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Hypothesis

The type-1 repeats of thyroglobulin regulate thyroglobulin degradation and T3, T4 release in thyrocytes

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Abstract Thyroglobulin (Tg) proteolytic steps are central phenomena in Tg processing and thyroid hormone release in thyrocytes. Based on recent literature data, we propose that the type-1 repetitive units present in the Tg sequence could act as binders and reversible inhibitors of the proteases implicated in Tg processing. The pH-dependent interactions of proteases with the repeats could permit (i) protection from degradation of low iodinated Tg to be recycled; (ii) restriction of early proteolytic attacks to N- and C-terminal hormone formation sites; (iii) increase of the half-time of acidic proteases necessary for the final, extensive degradation steps of Tg.

Key words: Thyroglobulin; Tg type-1 repeat; Proteolysis; Thyroid hormone release; Module

1. Introduction

Thyroglobulin (Tg) is the precursor of the thyroid hormones 3,3′,5-triiodo-L-thyronine (T3) and L-thyroxine (T4). Tg synthesis occurs exclusively in the epithelial cells (thyrocytes) of the thyroid gland and is stored in the lumen of thyroid follicles. There, iodination and intramolecular coupling of tyrosine residues from Tg take place. T3 and T4 secretion involves internalization, sorting of the most iodinated Tg molecule, and transport to lysosomes [1,2]. The thyroid hormone residues are released by early and limited alteration of the Tg structure [3]. Intensive Tg degradation is a delayed process, requiring several hours [4]. In spite of numerous reports on Tg proteolytic attacks by thyroid proteases [5–9], the physiological mechanisms for selective hormone release and sequential processing remain unclear. The amino acid sequence of Tg [10,11] encloses three kinds of cysteine-rich repetitive regions. In particular, there are 11 N-terminal repetitive sequences, which we recently characterized as Tg type-1 modules [12]. The function of these repetitive sequences in Tg is at present unknown. Tg type-1 modules were identified in 32 proteins belonging to seven different families. The different biological functions performed by these proteins apparently do not directly implicate their Tg type-1 modules [12], except in one case (see below). In a recent report, Yamashita and Konagaya [13] reported the isolation from chum salmon eggs of a 74 residue protein with extremely high sequence similarity with the Tg type-1 module. This protein is a cysteine protease inhibitor. This observation and two others to be described later have led us to hypothesize that the 11 Tg type-1 repeats could function as binders and reversible inhibitors of the proteases involved in the proteolytic processing of Tg.

2. The facts: Tg type-1 modules can interact with some proteases

2.1. ECI is a Tg type-1 module which inhibits papain and chum salmon cathepsin B

ECI is one of three isoforms of cysteine protease inhibitors found in chum salmon eggs [13]. The sequence of this newly isolated small molecule (74 amino acids) is the same as the Tg type-1 consensus sequence [12] except for an additional 10 residue N-terminal segment. ECI was found to display specific inhibitory activities against papain and chum salmon cathepsin B but not against *m*-calpain, bovine trypsin, or bovine chymotrypsin [13]. Thus, a protein which is extremely similar in its sequence to a repetitive unit of thyroglobulin functions as a specific protease inhibitor.

2.2. Cathepsin L is reversibly complexed and inhibited by the Tg type-1 module of Ii

Ii is a transmembrane glycoprotein associated with major histocompatibility complex (MHC) class II proteins that exists in two alternatively spliced forms, P31 and P41. The additional exon of the P41 form codes for a Tg type-1 module [14]. Both P31 and P41 seem to be functionally equivalent in most of their biological functions (MHC class II chaperone, targeting/retention signals, interference with peptide loading in the class II molecule [15]). There is only a slight difference between P31 and P41 in enhancing antigen presentation which is probably due to subtle differences in their proteolytic processing [16,17]. Two facts argue for a possible role of the Tg type-1 module in Ii degradation mechanisms. First, Ogrinc et al. [18] showed that the C-terminal part of P41 (containing the Tg type-1 module) is able to reversibly and specifically inhibit and stabilize cathepsin L in a pH-dependent manner; second, Fineschi et al. [19] proved that P31 and P41 have different patterns of proteolytic processing. Therefore, in transfected cells expressing only the P41 form of Ii, degradation of P41 generates a 12 kDa N-terminal fragment of Ii (P12) that remains associated with MHC class II for an extended time; in contrast, when P31 is expressed alone it is subjected to extensive degradation without an intermediate fragment associated with MHC class II. In cells expressing simultaneously both forms of Ii, P31 degradation also generates P12. To explain this differential sensitivity to proteases, the authors offered two possibilities: either the C-terminus (containing the Tg type-1 module) of P41 induces conformational changes which

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could alter the configuration of protease-sensitive sites, or released P41-specific fragments directly change the specificity or efficiency of endosomal proteases. These results show that a protein containing a Tg type-1 module is both capable of binding a protease [18] and of specifically controlling some of the proteolytic events that the protein containing the module undergoes [19].

