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Role of Protein Disulfide Isomerase in Molecular Fate of Thyroglobulin and its Regulation by Endogenous Oxidants and Reductants

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The molecular fate of thyroglobulin (Tg) is controlled by oligomerization, a means of storing Tg at high concentrations, and deoligomerization. The oligomerization of bovine Tg are intermolecular reactions that occur through oxidative processes, such as disulfide and dityrosine formation, as well as isopeptide formation; disulfide formation is primarily responsible for Tg oligomerization. Here, the protein disulfide isomerase (PDI) and/or peroxidase-induced oligomerization of unfolded thyroglobulins, which were prepared by treating bovine Tg with heat, urea or thiol/urea, was investigated using SDS-PAGE analyses. In addition, the enzymatic oligomerization was compared with non-enzymatic oligomerization. The thermally-induced oilgomerization of Tg, dependent on glutathione redox state, was affected by the ionic strength or the presence of a surfactant. Meanwhile, PDI-catalyzed oligomerization, time and pH-dependent, was the most remarkable with unfolded/reduced Tg, which was prepared from a treatment with urea/DTT, while the thermally-unfolded Tg was less sensitive. Similarly, the oligomerization of unfolded/reduced Tg was also mediated by peroxidase. However, PDI showed no remarkable effect on the peroxidase-mediated oligomerization of either the unfolded or unfolded/reduced Tg. Additionally, the reductive deoligomerization of oligomeric Tg was exerted by PDI in an excessively reducing state. Based on these results, it is proposed that PDI catalyzes the oligomerization of Tg through the disulfide linkage and its deoligomerization in the molecular fate, and this process may require a specific molecular form of Tg, optimally unfolded/reduced, in a proper redox state.

Key words: Thyroglobulin, Oligomerization, PDI, Peroxidase, Deoligomerization

INTRODUCTION

Thyroglobulin (Tg) is a precursor protein of thyroid hormones such as 3,5,3'-triiodothyronine (T_3) and 3,5,3',5'-tetraiodothyronine (thyroxine, T_4). It is the most abundant protein in the thyroid gland, constituting up to 75% of the total protein content (Chernoff and Rawitch, 1981; Gentile *et al*, 1995; Venkatsh and Deshpande, 1999). The storage of Tg in the follicular lumen is aided by a compaction process that is involved a tight packing of Tg molecules to reach a high lumenal concentration (~590 mg/ml). Recently (Berndorfer *et al.*,1996; Klein *et al.*, 2000), the packing mechanism was suggested to include the predominant formation of intermolecular disulfide bonds in human Tg, and the additional action of transglutaminase resulting in the formation of intermolecular isodipeptide cross-links (Saber-Lichtenberg *et al.*, 2000). Meanwhile, approximately half of the nascent Tg was detected as disulfide-linked protein aggregates during the process of Tg maturation in the endoplasmic reticulum (ER) (Kim and Arvan, 1991; Kim *et al.*, 1992; Kim *et al.*, 1993). The formation of Tg aggregates has been suggested to be regulated by some chaperone proteins (Delom *et al.*, 1999). Therefore, there is a need to determine how the formation of the disulfide-linked Tg aggregates can be regulated in the follicles as well as in ER.

A structural characteristic of Tg molecule is represented by the presence of 122 cysteinyl residues per Tg monomer, most of which are involved in intra-chain disulfide bonds and are enriched in three types of cysteine-rich tandem repeats (Veneziani *et al.*, 1999). It should be noted that

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some of the cysteine residues are present in the region compatible with the thioredoxin box (CXXC) sequence. Tg from various mammalian species contains three highly conserved thioredoxin boxes (CXXC). Recently (Klein *et al.*, 2000), it was observed that a human recombinant Tg fragment containing three thioredoxin boxes exhibited redox activity corresponding to PDI activity at the redox conditions that are present in the extracellular space of thyrocytes. This redox activity was suggested to lead to the intermolecular cross-linking of lumenal thyroglobulin. In addition, a recent study (Delom *et al.*, 1999) proposed that PDI might participate in the retardation of peroxidase-catalyzed oligomerization of Tg molecule in the follicles. Moreover, the action of PDI was suggested to be due to its interaction with the N-terminal domain of Tg (Delom *et al.*, 1999). Thus, the degree of oligomerization may differ according to the molecular form of Tg. This study attempted to evaluate the effect of PDI or peroxidase on the oligomerization of either the unfolded form or unfolded/reduced form of Tg molecule, which were employed to mimic the unfolded nascent Tg in the ER or goiter Tg in the folicles. Furthermore, the deoligomerization of multimerized Tg was investigated in different redox states.

