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NOTE

A Misfolded Thyroglobulin is Retained by Molecular Chaperones in the Endoplasmic Reticulum

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Abstract Deficient thyroglobulin is one of the important causes of congenital hypothyroid goiter with a prevalence of ~1/40,000 humans. We now demonstrate that in *cog/cog* mice showing hypothyroidism, four endoplasmic reticulum-molecular chaperones stably bind to thyroglobulin, providing insight into physiologic regulation of endoplasmic reticulum storage diseases.

Key words: Thyroglobulin, Hypothyroidism, Endoplasmic reticulum, Molecular chaperones.

The endoplasmic reticulum (ER) contains mechanisms to monitor the fidelity of early biosynthetic events in the protein export pathway to prevent premature export of incompletely or improperly folded proteins from ER, as well as machinery intended to initiate the removal of misfolded, incompetent proteins [6]. In case of a critically impotent secretory protein is accumulated in the ER by misfolding and unfolding, a target protein is unable to perform its physiologically intended function in the tissue [2], which is called ERSD (endoplasmic reticulum storage disease). Morphological studies of cells showing ERSD routinely reveal expansion and dilation of the ER compartment, which may in part be due to accumulation of misfolded exportable proteins [8]. ER-molecular chaperones are constantly expressed under normal conditions in the cells, and the enhanced expressions of those are observed in the cells stimulated or stressed by various factors, such as heat, chemical drugs, heavy metals and physiological stimulations.

The major secretory product of the thyroid epithelial cell is thyroglobulin (Tg), an extremely large glycoprotein that accounts for 13% of the total protein synthesis [13], contains 10% carbohydrate and servers as the matrix for thyroid hormone synthesis and iodine storage [3]. Tg encoding a single 2,750 aa, which undergoes extensive posttranslational modification in the endoplasmic reticulum before secretion as a form of 660 kDa homodimer [10]. Despite cloning of Tg gene ten years age, the causes of hypothyroidism is relatively little investigated and unclear its molecular mechanism is still in a rudimental stage. With in this mind, in this study, we have determined association between misfolded Tg and four ER-molecular chaperones using homozygous *cog/cog* mice that suffer congenital hypothyroidism [1,11, 12].

Minced thyroid from both normal and *cog/cog* mice was labeled for 30 min with a mixture of [^{35}S]Met and [^{35}S]Cys (Expre^{35}S^{35}S, Du Pont-New England Nuclear) and chased in the presence of excess unlabeled methioine/cysteine plus cycloheximide (500 μM) at 37℃, unless otherwise indicated. At the end of each chase, tissues were quickly chilled to 4℃ and treated with 50 mM iodoactamide in PBS for 10 min to alkylated intracellular sulfhydryls. Tissues were washed tree times in cold PBS and lysed in 0.5 mℓ of buffer containing 0.1 M NaCl, 25 mM Tris, pH 7.5, 5 mM EDTA, 1% Triton X-100, 10 units/mℓ apyrase to enzymatcally deplete ATP, and a mixture of protease inhibitors (0.1 mM leupeptin, 10 mM pepstatin, 1 mM DFP, 1 mU/mℓ aprotinin) by brief sonication using a sonifier with cup-horn (Branson Ultrasonics Corp., Danbury, CT). Finally, after exposure to apyrase for 90 min, 10 mM iodoactamide was added. Equal aliquots of lysates were first precleaned with Zysorbin and then immunoprecipitaed with each anti-chaperone antibody (GRP94, Bip, Erp72, and calnexin) [4,5,7,9] for 2 h at 4℃, before analysis by reducing SDS-PAGE. In most instances, before gel loading, samples were normalized either to DNA content or to comparable amounts of labeled Tg. Quantitation of radiolabeled Tg bands were performed by phos-

