Structural biology

Molecular architecture of thyroglobulin revealed

Nancy Carrasco

The structure of thyroglobulin, the enormous protein that acts as a precursor for thyroid hormones, has been determined, and its hormone-forming tyrosine amino-acid residues have been identified. **See p.627**

The thyroid hormones thyroxine and triiodothyronine are small molecules that have a big biological impact. They regulate metabolism in almost all cells, and are essential for the development and maturation of the central nervous system, the musculoskeletal system and the lungs. They are also the only hormones that contain iodine and that are synthesized partly inside and partly outside cells. An enormous dimeric glycoprotein called thyroglobulin (each identical monomer of which has a mass of about 330,000 daltons) serves as the thyroid hormones' precursor, scaffold and reservoir[1]. On page 627, Coscia *et al.*[2] report the first structure of full-length human thyroglobulin and identify its hormone-forming tyrosine amino-acid residues, thereby filling a crucial gap in our knowledge of the biosynthetic pathway of the thyroid hormones.

The thyroid gland is made up of spherical structures called follicles, which consist of a single layer of follicular cells surrounding a fluid known as the colloid, where thyroglobulin is stored. The complex biosynthesis of thyroid hormones[1,3] takes place in the follicles. Thyroglobulin is synthesized in an intracellular organelle of the follicular cells, called the endoplasmic reticulum, where it forms a dimer before being secreted into the colloid.

Iodide (I^-) in the bloodstream around the follicles is actively taken up by the follicular cells through a cell-membrane protein, the Na^+/I^- symporter[4], and then transported into the colloid. Here, I^- is oxidized to iodine by the thyroperoxidase (TPO) enzyme, using hydrogen peroxide produced by dual oxidase proteins, and then covalently incorporated into tyrosine residues in thyroglobulin in the colloid. This produces biosynthetic intermediates known as 3-monoiodotyrosine (MIT) and 3,5-diiodotyrosine (DIT) bound to thyroglobulin. MIT then reacts with DIT to form triiodothyronine, or two DITs react to produce thyroxine, still bound to thyroglobulin.

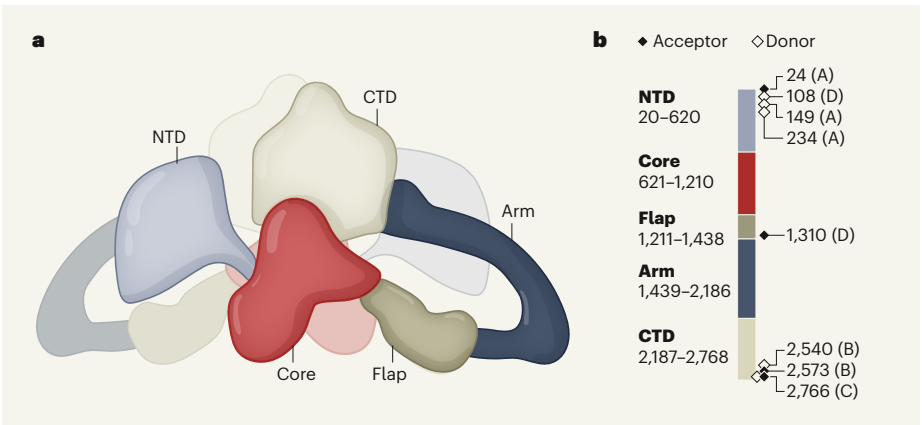

Figure 1 | Structure of human thyroglobulin. a, Coscia *et al.*[2] report the structure of the dimeric thyroglobulin protein, which is the precursor for thyroid hormones. Each identical monomer contains five domains: the amino-terminal domain (NTD), core, flap, arm and the carboxy-terminal domain (CTD). In this illustration, the darker domains comprise one monomer and the pale domains are in the second monomer, behind the first. **b**, This diagram shows which amino-acid residues are found in each domain. Coscia and colleagues identified the four hormone-forming sites (A–D) that are conserved across species. Each site corresponds to the position of a tyrosine residue known as the acceptor; tyrosine residues that react with acceptors during hormone biosynthesis are called donors. Here, the labels for acceptors and donors indicate the number of the amino-acid residue, and the site to which it contributes (in parentheses). The acceptor and donor for site C are probably the same residue (tyrosine 2766) from each monomer.

