

THE METABOLISM, STRUCTURE, AND FUNCTION OF PLANT LIPIDS

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PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

International Symposium on Plant Lipids (7th: 1986: University of California, Davis)
The metabolism, structure, and function of plant lipids.

"Proceedings of the Seventh International Symposium on Plant Lipids, held July 27-
August 1, 1986, at the University of California, Davis, Davis, California" - T.p. verso.

Includes bibliographies and index.

1. Plant lipids-Congresses. I. Stumpf, Paul K. (Paul Karl), 1919- . II. Mudd, J.
Brian (John Brian), 1929- . III. Nes, W. David. IV. Title. [DNLN: 1. Lipids-metab-
olism-congresses. 2. Plants-metabolism-congresses. W3 IN921BU 7th 1986b / QK
898.L56 I61 1986b]

QK898.L56I556 1986

581.19'247

87-1749

ISBN 0-306-42492-4



Proceedings of the Seventh International Symposium on Plant Lipids,
held July 27-August 1, 1986, at the University of California, Davis,
Davis, California

© 1987 Plenum Press, New York
A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

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Printed in the United States of America

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SYNTHESIS OF PLASTOQUINONE-9, α -TOCOPHEROL AND PHYLLOQUINONE (VITAMIN K₁)
AND ITS INTEGRATION IN CHLOROPLAST CARBON METABOLISM OF HIGHER PLANTS[#]

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INTRODUCTION

Plastoquinone-9, α -tocopherol and phylloquinone are known as plastidic prenylquinones fulfilling important functions: Plastoquinone-9 acts as mobile electron and proton carrier in photosynthetic electron transport and is involved in building up the electrochemical proton potential at the chloroplast cytochrom b₆/f complex /1,2/. α -Tocopherol is involved in inactivating energized oxygen species, formed in the light, by scavenging radicals and quenching singlet oxygen /3/. Phylloquinone is known as obligatory constituent of PS I (K₁/chlorophyll of PS I ratio about 1:100 /4/).

This report deals with the synthesis of α -tocopherol and plastoquinone-9 from homogentisate and of phylloquinone from 1,4-dihydroxy-2-naphthoate in chloroplasts. Furthermore, experimental data are presented to support earlier findings on the autonomic role of chloroplast carbon metabolism in forming plastidic isoprenoids. - The methods applied are described in /5,6,7,8,9/.

RESULTS AND DISCUSSION

Synthesis of α -Tocopherol and Plastoquinone-9 from Homogentisate at the Chloroplast Envelope Membrane

Homogentisate represents the aromatic intermediate in the formation of α T and PQ /10/ (Fig. 1). It is formed from Tyr via 4-hydroxyphenylpyruvate by

Abbreviations: DAHP, deoxy-D-arabinoheptulosonate-7-phosphate; DHAP, dihydroxyacetone phosphate; E⁻ase, 2-phosphoglycerate hydrolyase, enolase; rev. NADP GAPDH, reversible NADP glyceraldehyde 3-phosphate dehydrogenase; GG, geranylgeraniol; GGPP, geranylgeranyl-pyrophosphate; GK, glycerate kinase; HPP, 4-hydroxyphenylpyruvate; IPP, isopentenyl pyrophosphate; α KGA, 2-oxoglutarate; Me-6-PhQ(H₂) and isomers, 2-methyl-6-phytylquinone (quinol) and isomers; 2,3-Me₂-PhQ(H₂), 2,3-dimethyl-5-phytylquinone (quinol); MITO, mitochondrion; PDC, pyruvate dehydrogenase complex; PEP, phosphoenolpyruvate; PER, peroxysome; 3-PGA and 2-PGA, 3- and 2-phospho-D-glycerate; PK, pyruvate kinase; PGM, phosphoglycerate mutase; PQ(H₂), plastoquinone-9 (quinol-9); PS I, PS II, photosystem I and II; SAM, S-adenosylmethionine; SKA, shikimate; SORase, shikimate oxidoreductase; α , β , γ and δ T, α -, β -, γ - and δ -tocopherol

[#] The author's work was supported by the Deutsche Forschungsgemeinschaft.

