The Biosynthesis of Tocopherols and Related Compounds in the Blue-Green Alga *Anabaena variabilis*

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Tyrosine has been shown to provide the nucleus and one nuclear methyl group of the tocopherols and plastoquinone through the homogentisate pathway (Whistance & Threlfall, 1967). The 8-methyl group in the tocopherols arises from the β -carbon atom of tyrosine, and the 5- and 7-methyl groups appear to be derived from methionine (Threlfall, Whistance & Goodwin, 1968). Studies reported so far have been on α - and γ -tocopherol and to a lesser extent δ -tocopherol, but no work has been carried out on β -tocopherol. The blue-green alga *Anabaena variabilis* contains both α - and β -tocopherol (Powls & Redfearn, 1967), and this communication shows that tyrosine and methionine are incorporated into β -tocopherol, which does not appear to be a precursor of α -tocopherol in this organism.

A. variabilis was grown in 100 ml batches in the medium C of Kratz & Myers (1955) at 34°C in air supplemented with 5% of CO₂. Approx. 5 μ Ci of DL- $[\beta$ -¹⁴C]tyrosine or L- $[Me$ -¹⁴C]methionine was added to each flask before inoculation and two such flasks were used in each experiment. The algae were harvested at about 4 days and were extracted with acetone and boiling ethanol. The combined extracts were diluted with water and extracted with diethyl ether–light petroleum (b.p. 40–60°C) (1:2, v/v) and the solvent was removed. The mixture was applied to a Brockmann grade 3 acid-washed alumina column. Elution with diethyl ether–light petroleum (1:3, v/v) yielded the required fraction. The lipids were examined for radioactivity by radioautography after two-dimensional t.l.c. on silica gel G adsorbent, with chloroform followed by diethyl ether–light petroleum (1:3, v/v) as solvent, or by purifying individual compounds by adsorption and partition t.l.c. in several solvent systems and counting in a scintillation counter (Whitte, Dunphy & Pennock, 1966.) Chromatograms were also examined at each stage with a radioactivity scanner.

The radioautogram showed that tyrosine had been incorporated into four compounds only, α - and β -tocopherol, α -tocopherolquinone and plastoquinone (Table 1). The incorporation of label from tyrosine into α -tocopherol, α -tocopherolquinone and plastoquinone in higher-plant tissues has been shown by Whistance & Threlfall (1967), but radio-

activity in β -tocopherol has not been reported previously. β -Tocopherol is relatively rare in the green tissue of higher plants, the isomeric γ -tocopherol being more common, but β -tocopherol can be found in quite large amounts in some cereal seeds (Green, 1958). A small quantity of β -tocopherol can be detected in wheat seedlings (Hall & Laidman, 1966), but it does not seem to be synthesized in the shoot and is presumably transported there from the seed (I. R. Peake & J. F. Pennock, unpublished work). Measurements on another batch of *A. variabilis* cells revealed 8.95 μ g of α -tocopherol and 1.09 μ g of β -tocopherol/mg. of chlorophyll *a*, so that the specific radioactivity of β -tocopherol is slightly higher than that of α -tocopherol (cf. Table 1). These results are consistent with β -tocopherol being methylated in the cell to α -tocopherol.

When $[\beta$ -¹⁴C]tyrosine is given to higher plants labelling can be found in many terpenoids (into which tyrosine is not specifically incorporated) and in some cases fatty acids, the radioactivity presumably arriving via the pathway tyrosine $\rightarrow$ *p*-hydroxyphenylpyruvate $\rightarrow$ homogentisate $\rightarrow$ acetoacetate and fumarate. Acetoacetate can be incorporated into fatty acids or form mevalonate. Terpenoids present in *A. variabilis*, such as sterols, carotenoids and phylloquinone, were not labelled, and thus the metabolism of homogentisate to acetoacetate and fumarate may not occur in this organism.

