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Nature and Distribution of Terpene Quinones

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The terpene quinones observed in Nature are, according to their quinone component, divided into phyloquinone, menaquinones, ubiquinones and plastoquinones. Variations within the particular groups are caused by the length of the side chain, the number of double bonds and, for a few of them, the oxygen function within the side chain.

Plants for phyloquinone and micro-organisms for menaquinones were identified exclusively as places of origin. However, animals, micro-organisms and plants are able to synthesize ubiquinones.

Animals also ingest terpene quinones with their food. Thus the question arises how the administered quinones are distributed, metabolized and excreted. Two methods are particularly suitable for observing the distribution in the various organs and tissues:

- (1) administration of labelled compounds and localization of the radioactivity in the tissues after combustion;
- (2) radioautographic studies in the whole animal.

Results obtained up to now in the rat show that distribution and excretion in certain organs depend mainly on the length of the side chain. The degree of saturation of the side chain, too, seems to exert a certain influence.

Excretion takes place via bile and urine. The radioactive quinones are apparently excreted unchanged in the bile, but metabolites are found in the urine. Independent of the nature of the quinone component and the length and degree of saturation of the side chains, the latter are shortened to seven carbon atoms, and γ -lactones are formed.

Ubiquinones and plastoquinones were also studied with respect to their intracellular distribution. In plants as well as in animal cells, mitochondria are enriched with ubiquinone; however, plastoquinones are found in the chloroplasts.

The Biosynthesis of Terpenoid Quinones†

By HARRY RUDNEY. (*Department of Biological Chemistry, College of Medicine, University of Cincinnati, Cincinnati, Ohio, U.S.A.*)

Most of the work on this subject before 1966 has been well documented in monographs and reports of the proceedings of several symposia (see, e.g., Morton, 1965; Pridham, 1967; Harris, 1966). This presentation will deal mainly with the synthesis of ubiquinone and reference will be made to other quinones where significant differences appear.

The methyl groups of ubiquinone, both ring methyl and *O*-methyl, have been shown to arise from *S*-adenosylmethionine and the polyprenyl side chain from derivatives of mevalonate. The latter can exist in a variety of chain lengths with one to twelve isoprenoid unit groups in the side chain, depending on the source from which it is derived. The benzoquinone ring, however, has been shown to arise via POB,* and it is presumed that the latter is generated via the shikimate and chorismate pathways. The use of POB has provided a unique probe for tracing the metabolic sequences in ubiquinone biosynthesis, and has played the same role that mevalonate has in steroid and polyisoprenoid synthesis. The use of *p*-hydroxybenzaldehyde and POB as precursors of ubiquinone in *Rhodospirillum rubrum* (Parson & Rudney, 1965a,b)

* Abbreviations: POB, *p*-hydroxybenzoate; IPP, isopentenyl pyrophosphate.

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has recently been reviewed (Rudney & Raman, 1966). It is of significance that in all tissues thus far examined, ranging throughout bacterial, plant, yeast, mould and animal species, the benzoquinone portion of ubiquinone is derived from POB. This is true even in moulds where the acetate-malonate pathway exists for the synthesis of other quinonoid materials (Bentley & Lavate, 1965).

POB was not incorporated into the nuclei of other terpenoid quinones, e.g. plastoquinone, tocopherols and naphthaquinones, which, however, do arise from shikimate. Whistance & Threlfall (1968a,b) have clarified the origin of the benzoquinone portion of plastoquinone and tocopherol by their demonstration that homogentisate derived from tyrosine is the precursor of the ring and one of the attached methyl groups. Other methyl groups are derived from methionine and the polyprenyl chain from mevalonate. 3,4-Dihydroxybenzaldehyde and 3,4-dihydroxybenzoate, which were considered to be possible intermediates in naphthaquinone biosynthesis, are not involved (Whistance & Threlfall, 1968b; Campbell, Cosica, Kelsey & Bentley, 1967), and the question of naphthaquinone ring formation in relation to the shikimate pathway remains to be clarified.

