Thyroid Function

Thyroglobulin

Analyte Information

Thyroglobulin

Introduction

Thyroglobulin (Tg) is a big dimeric protein consisting of two identical subunits. It has 2,748 amino acids in total, and a molecular weight (Mr) of approximately 670 kDa.

It has 20 asparagine-linked glycosylation sites that bear highly branched oligosaccharide chains. These oligosaccharides, as well as their underlying peptide residues, are variably phosphorylated and sulphated, resulting in a considerable degree of complexity and variability in the thyroglobulin molecule. In addition, iodination of tyrosine residues in the protein may vary depending on such independent processes as dietary iodine availability, iodide transport into the follicular cell, and catalytic effects of thyroperoxidase and others.

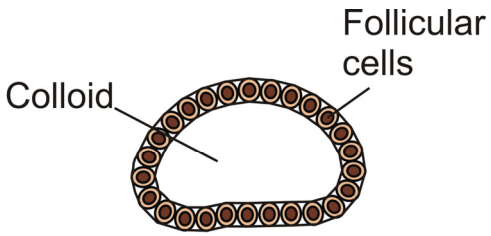
Carbohydrate content is approximately 8% to 10%, comprising mainly galactose, mannose, fucose, N-acetyl glucosamine and sialic acid residues. Iodine is bound via 140 tyrosines and its content varies between 0.2 and 1%.

Thyroglobulin is the unique product of thyroid follicular cells or malignantly transformed cells originating in the thyroid. It plays a key role in the synthesis of thyroid hormones.

Biosynthesis

The main functional unit of the thyroid gland is the thyroid follicle (fig.1). Each follicle is formed of a single layer of epithelial (follicular) cells and is filled with a secretory substance called colloid, which contain a large proportion of proteins, especially thyroglobulin. Biosynthesis itself takes place inside the follicular cells.

Fig.1: Structure of thyroid follicle



The protein is synthesised by polyribosomes located on the rough endoplasmic reticulum of thyroid follicular cells, and then undergoes post-translational modification by glycosylation in the Golgi apparatus. The molecules are then released and stored in the thyroid follicular colloid, where they comprise 75% of the total protein. Once the synthesis of polypeptide chains and the glycosylation of thyroglobulin is complete, synthesis of thyroid hormones occurs within the matrix of the molecule under the influence of the pituitary hormone thyrotropin (TSH).

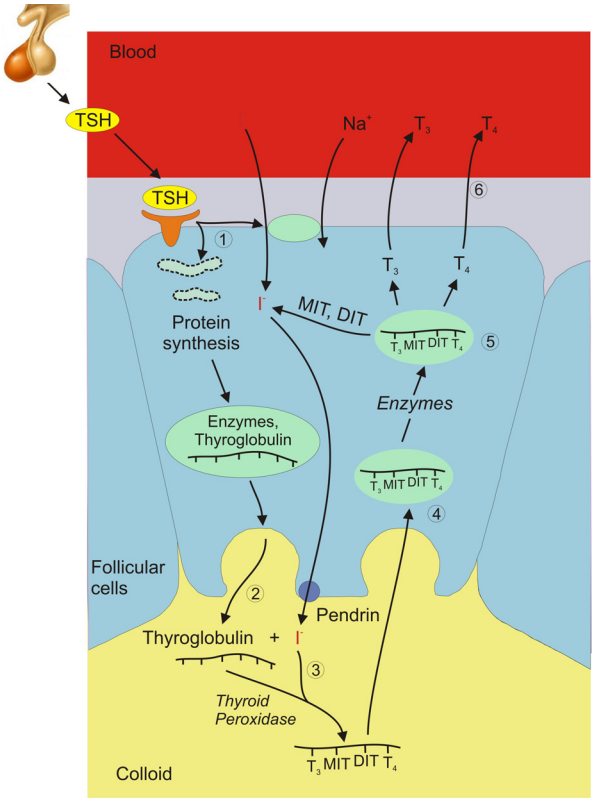
Metabolism

Once secreted into blood, thyroglobulin is cleared mainly by the liver, with a half-life of approximately 65 hours.

Physiological function

The physiological function of Tg is to provide the substrate for the synthesis of the thyroid hormones – thyroxine (T4) and triiodothyronine (T3). The mechanism of thyroid hormone synthesis is shown on fig.2 and fig. 3.