MATERIALS AND METHODS

Materials

Thermolysin (Bacillus thermoproteolyticus rokko, EC 3.4.24.4), Sephadex G 25, Sephacryl S-400 HR, EDTA, urea, mercaptoethanol, DTT, GSH, glycerol, Triton X 100, glucose oxidase, lactoperoxidase, deoxycholate, and Bicinchoninic acid Protein Assay Reagent were purchased from Sigma Chemical Co (St. Louis, MO, USA). Endoproteinase Asp-N (Pseudomonas fragi, EC 3.4.24.33) was obtained from Roche Molecular Biochemicals (Indianapolis, IN, USA). Various other products for electrophoresis were purchased from Bio-Rad Laboratories (St. Louis, MO, USA).

Preparation of thyroglobulin (Tg)

Bovine Tg was purchased from a local slaughterhouse through the Ohjungdong market, (Taejon, Korea). The tissue was finely minced with scissors on ice. Tg was extracted briefly on ice in 0.1 M sodium phosphate buffer, pH 7.2, and purified by fractional precipitation with 1.4-1.8 M ammonium sulfate, 50 mM Tris-HCl, pH 7.2, and gel filtration on Sephacryl S-400 HR in 50 mM Tris-HCl, pH 7.2 containing 130 mM NaCl at 4°C (Gentile *et al.*, 1997; Sok and Sih, 2001).

Purification of protein disulfide isomerase (PDI)

Purification of PDI was carried out according to the procedure published previously (Freedman *et al.*, 1995). Briefly, the porcine liver homogenate, prepared in the presence of Triton X 100, was frozen and thawed three times. The supernatant, after heat treatment, was fractionated by ammonium sulfate fractionation. The precipitate was dissolved in Tris-HCl buffer. After dialysis, the enzyme solution was purified by CM sephadex chromatography and subsequently DEAE sephacel chromatography. The purified PDI was found to be relatively pure by SDS-PAGE analysis (Laemmli, 1970).

Proteolysis of Tg by thermolysin

Limited proteolysis of Tg with thermolysin was carried out, as described previously (Gentile *et al.*, 1997). Tg (1 mg/ml) in 50 mM Tris-HCl (pH 8.0) containing 130 mM NaCl was incubated with thermolysin at the enzyme/substrate ratio of 1/50 (w/w) at 30 for 100 min. The digestion was stopped by adding EDTA (final concentration, 10 mM), and the mixture was then subjected to SDS-PAGE.

GSH/GSSG-induced oligomerization of Tg

Tg (0.7 mg/ml) was incubated in 300 μl of 25 mM Tris-HCl (pH 7.6) containing EDTA (1 mM), GSSG (1 mM) and GSH (2 mM) for 30 min at various temperatures (37°C-60°C). Independently, Tg (1.0 mg/ml) was incubated in 100 μl of 25 mM Tris-HCl (pH 7.6) containing EDTA (1 mM), GSSG (1 mM), and GSH (2 mM) in the presence of each detergent for 30 min at 45°C. Prior to loading into a 6% SDS-PAGE gel, the samples were treated with a non-reducing loading buffer.

GSH/GSSG-induced oligomerization of Tg fragments

Tg fragments (1.0 mg/ml), prepared from the treatment of Tg with thermolysin, was incubated in 1 ml of 25 mM Tris-HCl (pH 7.6) containing Triton×100 (1%), GSSG (1 mM), and GSH (2 mM) for 30 min at 50°C. Separately, the effect of KCl (1 mM) and/or PDI (60 μg/ml) was included in the above incubation mixture.

Effect of PDI on peroxidase-induced oligomerization of thermally-unfolded Tg or Tg pretreated with urea and DTT

Tg (3 mg/ml) was incubated in 50 ml of 50 mM Tris-HCl (pH 7.6) for 60 mim at 65°C. The thermally-unfolded Tg (0.6 mg/ml) was incubated with GSSG (0.05 mM), and GSH (0.2-2 mM) in the presence or absence of PDI (60 μg/ml) in 50 mM Tris-HCl (pH 7.2) containing glucose (1 mg/ml), KI (20 μM), lactoperoxidase (PO, 5 μg/ml) and glucose oxidase (GO, 2.5 μg/ml) for 30 min at 38°C.

Separately, unfolded/reduced Tg (0.6 mg/ml), which was prepared with either 50 mM DTT or 50 mM DTT/3 M urea, was incubated with GSSG (0.05 mM) and GSH (0.2 mM) in the presence or absence of PDI (60 μg/ml) in 50 mM Tris-HCl (pH 7.2) containing glucose (1 mg/ml), KI (20 μM) actoperoxidase (5 μg/ml), and glucose oxidase (2.5 μg/ml) for 30 min at 25°C.