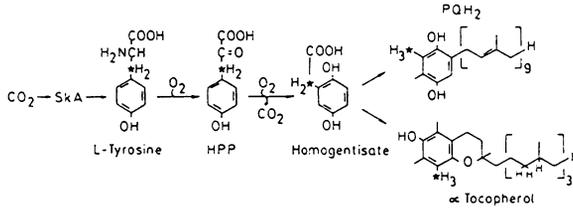
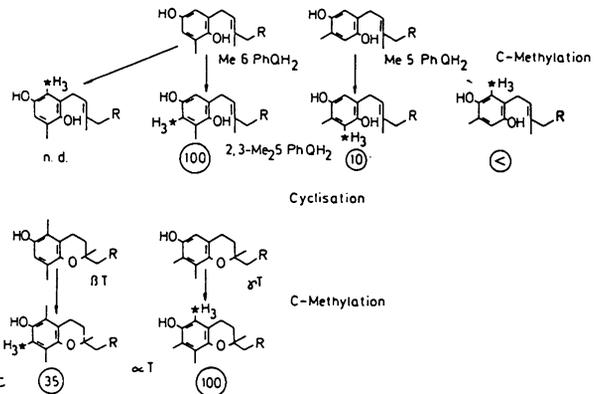


Fig. 1 . Incorporation of $3-^{14}\text{C}$ /tyrosine into plastoquinol and α -tocopherol in higher plant chloroplasts. Asterisks indicate ^{14}C label.

oxidases at the stromal face of chloroplast membranes /11/. All the following reactions of prenylquinone synthesis occur solely at the inner chloroplast envelope membrane /8/ (Fig. 4); chloroplast stroma or thylakoids are inactive /6,12/ (Fig. 4). In the prenyltransferase reaction, the carboxyl group of homogentisate is eliminated and a methylquinol is formed which is prenylated specifically at position 6 /6/. The prenyl side chain is always in trans configuration /13/. In this and the following methylation reaction only the quinol and not the quinone stage of the aromatic compound is active /5/. Phytol-PP is the only prenyl-PP suited for α T synthesis in chloroplasts and nonaprenyl- (solanesyl-) PP in PQ synthesis /6/. Consequently, the introductory step of prenylquinone synthesis is strictly specific in respect to substrate and position of attack, and the following reactions are strongly directed to a homogenous pattern of products.

As can be seen from the methylation step by SAM in Fig. 2, 2-methyl-6-phytylquinol is strongly preferred to its isomers. Thus, the main product is 2,3-dimethyl-5-phytylquinol which undergoes ringclosure (only verified in intact chloroplasts /5/) to form β T which is methylated by SAM to form α T. The chromanol stage is prerequisite for the second methylation reaction; no trimethylphytylquinol was formed from dimethylphytylquinols /5,14/. The β -tocopherol methyltransferase of *Capsicum annuum* was purified to homogeneity by d'Harlingue and Camara 1985 /15/. M_r has been determined to 33 kDa, K_m for β T to 13.7 μM and for SAM 2.5 μM . Thus the main sequence for α T synthesis in spinach is: Homogentisate \rightarrow 2-Methyl-6-phytylquinol \rightarrow 2,3-Dimethyl-5-phytylquinol \rightarrow β T \rightarrow α T (Fig. 3). Another sequence caused by changing methylation and cyclization reaction may occur additionally but at lower rates /16/: Homogentisate \rightarrow 2-Methyl-6-phytylquinol \rightarrow δ T \rightarrow β T \rightarrow α T.

Fig. 2 Substrate specificity of the first (upper series) and second methylation reaction (lower series) of α -tocopherol synthesis in spinach chloroplasts /5/. ^{14}C -Methyl from $^3\text{Me-}^{14}\text{C}$ /SAM was incorporated for labelling the substrates. The products were purified by co-chromatography adding reference substances and identified by radioscan. Asterisks indicate ^{14}C -label; the encircled figures indicate the ratio of products formed from different quinols (upper series) and tocopherols (lower series).



