CYTOCHEMICAL ASPECTS ON THE SECRETION MECHANISM OF THE THYROID GLAND

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1. *Orientation*

In the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) are not peptides but characteristic amino acid derivatives, derived from only two molecules of iodotyrosine linked by ether bond (-o-). In all the peptide-secreting cells, the secretory granule, which originates from the rough endoplasmic reticulum and Golgi apparatus system, is released into the connective tissue space. However, formation of T_3 and T_4 in the thyroid requires more complicated steps. The hormones are not synthesized directly from their constituents, tyrosine and iodine in the cell, but a part of their synthetic process takes place in the extracellular lumen, called the follicle lumen. The secretory process of T_3 and T_4 is as follows: (i) synthesis of a large molecular glycoprotein (MW 660,000), called thyroglobulin in the follicle epithelial cell, (ii) release of thyroglobulin into the follicle lumen, (iii) iodination of the tyrosyl residues of thyroglobulin, and coupling of iodotyrosine residues to make T_3 or T_4 in thyroglobulin, (iv) reabsorption of thyroglobulin from the follicle lumen into the follicle epithelial cell, (v) hydrolysis of thyroglobulin for the liberation of T_3 and T_4 , and (vi) release of T_3 and T_4 from the follicle epithelial cell into the connective tissue space. The present paper deals with the outline of cytochemical aspects on the secretion mechanism of the thyroid gland, except for the parafollicular cell, which secretes a peptide hormone called thyrocalcitonin.

2. *Autoradiography of 3H -amino acid*

The follicle epithelial cell has to synthesize a high-molecular-weight glycoprotein, called thyroglobulin. As in other protein secretory cells, the proteinous part of thyroglobulin is synthesized at the attached ribosomes of the well developed rough endoplasmic reticulum and is stored in the cisternae of this organelle. Then the synthesized protein is transported to the Golgi apparatus, where the secretory granules, about 200 nm in diameter, arise. By the electron microscopic autoradiography of 3H -amino acid, the intracellular route for the synthesis and release of thyroglobulin has been clarified (3, 6, 18). In our data, the labeled amino acid first appears in the cisternae of rough endoplasmic reticulum within 15 min after the intraperitoneal injection of 3H -leucine. Then the silver grains begin to appear in the Golgi apparatus and subapical vesicles (secretory granules) after 30–45 min and in the follicle lumen after 45 min (3, 6).

The proteinous part of thyroglobulin has to be bound to carbohydrate elements to become a glycoprotein. Using electron microscopic autoradiography of 3H -mannose, 3H -galactose, and 3H -fucose, the problem concerning the site of incorpo-

ration of carbohydrates into thyroglobulin has been solved (13, 22, 32). It is now believed that mannose is bound to protein in the rough endoplasmic reticulum, while galactose and fucose are bound to protein in the Golgi apparatus.

3. *Silver methenamine method for carbohydrates*

The silver methenamine method is useful for detection of the ultrastructural localization of carbohydrates and glycoproteins. This method was applied for the thyroid gland by several authors (14, 23, 29). In our data (29), the cisternae of the rough endoplasmic reticulum show a weak, but apparently positive reaction. The cisternae of 1–3 inner saccules (lamellae of trans-side) of the Golgi apparatus are intensely stained, while those of 1–3 outer saccules (lamellae of cis-side) are not so heavily stained. Numerous globules of various sizes, considered to be colloid droplets, lysosomes and secretory granules, are strongly positive to this reaction. The Golgi apparatus may have an internal polarity and its inner saccules may have a stronger ability for binding carbohydrates to thyroglobulin. Luminal colloid is also positive with the same intensity as the globules. These findings support the autoradiographic data referred to above that mannosylation of thyroglobulin occurs initially in the rough endoplasmic reticulum, and galactosylation and fucosylation takes place in the Golgi apparatus. It is known that the enzymes necessary for glycosylation such as mannosyl transferase and N-acetylglucosaminyl transferase have been demonstrated biochemically in the rough endoplasmic reticulum, and the galactosyl transferase activity in the Golgi-rich fraction (1).