PDI-induced oligomerization of Tg pretreated with urea and DTT

In order to prepare the unfolded/reduced Tg, Tg (15 mg/ml) was suspended in 1 ml of 50 mM Tris-HCl (pH 7.6) containing urea (3 M) and DTT (10 mM). The mixture was then incubated for 30 mim at 38°C, and passed through sephadex G-25. The unfolded/reduced Tg (0.6 mg/ml) was incubated in 50 mM Tris-HCl (pH 7.2) containing GSSG (0.1 mM) and/or GSH (1 mM) in the presence or absence of PDI (60 μg/ml), and the reaction was continued for 30 min at 25°C.

Deoligomerization of Tg oligomers by GSH/PDI

The unfolded/reduced Tg (0.6 mg/ml), which was prepared from the pretreatment of Tg (15 mg/ml) with 0.01% SDS/10 mM DTT in 50 mM Tris-HCl (pH 7.6), was incubated in 50 mM Tris-HCl (pH 7.2) containing GSSG (0.03 mM) and PDI (60 μg/ml) for 30 min at 25°C in order to generate the oligomerized forms of Tg. GSH (2 mM) was then added to the above mixture to give rise to the deoligomerization of the oligomerized Tg.

RESULTS

While human goiter Tg is known to transform to multimeric forms *in vitro* (Berndorfer *et al.*, 1996; Delom *et al.*, 1999), there have been no reports on the molecular fate of the normal bovine Tg molecule *in vitro*. In an attempt to elucidate the role of peroxidase or PDI in the process of oligomerization of Tg molecule and its deoligomerization, this study examined the molecular conversion of the bovine Tg molecule, which was unfolded using different procedures.

Initially, the thermally-induced oligomerization of bovine Tg during the exposure to glutathione redox system in 25 mM Tris-HCl buffer (pH 7.6) was examined. According to previous studies (Berdorfer *et al.*, 1996), the molecular form of Tg is broken down mainly into three types according to SDS-PAGE analyses (Fig. 1); monomer form (330 kDa), dimer form (660 kDa) and oligomer forms. Well consistent with this, the Tg prepared from the bovine thyroid exhibited the same pattern of Tg forms (Fig. 1, lane 2). Even gel chromatography of the thyroid Tg in 0.1% SDS showed the same pattern of Tg forms in SDS-PAGE analysis. Moreover, an analysis of the same sample by reducing SDS-PAGE caused the higher molecular weight bands to move to the 330 kDa band (unpublished data).

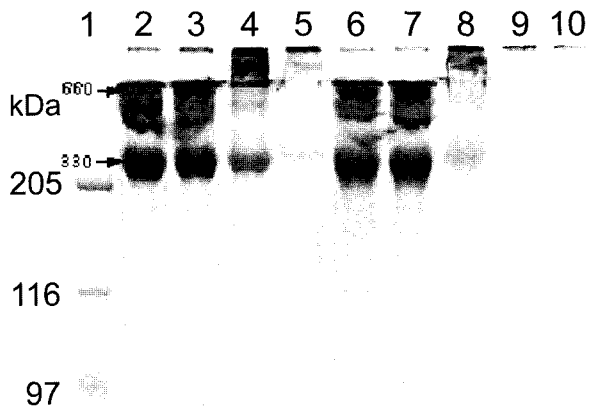

Fig. 1. Temperature-dependent oligomerization of Tg in the presence of GSH/GSSG.Tg (0.7 mg/ml) was incubated in 300 μl of 25 mM Tris-HCl (pH 7.6) for 30 min at various temperatures (25°C-60°C). Lane 1, marker proteins; Lane 2, Tg at 37°C; Lane 3, Tg at 50°C; Lane 4, Tg at 60°C; Lane 5, Tg boiled; Lane 6, Tg with GSH (2 mM)/GSSG (1 mM) at 25°C; Lane 7, Tg with GSH/GSSG at 37°C; Lane 8, Tg with GSH/GSSG at 50°C; Lane 9 with GSH/GSSG at 60°C; Lane 10, Tg boiled with GSH/GSSG.