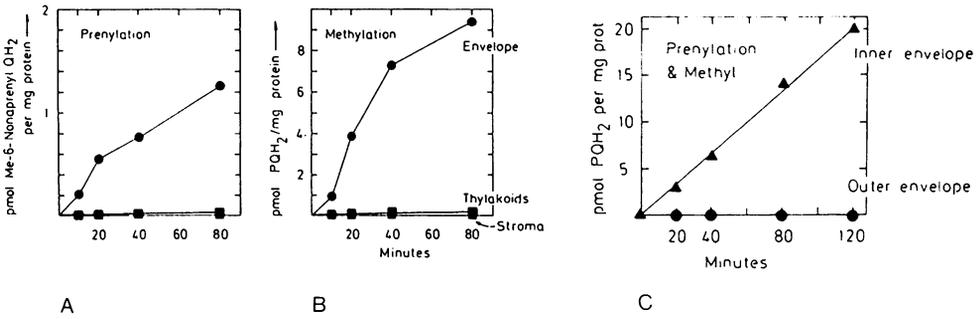


Fig. 4 Synthesis of plastoquinol-9 in spinach chloroplasts (calculated from /6,8/). (A) Prenylation reaction: Homogentisate + Nonaprenyl-(solanesyl)-PP \rightarrow 2-Methyl-6-nonaprenylquinol; (B) Methylation reaction: The quinol of (A) + SAM \rightarrow Plastoquinol-9. (C) Overall reaction: Homogentisate + Nonaprenyl-PP + SAM \rightarrow Plastoquinol-9. The reactions occur at the inner membrane (C) /8/ of the chloroplast envelope (A, B) /6/; stroma and thylakoid membranes are inactive.

Synthesis of Phylloquinone (Vitamin K₁) in Higher Plants

Feeding experiments using total plants revealed that phylloquinone is formed in leaves from shikimate /20/ and 2-succinylbenzoate /21/. Just recently Leistner's group provided evidence from studies on *E. coli* /22/ that isochorismate and not chorismate reacts with 2-oxoglutarate to form 2-succinylbenzoate. The results from studies on cell cultures /23,24/ and chloroplasts /25,26/ are summarized in Fig. 5. The chloroplast envelope is the site of prenylation /25/ and the thylakoid membrane of methylation reaction /26/, however, compartmentation of the other reactions remains still unclear. The synthesis in plants resembles the microbial one /27/ though phytyl-PP is preferred as prenyl donor in plants /25/.

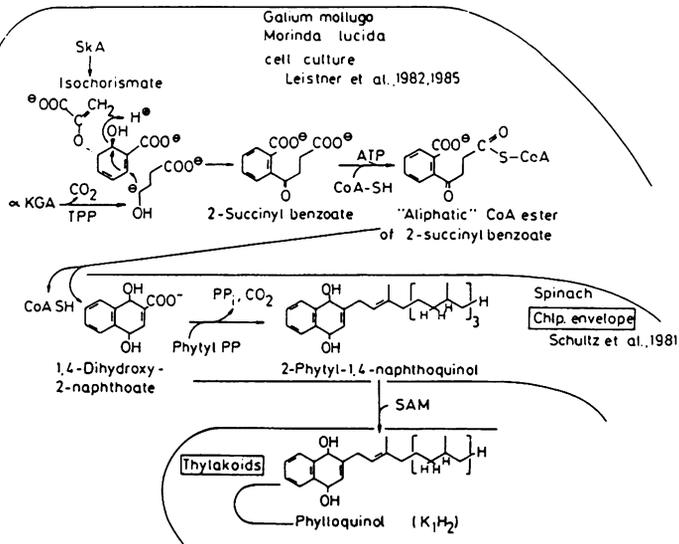


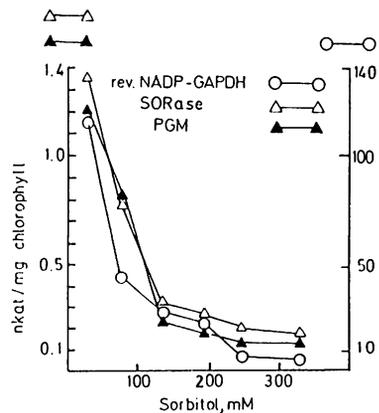
Fig. 5 Phylloquinone (K₁) synthesis in Higher Plants /23-26/. Probably isochorismate as in *E. coli* /22/ and 2-oxoglutarate (α -KGA) forms 2-succinylbenzoate.

Role of Chloroplast Carbon Metabolism in Plastidic Prenylquinone Synthesis

The carbon flow from 3-phosphoglycerate, phosphoenolpyruvate, pyruvate and acetyl-CoA. Even if the synthesis of aromatic amino acids by shikimate pathway /28,29,30,31/ and also prenyl-PP synthesis via mevalonate /32,33,34/ has been established in chloroplasts by identification of respective plastidic enzymes, it is still a matter of discussion from where PEP originates to supply DAHP synthesis of the shikimate pathway and from where pyruvate is delivered to supply the plastidic pyruvate dehydrogenase complex (for isolation see Treede and Heise, this Conference). Because phosphoglycerate mutase (PGM) to form 2-PGA from 3-PGA could not be detected in chloroplasts /35/ and acetyl-CoA is preferably synthesized from added acetate by the acetyl-CoA synthetase /36/, particularly in spinach chloroplasts, it was argued that chloroplasts are dependent on import of these substrates from the external site. Evidence for PEP formation from 3-PGA within the chloroplast could be obtained by three different approaches (D. Schulze-Siebert, A. Heintze and G. Schultz, in preparation; D. Schulze-Siebert and G. Schultz, in preparation, for plastidic isoenzyme of PGM in Ricinus see /37/ and in Brassica /38/).

