INCORPORATION OF [G-^{14}C]SHIKIMATE AND [U-^{14}C]PARA-HYDROXYBENZOATE INTO PHYTOQUINONES AND CHROMANOLS*

G.R. Whistance, D.R. Threlfall and T.W. Goodwin

Department of Biochemistry and Agricultural Biochemistry,
University College of Wales, Aberystwyth

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Para-hydroxybenzoic acid (PHBA) and p-hydroxybenzaldehyde, compounds which in microorganisms and higher plants can be formed by the shikimic acid (SKA) pathway of aromatic biosynthesis, are effective precursors of the ubiquinone (Q) ring in a variety of organisms (see Miller 1965). An intermediate in the conversion of PHBA to Q has been isolated from Rhodospirillum rubrum and characterised as 2-decaprenylphenol (Parson and Rudney, 1965; Olsen et al., 1965). Cox and Gibson (1964) showed that in Escherichia coli the Q and menaquinone rings are derived from shikimic acid, PHBA and protocatechuic acid, respectively, appearing to be obligate intermediates.

In this communication we present evidence which indicates that in maize shoots the nuclei of phylloquinone (K), plastoquinone (PQ), ubiquinone (Q), α-tocopherolquinone (α-TQ), α-tocopherol (α-T) and γ-tocopherol (γ-T) are all formed from SKA. In this and all other plant tissues examined PHBA was incorporated only into Q and an uncharacterised prenyl-containing phenol which may be a Q-precursor.

EXPERIMENTAL

Etiolated (dark grown) six day old maize shoots were excised and the cut ends dipped in an aqueous solution of the radiosubstrate. The plants were then illuminated for 24-hours. Under these conditions a marked synthesis of K, PQ and α-TQ takes place (Griffiths et al., 1966).

* G and U indicate generally and uniformly labelled compounds respectively.

Other higher plant tissues (5-10 g fresh weight) were sliced into small pieces and dampened with an aqueous solution of the substrate. Ochromonas danica (light grown) was harvested and resuspended in a solution of the substrate.

At the end of the experimental period the lipid was extracted and chromatographed on a column of Brockmann Grade III acid-washed alumina (Woelm) (Threlfall et al., 1965). Quinones and other terpenoids (chromanols, phytosterols and β-carotene) from the various column fractions were purified by adsorptive and reversed phase chromatography on thin layers of Kieselgel G (Griffiths et al., 1966).

Quinones and chromanols were estimated spectrophotometrically (Griffiths et al., 1966) and their [^{14}C] activity determined by liquid scintillation counting. Chromatograms were tested for [^{14}C] activity by scanning or autoradiography.

STUDIES WITH [U-^{14}C]PHBA

(a) Maize shoots

Etiolated shoots (300) were exposed with continuous illumination for 24-hours to 2μC [U-^{14}C]PHBA (prepared from [U-^{14}C]L-tyrosine (5.5μC/μmole) by alkaline fusion (Parson and Rudney, 1964)). On [^{14}C] assay of the various fractions obtained following column chromatography activity was found only in the Q-containing fraction (2% administered dose). K, PQ, T, α-TQ and phytosterol-containing fractions were essentially inactive; further purification and [^{14}C] assay of larger samples of these components demonstrated that they were completely inactive.

Q (407 μg; 169,000 disintegrations/min/mg) was purified by repeated chromatography on thin layers of Kieselgel G with benzene, chloroform and benzene - chloroform ($^{1}/_{1}$, $^{v}/_{v}$) as developing solvents. In all systems the specific activity of the recovered Q was constant (171,000 disintegrations/min/mg) and radioscans and radioautographs

