Alterations in the Enzyme Activity and Protein Contents of Protein Disulfide Isomerase in Rat Tissues During Fasting and Refeeding

Tomoko Mikami, Rieko Genma, Kozo Nishiyama, Shinichiro Ando, Akira Kitahara, Hiroko Natsume, Teruya Yoshimi, Ryuya Horiuchi, and Hirotoshi Nakamura

Protein disulfide isomerase (PDI) is an enzyme that participates in the formation of disulfide bonds. It is also known to be the subunits of some enzymes and the membrane-associated thyroid hormone–binding protein. In this study, we measured the quantitative distribution of PDI protein in rat tissues and examined the relationship between protein level and enzyme activity in PDI during fasting and refeeding. Western blotting with specific anti-PDI antiserum detected the PDI protein band of 55 kd. Among several tissues, liver contained the largest amount of PDI protein, followed by kidney and fat, in which one-third to one-fourth of the hepatic PDI protein existed. The PDI protein band was also detected in heart and muscle. Fasting for 3 days decreased PDI protein levels in rat liver by 40%; control levels were recovered after 3 days of refeeding. The same change was observed in kidney. PDI activity, measured by the scrambled ribonuclease method, did not show the parallel alteration to PDI protein level in liver and kidney. Isomerase activity decreased to 50% of control values during fasting, but did not recover by refeeding. Thyroidal status did not affect either PDI protein level or isomerase activity. These findings show that fasting and refeeding affect PDI protein and enzyme activity, and that PDI protein level does not always reflect PDI activity.

THE FORMATION OF disulfide bonds in secretory and cell-surface proteins occurs in the lumen of the endoplasmic reticulum (ER) in eukaryotic cells. Protein disulfide isomerase (PDI), the enzyme that locates abundantly in ER, is considered to catalyze the disulfide bond formation of newly synthesized proteins. A close correlation has been shown between the levels of PDI activity and the extent of synthesis of disulfide bond–containing proteins such as immunoglobulins.[1] Bulleid and Freedman[2] prepared PDI-deprived dog pancreas microsomes, which function in processing protein synthesis. Using the formation of intramolecular disulfide bonds during the in vitro synthesis of γ-gliadin, a plant storage protein, as a model, they showed the critical participation of PDI in cotranslational formation of disulfide bonds.

In addition to the role in disulfide bond formations, PDI also functions as the subunits of prolyl-4-hydroxylase[3] and the microsomal triglyceride transfer protein (MTP). Prolyl-4-hydroxylase is a heterotetramer enzyme composed of two α- and two β-subunits and catalyzes the posttranslational hydroxylation of peptidyl proline residues in procollagen.[3] PDI itself does not have the enzyme activity of prolyl-4-hydroxylase, but as the β-subunit, it functions to keep the active form of the enzyme by preventing the α-subunit aggregation.[4] MTP, a heterodimeric lipid transfer protein composed of a unique, large, 97-kd subunit and PDI, is essential for the assembly and secretion of apolipoprotein B–containing lipoproteins.[5] PDI is again considered to prevent MTP aggregation.[5] PDI also functions as glutathione-insulin transhydrogenase, which degrades insulin by cleaving the disulfide bonds.[6]

Furthermore, PDI has been shown to be a triiodothyronine (T_3)-binding protein. The ligand-binding assays[7] and affinity-labeling studies[8] suggested the existence of T_3-binding protein in plasma membranes. Cheng et al[9] and Horiuchi et al[10] purified and characterized this membrane-associated T_3-binding protein. The apparent molecular size of the protein was 55 kd, and it localized primarily in the ER and nuclear envelopes. The cDNA isolation for this protein revealed that the 55-kd protein was highly homologous to PDI.[11] Horiuchi et al[10] demonstrated that the purified membrane-associated T_3-binding protein contained the PDI activity.

Thus, PDI is a multifunctional protein. The physiological functions of this protein are still not fully clarified. The quantitative analysis of the PDI protein in various tissues has not been determined, although the tissue distribution has been studied by immunohistochemistry,[12] enzyme assay,[13] and Northern blotting.[14] The expression of PDI is influenced by the extent of protein biosynthesis[15] and "stress conditions,"[16] which induce the synthesis of other ER proteins such as BiP and GRP94. In this study, we assayed PDI protein contents in various rat tissues using Western blot analysis and studied the changes in PDI protein levels and isomerase activities in rat liver and kidney during fasting and refeeding. We also examined the influence of thyroid function on PDI.

MATERIALS AND METHODS

Preparation of Samples

Male Sprague-Dawley rats (6 to 7 weeks of age, 150 to 180 g body weight) were killed by exsanguination through the abdominal aorta. Liver, kidney, brain, heart, femur muscles, and abdominal fat were removed and stored at −70°C. The enzyme activity of PDI in the tissues was retained for at least 6 months under this condition (data not shown). A portion of the liver was homogenized using a Teflon-pestle/glass-body homogenizer with 10 up-and-down strokes in 4 vol of 0.25 mol/L sucrose and 5 mmol/L $MgCl_2$ on ice. Other tissues were homogenized in 2.5 vol of the same solution. Protein concentration in the homogenate was measured by the Bradford procedure[17] (Bio-Rad protein assay; Bio Rad, Hercules, CA). Aliquots of the homogenates were used for Western blotting and isomerase activity assays.