Fig.2: Follicular cell and thyroid hormone synthesis



1. TSH binds to its receptor and thus stimulates intake of iodide and synthesis of thyroglobulin

2. Enzymes and thyroglobulin are transported into the colloid by exocytosis

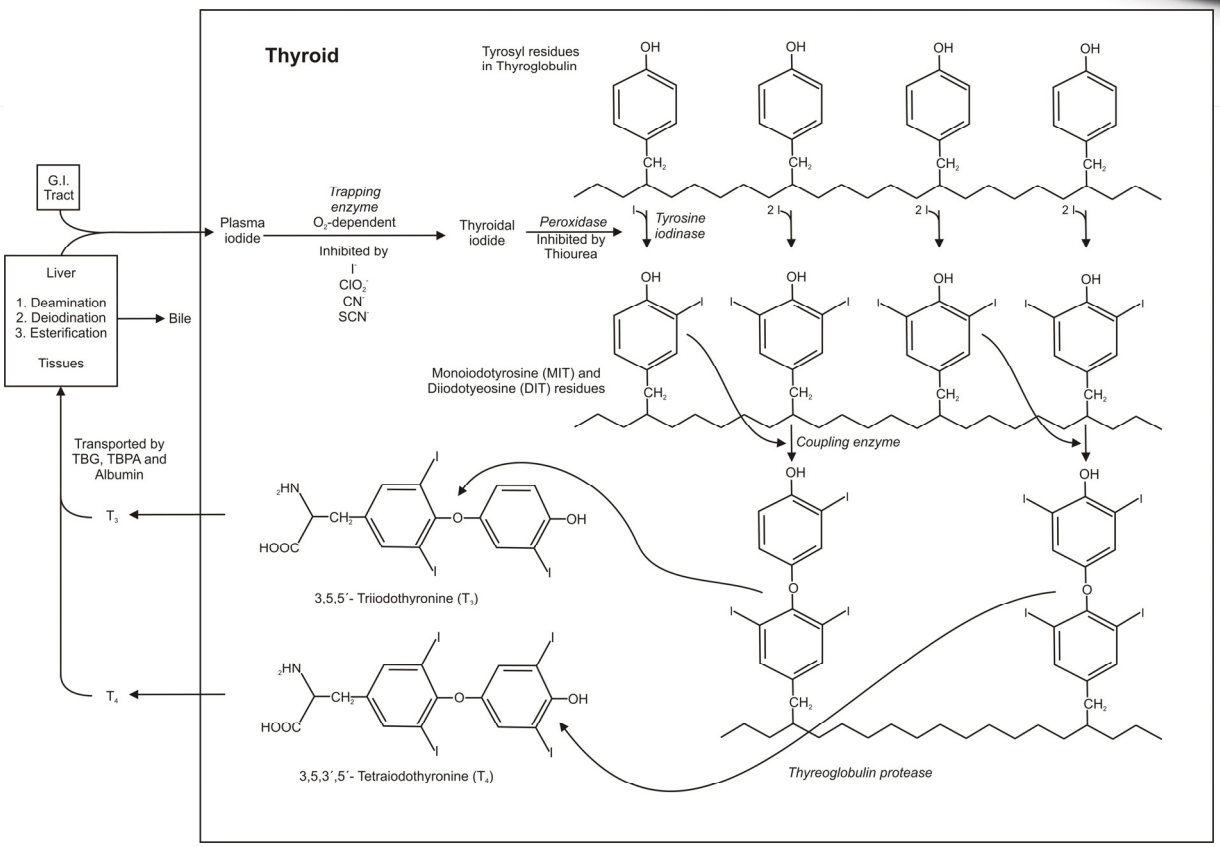
3. Iodide is added to the thyroglobulin molecule to create T3 and T4

4. Thyroglobulin is taken back into the cells by endocytosis of the colloid

5. Globules with colloid merge with lysosomes; lysosomal proteases release T3 and T4 from Tg

6. T3 and T4 are transported across the cell membrane and enter circulation

Fig.3: Formation of thyroid hormones[2]



The thyroid is remarkably efficient in its use of iodide (I^-). In the process of removing it from the blood and storing it for future use, iodide is pumped into follicular cells against a concentration gradient. Iodide is transported from the blood across the basement membrane of the thyroid cells by an intrinsic membrane protein called the Na^+/I^- symporter (NIS), stimulated by TSH. On the other side of the membrane, a second I − transport protein called pendrin moves iodide into the colloid, where it is involved in hormonogenesis.

As a result, the concentration of iodide in a normal thyroid gland is approximately 40 times higher than in the blood.

Daily absorption of 150 to 200 µg of dietary iodine (I) is sufficient to form normal quantities of thyroid hormone. If there is a deficiency of dietary iodine, the thyroid enlarges in an attempt to trap more iodine, resulting in goitre.

As iodide is taken in, TSH stimulates the synthesis of thyroglobulin and other enzymes in the follicular cells as well as their transport into the follicular colloid.

