SYNTHESIS OF 3:3' DIIODOTHYRONINE BY BEEF THYROID MICROSOMES

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Limited enzyme studies have been performed to show the in vitro synthesis of iodinated thyronines. Igo and Mackler (1961) employed modified beef thyroid mitochondria and reported the incorporation of I^{131} and tyrosine into products which appeared by paper chromatography to be monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (TH_3) and thyroxine (TH_4). The formation of TH_4 from DIT catalyzed by a cell-free beef thyroid preparation has been reported in yields of 0.05 - 0.25% (Yip and Klebanoff, 1963). It is the purpose of this communication to report the biosynthesis of radioactive 3:3'-diiodothyronine (3:3'-TH_2) from U-C^{14}-MIT by beef thyroid microsomes.

The C^{14}-MIT was prepared from U-C^{14} -L-tyrosine by a modification of Gemmill's procedure (1955) and was purified by column chromatography on Dowex-1 in the acetate form (Lewallen, 1963). Microsomes were prepared by homogenizing bovine thyroid glands in 0.25 M sucrose, 0.05 M tris-(hydroxymethyl)aminomethane (Tris), pH 7.5, and 0.002 M ethylenediaminetetraacetate (EDTA), pH 7.5. The cell debris, nuclei and mitochondria were sedimented at 12,000 x g for 10 minutes. The supernatant was decanted and centrifuged at 105,000 x g for 60 minutes. The resultant pellet was suspended by gentle homogenization in the sucrose-buffer solution, pH 7.5.

The incubation medium consisted of 10 mM U-C^{14}-MIT, 3.3 mM $MnCl_2$ 3.3 mM NAI, 33.3 mM glucose, 40 mM sucrose, 0.33 mM EDTA, 8 mM Tris, and 200 ug of glucose oxidase. The osmolarity of each flask was adjusted to 0.25 M sucrose by the addition of KCl. In the various experiments, each flask contained from 15 to 25 mg of microsomal protein. After a two hour incubation period at 30° C, the reaction was terminated by exhaustive extraction of the incubation medium with 1-butanol. The extracted material was chromatographed on Whatman No. 1 paper in 1-butanol: 2-butanone: H_2O (10:10:5). The C^{14}-material with an R_f value corresponding to authentic 3:3'-TH_2 was eluted from the paper with ethanol and rechromatographed on Whatman No. 1 paper in 1-butanol: dioxane: 2 N ammonia (4:1:5). In the second solvent system, all of the radioactivity migrated with an R_f identical with 3:3'-TH_2. The C^{14}-material was eluted from the paper with ethanol.

The difference spectra of the eluted material and authentic 3:3'-TH_2 in 0.1 N NaOH and ethanol are shown in Figure 1. Both curves show absorption peaks at 322 mμ and 255 mμ. These difference spectra are clearly distinct from those of thyronine and other iodinated thyronines. Comparison of the absorption spectra of the derivatives from the ninhydrin reaction of 3:3'-TH_2 and the eluted material also suggest that the two compounds are identical (Figure 2). To further substantiate the identification of the C^{14}-material as 3:3'-TH_2, 20 mg of carrier 3:3'-TH_2 were added and crystallized to a constant specific activity of 3.24 mμc/mmole. The yield of 3:3'-TH_2 synthesized in different experiments ranged from 1.5 to 2.5%.

Omission of microsomes or the hydrogen peroxide generating system from the incubation medium prevented the synthesis of 3:3'-TH_2. Horseradish peroxidase could not replace the microsomes in this system. The biosynthetic reaction was inhibited by 0.33 mM KCN. This concentration of cyanide has been reported to have no inhibitory effect on glucose

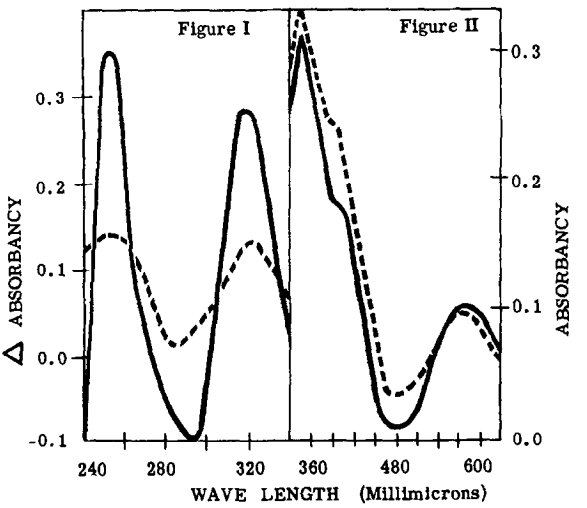

Figure 1. Change in absorbancy of authentic 3:3'-TH_2 (solid line) and synthesized 3:3'-TH_2 (Broken line) in 0.1N NaOH and ethanol.

Figure 2. Spectra of the ninhydrin derivative of authentic 3:3'-TH_2 (solid line) and synthesized 3:3'-TH_2 (broken line). The method of Troll and Cannan (1953) was employed.

oxidase (van Bruggen et al, 1943). If MIT was replaced by tyrosine no enzyme coupling to thyronine occurred.

Preliminary experiments using DIT as substrate resulted in TH_4 synthesis. Pitt-Rivers (1948) has reported the yield of thyroxine to be increased when the charge on the amino and/or carboxyl group of the precursor has been obviated by formation of derivates. When N-acetyl-3:5-DIT or the corresponding carboxyl methyl ester were substituted for MIT, no corresponding TH_4 derivative could be detected.

These results demonstrate that the enzymic coupling of MIT to give 3:3'-TH_2 is catalyzed by a thyroid microsomal preparation. Since a hydrogen perioxide generating system is essential, our results are consistent with the proposal of Johnson and Tewkesbury (1942) that the coupling proceeds via a free-radical mechanism initiated by hydrogen peroxide.

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