THE NATURAL OCCURRENCE OF UBICHROMENOL

F. W. Hemming, D. L. Laidman*, R. A. Morton and J. F. Pennock

Johnston Laboratories, Biochemistry Department, The University of Liverpool.

Received March 17, 1961

Ubichromenol, a cyclic isomer of ubiquinone, has been isolated from animal tissues (Laidman, Morton, Paterson and Pennock, 1960) but doubts have been raised subsequently concerning its natural occurrence (Links, 1960; Draper and Csallany, 1960). We have investigated these claims and now report our results.

Conversion of ubiquinone to ubichromenol on alumina

Ubiquinone (50) left on a column of alumina (as purchased) for 24 hr. was converted partially to ubichromenol (Links, 1960). Links concluded that the ubichromenol isolated in this Department is probably not a natural substance, but is an artifact produced in the course of chromatography of ubiquinone on alumina. If the alumina was pretreated with acid or weakened by the addition of water the rate of conversion was decreased. We have reported elsewhere (Hemming, Morton and Pennock, 1961) that ubiquinone (50) adsorbed on acid-washed Brockmann Grade 2 alumina for 1 hr. can be recovered quantitatively. After 2 hr. less than 0.6% of the ubiquinone is isolated as ubichromenol while 98% is recovered unchanged. Longer periods of adsorption increase the amount of ubichromenol formed. Therefore, providing that the ubiquinone is not left on the column for a longer period than is necessary, it can be chromatographed on acid-washed Brockmann Grade 2 alumina with negligible losses.

* In receipt of a Research Studentship from the Agricultural Research Council.

Formation of ubichromenol during standard isolation procedures

The isolation of ubiquinone and ubichromenol from tissue involves saponification of that tissue followed by chromatography of the unsaponifiable lipids on alumina. Draper and Csallany (1960) found that ubichromenol was formed on refluxing ubiquinone in ethanolic KOH. They then isolated ubichromenol from pig kidney by the method of Mervyn and Morton (1959). If ubiquinone was added to the tissue before saponification, 35% of that added was recovered as ubichromenol. No ubichromenol was found in lipid extracted from pig kidney without saponification. They concluded from these results that ubichromenol is not a natural constituent of animal lipids. This work also suggested that the recovery of ubiquinone after saponification of tissues is lower than has hitherto been supposed.

(a) The loss of ubiquinone during tissue saponification

A sample of pig liver was minced and divided into two 20 g. portions. One portion was saponified by the method described later and yielded 0.75 μmole of ubiquinone, estimated after chromatography by borohydride reduction in ethanol (Crane, Lester, Widmer and Hatefi, 1959). The other sample of pig liver was extracted 6 times with a hot mixture of equal volumes of chloroform and methanol. Chromatography of the lipid yielded 0.86 μmole of ubiquinone. Therefore, during saponification, 0.11 μmole of the ubiquinone was lost i.e. 13 % of that originally present.

(b) Ubichromenol in human kidney unsaponifiable lipids

Human kidney contains ubiquinone (50) and the isomeric ubichromenol (50) (Laidman et al., 1960). Ubichromenols (50) and (30) can be distinguished quite easily by reversed-phase partition chromatography on paper using 80% n-propanol/water as mobile phase, according to the method of Eggitt and Ward (1953) for chromatography of tocopherols. Thus if ubiquinone (30) is added to human kidney tissue any ubichromenol

(30)formed in the ensuing isolation procedure can be separated from the ubichromenol (50) present.

Minced human kidney was divided into two 200 g. portions. One portion was saponified by refluxing with 200 ml. of 0.25 % methanolic pyrogallol and 100 ml. of 60 % (w/v) aqueous KOH for 1 hr. The unsaponifiable lipids were extracted with ether and chromatographed on 50 g. of acid-washed Brockmann Grade 2 alumina. The fraction eluted by 6 % ether/petrol contained 15.17 μmoles of ubiquinone (50) and the 8 and 10 % ether/petrol fractions yielded, after rechromatography, 2.00 μmoles of ubichromenol. Reversed-phase partition chromatography of the latter showed only ubichromenol (50)to be present.

To the other portion of minced human kidney tissue, ubiquinone (30) was added (17.43 μmoles)and the isolation procedure repeated. Spectrophotometric assay indicated a recovery of 27.30 μmoles of ubiquinone and 2.40 μmoles of ubichromenol. Since the ubiquinone fraction should contain 15.17 μmoles of ubiquinone (50), 12.06 μmoles of ubiquinone (30) must be present. The ubichromenol fraction was chromatographed as a line using the reversed-phase technique on paper with authentic ubichromenols (50) and (30) as markers. The corresponding ubichromenol bands were extracted and, after removal of paraffin by chromatography on alumina, were assayed spectrophotometrically. The ratio of ubichromenol (50) to (30) was 7:1. Thus of the total ubichromenol (2.40 μmoles), ubichromenol (30) accounts for 0.30 μmoles and this indicates that 1.72 % of the added ubiquinone (30) was recovered as ubichromenol (30).

Since the ubiquinone (50) isolated from human kidney (15.17 μmoles) represents only 87% of that originally present (see section a) then the tissue before saponification must have contained 17.44 μmoles of ubiquinone (50). On a molecular basis this is the same as the amount of ubiquinone (30) added. If it is assumed that, during saponification, both

endogenous and exogenous ubiquinone are converted to ubichromenol at approximately the same rate, then 0.30 μmole of ubichromenol (50) will have been produced. This represents 14-15% of the total ubichromenol (50) isolated.

(c) Ubichromenol in human kidney lipids

The minced tissue (200 g.) from another batch of human kidneys was ground to a dry powder with silver sand and anhydrous sodium sulphate. This was extracted several times with hot ether and methylal. Chromatography of the lipid, as in the above experiments, yielded a ubiquinone fraction (11.05 μmoles) and an impure ubichromenol fraction. The latter was further purified by acetylation followed by chromatography on alumina. Gentle saponification of the ubichromenyl acetate fraction yielded 0.96 μmole of ubichromenol, assayed spectrophotometrically. The ubichromenol value is lower than expected. However, since the ubiquinone level is also low, the extraction of lipid appears to be incomplete and indicates that the method used does not work well with very fibrous tissues. Furthermore a more rigorous purification procedure is needed to obtain the ubichromenol free of lipid contaminants, resulting in further loss of ubichromenol.

These results show that ubichromenol is a natural component of human kidney but also a small amount may be formed during the isolation procedure. The values obtained indicate that 14-15% of the ubichromenol isolated is an artifact of isolation, while the remaining 85% is of natural origin. In some tissues e.g. pig heart and liver, the ubiquinone concentration is relatively high while no ubichromenol is detected and this supports the claim that the ubichromenol isolated above is mainly of natural origin. It has also been shown that ubichromenol can be isolated from human kidney by a procedure not involving saponification of the tissue.

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