(i) The only enzyme of carbon metabolism hitherto questioned to be present in spinach chloroplasts, PGM, could be identified by the latency method. The enzyme thus identified exhibits an identical behaviour in comparison to reversible NADP D-glyceraldehyde 3-phosphate dehydrogenase and shikimate oxidoreductase as plastidic marker enzymes (Fig. 6).

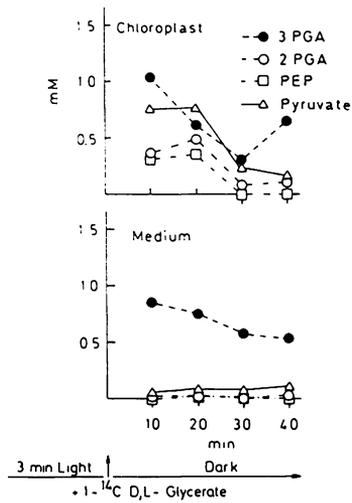
Fig. 6 Phosphoglycerate mutase (PGM) in spinach chloroplasts identified by the latency method. Purified, intact chloroplasts were subjected to step-wise osmotic shock by lowering the sorbitol conc. of the medium. The enzyme activity released from chloroplast was determined in the supernatant. In the latency studies, PGM exhibits an identical behaviour compared to reversible NADP-GAPDH and shikimate oxidoreductase (SORase) as marker enzymes for chloroplasts.



(ii) The carbon flow from 3-PGA to PEP and pyruvate was demonstrated within chloroplasts by adding labelled glycerate which is known to be phosphorylated by the glycerate kinase localized solely in the chloroplast stroma /39/. After spinning down the chloroplasts (Fig. 7), 3-PGA, 2-PGA, PEP and pyruvate were found at considerable amounts only in the chloroplast pellet and thus support above findings on plastidic PGM. Only 3-PGA was nearly equally distributed between chloroplast and suspension medium which can be attributed to action of the phosphate translocator. To reduce the activity of the translocator /40/ only 0.5 mM P_i was applied to the medium.

(iii) The increase of the synthesis of amino acids and prenylquinones in chloroplasts by omission of P_i and deminishing the exchange of triose-phosphates could be demonstrated earlier /41/.

Fig. 7 Experiment proving the carbon flow from 3-PGA to pyruvate via 2-PGA and PEP in spinach chloroplasts. Purified, intact chloroplasts were incubated with $1\text{-}^{14}\text{C}$ /glycerate which is phosphorylated by the glycerate kinase localized solely in the stroma /39/. 3-PGA thus formed served as substrate for C_3 -metabolism within the chloroplast. Aliquots taken at indicated time were centrifuged for 1 min and 3-PGA, 2-PGA, PEP and pyruvate determined by modified enzymatic analysis (D. Schulze-Siebert, A. Heintze and G. Schultz, in preparation).



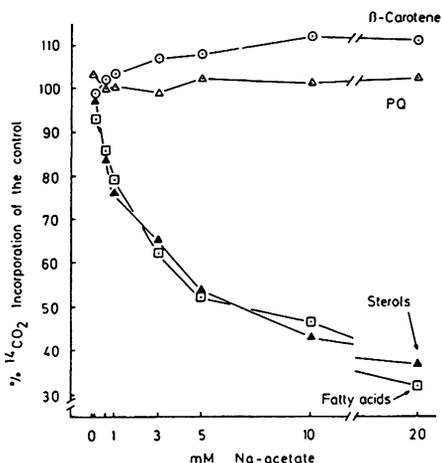
An external site of synthesis of PEP from photosynthetically formed 3-PGA can be detected by rising the P_i concentration up to 5 mM and adding exogenously excessive amounts of rabbit PGM and enolase to intact chloroplasts (10 and 2.5 units/50 μg chlorophyll) similar as in /42/. The activity of the shikimate pathway (measured as nmol Phe and Tyr formed) was considerably enhanced as a result of supply of high amounts of PEP by this enzyme reaction and re-import by the phosphate translocator. Consequently, this way might be considered as an additional site of supply optimized under in vitro conditions by adding enzymes in excess. Under in vivo conditions, the carbon flow from triosephosphates of photosynthetic carbon fixation is preponderantly directed to sucrose synthesis /43/ and less to PEP.

