation of ^{14}C-labeled CO_2, phosphoglycerate, phosphoenolpyruvate, pyruvate, acetate and mevalonate into terpenoids and acyllipids of isolated intact spinach chloroplasts
K.H. Grumbach 421

Site of synthesis of geranylgeraniol derivatives in intact spinach chloroplasts
M.A. Block, J. Joyard and R. Douce 427

Latency of uridine diphosphate glucose-sterol-β-D-glucoysl-transferase and uridine diphosphatase in purified membrane fractions from maize coleoptiles
M. M'Voula-Tsiéri, P. Benveniste, E. Martenot and
M.-A. Hartmann-Bouillon 431

Biosynthesis and cellular localisation of terpene hydrocarbons in maritime pine
C. Bernard-Dagan, M. Gleizes, G. Pauly, J.P. Carde and A. Marpeau 437

Membrane systems involved in synthesis and transport of monoterpene hydrocarbons in pine leaves
J.-P. Carde, C. Bernard-Dagan and M. Gleizes 441

Presence of steryl glycosides and amyrin glycosides in a blue-green alga: *Nostoc commune* and a red alga: *Porphyridium sp.*
R. Duperon, P. Doireau, A. Verger and P. Duperon 445

Author index 449

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BIOSYNTHESIS OF α-TOCOPHEROL AND PLASTOQUINONE-9 IN SPINACH CHLOROPLASTS

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ABSTRACT

Prenylation and methylation reaction in αT biosynthesis is localized in the envelope membranes of the chloroplasts, while PQ-9 biosynthesis takes place in the envelope membranes and also in the thylakoid membranes. The sequence in α-T biosynthesis in spinach is (see also Figure 1): Homogentisate + Phytyl-PP $\longrightarrow$ Me-6-PQH$_2$ $\longrightarrow$ 2,3-Me$_2$PQH$_2$ $\longrightarrow$ γT $\longrightarrow$ αT; for the PQ-9 biosynthesis it is: Homogentisate + Solanesyl-PP $\longrightarrow$ Me-6-SQH$_2$ $\longrightarrow$ PQH$_2$.

RESULTS

The two major prenylquinones of the chloroplast are αT and PQ-9. The aromatic moiety of both derives from homogentisate[1]; the prenyl sidechain originates from a polyprenyl-PP (C_{20} in the case of αT, C_{45} in that of PQ-9). Homogentisate is formed by an intraplastidic occurring shikimate pathway[2,3].

α-Tocopherol biosynthesis

The only site of αT biosynthesis in spinach chloroplasts are the envelope membranes[4,5]. Phytyl-PP derives by reduction from GGPP which is synthesized by a recombinated system of chloroplast envelope or thylakoid membranes with stroma (soluble fraction)[6]. Homogentisate is solely prenylated with phytyl-PP to form Me-6-PQH$_2$[5]. There is no stimulation by other chloroplast fractions like stroma or thylakoid membranes[5]. The prenyltransferase in spinach shows a strong specifity for phytyl-PP (26 pmol/h. mg protein); GGPP is inactive in this system[5]! From the possible position isomers only Me-6-PQH$_2$ but not Me-5- or Me-3-PQH$_2$ are formed[5]. A kinase which forms phytyl-PP from phytol + ATP is localized in the stroma[5].

The following two methylation steps with SAM as methyl-group donor to form

---

Abbreviations: GGPP - geranylgeranyl-PP; Me-6-GGQH$_2$ - 2-methyl-6-geranylgeranylquinol; Me-6-PQH$_2$ and isomers - 2-methyl-6-phytylquinol and 5- and 3-phytyl-isomers; 2,3-Me$_2$PQH$_2$ - 2,3-dimethyl-5-phytylquinol; Me$_3$PQH$_2$ - trimethylphytylquinol; Me-6-SQH$_2$ - 2-methyl-6-solanesylquinol; PQ-9 - plastoquinone-9; PQH$_2$ - plastoquinol-9; SAM - S-adenosylmethionine; αT, γT, δT - α, γ, δ-tocopherol; γT$_3$ - γ-tocotrienol.