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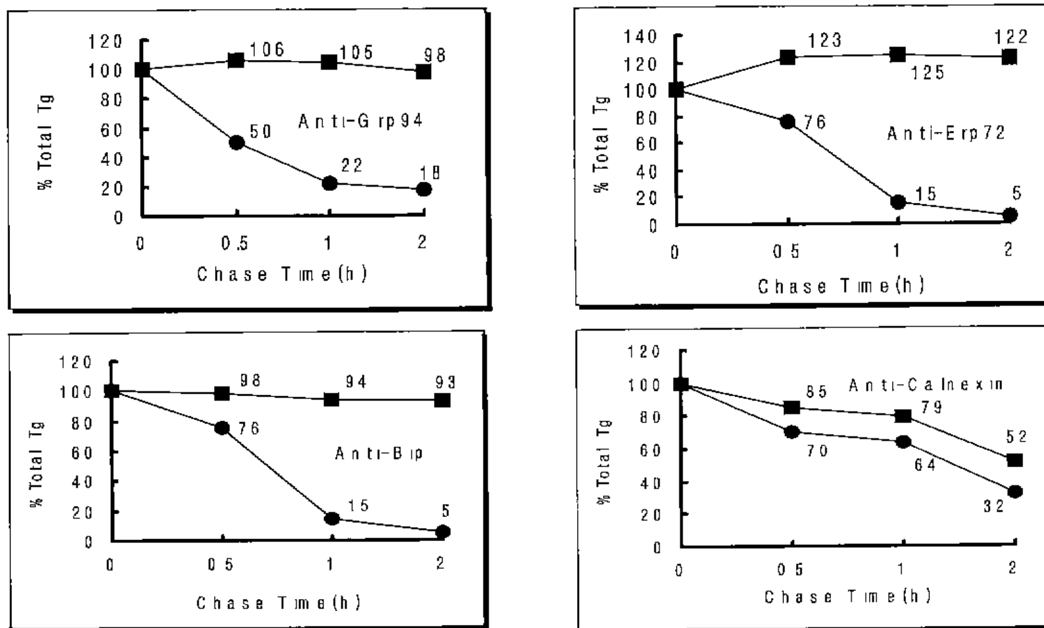

Fig. 1. Stable association between misfolded cog/cog Tg and ER resident molecular chaperones. Lysates described above were also immunoprecipitaed using anti-chaperone antibodies (GRP94, Bip, Erp72, and calnexin) and analyzed by 4% reducing SDS- PAGE. Co-precipitated Tg was quantitated by phosphorimaging. Symbol of ● represents Tg of normal thyrocytes and ■ is *cog/cog* Tg.

phorimaging coupled to the ImageQuant software package (Molecular Dynamics, Inc., Sunnyvale, CA).

Structurally-deficient Tg from *cog/cog* mice exhibits long-term association of with four ER-resident molecular chaperones including GRP94, Bip, Erp72, and calnexin. For analysis of Tg co-precipitation with the four chaperones, pulse-labeled thyroid tissues as described above were immunoprcipitaed with anti-chaperone antibodies, respectively. Recovery of newly-synthesized Tg was analyzed by SDS-PAGE and quantitated by phosphorimaging. Normal Tg (●) in Fig. 1 shows maximal interaction with GRP94, Bip, Erp72, and calnexin at the earliest chase time, with progressive dissociation from these chaperones during structural maturation of the secretory protein. The normal decline of Tg association is occurred with 60 min. By contrast in the mutant (*cog/cog*), misfolded Tg (■) exhibits continued interaction with these chaperones even at late chase times in Fig. 1, in which Tg binding did not diminish over 2 h. Since accumulation of mutant Tg in the ER is not a lethal condition for thyrocytes, at present, little or nothing is known about *cog/cog* mice's Tg metabolism. One of the possible hypotheses is that a fragment of misfolded Tg induced by a chaperone enters into the blood system, which may act like bioactive Tg against target tissues. Indeed, degrading misfolded Tg may be occurred by Erp72, which based on the result of increasing association with misfolded Tg and Erp72 as shown in Fig. 1.

We conclude that exportable Tg may experience both simultaneous and sequential interactions with different ER resident molecular chaperones which play unique roles in protein folding and assembly in normal thyroid physiology. It is also suggested that this process has evolved via cotranslational domain-dependent folding in conjunction with the actions of compartment-specific molecular chaperones.

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