confirmed that all the [^{14}C] activity in the original fraction had remained with Q. However, on running the sample on reversed phase thin layer a sharp drop in specific activity was obtained (116,500 disintegrations/min/mg) indicating the presence of other [^{14}C] components. Radioscans and radioautographs showed the presence of a second radioactive compound (compound X) running ahead of Q-9 (the homologue found in maize) with R_F similar to Q-8 (R_F Q-9 = 0.54; R_F Q-8 = 0.63), and accounting for some 30% of the [^{14}C] activity in the fraction. The presence of [^{14}C] activity in Q-9 was confirmed by converting the quinone into the quinol (with $NaBH_4$) and rerunning in the reversed phase system, when the [^{14}C] activity migrated with the quinol. The absence of [^{14}C] activity in phytosterols, the skeleton and supernumerary methyl groups of which are derived from the same precursors as the prenyl side chains and ring and O-methyls, respectively, of phytoquinones, lead to the conclusion that PHBA has been incorporated *in toto* (apart from probable loss of the carboxyl group) into the nuclear portion of Q.

Further experiments showed that compound X has selective absorption bands in the u.v. at λ_{max} 276 and 283 mμ, is phenolic in character (positive Emmerie-Engel reaction) and has a terpenoid side chain ([2-^{14}C]MVA is incorporated into compound X). The possibilities that compound X is 2-nonaprenylphenol, ubichromenol (which has similar properties) or an artifact have been eliminated. In view of its apparently high specific activity, compound X may well be a Q-precursor; some support for this has been obtained from a pulse-labelling experiment.

(b) *Other plant tissues*

In short term incubation experiments using maize roots, french bean leaves and cotyledons, and the alga *O. danica* we have been able to demonstrate the incorporation of [^{14}C] activity from [U-^{14}C]PHBA only into Q and compound X. It is significant that in tissues containing

Q-10, the next higher homologue to that found in maize, X also behaves as the next higher homologue i.e. runs with R_F similar to Q-9 instead of Q-8. In these experiments, with the exception of O. danica, incorporations of the order 2-3% were obtained in 3-6 hours. The incorporation into the alga was 0.4%.

STUDIES WITH [G-^{14}C]SKA

Etiolated maize shoots (300) were exposed to 4μC [G-^{14}C]SKA with continuous illumination for 24-hours. Table I summarizes the incorporation of [^{14}C] activity into the components examined.

TABLE I

Component	K, α-TQ and X	PQ	Q-9	α-T	γ-T
[^{14}C] activity (d./min./mg.)	All active	3,340	7,860,	4,250,	13,370

The incorporation of [^{14}C] activity into all components was low, for example, Q + X accounted for only 0.055% of the available activity compared to 2% when [U-^{14}C]PHBA was administered. This is to be expected when such a distal precursor as SKA is used, since it can enter many alternative biosynthetic sequences. Radioautographs of the thin layer chromatograms used in the purification procedures showed the presence of bands, containing small amounts of [^{14}C]activity, which could not be correlated with known compounds and thus may represent possible quinone precursors. Phytosterols and β-carotene were unlabelled, which demonstrates the specific incorporation of SKA into the compounds listed in Table I.

SUMMARY

[^{14}C] Activity from [G-^{14}C]SKA was incorporated into K, PQ, Q, α-TQ, α-T and γ-T by maize shoots. When [U-^{14}C]PHBA was used, however, [^{14}C] activity was found only in Q and an uncharacterised prenyl-containing phenol; this was shown for all tissues examined.

These results suggest that in higher plant tissues the nuclear portions of the biologically important terpenoid quinones and their derivatives are formed by the shikimic acid pathway of aromatic biosynthesis. In this connection it will be interesting to see which portion of the naphthoquinone ring of K arises from SKA.

PHBA, in agreement with other observations, was incorporated into Q. A phenolic compound, which is not a 2-prenylphenol, was also labelled and this may prove to be a Q-precursor. The failure of PHBA to be incorporated into other quinones could reflect either a specific requirement of PHBA for Q synthesis, or the failure of this compound to penetrate the chloroplast membrane and reach the proposed site of plastidic terpenoid quinone synthesis (Threlfall and Griffiths, 1966).

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