While the molecular forms of Tg were not altered during incubation at either 37°C or 50°C, 6% SDS-PAGE analyses showed that incubation at 60°C resulted in the conversion of a large part of Tg monomer and dimmer forms to Tg multimers. The form of the boiled Tg was found to be a multimeric form as observed previously (Lee and Sok, 2000). This showed that most of Tg molecule appearing as a high-molecular weight complex was unable to enter the running gel under the denaturing conditions. Based on this, it was assumed that an intermolecular thiol disulfide exchange reaction may be responsible for the oligomerization of Tg, which had been known to contain 122 cysteinyl residues per Tg monomer. Therefore, the thermal oligomerization of Tg was carried out in the presence of 2 mM glutathione and 1 mM GSSG in 25 mM Tris-HCl (pH 7.6) from 37°C to 60°C in an attempt to test this assumption. As shown in Fig. 1, while the molecular change at 37°C was not remarkable, incubation at 50°C in the presence of the same redox buffer caused a remarkable transformation of the monomer and dimmer forms to Tg multimer forms (lane 8). Moreover, the incubation at 60°C produced a much higher degree of oligomerization, which is similar to the observation with Tg boiled in the glutathione redox buffer (lane 10). In particular, the effect of the redox buffer on the molecular pattern of Tg at 50°C or 60°C supports the notion that thiol-disulfide exchange reaction may be

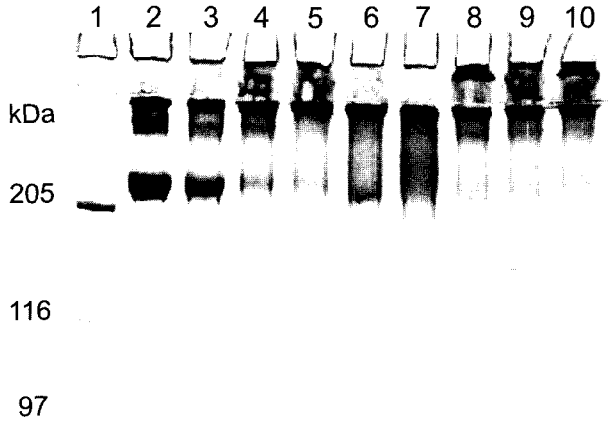

Fig. 2. Effect of surfactants on GSH/GSSG-induced oligomerization of Tg.Tg (1.0 mg/ml) was incubated in 100 μl of 25 mM Tris-HCl (pH 7.6) in the presence or absence of GSH (2 mM)/GSSG (1 mM) for 30 min at 45°C. Lane 1, marker proteins; Lane 2, Tg in the absence of redox system; Lane 3, GSSG/GSH; Lane 4, + 0.1% deoxycholate; Lane 5, + 0.3% deoxycholate; Lane 6, + 1.0% deoxycholate; Lane 7, + 0.1% Triton × 100; Lane 8, + 0.3% Triton × 100; Lane 9, + 1% Triton × 100; Lane 10, + 2% Tritoxn × 100.

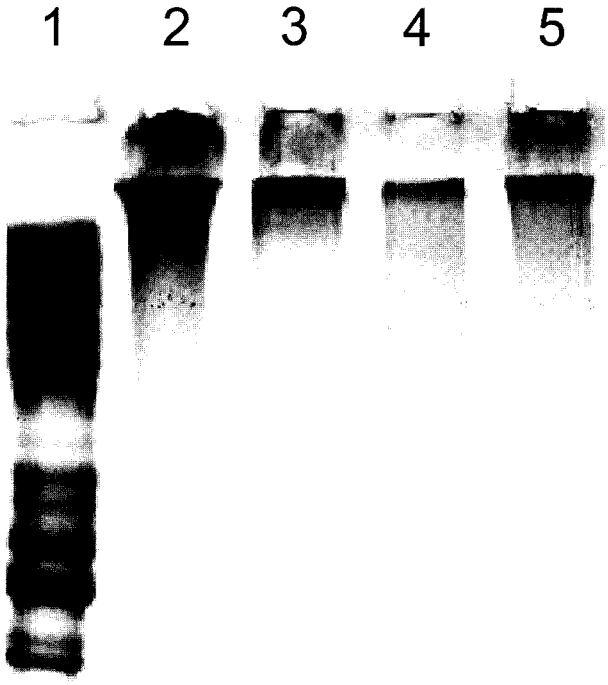

Fig. 3. GSH/GSSG-induced oligomerization of Tg fragments. Thyroglobulin fragments (1.0 mg/ml) was incubated in 1 ml of 25 mM Tris-HCl (pH 7.6) containing Triton × 100 (1%) for 30 min at 50°C. Lane 1, Tg fragments after treatment with thermolysin; Lane 2, with GSH/GSSG + Triton × 100; Lane 3, after gel filtration of lane 2 sample; Lane 4, lane 3 sample with GSH/GSSG + KCl; Lane 5, lane 4 sample + PDI (60 μg/ml).