4. *Localization of ATPase activity*

It has been well known that inorganic iodide is quickly taken up into the thyroid follicle from the blood capillary. This phenomenon is called iodide trapping. Active transport has been proposed for this mechanism and ouabain-sensitive ATPase has been considered to be implicated for iodide trapping. In our data, ATPase activity is demonstrated in the basal as well as the apical plasma membrane in the follicle epithelial cell of the guinea pig (8). The reaction for ATPase in the apical plasma membrane becomes markedly stronger after the injection of TSH.

ATPase activity in the basal plasma membrane of the follicle epithelial cell is considered to have a role in iodide trapping, but it is difficult to determine whether or not the enzymic activity in the apical plasma membrane is related to the transport of inorganic iodide into the follicle lumen. This enzyme might play a role in the absorption of colloid from the follicle lumen into the cytoplasm. The problem remains as to the mechanism by which iodide ion is accumulated selectively in the thyroid gland.

5. *Autoradiography of ^{125}I*

Electron microscopic autoradiography of ^{125}I is a powerful tool for detecting the site of iodination of tyrosine residues in thyroglobulin. Many researchers, using this method, showed that silver grains appear almost entirely in the follicular lumen within a few min or a few hr after the injection of ^{125}I into the mouse, rat, frog, and fish (2, 4, 5, 6, 11, 20). In the mouse thyroid cultured in the medium containing ^{125}I , silver grains appear only in the follicle lumen within 30 sec, and increase rapidly in number with time. However, very few grains are recognized

in the cell cytoplasm. It is considered that the iodination of thyroglobulin takes place almost entirely in the follicle lumen especially at the periluminal region (4). The most primitive thyroid gland in the hagfish also shows similar patterns for the site of iodination of tyrosine in thyroglobulin-equivalent protein (11). In the thyroid-homologous organ called endostyle in the larval lamprey and tunicate (*Ciona intestinalis*), the main site of the iodination of tyrosine in thyroglobulin-equivalent protein is an apical plasma membrane region and may be also in the endostylar lumen (7, 9).

Light microscopic autoradiogram of freeze-dried sections shows that silver grains for iodide are accumulated chiefly over the follicle lumen 2 hr after the injection of ^{131}I into mercaptoimidazole-treated rats, and the silver grains disappear in the fixed, dehydrated, and paraffin-embedded sections obtained from the same animal. This fact means that most inorganic iodide taken up is transported to the follicle lumen.

6. *Localization of peroxidase activity*

It has been believed that endogeneous peroxidase is important for iodination of tyrosyl residues in thyroglobulin. The fine structural localization of endogeneous peroxidase activity in the thyroid has been demonstrated using the DAB method. Reaction products are recognized in the perinuclear cisternae, cisternae of rough endoplasmic reticulum, Golgi saccules, subapical vesicles, and around the external surface of the microvilli in the apical lumen of rat thyroids (6, 19). Similar data has been published by several authors (6, 10, 28, 30). In addition, in the thyroid of lower vertebrates such as frog and fish, and the endostyle of *Ciona intestinalis*, the enzyme activity localizes principally in a similar region to that of rats (10, 21). Periluminal region, subapical vesicles and Golgi apparatus are always positive for this reaction in these animals. This data indicates the possibility that the iodination of tyrosine in thyroglobulin could occur not only in the follicle lumen but also in the cytoplasm, such as rough endoplasmic reticulum, Golgi apparatus and subapical vesicles. Secretory granules for thyroglobulin contain also peroxidase activity (24). As to the data obtained from the ^{125}I autoradiography showing that iodination of tyrosine takes place almost entirely in the follicle lumen, the author considers that thyroglobulin in the follicle lumen is far larger in quantity than that in the cell cytoplasm and numerous molecules of thyroglobulin in the follicle lumen might have not yet been completely iodinated and therefore injected iodine is considered to be combined with luminal colloid preferentially (4). There is an opinion that synthesized peroxidase in the rough endoplasmic reticulum is condensed and matured during the transportation via the Golgi apparatus and subapical vesicles to the follicle lumen (28).

By the data obtained from the autoradiography of ^{131}I in the paper chromatogram of the mouse thyroid, it is clear that iodination of tyrosine residues takes place before the coupling of tyrosine residues to become thyronine in thyroglobulin. Since the iodination of tyrosine is considered to occur almost entirely in the follicle lumen, it is easy to understand that the coupling of iodotyrosine residues to make iodothyronine in thyroglobulin takes place in the follicle lumen.

