IODINE CONTENT OF SUBMITOCHONDRIAL PARTICLES PREPARED FROM RAT LIVER BY DRASTIC SONICATION

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1. Introduction

Injection of hypothyroid rats with L-thyroxine produces significant changes toward normal respiratory control in liver mitochondria within two minutes [1]. These early changes were reversible and thus were more likely primary functional changes due to the presence of the hormone rather than secondary effects also attributed to thyroxine [2]. If the primary action of thyroxine is at the site of oxidative phosphorylation in the mitochondria, the inner membrane, iodine should be present at that locus. Measurements of the amount of iodine in submitochondrial particles prepared by drastic sonication were made to determine if the hormone was present, and to compare with the iodine content in the intact mitochondria.

2. Materials and methods

Iodine was determined by a highly sensitive method based on that of Benotti et al. [3]. To a sample of 0.5 ml was added 0.2 ml of 0.5% Na_2CrO_4 plus 3 ml of 28% chloric acid prepared as in [3]. The mixture was digested thirty minutes at 90°C in an oil bath and then at 145°C for a total time of 3 hr. The digest was shaken with 1 ml of distilled water, then 2.0 ml of 0.3% arsenious acid was added, and incubated 30 min at 37°C. After adding 0.5 ml of 0.5% ceric ammonium sulfate, incubation was continued in a constant temperature water bath at 37°C for exactly 30 min. The disappearance of the yellow color, catalyzed by iodine, was read in a Klett colorimeter at 420 mμ. Calculations were based on an internal standard of 5 mμg of iodine, added as potassium iodate, prepared from the dried analytical reagent (Mallinckrodt). The method measured 5 mμg of iodine with a precision expressed by a 21% coefficient of variation in 13 consecutive samples.

Rat liver mitochondria were prepared [4] in 0.25 M sucrose. Submitochondrial particles were prepared from the mitochondria by a method similar to that of Gregg [5], in tris-HCl buffer prepared from Trizma base (Sigma). The mitochondria were cooled in a chamber by an alcohol flow at 263°K and disrupted with a 60 Kc sonifier (Heat Systems, Inc., Model W 185 G), for 15 min. The sonicated suspension was centrifuged at 110 000 × *g* for 40 min, and finally suspended in 2 ml of water. Three rat livers were used to prepare each submitochondrial assay and two for each mitochondrial assay. Thyroxine-injected rats received 2.5 μg of L-thyroxine sodium pentahydrate (a gift from Smith, Kline and French Laboratories) intraperitoneally per gram body weight per day for three days. All rats were then starved overnight and sacrificed the next day. Protein was determined by a biuret method [6] using Sigma biuret reagent and deoxycholate, with bovine serum albumin (Sigma) as the standard.

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Table 1

Iodine contents of rat liver mitochondria and of particles prepared therefrom by drastic sonication.

	μg iodine/ g protein
Rat liver mitochondria [6]	0.50 ± 0.08
Sonicated submitochondrial particles [5]	1.46*± 0.19
Sonicated submitochondrial particles from 2 groups of 3 rats, each + LT4, 2.5μg/g/d X 3d	27 69

* $p < 0.001$.

The number of assays appears in parentheses; each mitochondrial assay was performed using the livers of two rats, and each particle assay, three rats; the values for particles prepared from two groups of three L-thyroxine-injected rats are shown. Mean iodine contents are given ± SE.

3. Results

The iodine content of mitochondria (table 1) varied from 0.25 to 0.80 μg I/g protein with a mean value of 0.50 ± 0.08 (SE) μg I/g protein. Submitochondrial particles prepared from these mitochondria contained 1.1 to 2.1 μg I/g protein with an average of 1.46 ± 0.19 μg I/g protein. Submitochondrial particles from two groups of thyroxine-injected rats contained 18 and 47 times as much iodine per g protein as those from normal rats. From 13 to 17% (mean = 15%) of the mitochondrial protein was recovered in the particles, whereas iodine recovery was higher, with a range of 31 to 66% and an average of 46%. Thus the three-fold higher iodine content in the particles as compared with the mitochondria is due to iodine accumulation.

4. Discussion

The iodine in mitochondria obtained from the livers of normal rats, or rats previously injected with L-thyroxine, represents mainly thyroid hormone, about 75% being butanol-extractable or -insoluble [7]. The present results demonstrate that iodine, presumably as thyroxine, is associated with the particles that are prepared from the inner membrane of mitochondria by drastic sonication. Since such particles contain the electron-transport and phosphorylative apparatus, the present results are consistent with a direct action of the hormone upon oxidative phosphorylation, as postulated from previous measurements of mitochondrial function and iodine content [1, 2].

The degree of association between mitochondrial iodine and the oxidative phosphorylation apparatus, i.e. whether all the hormone or only part of it is in the particles, is not determined by the present data. In similar submitochondrial particles prepared from beef heart [8] and *S. cerevisiae* [9], the protein represented 67% and 77% of the mitochondrial protein. We recovered 46% of the total iodine in the particles. Measurements of the cytochrome contents of our mitochondria and particles should indicate whether the number of molecules of hormone per respiratory assembly, calculated as 1:200 in mitochondria [7], is different in the particles. The three-fold accumulation of iodine in the normal particles, and the 33-fold increase in the particles from hormone injected rats, speak for at least the same or perhaps a higher stoichiometry between hormone and respiratory assembly.

The association of thyroid hormone with submitochondrial particles that show no respiratory control [5] has implications that will be discussed elsewhere (Hoch, F. L., in preparation).

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