involved in the oligomerization according to the isomerization mechanism. In a related study to examine the effect of PDI on the thermal oligomerization in the presence of a glutathione redox buffer, PDI was included in the incubation with Tg in the glutathione redox buffer at 45°C, where the PDI activity was maintained for the incubation time used. However, PDI failed to further enhance the thermal oligomerization of Tg molecule. In an additional experiment (Fig. 2), aimed at evaluating the effect of detergents on the thermal oligomerization of the Tg molecule, Triton × 100 was found to enhance the oligomerization in a concentrationdependent manner in the glutathione redox buffer. In addition, deoxycholate was found to enhance Tg oligomerization at low concentrations (0.1%), although it had no effect at higher concentrations (1.0%). Therefore, the partial and mild unfolding of Tg molecule at a local region of the molecule appears to be necessary for the effective oligomerization of Tg molecule. The interfering effect of deoxycholate at higher concentrations appears to be due to the possible reductive reversal caused by the extensive thiol disulfide exchange. Additionally, the effect of PDI on the thermally-induced oligomerization of Tg fragments, which were prepared from the treatment of Tg with thermolysin in the presence of redox buffer at 50°C, was examined in the presence of 1% Triton × 100. As shown in Fig. 3, the oligomerization of Tg fragments was successfully achieved by GSH/GSSG in the presence of Triton × 100 (lane 2). The oligomerization of Tg fragments after gel filtration appeared to be somewhat reduced by the inclusion of KCl (dipersed trace at lane 4 in Fig. 3). In contrast, the addition of PDI was successful in enhancing the oligomerization, indicating that PDI appears to enhance the intermolecular disulfide linkage between the Tg fragments at 50 °C.

Subsequently, the peroxidase-induced oligomerization of Tg molecule was examined. In contrast to human goiter Tg (Delom *et al.*, 1999; Klein *et al.*, 2000), which exhibited oligomerization after treatment with a peroxidase system, normal bovine Tg showed no oligomerization at 38°C. Therefore, the structurally-unfolded Tg molecule was employed for the peroxidase-catalyzed oligomerization. For this purpose, Tg (3 mg/ml) was thermally unfolded by preincubating it at 65°C in 50 μl of 50 mM Tris (pH 7.6) for 60 min. The thermally-unfolded Tg (0.6 mg/ml) was then exposed to various oxidation conditions as shown in Fig. 4. Overall, the extent of oligomerization was lower in 50 mM Tris-HCl buffer (Fig. 4, lane 1) than in 25 mM Tris-HCl buffer (Fig. 1, lane 4). In particular, the thermally-unfolded Tg exhibited partial dimerization under the conditions used; some part of the 330 kDa Tg moved to the 660 kDa Tg molecule. The treatment of the thermally-unfolded Tg with HOI-generating system (peroxidase/glucose oxidase/

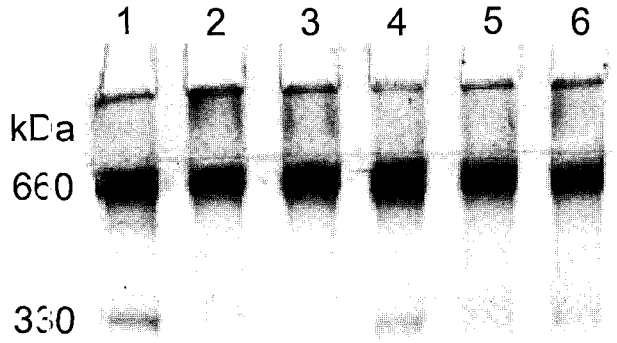

Fig. 4. Effect of PDI on the peroxidase-induced oligomerization of thermally unfolded Tg.Thermally-unfolded Tg (0.6 mg/ml) was incubated in 50 mM Tris-HCl (pH 7.2) for 30 min at 38°C. Lane 1, Tg after pretreatment at 65°C; Lane 2, + glucose/ PO/GO; Lane 3, + glucose/PO/GO/KI. Lane 4, + glucose/PO/GO/GSSG/GSH; Lane 5,+ glucose/PO/GO/KI/GSSG/GSH; lane 6, + glucose/PO/GO/GSSG/GSH/PDI.