2.3. IBP-3 acts as a reversible inhibitor of IBP-4 proteolysis

Insulin-like growth factor binding proteins (IBP) bind insulin-like growth factor I (IGF I) and modulate the interaction between IGF I and IGF I receptor [20]. This modulation is dependent on different biochemical events comprising proteolysis of IBP. The sequence of IBP-3 contains a Tg type-1 module. Fowlkes et al. [21] showed that IBP-3 functions as a reversible inhibitor of IBP-4 proteolysis. The protease seems to be cation-dependent and is inhibited by a synthetic peptide contained in the sequence of the Tg type-1 module. Thus the amino acid sequence of the Tg type-1 repeat from IBP-3 is endowed with the capacity to inhibit the cation-dependent protease activity that contributes to the degradation of IBP-4.

All these recent experimental data indicate again that some Tg type-1 modules have reversible binding and/or inhibitory properties for proteases and/or could interfere with the proteolysis of the protein which contains these modules. Due to sequence variations of Tg type-1 modules, it is possible that each of the Tg type-1 repeats could be specific for one type of protease, which may explain why Yamashita and Konagaya [13] did not find any inhibitory activity of Tg against papain, which is not present in thyrocytes.

3. The hypothesis: The Tg type-1 modules of thyroglobulin control the sequential proteolytic events of thyroglobulin degradation

The arguments mentioned above raise the possibility that

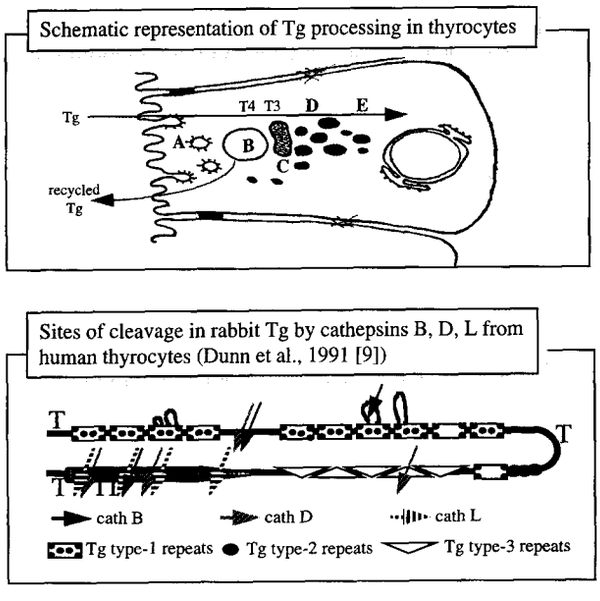

Fig. 1. Upper panel: Schematic drawing of Tg processing in thyrocytes; steps A–E correspond to those described in Fig. 2. Lower panel: Major sites of cleavage of rabbit Tg by cathepsins B, H, L from human thyrocytes.

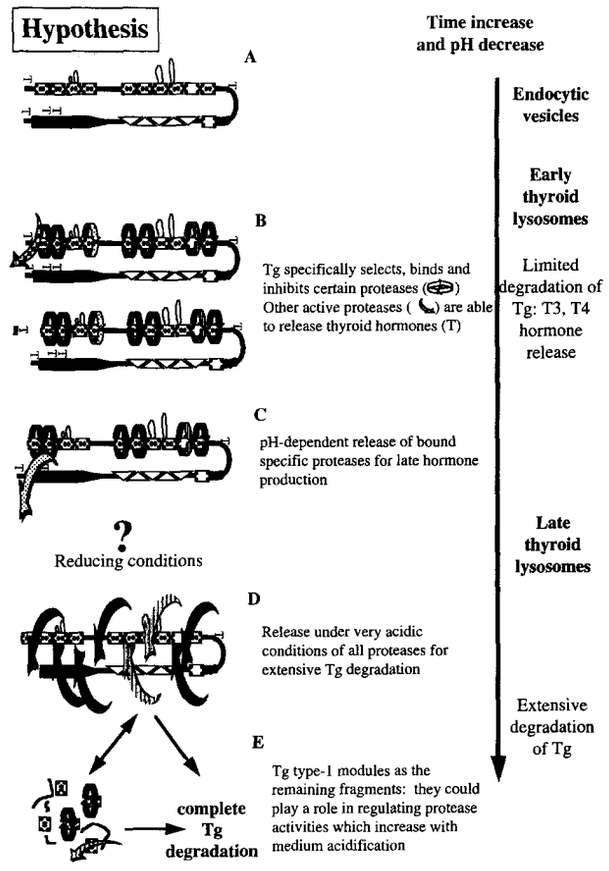

Fig. 2. Schematic representation of the hypothetic proteolytic processing of Tg.

the 11 Tg type-1 repeats in Tg itself could control the activities of the proteases involved in its degradation. Indeed several of the molecular mechanisms of the sequential proteolytic processing of Tg remain obscure. In brief, in the first step after endocytosis, hormone-containing Tg is transferred to lysosomes where it undergoes rapid (few minutes) and specific proteolytic cleavage that selectively generates free thyroid hormones (Fig. 1, upper panel, steps A, B, C). Incompletely iodinated Tg goes back into the lumen for recycling without degradation [22]. In a late and long (1–4 h) second step (Fig. 1, upper panel, steps D and E) the remainder of the Tg molecule is extensively degraded to small fragments and/or amino acids [3].

