Vol. 91, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 14, 1979 Pages 715-720

COMPARISON OF GERANYLGERANYL AND PHYTYL SUBSTITUTED METHYLQUINOLS IN THE TOCOPHEROL SYNTHESIS OF SPINACH CHLOROPLASTS

Jürgen Soll[1] and Gernot Schultz[2]
[1]Institut für Klinische Biochemie und Physiologische Chemie, Medizinische Hochschule Hannover, and
[2]Institut für Tierernährung, Arbeitsgruppe für Phytochemie und Futtermittelkunde, Tierärztliche Hochschule Hannover, D-3000 Hannover 1

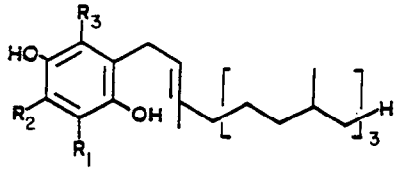
Received October 8, 1979

SUMMARY: Geranylgeranyl substituted methylquinols are shown to be precursors of tocopherol biosynthesis in spinach chloroplasts as well as phytyl substituted ones. The geranylgeranyl substituted quinols are methylated even to a greater extent than the phytyl substituted ones. The connection to the so far known biosynthetic origin of α-tocopherol is probably -tocotrienol which is hydrogenated to γ-tocopherol and then further methylated to α-tocopherol.

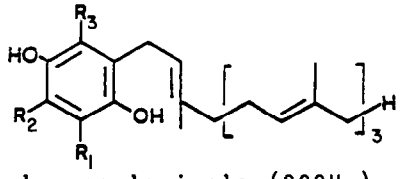
INTRODUCTION: Tocopherols are synthesized from homogentisic acid and a C_{20} prenol unit to form the 2-methyl-6-prenylquinols (1). Using 2-methyl-6-phytylquinol as precursor for the tocopherol synthesis by chloroplasts the following sequence of products has been found: Me-6-PQH_2 → 2,3-Me_2PQH_2 → γT → αT (2). Since tocotrienols have been found in several plant species (3,4) and hydrogenation of tocotrienols to tocopherols by $NADPH_2$ has been shown (5) it seems possible that the precursors of αT are geranylgeranyl and not phytyl substituted methylquinols. So it has been examined whether the enzymatic methylation of 2-methylprenylquinols by SAM to form αT exhibit a specifity for the different prenyl side chains.

METHODS: The geranylgeranyl and phytyl substituted methylquinols and quinones were prepared from the corresponding methylquinol with trans-geranyllinalool and isophytol by the procedure described in (2), γT_3 was synthesized according to (6). αT_3 was a gift from Dr. F. Weber, Fa. Hoffmann-La Roche, Basle. ^{14}C/Me-SAM (sp. act. 50 mCi/mmol) was purchased from Amersham Buchler, Braunschweig.

0006-291X/79/230715-06$01.00/0



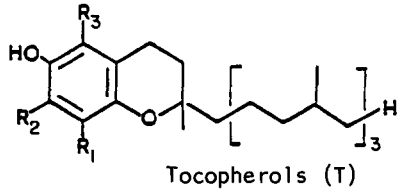
Methyl-phytylquinols (PQH_2)



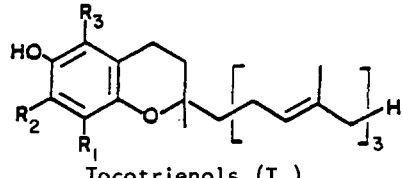
-geranylgeranylquinols ($GGQH_2$)

R_1	R_2	R_3					
CH_3	H	H	Me-6-PQH_2	2-Methyl-6-	} PQH_2	Me-6-$GGQH_2$	6-Methyl-	} $GGQH_2$
CH_3	CH_3	H	2,3-Me_2PQH_2	2,3-Dimethyl-5-		2,3-Me_2GGQH_2	2,3-Dimethyl-5-	
CH_3	CH_3	CH_3	Me_3PQH_2	Trimethyl-		Me_3GGQH_2	Trimethyl-	

The corresponding quinones are signified in the text with Q instead of QH_2.