7. *Freeze-fracture images of apical plasma membrane*

Thyroglobulin stored in the follicle lumen is reabsorbed into the follicle epithelial

cell by phagocytosis as well as micropinocytosis. After injection of TSH, cytoplasmic pseudopods suggesting an engulfment of the luminal colloid appear on the apical surface of the cell and the large colloid droplets are increased in number in the cytoplasm (5, 6, 31). In addition it has been shown that the luminal colloid is reabsorbed by micropinocytosis (26).

In the freeze-fracture image of the apical plasma membrane, intramembrane particles (IMP) are distributed randomly on the protoplasmic face (PF). The particles on the PF of the apical plasma membrane often form aggregates especially in TSH-treated mice (16). Each aggregate, about 200 nm in diameter, and consisting of 15–25 particles, corresponds to a depression of the apical cytoplasm, which is considered to be an initial site for the micropinocytosis of the luminal colloid (16).

In sections obtained from the block stained with tannic acid, it is clear that most of micropinocytotic pits are coated. Some coated vesicles containing reabsorbed thyroglobulin fuse with one another or with a reabsorbed colloid droplet to be a larger colloid droplet.

8. *Localization of AcPase activity*

Thyroid hormones, T_3 and T_4 are liberated by the hydrolysis of reabsorbed thyroglobulin. Acid phosphatase activity is localized in primary and secondary lysosomes, small vesicles, multivesicular bodies, rigid lamellae and the cisternae of 1–2 saccules of trans-side of the Golgi apparatus (24). Small lysosomes, 100–300 nm in diameter and homogeneously dense, are primary lysosomes derived from the Golgi apparatus. These lysosomes are fused with the reabsorbed colloid (thyroglobulin) droplets. AcPase activity is positive in some large colloid droplets which could be called secondary lysosomes. The large colloid droplets change from low to high in electron density, and from negative to positive in AcPase reaction. In the colloid droplet showing AcPase activity, hydrolysis of thyroglobulin takes place for liberating T_3 and T_4 . These hormones, which are amino acid derivatives and very small in molecular weight, may not show any granule form. So the hormones produced by hydrolysis of thyroglobulin are considered to be liberated into the cytoplasmic matrix from the secondary lysosome (AcPase positive colloid droplet). The release mechanism of T_3 and T_4 from the basal part of the cell into the connective tissue space (interfollicular connective tissue) is also obscure.

In mice over 15 months old, heterogeneously dense lysosomes containing lipid-like droplets, appear and increase in number with age. The specific activities of AcPase, β -glucuronidase and β -galactosidase show a relative decrease. Some of the lysosomes observed in such older animals may be residual bodies (12).

In addition to AcPase activity, thiamine pyrophosphatase (TPPase) activity is localized in the cisternae of 1–2 stacks of trans-side of the Golgi apparatus (24). This fact is common to the Golgi apparatus of all kinds of cells. The author has the opinion that the GERL overlaps the Golgi apparatus also in the thyroid follicle epithelial cell.

9. *Freeze-fracture images of capillary endothelium*

The number of fenestrations and their population density in the capillary endothelium are easily observed and calculated using freeze-fracture images. They are increased in TSH-treated mice which are in a hyperactive state and decreased in

Thyradin (thyroid powder)-treated animals which are in a lower active state (17). It is considered that the endothelial fenestrations are changed in number and population density according to the functional activity of the gland.

10. *Epilogue*

The author wishes to emphasize that the follicle epithelial cells, which are arranged making a simple epithelium around the follicle lumen, show quite exocrine cell structures, because of the occurrence of a well organized zonula occludens, of an exocrine cell polarity, and of an exocrine-like secretory activity of thyroglobulin into the lumen. In addition, the cell has also an absorption activity of thyroglobulin from the follicle lumen, and a release activity of the hormones, T_3 and T_4 from the basal part of the cell. These structural peculiarities of this organ are considered to be due to its functional properties in that the hormones produced are not peptides but characteristic amino acid derivatives. The follicle lumen is considered to be a site for the storage of thyroglobulin, for the iodination of tyrosine residues in thyroglobulin, and for the coupling of iodotyrosine residues in thyroglobulin.

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