ACCUMULATION OF NON-IODINATED THYROGLOBULIN IN THE THYROID OF GOITROGEN-TREATED HOGS

Osamu Tarutani and Nobuo Ui

Institute of Endocrinology, Gunma University

Maebashi, Japan

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Recent biochemical studies both *in vitro* (Lissitzky *et al.*, 1964 & 1965; Seed and Goldberg, 1965a; Nunez *et al.*, 1965) and *in vivo* (Cavalieri and Searle, 1967) have demonstrated that the iodination of thyroglobulin in the thyroid is a process independent of the protein biosynthesis and occurs following the completion of the polypeptide backbone. The presence of immature thyroglobulin essentially free of iodine was shown in tracer experiments using labeled amino acids. The reported sedimentation coefficient of this newly-formed thyroglobulin 16 ~ 17 S in rats (Seed and Goldberg, 1965b; Cavalieri and Searle, 1967) and 17 ~ 18 S in sheep (Goldberg and Seed, 1965; Nunez *et al.*, 1965), was apparently lower than that of the pre-formed, iodine-containing thyroglobulin (19 S). Difference in stability toward alkali or detergents between iodinated and non-iodinated thyroglobulins has also been indicated (Lissitzky *et al.*, 1964 & 1965; Seed and Goldberg, 1965a; Sellin and Goldberg, 1965).

On the other hand, fractionation studies on purified thyroglobulin have revealed that thyroglobulin consists of a spectrum of molecules with varying iodine content (Ui *et al.*, 1961; Robbins, 1963). However, thyroglobulin without containing iodine has never been isolated from the thyroid at least in an appreciable amount. Only reported was the fact that the mean iodine content of thyroglobulin obtained from the thyroid of goitrous patients or goitrogen-treated animals was considerablly low (Pierce *et al.*, 1965; Perelmutter *et al.*, 1965; de Crombrugghe *et al.*, 1967).

The purpose of this communication is to report our finding that a fairly large quantity of completely iodine-free thyroglobulin could be accumulated in the thyroid of hogs when the animals had been treated with a goitrogen, methylthiouracil. Characterestics of this unusual protein are briefly described.

EXPERIMENTAL PROCEDURES

Animals used in this investigation were castrated male hogs of the White Yorkshire strain and were maintained on a standard diet at the Gunma Zootechnical Experiment Station by the courtesy of Mr. Y. Ishii. The majority were young adults and weighed approximately 90 Kg. In a few experiments much younger hogs were used. On treating animals, they were injected daily with 25 or 30 ml of 20 % methylthiouracil solution ("Methiocil" manufactured by Chugai Pharmaceutical Co., Ltd., Tokyo) for varying periods before sacrifice. After the animal was killed by an electric shock, the thyroid gland was dissected quickly and chilled.

Proteins were extracted from the thyroid gland(s) as far as possible with 0.9 % sodium chloride at pH 6.8 and the extract was subjected to examination in an analytical ultracentrifuge to measure the contents of soluble thyroidal proteins. Purification of thyroglobulin was carried out by salting-out with ammonium sulfate, followed, in most cases, by DEAE-cellulose chromatography in a manner reported previously (Ui et al., 1961; Ui and Tarutani, 1961). Iodine content of thyroglobulin preparations was determined by a modified method of Gross et al. (1948) and corrected for impurity, if present, by assuming that the components other than 18 ~ 19 S protein were free of iodine.

RESULTS AND DISCUSSIONS

Effects of methylthiouracil injection on the tissue contents of thyroidal proteins and iodine content of thyroglobulin are shown in Table 1. The data obtained with untreated hogs (body weight: approximately 90 Kg) which served as control are also tabulated. These animals included the litters of those used for treatments.

From this table, it is seen that increasing the duration of treatment with methylthiouracil caused a gradual decrease in the iodine content of thyroglobulin.

Table 1.

Accumulation of non-iodinated thyroglobulin (TG)

by treatment with methylthiouracil (MTU)

Group*	Body wt. (Kg)	MTU treatment (days)	Wt. of thyroid (g/animal)	Contents of thyroidal proteins			Iodine content in TG (%)
				F-comp.†	TG	Slow comp.	
Control(8)	86.7 ± 2.3	—	8.2 ± 1.7	3.7	57.4	27.0	0.38
Treated(1)	86.0	6	10.8	5.5	86.6	23.1	0.34
Treated(1)	86.5	13	9.6	4.4	83.3	15.4	0.17
Treated(1)	87.0	18	12.0	0.7	32.4	35.8	0.06
Treated(2)	88.3	21	9.5	0.0	2.5	22.2	< 0.01
Treated(1)§	85.5	21	9.0	0.0	8.8	21.6	0.009
Treated(3)	33.8	23	5.3	0.0	3.9	61.6	< 0.01
Treated(3)§	42.8	23	6.3	0.0	17.3	46.8	0.005

* The number of animals employed for each group is indicated in parentheses.