Tocopherols (T)



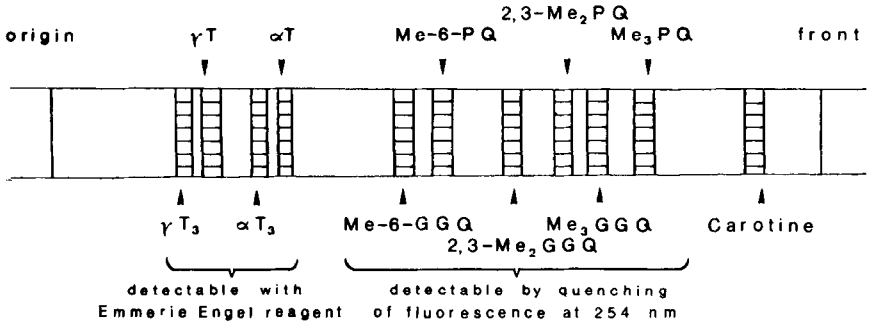
Tocotrienols (T_3)

Tocochromanols

R_1	R_2	R_3		
CH_3	H	H	δT	δT_3
CH_3	CH_3	H	γT	γT_3
CH_3	CH_3	CH_3	αT	αT_3

"Broken chloroplasts" were prepared from intact spinach chloroplasts isolated according to ref. (7) as modified by Prof. U. Heber (personal communication). Several times intact chloroplasts were used directly with similar results. To get "broken chloroplasts" the pellet resulting from centrifugation of medium B chloroplasts suspension (7) was suspended with dist. water for 10 min at 0°. The chlorophyll content was at least 2 mg/ml. Only nondifferentiated leaves from young plants are able to synthesize prenylquinols to a higher extent.

The application of substrates to chloroplast suspension for methylation and cyclization reaction in tocopherol synthesis was performed as follows: 1000 µl chloroplast suspension in medium C (7) (about 1 mg chlorophyll/ml) or 500 µl "broken chloroplasts" suspension diluted with 500 µl medium C, 100 µl 0,2 M $NaHCO_3$, 50 µl /Me-^{14}C/ SAM (= 2,5 µCi, soluted in 0,1 N H_2SO_4), substituted methylprenylquinol as substrate (10^{-7} and 10^{-8} mol., respectively, evaporated from etheral solution) were added into a test vial. The resulting suspension was illuminated by halogen lamps (0,1 J/cm^2 x s) in a water bath at 20° ± 2° for 30 min.



Scheme 1: Sequence of prenylquinones and tocochromanols on TLC system I

Isolation and purification of investigated substrates were performed as in ref (2). All quinols were oxidized for a more efficient purification by air supply before TLC on system I (for sequence of detectable substances see Scheme 1). All substances were cochromatographed with reference substances. All methylprenylquinones could be rechromatographed as single substances on system II, whereas tocopherols and tocotrienols could be rechromatographed only in couples of γT + γT_3 and αT + αT_3, respectively.

The following TLC-systems were used: System I - silicagel with petrol (b.r. 60 - 80°)/Et_2O (7 : 1); system II - reversed phase system (= cellulose layers impregnated with 7 % paraffin in petrol (b.r. 100 - 140°)) with Me_2CO/H_2O (85 : 15). All thin layers (Schleicher & Schüll, Germany) on glass contained a fluorescent indicator F 254. System I was developed to 20 cm height, system II to 10 cm.