The enzyme thyroid peroxidase catalyses covalent binding of iodine to tyrosine residues in the thyroglobulin molecule, forming monoiodotyrosine (MIT) and diiodotyrosine (DIT). Thyroxine is created by combining two molecules of DIT; triiodothyronine is created by combining one molecule of MIT and one molecule of DIT. This occurs mainly at the interface between the follicular cell and the colloid, but also within the colloid.

Small globules of follicular colloid are endocytosed under the influence of TSH. These globules merge with lysosomes and proteases present in lysosome digest iodinated thyroglobulin and release T3 and T4 from binding. TSH also mediates the transport of T3 (10%) and T4 (90%) across the thyrocyte membrane into circulation, while the lysosome is recycled back into the follicular lumen.

Levels

Although thyroglobulin is produced solely in the thyroid gland, a certain amount is released into circulation and may be detected there. Thyroglobulin levels thus reflect the condition of the thyroid gland.

Levels are elevated in neonates and decrease significantly during the first two years of life.

After total thyroidectomy, levels should fall to an undetectable level.

Typical thyroglobulin levels[2] are given in table 1.

For each assay, relevant reference values are given in the appropriate Instructions for Use (IFU).

Tab.1: Typical thyroglobulin levels

Specimen (serum)	**Reference interval** (ng/mL)
Adult euthyroid	3 - 42
Athyroidic patients	< 5

Analytical problems in thyroglobulin determination

There is evidence that stimulation of thyroglobulin by TSH may affect thyroglobulin structure, particularly the structure of carbohydrates. As a result, the thyroglobulin molecule may be highly heterogeneous in its composition, varying significantly in antigenicity and altering the effectiveness of immunological methods for quantification and assessment. This is especially true for thyroglobulin produced by thyroid carcinoma cells, which is often poorly iodinated compared to thyroglobulin from benign follicular cells, and there are complex changes of carbohydrate chains.

Consequently, results vary greatly across different assays in different clinical conditions. This is enhanced by the fact that international thyroglobulin reference preparation (CRM-457, BCR Brussels) used to calibrate thyroglobulin immunoassays is derived from normal thyroid tissue, what may lead to different results when samples from oncology patients are analyzed.

Another complication is the presence of heterophile antibodies and anti-Tg antibodies.

Heterophile antibodies appear in as many as 3% of thyroid cancer patients and may cause spurious increases in thyroglobulin levels.

Anti-Tg antibodies may be present in approx. 25% of thyroid cancer patients (compared to approx. 10% in the general population). These antibodies may cause falsely decreased results in "sandwich" immunoassay methods, and falsely increased results in competitive immunoassay methods. For correct interpretation, it is always necessary to determine anti-Tg antibodies, or to perform a recovery test, in order to exclude the possibility of antibody interference.

Diagnostic utility – prospects and possibilities

Serum thyroglobulin level is a useful indicator of thyroid gland condition. Increased levels are typical for thyroid cancer, but they may be found also in hyperthyroidism and other non-neoplastic conditions. Decreased values may be found when thyroid function is diminished.

Elevated thyroglobulin levels

- papillary or follicular thyroid cancer (but not medullary thyroid cancer)
- goitre
- hyperthyroidism
- thyroid adenoma
- subacute thyroiditis
- Hashimoto's thyroiditis
- Graves disease

Diminished thyroglobulin levels

- thyroidectomy and radioiodine ablation
- disgenesis of thyroid gland
- thyrotoxicosis factitia

Diagnostic utility – practical applications

Monitoring of differentiated thyroid carcinomas after surgical resection of thyroid gland and radioisotope ablation

Thyroid carcinoma represents 1.6% of cancers of all ages and 3.8% of cancers that appear before 20 years of age[3] (data from US). Women are affected more than men, in a ratio of approximately 3 to 1.

Thyroid carcinomas, mostly derived from thyroid follicular cells, are frequently responsive to treatment with surgery and radioactive iodine. Nevertheless, life-long follow-up is necessary as certain number of patients develop late recurrent disease despite its apparent initial resolution.

Since no other tissues have been found to produce thyroglobulin, this protein may serve as a specific marker for papillary or follicular thyroid cancers after complete thyroidectomy.

The determination is not used for primary diagnosis as increased values may also appear due to benign etiology.

It is possible to follow-up with a simple serial determination of thyroglobulin or, favourably, after TSH stimulation. Such stimulation may be achieved either by administration of recombinant TSH or by thyroid hormone withdrawal (THW). Currently, THW seems preferable due to its higher potential to stimulate thyroglobulin production.

Potential interference with anti-Tg antibodies and heterophile antibodies has to be taken into account. Either parallel determination of anti-Tg, or performance of a recovery test is highly recommended.

Investigation of congenital hypothyroidism etiology in infants

Confirmation of diagnosis of thyrotoxicosis factitia

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