† A faster sedimenting component with $s^{\circ}_{20,w}$ of 27 ~ 28 S.

§ Thyroxine was injected once one or two days before sacrifice.

No appreciable change in the wet weight of the thyroid gland was observed. After methylthiouracil was injected more than three weeks, the isolated thyroglobulin showed an iodine content definitely lower than 0.01 %. This means that the thyroglobulin molecule in these preparations contained less than 0.5 atom of iodine on the average. Very low yield of non-iodinated thyroglobulin was also observed. At this time, histological examination of the gland (by the courtesy of Dr. H. Takahashi of this Institute) indicated that the epithelial cells were hyperplastic and the colloid was almost lost.

A remarkable effect of thyroxine injection at the final stage of goitrogen treatment on the tissue content of non-iodinated thyroglobulin is to be noted. When 20 mg of L-thyroxine was injected to methylthiouracil-treated hogs once one or two days before sacrifice, a three- to five-fold increase in the content

of non-iodinated thyroglobulin per gram of the wet tissue was observed as shown in Table 1. This increase was clearly reflected in the reappearance of colloid as revealed by microscopic observation.

On DEAE-cellulose chromatography at pH 6.5 (for experimental condition, see Ui et al., 1961), non-iodinated thyroglobulin was eluted largely in one fraction at a lower ionic strength. This confirms the previous proposition (Ui et al., 1961) that normal thyroglobulin can be fractionated by DEAE-cellulose chromatography on the basis of difference in the iodine content. After being purified, the preparation of non-iodinated thyroglobulin revealed a single, symmetrical boundary in an ultracentrifuge. The sedimentation coefficient at infinite dilution ($s^{\circ}_{20,w}$) was calculated to be 18.4 S. This value was slightly but definitely lower than that of normal, iodine-containing hog thyroglobulin which showed 18.8 S or a little higher value. In the double diffusion method in agar gel, non-iodinated thyroglobulin and normal thyroglobulin did not show any immunochemical difference.

In accordance with the observation with newly-formed, immature thyroglobulin as revealed by tracer experiments, non-iodinated thyroglobulin obtained in this study was very labile toward treatment with sodium dodecyl sulfate (SDS). It readily dissociated into subunits (S-12) even in 1 mM SDS at pH 6.5, and complete dissociation was attained when the concentration of SDS was increased. This can be compared with the observation with normal thyroglobulin (Tarutani and Ui, to be published) in which a definite percentage of the protein remained undissociated and partially unfolded (S-17) even in 10 mM SDS (heterogeneity of 19 S thyroglobulin with respect to subunit structure was thus suggested). Non-iodinated thyroglobulin, in contrast to normal thyroglobulin, seems to consist entirely of dissociable type of molecules in which two subunits (S-12) are held together by non-covalent bonds.

From these results, it appears likely that non-iodinated thyroglobulin found in goitrogen-treated hogs is equivalent to newly-formed, immature thyroglobulin, the presence of which has been indicated only by radioactivity. Presumably

the occurrence of this non-iodinated thyroglobulin is due to the unusual accumulation of the immediate intermediate protein involved in the thyroglobulin biosynthesis under the prolonged influence of the inhibitor for iodination, methylthiouracil. In view of the well-known effect of goitrogens in increasing the level of thyroid-stimulating hormone (TSH) in blood (see Greer et al., 1964) as well as the present finding on the effect of thyroxine injection on the degree of non-iodinated thyroglobulin accumulation, it might be reasonable to assume that the unbalanced formation and proteolysis of thyroglobulin in the thyroid caused by TSH would also be responsible for the occurrence of non-iodinated thyroglobulin. The detailed mechanism, however, remains to be seen.

It is also to be noted that the result of this investigation not only supports the presumption that iodination of thyroglobulin occurs posterior to the biosynthesis of the polypeptide backbone of this protein, but also suggests that immature thyroglobulin can be released into the colloid without being iodinated previously. The latter statement does not preclude the intracellular iodination of newly-formed thyroglobulin which occurs presumably within the complex network of endoplasmic reticulum. It does, however, support the view that the iodination of thyroglobulin takes place even after it is transfered from the epithelial cell to the follicular lumen. This possibility has also been suggested by radioautographic studies of Nadler et al. (1964).

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