Our hypothesis is as follows. The hormone-containing Tg is internalized in endocytotic vesicles. The fusion between these Tg-containing endocytic vesicles and primary lysosomes forms the thyroid lysosomes [23] where proteases and peptidases required for Tg processing are present. The evolution of early thyroid lysosomes to late thyroid lysosomes [24] is marked by progressive acidification. We propose that using the pH gradient as a signal, one or several Tg type-1 modules specifically and reversibly bind and inhibit some of the proteases and thus regulate the proteolytic activities in the thyroid lysosomes. Due to the sequence variation in the Tg type-1 repeats, it is possible that the 11 copies in Tg may have specificities for different proteases activities. In step A (Fig. 2), hormone-containing Tg (shown as a monomer for clarity) in endocytic vesicles is naked, ready to meet lysosomal proteases (espe-

cially cathepsins [5–9]). In step B, a few minutes later, early thyroid lysosomes are formed. Some or all of the 11 Tg type-1 modules bind and inhibit one or more (membrane-associated or not) of the cathepsins. The Tg-associated cathepsins are thus inhibited and stabilized, so as to increase their half-life, and remain available for the late degradation steps. In addition, the immature incompletely iodinated Tg could be more resistant to degradation in its way back for recycling in the lumen [22]. The drawing in Fig. 1 (lower panel) shows the sites of proteolytic cleavage of rabbit Tg by cathepsin B, D, L [9]. All type-1 repeats, except one, are insensitive to cathepsin action. This could be consistent with protection from proteolysis by the inactivated masking proteases. The unprotected parts of Tg, in particular the N- and C-termini, are, however, available to attack by other free and active proteases. This is consistent with the observation that N-terminal and C-terminal hormone-containing extremities of Tg are cleaved in these early stages [3]. In step C (Fig. 2), the progressive pH decrease could specifically induce the release of a protease specific for C-terminal cleavage of Tg from a Tg type-1 repeat. This could explain the observation that, in mouse macrophages, T3 release occurs later than T4 release, in more acidic compartments [4], and may be due to the action of different enzymes [8]. In step D, the very acidic conditions in the late lysosomes constitute the signal for the extensive Tg degradation. The release of a second wave of proteases then occurs under optimal acidic conditions (during this step disulfide bond reduction should occur. Our hypothetical Tg degradation mechanism does not explain the nature of the reduction of disulfide bridges. As of now, the reducing agent is still unknown but a high concentration of free cysteine in the late lysosomes is thought to play this role [3]). The complete degradation of Tg (step E) takes a long time (up to 3 h). Since Tg type-1 modules are probably more resistant to proteolytic attacks [25], they could also participate, as proteolytic fragments, in the control of an equilibrium between free active proteases and inactive bound proteases. In any case, the final step will be the extensive Tg degradation with or without Tg type-1 module recycling.

4. Conclusion

Several recent findings in the literature indicate that Tg type-1 repeats could have a common function. A Tg type-1 repeat could act as an internal, selective and reversible protease inhibitor included in the protein sequence of various proteins.

In this report we propose a new hypothesis which could enlighten our understanding of how proteolytic events lead first to selective release of thyroid hormones and then to complete Tg degradation. These proteolytic events are key steps in the single known function of Tg: the release of T3 and T4 hormones. These proteolytic events must be regulated to participate in a concerted processing. We propose that the 11 type-1 repeats from Tg could drive this regulation in thyrocytes by selectively and reversibly inhibiting endosomal and/or lysosomal proteases following the gradual pH decrease (Fig. 2) which characterizes lysosomes evolution. Indeed, Tg type-1 repeat interactions with proteases could permit (i) protection from degradation of low iodinated Tg to be recycled, (ii) restriction of early proteolysis to N- and C-terminal hormone formation sites, (iii) increase of the half-life of acidic proteases necessary for extensive, final, degradation steps. The role we tentatively assign to the 11 type-1 repeats of Tg (which together correspond to almost half of the amino acid sequence of Tg) allows us to understand why molecular evolution has conserved such a giant gene coding for a 2748 amino acid protein as a precursor of very small hormones.

Our hypothesis could help us understand the role of the Tg type-1 module present in numerous proteins.

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