Table 1. *Incorporation of radioactive tyrosine and methionine into terpenoids in Anabaena variabilis*

Anabaena variabilis was grown for 4 days in the presence of the radioactive precursor (10 μ Ci). The lipids were extracted and compounds isolated after adsorption and partition t.l.c. and their radioactivities were then counted in a scintillation counter.

	Radioactivity incorporated (c.p.m.)	
	DL- $[\beta$ - ¹⁴ C]- Tyrosine	L- $[Me$ - ¹⁴ C]- Methionine
α -Tocopherol	3 566	2 394
α -Tocopherolquinone	14 370	2 054
β -Tocopherol	515	898
Plastoquinone	6 510	12 602
Phylloquinone	—	10 166

With methionine as precursor radioactivity was detected in phylloquinone, as well as in α - and β -tocopherol, α -tocopherolquinone and plastoquinone as expected (Threlfall *et al.* 1968). However, most of the label was found in two chromatographically non-polar compounds that were not investigated further at this stage since they were not labelled from tyrosine. The incorporation of methionine into β -tocopherol is significant in that it gives strong confirmation that the 5-methyl group of tocopherols arises from methionine. It has been reported that the 8-methyl group of tocopherols does not come from methionine, since δ -tocopherol (8-methyltolcol) is not labelled from methionine (Threlfall *et al.* 1968), and since γ -tocopherol (7,8-dimethyltolcol) is labelled then clearly the 7-methyl group originates from methionine. As α -tocopherol (5,7,8-trimethyltolcol) contains a 7-methyl group radioactivity is expected in it from labelled methionine and no information can be gained about the 5-methyl group. The labelling of β -tocopherol (5,8-dimethyltolcol) in experiments reported here with *A. variabilis* indicates that the 5-methyl group comes from methionine.

It has been suggested that γ -tocopherol could be the precursor of α -tocopherol in some tissues (Whistance & Threlfall, 1968; Threlfall *et al.* 1968). but in *A. variabilis*, where there is no γ -tocopherol, β -tocopherol may be the more likely precursor. To investigate this possibility, cells of *A. variabilis* were grown for 4 days and then the culture (5 litres) was divided into two portions and the cells were harvested. After being washed in 0.05M-potassium

phosphate buffer, pH 7.4, the cells were resuspended in phosphate buffer containing [β - 14 C]tyrosine (7 μ Ci in each half). The flasks were incubated at 34°C, one for 6 h and the other for 24 h, after which the lipids were isolated as described above and the radioactivity was measured. At 6 h the values were β -tocopherol 34 c.p.m. and α -tocopherol 1170 c.p.m., and after 24 h 446 c.p.m. and 603 c.p.m. respectively. β -Tocopherol is formed more slowly than α -tocopherol and it is therefore unlikely to be a precursor of α -tocopherol. The fall in radioactivity in α -tocopherol in the 24 h relative to the 6 h experiment may reflect its relatively rapid turnover, since the concentration of [14 C]tyrosine in the medium is likely to be much lower at 24 h than at 6 h. Thus, other than having a common precursor, α - and β -tocopherol are not linked biosynthetically.

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- Green, J. (1958). *J. Sci. Fd Agric.* **9**, 801.
Hall, S. G. & Laidman, D. L. (1966). *Biochem. J.* **101**, 5r.
Kratz, W. A. & Myers, J. (1955). *Am. J. Bot.* **42**, 282.
Powls, R. & Redfearn, E. R. (1967). *Biochem. J.* **104**, 24c.
Threlfall, D. R., Whistance, G. R. & Goodwin, T. W. (1968). *Biochem. J.* **106**, 107.
Whistance, G. R. & Threlfall, D. R. (1967). *Biochem. biophys. Res. Commun.* **28**, 295.
Whistance, G. R. & Threlfall, D. R. (1968). *Biochem. J.* **109**, 577.
Whittle, K. J., Dunphy, P. J. & Pennock, J. F. (1966). *Biochem. J.* **100**, 138.