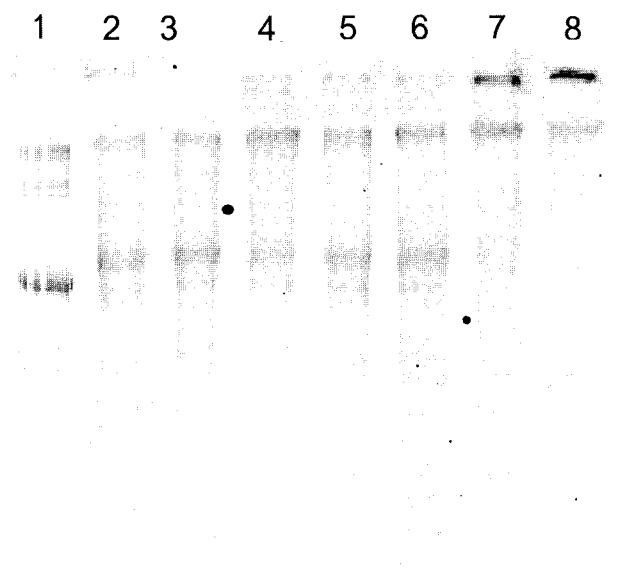

Fig. 5. Effect of PDI on oligomerization of Tg treated with urea/DTT. Unfolded/reduced Tg (0.6 mg/ml) was incubated in 50 mM Tris-HCl (pH 7.2) for 30 min at 25°C. Lane 1, Tg; Lane 2, after gel filtration of Tg treated with urea/DTT (10 mM); Lane 3, lane 2 sample + GSH; Lane 4, lane 2 sample + GSH/PDI; Lane 5, lane 2 sample + GSSG (0.01 mM); Lane 6, lane 2 sample + GSSG (0.1 mM); Lane 7, lane 2 sample + GSSG (0.01 mM)/PDI (60 μg/ml); Lane 8, lane 2 sample + GSSG (0.1 mM) PDI.

KI) caused the remaining Tg of 330 kDa to move to the dimeric form. Thus, the thermally-unfolded Tg appears to expose some part of the cysteine residue to oxidative

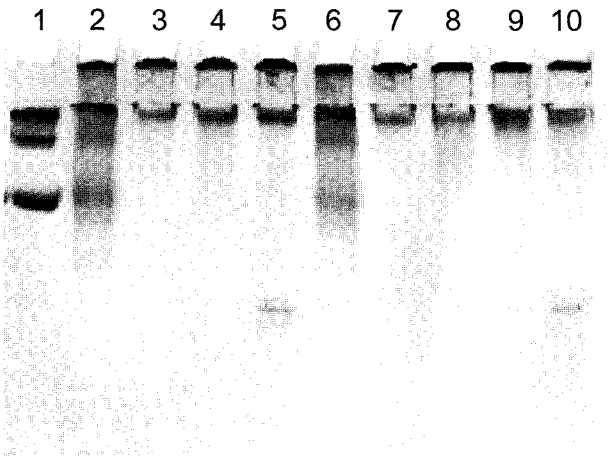

Fig. 6. Effect of PDI on peroxidase-induced oligomerization of Tg treated with DTT or DTT/urea.Unfolded/reduced Tg (0.6 mg/ml) was incubated in 50 mM Tris-HCl (pH 7.2) for 30 min at 25°C. Lane 1, Tg; Lane 2, after gel filtration of Tg pretreated with 50 mM DTT; Lane 3, + glucose/PO/GO/KI; Lane 4, + glucose/PO/GO/KI/GSSG/GSH; Lane 5, + glucose/PO/GO/KI/GSSG/GSH/PDI; Lane 6, after gel filtration of Tg pretreated with 50 mM DTT/3 M urea; Lane 7, + glucose/PO/GO/KI; Lane 8, + glucose/PO/GO/ KI/GSSG/GSH; Lane 9, + glucose/PO/GO/ KI/GSSG/GSH/PDI; Lane10, +glucose/PO/GO/KI/GSSG/GSH/PDI.