The origin of acetyl-CoA for isoprenoid synthesis. As shown in Table 1, highest amounts of PQ and β -carotene were formed from added bicarbonate by spinach protoplasts in the light and only lower ones from added acetate. On the other hand, saturation of fatty acid formation is only achieved by adding acetate. Therefore, the hypothesis was raised that a more or less channelling of pyruvate dehydrogenase complex and isoprenoid synthesis in chloroplasts may exist. To prove this the dilution of ^{14}C /bicarbonate by increasing amounts of added acetate was studied. In spite of increasing amounts of added acetate no dilution of ^{14}C activity in β -carotene and PQ could be observed. Only fatty acids formed inside and sterols formed outside the chloroplasts were diluted as expected (Fig. 8). Also additional vice versa experiments (data not shown) pointed at the same direction.

Table 1 Formation of plastoquinone-9, β -carotene, fatty acids (F.A.) and sterols from ^{14}C /bicarbonate, ^{14}C /acetate and ^{14}C /mevalonate using spinach protoplasts at pH 7.6. Note the preference of bicarbonate in the isoprenoid and of acetate in the fatty acid synthesis. Plastoquinone-9 and β -carotene are not formed if mevalonate is applied from the external site.

| | nmol Acetate units formed per mg chlorophyll x h | | | | |
|-------------------------------------|--|---------|-------|----------|------|
| | Lipids | Sterols | F.A. | Carotene | PQ |
| $\text{NaH}^{14}\text{CO}_3$ 0.5 mM | 5.1 ± 0.3 | 0.67 | 1.58 | 1.18 | 1.12 |
| ^{14}C /Acetate 0.1 mM | 33.3 ± 6.0 | 2.00 | 28.64 | 0.33 | 0.67 |
| ^{14}C /Mevalonate | 3.5 ± 0.6 | 1.80 | 0 | 0.03 | 0.03 |

Fig. 8 Demonstration of the metabolic channelling of carbon metabolism and isoprenoid synthesis in chloroplasts by a competition experiment adding ^{14}C /bicarbonate and increasing amounts of acetate to spinach protoplasts. A competition effect could only be observed in fatty acid and sterol synthesis. Plastoquinone-9 and β -carotene synthesis remained unaffected.



The scheme in Fig. 9 summarizes the above results.

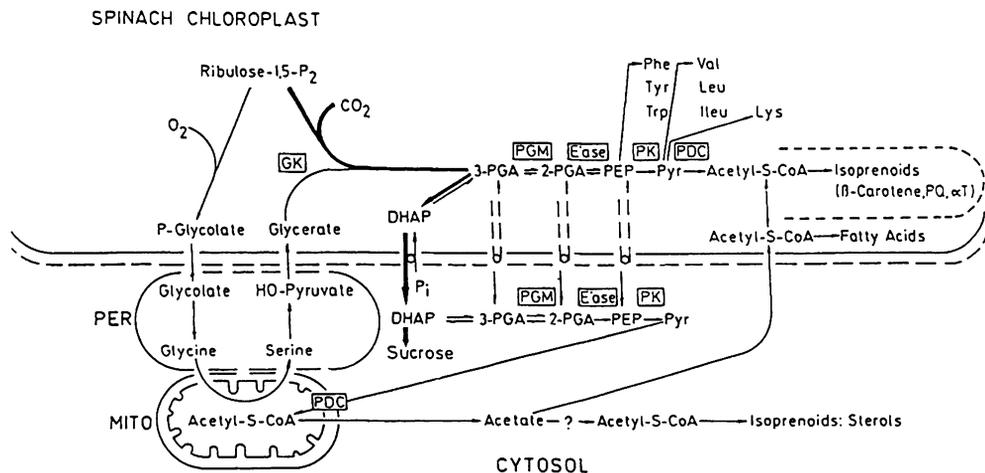


Fig. 9 Proposed scheme of carbon flow from primary photosynthetic products to isoprenoid compounds, fatty acids, amino acids and other compounds in spinach chloroplasts. Based on recent findings the scheme demonstrates the following points: (i) A carbon flow from 3-PGA to 2-PGA, PEP, and pyruvate within the chloroplast (and to some extent outside the chloroplast) exists to provide substrates for the synthesis of amino acids, isoprenoids and prenylquinones. (ii) Plastidic pyruvate dehydrogenase complex and plastidic isoprenoid synthesis are more or less channelled systems. (iii) The supply with acetate for fatty acid synthesis predominantly occurs from the external site.

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