

The Isolation and Identification of 2,6-Diiodohydroquinone from the Thyroid Gland

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¹³¹I-labelled 2,6-diiodohydroquinone has been isolated from the thyroid gland of rabbits after a single injection of ¹³¹I, and identified by the use of the reversed isotope dilution technique and by radiochromatography.

The recovery of ¹³¹I in the ¹³¹I-labelled diiodohydroquinone fraction was about 0.2 % of the total ¹³¹I in the thyroid.

The results are discussed with respect to the compound being an intermediate between diiodotyrosine and thyroxine.

The formation of 2,6-diiodobenzoquinone from 3,5-diiodotyrosine by the action of peroxidase has been described previously¹. It was suggested that 2,6-diiodobenzoquinone, or 2,6-diiodohydroquinone, might be an intermediate in the *in vivo* conversion of diiodotyrosine into thyroxine.

Lissitzky and Krottemberg² have reported that 2,6-diiodohydroquinone, or a related compound, could be isolated from the thyroid gland under certain conditions, but no detailed experimental data were given. Allegretti³ has claimed that incubation of thyroxine with liver homogenate leads to the formation of 2,6-diiodo-*p*-benzoquinone and 3,5-diiodophenylalanine.

In this investigation 2,6-diiodobenzoquinone and 2,6-diiodohydroquinone have not been isolated separately. Before the isolation was begun the 2,6-diiodobenzoquinone was reduced to 2,6-diiodohydroquinone.

EXPERIMENTAL

Materials

The following chemicals and apparatus were used:

¹³¹I, Catalogue number IBS 2, The Radiochemical Centre, England.

2,6-Diiodohydroquinone was synthesized according to Elbs and Volk⁴. A commercial product was also used. The melting point of the crystals was 144°C.

Trypsin. Unfractionated trypsin with an activity of 1.14 units/g^{5,6}.

All commercial chemicals were of analytical reagent quality. The solvents used for paperchromatography were freshly distilled before use.

Homogenizer. Glass homogenizer with a Teflon plunger. Catalogue number, 4288-B, Arthur H. Thomas Comp., U.S.A.

Counting equipment. ^{131}I -Labelled compounds measured were in a well-scintillation counter (type-number P-20B, Tracerlab, Inc. U.S.A.) connected to a Single Pulse Height Analyzer (type-number RLI-4, Tracerlab.) and a Superscaler (type-number SC 18 A, Tracerlab.). The high voltage was fed from a Superstable High Voltage Power Supply (type-number 312, Baird Atomic Inc., U.S.A.). The pulse-height analyzer was adjusted to count the gamma emissions of the 0.36 MeV photopeak of ^{131}I . The background was about 24 counts/min.

Methods

The following technique was routinely employed.

Isolation procedure. 100 μC ^{131}I was injected intraperitoneally into each of two rabbits. After 48 h, the animals were sacrificed by injection of air in the ear veins, and their thyroid glands were removed, minced with scissors and transferred to a chilled motor driven homogenizer. The homogenizer tube was surrounded by a beaker of ice during the grinding procedure. The glands were homogenized in 15 ml 0.05 M phosphate buffer pH 7.4 and then incubated in the same solution for 72 h at 37°C with 25 mg unfractionated trypsin and one drop of toluene during continuous stirring. After hydrolysis the pH was adjusted to 2 with 0.1 N sulfuric acid and SO_2 -gas was bubbled through for 20 min. The homogenate was then extracted three times with 10 ml diethyl ether and the ether extracts were combined.

Identification procedure. The compound was identified by the reversed isotope dilution technique and by radiochromatography.

1. *The reversed isotope dilution technique.* To the combined ether fractions was added 200 mg of synthetic 2,6-diiodohydroquinone and 20 ml of water. Before adding, the pH of the water had been adjusted to 2 with 0.1 N sulfuric acid and SO_2 -gas had bubbled through for 20 min at 20°C. The system was then slowly heated on a waterbath, and stirred continuously until the ether evaporated and the diiodohydroquinone dissolved. The solution was filtered, and allowed to stand at 0°C to crystallize the product. The crystals were collected, and washed three times with distilled water (pH 2, SO_2 -saturated, 0°C). An aliquot of the crystals was dried in a desiccator and analyzed as described below. The remaining crystals were redissolved in 15 ml distilled water (pH 2, SO_2 -saturated), heated, filtered and crystallized as described above. This recrystallization procedure was repeated 10 times. The volume of solvent was gradually decreased with each recrystallization. An aliquot of the crystals were, after each crystallization, analyzed by radiochromatography, and the melting point, and specific activity (counts/min/mg dry weight) were measured. The melting point was measured in order to make sure that the carrier material was not decomposed by the recrystallization procedure.

To determine if there was any exchange between free iodine and the iodine bound to the diiodohydroquinone, the following control experiment was conducted. 15 μC ^{131}I was added to 15 ml distilled water, the pH was adjusted to 2 with 0.1 N sulfuric acid and SO_2 -gas was bubbled through for 20 min. The solution was then extracted three times with 10 ml diethyl ether and the ether extracts were combined. 200 mg of synthetic 2,6-diiodohydroquinone was added, recrystallized and analyzed as described above.

2. *Radiochromatography.* The ether extract of the hydrolyzed homogenate was combined with 3 ml of distilled water. The pH was adjusted to 2 with 0.1 N sulfuric acid and SO_2 -gas was bubbled through for 5 min at 20°C. The system was then slowly heated on a waterbath and stirred continuously until the ether evaporated. The solution was filtered.