When levels of thyroid hormones circulating in the blood decrease and levels of thyroid-stimulating hormone (TSH) rise, thyroglobulin is internalized into the follicular cells through a process called endocytosis. Thyroglobulin is then digested in organelles called lysosomes, producing free triiodothyronine and thyroxine, which are finally released into the bloodstream. The ratio of thyroxine to triiodothyronine in humans is about 80:20 (ref. 1). MIT and DIT produced during the digestion process as a result of incomplete thyroid-hormone synthesis are metabolized in the follicular cells by an iodotyrosine dehydrogenase enzyme to produce I^- and tyrosine, ensuring that any I^- not incorporated into hormones is recycled.

Coscia *et al.* set out to determine the structure of human thyroglobulin using cryo-electron microscopy (cryo-EM), to deepen our understanding of thyroid-hormone biogenesis. They purified thyroglobulin from cultured cells that had been engineered to secrete the protein at a high concentration. Using the cryo-EM data, the authors built an atomic model of the protein that contained 93% of its amino-acid residues, and defined five regions in the structure (Fig. 1): the amino-terminal domain (NTD), core, flap, arm and carboxy-terminal domain. The model reveals that the two monomers are intertwined, and that the NTD of each monomer interacts with all five regions of the other monomer. The interface between the monomers is immense (29,350 square ångströms), and each monomer has 60 disulfide bonds (structural motifs that stabilize the 3D structure of proteins). All of these disulfide bonds connect residues in monomers, as previously reported[5], rather than between monomers.

Coscia and colleagues identified the four hormonogenic (hormone-forming) sites (A–D) that are known to be conserved across species, from the sea lamprey[6] to humans. Each site corresponds to the position of a tyrosine residue known as the acceptor; the tyrosine residues that react with acceptors during hormone biosynthesis are known as donors. At site A, the acceptor is tyrosine 24 (Tyr 24), and a donor (Tyr 149) had previously been discovered[7]. However, Coscia *et al.* find that a second residue (Tyr 234) also acts as a donor at site A. At the other sites, the acceptors were known[8] but some of the donors were not. The authors report that Tyr 2573 is the acceptor at site B, and Tyr 2540 is the donor; and that at site D, the acceptor is Tyr 1310 and the donor is Tyr 108 of the other monomer. Strikingly, the acceptor and donor for site C are probably the same residue (Tyr 2766) from each monomer – but

the resolution of this region of the protein structure is not high enough to be completely certain.

When Coscia and co-workers replaced all eight hormonogenic tyrosine residues with a different residue, they could not detect any thyroxine production from the resulting mutant in their *in vitro* assay. The authors therefore conclude that only these residues are hormonogenic, out of 67 tyrosine residues in each monomer. However, it could be that the lack of hormone was due to other, unidentified sites ceasing to produce thyroxine as a result of conformational changes induced by the tyrosine substitutions.

So, do the eight identified tyrosine residues have anything in common that explains their hormonogenic activity? They are all at least partly exposed to the solvent around thyroglobulin, and the side chains of the donor–acceptor pairs formed by these residues face each other in an approximately antiparallel configuration. These residues are also all in highly mobile regions of the protein — presumably to enable the substantial bond rearrangements that need to take place to generate thyroxine.