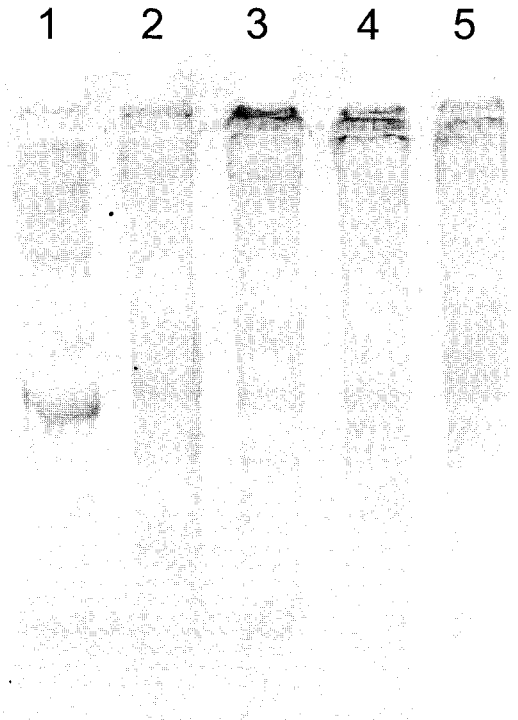

Fig. 7. Deoligomerization of oligomerized Tg by GSH/PDI. The unfolded/reduced Tg (0.6 mg/ml) was incubated in 50 mM Tris-HCl (pH 7.2) containing GSSG (0.03 mM) and PDI (60 μg/ml) for 30 min at 25°C. GSH was then included in the same mixture. Lane 1, Tg; Lane 2, after gel filtration of Tg pretreated with 0.01% SDS/10 mM DTT; Lane 3, lane 2 sample + GSSG (0.03 mM)/PDI (60 μg/ml); Lane 4, lane 3 sample + GSH (0.2 mM)/PDI (60 μg/ml); Lane 5, lane 3 sample + GSH (2 mM)/PDI (60 μg/ml).

disulfide formation by peroxidase. Since PDI had been previously reported to retard the peroxidase-induced multimerization of goiter Tg (Delom *et al.*, 1999), the effect of PDI on the peroxidase-induced dimerization of unfolded Tg was examined. Fig. 4 shows that whereas the glutathione redox system somewhat inhibited the peroxidaseinduced conversion of 330 kDa protein (lane 4), PDI did not cause any remarkable alteration in the profile of Tg forms (lane 5). In contrast to the effect of PDI on the peroxidase-induced oligomerization of goiter Tg (Delom *et al.*, 1999), the thermally-unfolded Tg was not adequate for the study on the role of PDI in peroxidase-induced oligomerization.

DISCUSSION

Previous *in vivo* results suggest that the Tg molecule can be multimerized by peroxidase catalysis (Berndorffer *et al.*, 1996; Delom *et al.*, 1999; Klein *et al.*, 2000). This event is limited largely to the thyroid follicles, where the peroxidase activity is responsible for the oxidative oligomerization (Berndorffer *et al.*, 1996). Most of these studies were carried out using human goiter Tg, which is sensitive to oxidative disulfide linking. Thus far, there has been no extensive study on the oxidative oligomerization of bovine Tg *in vitro*.

This study showed that thermally-unfolded bovine Tg is susceptible to a thiol disulfide exchange reaction, irrespective of the glutathione redox system, leading to non-enzymatic oligomerization. In addition, thermallyunfolded Tg appears to be sensitive to peroxidase. Normal Tg may be distinguished from goiter Tg at least by its sensitivity to oxidative disulfide linking by peroxidase. This might be due to differences in the susceptibility of the surface-exposed cysteine residue to peroxidase oxidation. In support of this, the peroxidase-catalyzed oligomerization of Tg was greater with Tg treated with urea and thiol, which can produce the unfolded/reduced form. This shows that the microenvironmental structure in the N-terminal domain or the thioredoxin box region of normal Tg, which had been reported to be involved in Tg multimerization (Delom *et al.*, 1999), may differ according to the type of Tg molecule or its source.

The enhancing effect of the glutathione redox state on the thermally-induced oligomerization of Tg, may be consistent with the idea that the thiol-disulfide exchange is responsible for the oligomerization. This is well supported by the apparent effect of the redox buffer at 55°C or 65°C, where protein unfolding may begin to occur. Nevertheless, PDI had no remarkable effect on the thermally-induced oligomerization of Tg via the thiol-disulfide exchange, which suggests that PDI catalysis may not be important for thiol disulfide exchange in proteins, where self-assisted thiol-disulfide exchange actively occurs. Therefore it is hypothesized that the excess glutathione redox buffer may suppress the PDI-catalyzed oligomerization of unfolded/oxidized Tg via thiol disulfide exchange. In this regard, it appears that PDI has a low sensitivity to unfolded/oxidized Tg.

Early in the maturing process of Tg, nascent Tg was detected as aggregates linked by interchain disulfide bonds in the thyrocytes (Kim *et al.*, 1993). In particular, nascent Tg is destined to form transient aggregates in the thyroid follicles. Furthermore, the extent of disulfide-linked aggregation was particularly altered by treating cells with a reducing agent, which is believed to perturb the folding of the exportable proteins by altering the redox potential in the ER lumen (Kim *et al.*, 1993). This *in vivo* study also reaffirms the apparent role of PDI in the oligomerization of unfolded/reduced Tg, which is structurally similar to the nascent Tg. The time-dependent course for the formation of Tg multimers suggests a gradual thiol disulfide exchange reaction, which is catalyzed by PDI. Moreover, the redox condition controlled the oligomerization of Tg, which was favorable in a relatively oxidizing environment. Therefore, the role of the glutathione redox state in the oxidase function of PDI should not be neglected, although recent data (Frand and Kaiser, 1999; Tu *et al.*, 2000) shows an inclination to emphasize the oxidant protein, Ero1.