Studies on the incorporation of POB into ubiquinone have revealed the presence of a compound that is a key intermediate in the pathway from POB to ubiquinone in *R. rubrum* (Parson & Rudney, 1965a,b). The structure of this compound was determined to be 2-decaprenylphenol by Olsen *et al.* (1966). This substance provided the first demonstration of the level at which coupling occurred between the polyprenyl side chain and the aromatic nucleus. Subsequently Olsen *et al.* (1966) isolated 6-methoxy-2-decaprenylphenol and presented evidence for its conversion into ubiquinone. On the basis of these findings, as well as others, a plausible scheme for the synthesis of ubiquinone from POB in *R. rubrum* was presented by Friis, Daves & Folkers (1966). The basic features of this pathway are the alkylation of POB by polyprenyl pyrophosphate to form 3-polyprenyl-4-hydroxybenzoate followed by a subsequent decarboxylation to polyprenylphenol. Subsequent steps involve hydroxylation and methylation until the desired structure of ubiquinone was obtained. Polyprenylphenol intermediates have been observed in bacterial species (Rudney & Raman, 1966). Whistance, Threlfall & Goodwin (1967) observed a prenylated derivative of POB in maize shoots, but were unable to detect polyprenylphenols. Similarly such intermediates have not generally been found in animal tissues. However, Olson & Aiyar (1966) observed a prenylated product convertible into ubiquinone in rat liver.

Inability to obtain ubiquinone synthesis in

cell-free systems has hampered clarification of the role of postulated intermediates in the biosynthetic pathway. By a combination of lysozyme and osmotic shock, cell-free systems of *R. rubrum* and *Escherichia coli* were obtained (Rudney & Raman, 1966), which had the capacity to synthesize ubiquinone. Further work has demonstrated that these preparations may have contained viable cells, which might have accounted for the ubiquinone synthesis. Recently, we obtained by ultrasonic treatment and lysis truly cell-free preparations from *R. rubrum* and *E. coli* that can carry out the first two steps of the postulated pathway (Raman, Rudney & Buzzelli, 1969). It was observed that these extracts were able to synthesize 2-polyprenylphenol in moderate amounts, but not ubiquinone. The incorporation of POB into 2-polyprenylphenol by both types of extracts was spectacularly stimulated by the addition of *Micrococcus lysodeikticus* extracts and IPP. This extract has been shown by Allen, Alworth, Macrae & Bloch (1967) to synthesize polyprenyl pyrophosphate compounds ranging from C₃₅ to C₅₀. It was completely inactive with regard to the incorporation of POB into polyprenylphenols. The extract from *E. coli* was also stimulated in the same manner by the *M. lysodeikticus* extract. Both extracts from *R. rubrum* and *E. coli*, especially when supplemented with *M. lysodeikticus* extracts and IPP, incorporated radioactivity from POB into another intermediate in ubiquinone synthesis. *p*-Hydroxy[carboxy-¹⁴C]benzoate was also incorporated into this intermediate, but [methyl-¹⁴C]methionine was not. The chromatographic characteristics of this compound were similar to those of 2-phytyl-4-hydroxybenzoate. Ultrasonically treated preparations of *R. rubrum* converted this compound into 2-polyprenylphenol. On this basis a reasonable structure to assign to this compound is 3-polyprenyl-4-hydroxybenzoate, the first intermediate in the pathway of incorporation of POB into ubiquinone. Nilsson, Farley & Folkers (1968) presented chromatographic evidence for its presence in *R. rubrum*.

The distribution of prenylogues of 2-polyprenylphenol observed in *R. rubrum* extracts supplemented with *Micrococcus* extracts and IPP was the same as that observed in whole cells. IPP without supplementation with *M. lysodeikticus* extracts did not stimulate incorporation of POB into polyprenylphenols. *R. rubrum* extracts are apparently capable of selecting the proper length of polyprenyl pyrophosphate made by *M. lysodeikticus* to meet their special needs.