The following solvent systems have been developed for paperchromatography of 2,6-diiodohydroquinone.

A. Heptane:*n*-propanol:acetic acid:0.001 M sodium thiosulfate = 100:50:1:100 v/v (organic phase).

B. Petroleum ether (boiling range 60–80°C):acetone:0.001 M sodium thiosulfate = 3:1:3 v/v (organic phase).

C. Benzene:0.001 M sodium thiosulfate = 1:1 v/v (organic phase).

D. Acetone:acetic acid:0.001 M sodium thiosulfate = 8:1:60 v/v.

E. *n*-Propanol:acetic acid:0.001 M sodium thiosulfate = 10:1:60 v/v.

Whatman No. 1 filter paper was used and the chromatograms were run at 20°C. The spots were visualized with diazotized sulfanilic acid ⁷. The diiodohydroquinone compound was also visualized with alkaline silver nitrate ⁸ and iodide with 0.1 N ferric nitrate + 3% hydrogen peroxide.

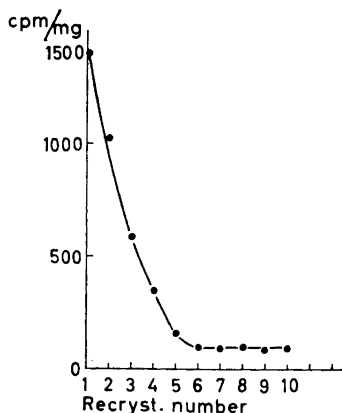


Fig. 1. The specific activity (counts/min/mg dry weight) of the diiodohydroquinone crystals from ten recrystallization experiments.

Synthetic 2,6-diiodohydroquinone was added to the solution before chromatography. In some experiments synthetic 2,6-diiodohydroquinone was not added to the solution but was placed beside the test spot. Both one- and two-dimensional systems were run. When two-dimensional chromatograms were run the order to the solvents was reversed. After visualizing, the papers were cut out in strips and their radioactivity measured. The colored and the radioactive spots were compared. The R_F values of other thyroxine precursors were also investigated.

RESULTS

The specific activity (counts/min/mg dry weight) of the diiodohydroquinone crystals obtained after each recrystallization was measured. It was found that the specific activity gradually decreased at first, but reached a constant value after the 6th recrystallization. The results, corrected for decay and background, are shown in Fig. 1.

Table 1. R_F -values of 2,6-diiodohydroquinone and other thyroxine precursors in system *A* (heptane:acetic acid:0.001 M sodium thiosulfate), *B* (petroleum ether:acetone:0.001 M thiosulfate), *C* (benzene: 0.001 M sodium thiosulfate), *D* (acetone:acetic acid:0.001 M sodium thiosulfate) and *E* (propanol:acetic acid:0.001 M sodium thiosulfate). The different systems are further described in the text.

System	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>
<i>Compound</i>					
Iodide	0	0	0	0.84	0.84
L-Tyrosine	0	0	0	0.80	0.79
3-Monoiodo-L-tyrosine	0	0	0	0.70	0.71
3,5-Diiodo-L-tyrosine	0	0	0	0.59	0.59
L-Thyroxine	0	0	0	0	0
3,5,3-Triiodothyronine	0	0	0	0	0
2,6-Diiodohydroquinone	0.73	0.47	0.60	0.51	0.52

In the control experiment, when ^{131}I was added to synthetic diiodohydroquinone and then recrystallized, the crystals could be completely freed of radioactivity after the first recrystallization experiment.

The melting points for the different crystals were the same (144°C).

The recovery of ^{131}I in the ^{131}I -labelled diiodohydroquinone fraction was about 0.2 % of the total ^{131}I in the thyroid, based on the total amount of ^{131}I in the gland, the specific activity of the crystals and the added amount of synthetic diiodohydroquinone.

The R_F values obtained for 2,6-diiodohydroquinone and other thyroxine precursors are shown in Table 1.

When the colored area on the chromatograms, given by the added sample of synthetic 2,6-diiodohydroquinone, were compared to the radioactive spots a complete coincidence was found.

DISCUSSION

The time interval between injection and sacrificing was 48 h in this experiment. This time interval was chosen according to Taurog and Chaikoff⁹. They investigated the distribution of ^{131}I in rat thyroids at different time intervals and found that after 50 h about 27 % of the total ^{131}I in the thyroid gland was in the thyroxine, about 72 % in the diiodotyrosine and about 1.5 % in the non-protein-bound fraction.

Air oxidation of iodide-131 in acid solution may easily produce $^{131}\text{I}_2$, and the latter might then iodinate organic compounds, especially those added in relatively large amounts as markers for chromatography¹⁰. Control experiments have excluded these types of artefacts in this investigation.

Unidentified ^{131}I -labelled compounds in the thyroid gland have been reported by many authors (*e.g.* Refs.^{11, 12}). It might, however, be possible that many of these unknown compounds are simple oxidation products of iodide¹³, but the possible identity of some of them with 2,6-diiodohydroquinone or 2,6-diiodobenzoquinone must not be disregarded.

The formation of 2,6-diiodobenzoquinone from 3,5-diiodotyrosine by the action of peroxidase has previously been described. It might be possible that 2,6-diiodobenzoquinone or 2,6-diiodohydroquinone, is an intermediate in the conversion of diiodotyrosine into thyroxine in the thyroid gland. This conversion is now being investigated.

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