Conversely, the Tg multimer was deoligomerized by PDI in the predominantly reducing condition, suggesting the reduction of PDI by the excessively reducing conditions. Thus, a remarkable reductive reversal of the oligomerized Tg was observed with the unfolded/reduced Tg, which was prepared bt treating it with deoxycholate/DTT, but not with urea/DTT. Other results, where GSH is replaced by reduced lipoic acid, suggests that reduced lipoic acid, a dithiol, was more effective than GSH, a monothiol, which is consistent with the higher reducing power of dithiol (unpublished data). This might be explained by the assumption that PDI is maintained in a reduced dithiol form for the effective reductive deoligomerization of Tg. The Tg bands at lower molecular weights, which were distributed along the running gel, indicate that the rate of aggregate dissolution may be relatively slow.

Overall, PDI acts as an oxidant for the unfolded/reduced Tg, while it may be an isomerase for the unfolded/oxidized form. However, the isomerase role of PDI may not be important for the multimerization of the unfolded/ oxidized Tg in relatively reducing redox conditions. In addition, it is possible to imagine that PDI may be a reductant for the Tg oligomers, which are folded/oxidized. Therefore, the role of PDI differs according to the molecular form of Tg as well as the glutathione redox state.

An incomplete tertiary structure creates a great

susceptibility for interactions between the not-yet-buried hydrophobic regions of the nascent Tg molecules (Kim *et al.*, 1992). This leads to intermolecular non-polar bonding. Tg molecule is known to generate the aggregated form through via non-polar binding (Kim *et al.*, 1992; Kim *et al.*, 1993; Muresan and Arvan, 1998), and some chaperones exert their function through the non-polar property. Since the promoting effect of Triton × 100 and deoxycholate on Tg multimerization might be ascribed to their interference with the non-polar binding, a non-polar interaction may be a factor to affect the oligomerization of Tg molecule. This might be related to an earlier report (Kim *et al.*, 1992) that Tg maturation required chaperones such as BiP in addition to PDI. PDI and BiP may act sequentially on the reduction of the intermolecular disulfide bridges, causing Tg molecules to gradually detach themselves.

Earlier, it was reported that the structure of Tg is characterized by the existence of a thioredoxin box (CXXC), a vicinal dithiol, which is conserved in Tg (Klein *et al.*, 2000). Therefore, the oligomerization of Tg in the presence of GSH was meant to proceed through the PDI-like redox activity present in the thioredoxin box of Tg. In this mechanism, the surface-exposed vicinal SH-groups may be employed to generate intermolecular disulfide bonds or isomerize an existing intramolecular disulfide bond to an intermolecular disulfide bond. Meanwhile, the N-terminal domain (NTD) of human goiter Tg was suggested to be responsible for the peroxidase-induced multimerization of Tg (Delom *et al.*, 1999). Since the interfering role of PDI in the polymerization of goiter Tg through the NTD region was contrary to the enhancing effect of PDI in oxidative multimerization of the unfolded/reduced Tg, it is highly likely that the NTD region might not be involved in the oligomerization. Instead, it is feasible that PDI-catalyzed oligomerization may be related to the thioredoxin box-rich region of Tg. Although the microenvironment of the thiredoxin region was not examined further, it is proposed that the thioredoxin box region may be hidden in the inner side of the Tg molecule. This might explain why Tg oligomerization required a primary unfolding. This may be analogous to an earlier report (Sok and Sih, 2001) that the tyrosine residues in the thioredoxin region may be protected from the thyroid peroxidase-generated HOI, a diffusible modifier of the tyrosine residue in other proteins. Overall, this study suggests that in order to be utilized by PDI, the Tg molecule needs to be at least partially unfolded.

In conclusion, it is proposed that PDI participates in the oxidative oligomerization of Tg molecule as well as its deoligomerization. Moreover, the PDI-catalyzed molecular change of Tg is governed by the glutathione redox state, since the isomerase action of PDI is also important for the continuous thiol disulfide exchange on Tg molecule. Further study to determine how the molecular form of PDI, oxidized or reduced, can be switched in the ER system needs to be done.

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