RESULTS: Monomethylcompounds as substrates: Studying the incorporation of /Me-^{14}C/ from /Me-^{14}C/ SAM into Me-6-$GGQH_2$ and Me-6-PQH_2, respectively, a strong preference of the geranylgeranyl-compound could be observed. The incorporation rates were up to 4,5 times higher as for the phytyl compound. In the case of Me-6-GGH_2 the total amount of fixed ^{14}C of applied /Me-^{14}C/ SAM was about 1 %. As described in (2) the first step was the methylation to the 2,3-dimethylprenylquinol (for stereospecifity of reaction see 2), the second step was the cyclization to the corresponding dimethyltocochromanol and the last step was the methylation to the trimethyltocochromanol. The novel point was the hydrogenation of the geranylgeranyl moiety to the phytyl ones. Only traces of 2,3-Me_2PQH_2 beside γT_3, γT and αT were found. Thus it might be concluded that hydrogenation occurs on the chromanol stage. Adding 10^{-4} M of Me-6-$GGQH_2$ the relation of /Me-^{14}C/ incorporation in these compounds were as follows:

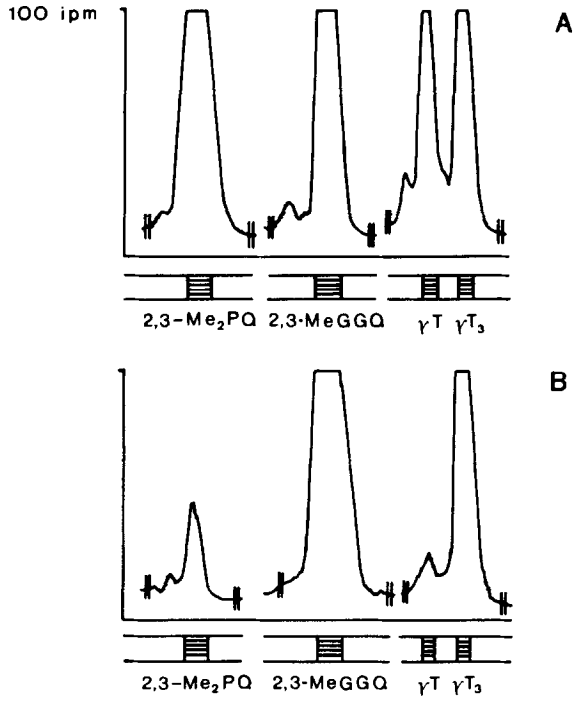


Figure 1: Radioscan of products obtained by incorporation of /Me-^{14}C/ from /Me-^{14}C/ SAM into Me-6-GGQH$_2$ plus Me-6-PQH$_2$ applied in different concentrations (competition experiment):
A. 10^{-5} M Me-6-GGQH$_2$ plus 10^{-4} M Me-6-PQH$_2$ as substrate;
B. 10^{-4} M Me-6-GGQH$_2$ plus 10^{-5} M Me-6-PQH$_2$ as substrate.
The figure shows the re-chromatography of products on system II. For further experimental details see Methods.

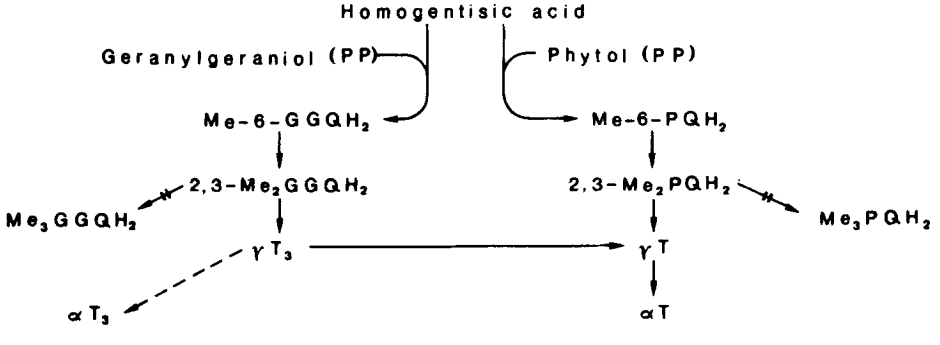
2,3-Me$_2$GGQH$_2$: 2,3-Me$_2$PQH$_2$: γT$_3$: γT : αT = 100 : traces : 59 : traces : 18. Using 10^{-4} M Me-6-PQH$_2$ as substrate the values were as follows: 2,3-Me$_2$PQH$_2$: γT : αT = 100 : 60 : 30. As it can be seen in Figure 1 a competition of Me-6-GGQH$_2$ and Me-6-PQH$_2$ could be observed if these substrates were combined in different concentrations in the chloroplast experiment.