αT from Me-6-PQH$_2$ are also only due to the envelope membranes[4]: (that is: Me-6-PQH$_2$ $\xrightarrow{\text{SAM}}$ 2,3-Me$_2$PQH$_2$ $\rightarrow$ γT $\xrightarrow{\text{SAM}}$ αT). Again no stimulation occurs by combining envelope membranes with stroma protein. In marked contrast to the prenylation enzyme, the methyl transferase exhibits a preference for Me-6-GGQH$_2$ (2 nmol/h. mg protein) in comparison to Me-6-PQH$_2$ (0,7 nmol/h. mg protein)[4,8]. In spinach, however only products of the pathway with phytyl-PP as substrate are found.

The cyclization of the prenylquinol to the corresponding chromanol (in this case: 2,3-Me$_2$PQH$_2$ $\rightarrow$ γT) which is a prerequisite for the second methylation step takes place only in intact chloroplasts[7,8] but not in isolated envelope membranes. This might be due to lack of cofactors in the medium used. It is also probable that the procedure to prepare envelope membranes[4,9] markedly effects the enzymes involved in the cyclization reaction.

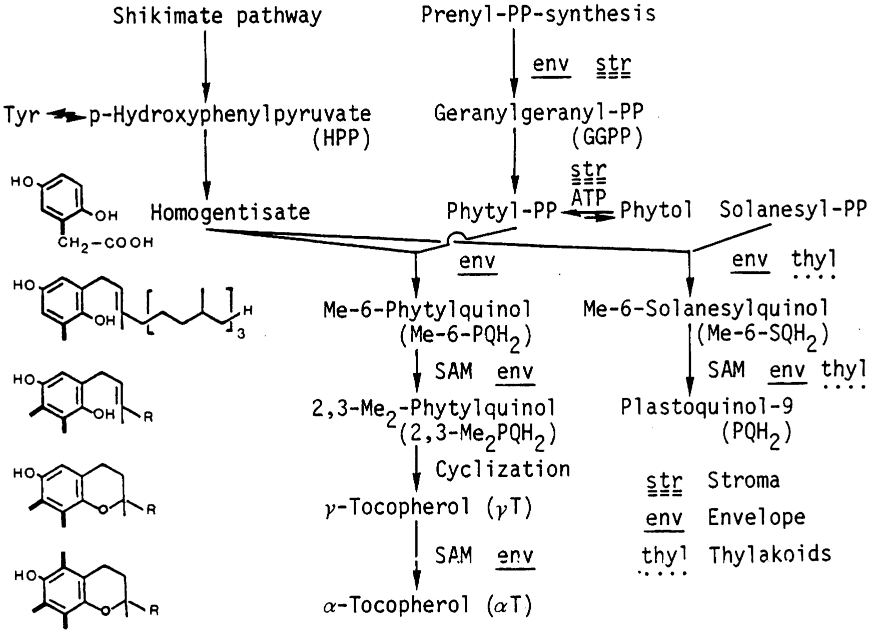

Fig. 1. Biosynthesis of αT and PQ-9 in spinach chloroplasts.

Plastoquinone-9 biosynthesis

PQ-9 biosynthesis, both prenylation and methylation is not only performed by the envelope membranes (1,2 pmol/h. mg protein and 10 pmol/h. mg protein) but also at low rates by the thylakoid membranes (0,o13 pmol/h. mg protein and 0,35 pmol/h. mg protein)[5]. However, if one takes into account the rate of thylakoid protein to that of envelope protein per mg chlorophyll the yields in total are not as different as they are calculated on the basis of protein itself. The sequence of reactions involved in the PQ-9 biosynthesis is similar to αT. Solanesyl-PP (C_{45}) serves as prenyl compound in the prenylation reaction to form Me-6-SQH_2 with homogentisate. In the following steps Me-6-SQH_2 is methylated with SAM to yield PQ-9. In either case, αT and PQ-9 biosynthesis, the quinol stage of the precursor is involved in the methylation reaction and not quinone stage[7].

CONCLUSIONS

Prenylquinones synthesized in chloroplasts operate in different ways in the photosynthetic mechanism. Whereas PQ-9 acts in the photosynthetic electron transport, αT inactivates energetisized oxygen species formed by light (by scavenging radicals and/or quenching of $^1O_2$ [11,12].

Both αT and PQ-9, are formed by enzyme systems of lipophilic membranes of the chloroplast being in direct contact to more hydrophilic areas of the stroma. The steps of the synthesis, both prenylation and methylation of the quinols, are conceivable as electrophilic substitution of aromatics, the mechanism is not yet clear.

In fewer cases, tocopherols and tocotrienols in plants occur also in laticifers[13] and oil seeds but compartmentation of synthesis in comparison to chloroplast remains of future interest.

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