The authors went on to show that thyroxine can be produced *in vitro* from a bacterial protein (maltose-binding protein; MBP) that has nothing to do with thyroid-hormone production. They found that either a pair of tyrosine residues found naturally in MBP, or a pair that was specifically introduced to have the same geometric arrangement and flexibility as the hormonogenic residues in thyroglobulin, produced thyroxine in the presence of an I^--oxidizing system and a peroxidase enzyme. Lactoperoxidase could be used instead of TPO, which is consistent with the previously reported observation that lactoperoxidase can promote the synthesis of thyroxine from thyroglobulin[9]. The observation that thyroxine can be produced using TPO and MPB indicates that the key requirement for generating thyroxine is the production of DIT, rather than the existence of a particular protein scaffold for the hormonogenic residues.

For reasons that are unclear, Coscia *et al.* did not detect the generation of triiodothyronine in any of their *in vitro* experiments. An earlier study[10] reported that triiodothyronine can be produced from thyroglobulin *in vitro*, and that the main site of hormonogenesis was Tyr 2766. It remains to be seen whether triiodothyronine was not observed in the current study because of the experimental conditions or because of the sensitivity of the assay used. More experiments are needed to understand not only normal triiodothyronine production, but also the mechanism that causes an increase in triiodothyronine biosynthesis in several situations: in Graves' disease (an autoimmune disease that affects the thyroid); in I^- deficiency; in people who have activating mutations of the TSH receptor; and when thyroid cells in culture are stimulated with sera from people with Graves' disease[1].

In addition to shedding light on details of the biosynthesis of thyroid hormones, Coscia and colleagues' determination of the 3D structure of thyroglobulin will probably also lead to a more thorough understanding of the effect of thyroglobulin mutations that cause congenital hypothyroidism — a deficiency of thyroid-hormone biosynthesis. It is a breakthrough as impressively big as the protein itself.

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Materials science

Metallic glasses that harden under strain

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Metallic glasses are much stronger than conventional metals, but form certain instabilities under stress that lead to fracture. A process known as rejuvenation has been shown to solve this problem. **See p.559**

Metallic glasses are formed by cooling melted alloys under conditions that prevent the melt from crystallizing[1]. They have remarkable mechanical properties — in particular, they can be subjected to high forces and undergo a large amount of deformation before they stop behaving elastically and start to deform permanently (plastically). However, they have one key weakness: they are prone to catastrophic failure under stress because they soften during plastic deformation, rather than hardening, as crystalline metals do. On page 559, Pan *et al.*[2] report a method for preparing metallic glasses that causes them to harden during plastic deformation, thereby avoiding the instabilities that lead to failure.

If you take a paper clip and bend it, you'll find that more force is needed as you bend it to an increasingly sharp angle. This is an example of work, or strain, hardening — the strengthening of a material through plastic deformation. At the atomic scale, the plastic deformation of metallic crystals in the wire is caused by the motion of 'dislocations'. These linear defects in the crystal structure multiply, intersect and entangle as deformation proceeds, thereby getting in each other's way and strengthening the material[3]. This makes work hardening one of the most complex problems in science: it needs to be understood at many length scales, from the atomic-scale lengths of the dislocation cores, through the nano- and micrometre scales involved in dislocation interactions and structures, to the macroscale lengths associated with crack propagation and the structural stability of bulk materials.

The mechanical behaviour of metallic glasses is fundamentally different. Because their atomic structure is not periodic, there are no dislocations. Plastic deformation instead occurs through shear, a mode of deformation that affects small groups of atoms (known as shear transformation zones; STZs) throughout the glass[4]. This shearing loosens (dilates) the atomic structure, and the resulting increase in volume facilitates the formation of new STZs. If the rate of deformation is sufficiently high, the atomic structure does not have time to relax and densify again. As a result, the local deformation rate continues to rise and finally becomes unstable, forming a narrow zone of intense shear strain (a type of deformation) known as a shear band.

Shear bands are macroscopic phenomena. They cause steps to form on the surfaces of materials and can therefore be suppressed by applying appropriate constraints — for example, by sandwiching metallic-glass layers