Dimethyl compounds as substrates: 2,3-Me$_2$GGQH$_2$ and 2,3-Me$_2$PQH$_2$ were incorporated into αT in similar rates. The optimal concentration of 2,3-Me$_2$GGQH$_2$ to form αT is about 10^{-5} M which fairly agreed with 2,3-Me$_2$PQH$_2$ (2). No radioavtivity could be proved in the trimethylprenylquinones. When 2,3-Me$_2$GGQH$_2$ was applied, no αT$_3$ could be detected. On the other hand, when γT$_3$ was added, αT$_3$ was found. The optimal concentrations of γT and γT$_3$ to form trimethyltocochromanols were similar to the above dimethylpre-

nylquinols. As could be deduced from ref. (5) additional $NADPH_2$ should result in a more complete hydrogenation of the unsaturated prenyl side chain. Under present conditions, however, an addition of 10^{-4} M $NADPH_2$ was not effective in case of Me-6-$GGQH_2$, 2,3-Me_2GGQH_2 and γT_3. Obviously $NADPH_2$ supply by photosynthetic NADP reduction in thylakoids of illuminated chloroplasts is sufficient for the - presumably saturated - prenyl-hydrogenation system.

If no substrate was added, only αT was obtained in reasonable amounts from the endogenous substrate pool. Application of dimethylprenylquinols increases the amount of labeled αT about twofold.

DISCUSSION: The higher methylation rate of Me-6-$GGQH_2$ in comparison to Me-6-PQH_2 indicates that even in spinach chloroplasts geranylgeranyl substituted methylquinols can be precursors for the tocopherol synthesis. In case of Me-6-$GGQH_2$ the resulting 2,3-Me_2GGQH_2 is converted to γT_3 and then hydrogenated to γT. The only yield of αT_3 by feeding γT_3 and not by 2,3-$MeGGQH_2$ also points to a saturation reaction on a step between 2,3-Me_2GGQH_2 and γT. This probably represents the connection to tocopherols. The absence of trimethylprenylquinones shows that the cyclization to the chromanol stage is restricted to the dimethylprenylquinol stage. The methyltransferase reactions has not only a fairly high specifity for the methyl position at the monomethyl prenylquinol (2), but also for the different prenyl side chains. This might be an indication that the isoprens both phytol and geranylgeraniol (or its pyrophos-



Scheme 2: Proposed scheme for the biosynthesis of tocopherols

phates) could be substrates for the prenyl-ligase to form 2-methyl-6-prenylquinol. From the results two possible pathways of tocopherol synthesis are proposed in Scheme 2.

ACKNOWLEDGEMENTS

Financial support by the Deutsche Forschungsgemeinschaft is thankfully acknowledged. We thank Mrs. D. Bodenstab for excellent technical assistence.

References

1. Whistance, G.R., and Threlfall, D.R. (1970) Biochem.J. 117, 593 - 600.
2. Soll, J., and Schultz, G. (1979) Phytochemistry, accepted for publication.
3. Chow, C.K., Draper, H.H., and Csallany, A.S. (1969) Anal. Biochem. 32, 81 -90.
4. Janiszowska, W., and Pennock, J.F. (1976) Vitam. Horm. (N.Y.) 34, 77 - 105.
5. Wellburn, A.R. (1970) Phytochemistry 9, 743 - 748.
6. Mayer, H., and Isler, O. (1971) Methods in Enzymology, Vol. 18 C, p. 241 - 348, Academic Press, N.Y.
7. Jensen, R.G., and Bassham, J.A. (1966) Proc. Natl. Acad. Sci. U.S. 56, 1095.