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Editors

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Biogenesis and Function of Plant Lipids
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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 ⟶ Me-6-PQH_2 ⟶ 2,3-Me_2PQH_2 ⟶ γT ⟶ αT; for the PQ-9 biosynthesis it is: Homogentisate + Solanesyl-PP ⟶ Me-6-SQH_2 ⟶ 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

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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_2PQH_2 - 2,3-dimethyl-5-phytylquinol; Me_3PQH_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 $\xrightarrow{SAM}$ 2,3-Me_2PQH_2 $\longrightarrow$ γT $\xrightarrow{SAM}$ 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_2PQH_2 $\longrightarrow$ 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.

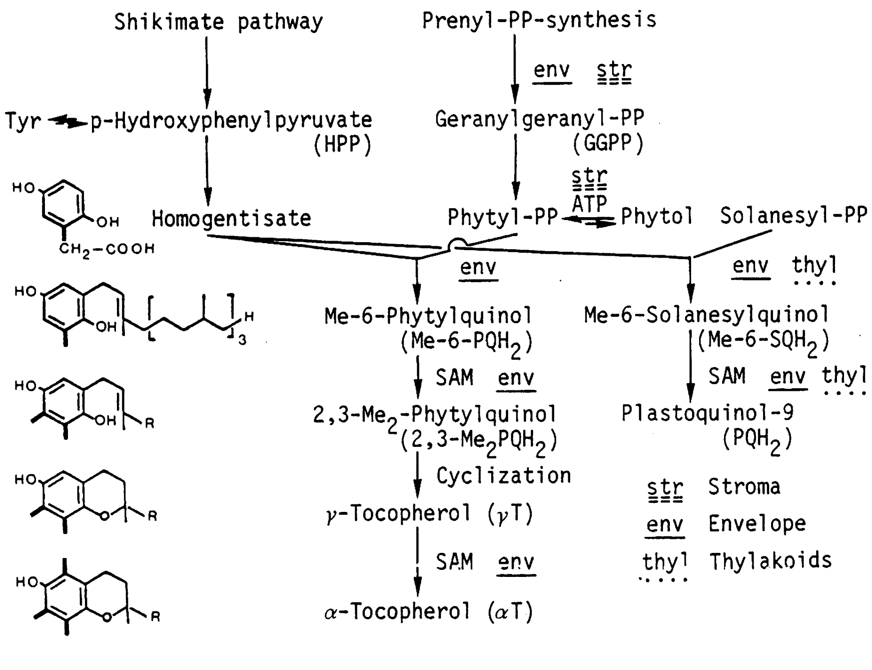

Fig. 1. Biosynthesis of αT and PQ-9 in spinach chloroplasts.

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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