Further studies on the properties and localization of the enzymes catalysing the first two steps in *R. rubrum* and *E. coli* are being made. Preliminary results indicate that the enzymes catalysing the alkylation and decarboxylation of POB are

membrane-bound. These activities can be solubilized by ultrasonic treatment under certain conditions, and work is under way to separate and purify these enzymes. Various factors that influence these reactions will be described. The formerly observed inhibition of ubiquinone biosynthesis by diphenylamine (Sugimura & Rudney, 1962) can now be located as an action on the first step, since it has been observed that the alkylation of POB is inhibited by this substance. Supplementation of other systems capable of synthesizing terpenoid quinones with this extract should be of great benefit in future work with cell-free systems and should help in clarifying some of the steps in the postulated pathways. This will be especially true where the rate-limiting step is the synthesis of polyprenyl pyrophosphates. We have obtained preliminary results with homogenates of rat kidney, liver and brain tissues which show the presence of a prenylated derivative of POB only after supplementation with the *M. lysodeikticus* extract.

Polyprenols

By F. W. HEMMING. (*Department of Biochemistry, University of Liverpool*)

Polyprenols are those compounds with the general structure $\text{H}-[\text{CH}_2-\text{C}(\text{CH}_3)=\text{CH}-\text{CH}_2]_n-\text{OH}$ in which n is greater than 5. There is a variety of size (n may reach 24) and the shape varies according to the number and location of *cis*, *trans* and saturated isoprene residues.

The prenols are almost certainly formed as pyrophosphates by the usual condensation of isopentenyl pyrophosphate. The endogenous pool size of prenyl pyrophosphate or phosphate is always very small and if a prenol accumulates it is usually as the free alcohol.

The pyrophosphates of all-*trans*-prenols are probably formed primarily as the precursors of side chains of polyisoprenoid quinones. The natural all-*trans*-prenols that have been studied correspond in size to the all-*trans*-polyisoprenoid side chain of the endogenous quinone. Surprisingly, however, there is still no direct evidence that prenyl pyrophosphates do condense with aromatic precursors of the polyisoprenoid quinones.

Of the prenols isolated from tissues the all-*trans* forms are quantitatively relatively minor, the predominant prenols having mixed stereochemistry. Thus the leaves of most angiosperms yield small quantities (<5mg./kg. of leaf) of solanesol (all-*trans*-prenol-9, $n=9$), but considerably more (up to 2g./kg. of leaf) of *cis-trans*-polyprenols. Leaves of angiosperms contain a family of *cis-trans*-polyprenols, the individual members of which are made up of 9, 10, 11, 12 or 13 isoprene residues, usually with the prenol-11 or -12 predominating (Wellburn & Hemming, 1966a; Fukawa, Toyoda, Shimiza & Murohashi, 1966; Dunphy, Kerr, Pennock, Whittle & Feeney, 1967). Each polyprenol contains 3 internal *trans*-isoprene residues, the other internal residues and the OH-terminal isoprene residue (the α -residue) being *cis* (Wellburn, Stevenson, Hemming & Morton, 1967; Stone, Wellburn, Hemming & Pennock, 1967; Feeney & Hemming, 1967; Dunphy *et al.* 1967). It seems likely that these prenols are formed by *cis*-addition to geranylgeranyl pyrophosphate. On the other hand, the wood of the silver birch tree yields polyprenols containing from 6 to 9 isoprene residues (betulaprenols-6 to -9) which contain only 2 internal *trans*-residues and are thus probably formed by *cis*-addition to *trans-trans*-farnesyl pyrophosphate (Lindgren, 1965; Wellburn & Hemming, 1966b).

Although it is known that most of the polyprenols in leaves occur in chloroplasts, and that that remaining is associated with particulate fractions of the cell (Wellburn & Hemming, 1966c, 1967), nothing positive is known of their function. The

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