From the Second Division, Department of Internal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka; and the Department of Pharmacy, Gunma University School of Medicine, Gunma, Japan.

Submitted September 29, 1997; accepted March 9, 1998.

Address reprint requests to Hirotoshi Nakamura, MD, Second Division, Department of Internal Medicine, Hamamatsu University School of Medicine, 3600 Handacho, Hamamatsu, Shizuoka, 431-31, Japan.

0026-0495/98/4709-0010$03.00/0

Western Blotting Analysis of PDI Protein

The homogenates were boiled at 100°C for 5 minutes in the sample buffer containing sodium dodecyl sulfate (SDS; 2.3% SDS, 5% β-mercaptoethanol, 10% glycerol, and 62.5 mmol/L Tris) before electrophoresis. Western blotting analyses were performed as we have described previously.[18] In brief, the samples (20 μg protein/lane of the liver homogenate and 100μg protein/lane of other tissue homogenates) were separated on SDS–polyacrylamide gel electrophoresis (PAGE) with 10% polyacrylamide gel and then electrophoretically transferred to nitrocellulose membranes (Trans-Blot; Bio-Rad) at 0.8 mA/cm^2 membrane at room temperature. After being blocked overnight with Block Ace (Yukijirushi-Nyugyou, Sapporo, Japan), the membrane was incubated with antibovine PDI antiserum[10] in 0.5% bovine serum albumin (BSA), 5% Block Ace, and 0.1% Tween-20 at 1:2,500 dilution overnight. The membrane was washed three times with 0.1% Tween-20 in phosphate-buffered saline (T-PBS) and then incubated with an alkaline phosphatase–conjugated goat antirabbit immunoglobulin (Tago, Burlingame, CA) in 5% Block Ace and T-PBS at 1:1,000 dilution for 2 hours at room temperature. After three washes with T-PBS, the blot was developed with nitro blue tetrazolium (NBT; Sigma, St Louis, MO) and 5-bromo-4-chloro-3-indolyl-phosphate *p*-toluidine salt (BCIP; Sigma) in 100 mmol/L Tris-HCl (pH 9.5), 100 mmol/L NaCl, and 5 mmol/L $MgCl_2$. Immunoblot was quantified by computerized densitometry using the National Institutes of Health image program on an Apple Macintosh computer (Cupertino, CA).

PDI Isomerase Activity Assay

Scrambled ribonuclease was prepared by the method of Hillson et al.[19] PDI isomerase activity was assayed by reactivation of RNase according to the method of previously described.[10] The homogenate of the liver (100 to 200 μg of protein) or kidney (100 to 600 μg of protein) was sonicated in an ice bath and incubated with 10 μg scrambled RNase in 200 μL of 100 mmol/L sodium phosphate (pH 7.5), 10 mmol/L EDTA and 3 μmol/L diothiothreitol (DTT) at 20°C for 20 minutes. After the incubation, 800 μL of 0.2 mol/L sodium acetate (pH 5.0) was added to stop the RNase reactivation. The reactivated RNase (200 μL) was incubated with 200 μL of 1% yeast RNA in 0.1 mol/L sodium acetate (pH 5.0) at 37°C for 4 minutes. The reaction solution was mixed with 200 μL of 0.75% uranyl acetate in 25% perchloric acid and centrifuged at 18,000 × *g* for 5 minutes at 4°C. Acid-soluble RNA in the supernatant was measured by absorbance at 260 nm. Absolute PDI activity was defined as $A = A' - A_0$ (A, PDI activity; A′, OD260 of the unknown sample; A_0, OD260 of the sample to which no tissue homogenate was added).

Experimental Designs for Fasting/Refeeding and Hyperthyroidism/Hypothyroidism

In the fasting and refeeding study, Sprague-Dawley rats (6 to 7 weeks of age, 150 to 180 g body weight) were separated into five groups. Rats were fasted for 24 hours (1-day–fasted group) or 3 days (3-day–fasted group) or were refed for 3 (3-day–refed group) or 6 days (6-day–refed group) after a 3-day fast. Control rats were fed on stock diet and water ad libitum. As compared with control rats, animals in the 3-day–fasted, 3-day–refed, and 6-day–refed groups, but not in the 1-day–fasted group, had significantly reduced body weights (233 ± 10 g in control rats, mean ± SD, n = 16; 217 ± 10 g in 1-day–fasted rats, n = 6; 188 ± 12 g in 3-day–fasted rats, n = 11, $P < .05$ *v* control rats; 205 ± 6 g in 3-day–refed rats n = 11, $P < .05$ *v* control rats; 210 ± 10 g in 6-day–refed rats, n = 6, $P < .05$ *v* control rats).

To observe the influence of thyroid hormone status, rats were rendered into a hyperthyroid state by injecting 2.5 μg T_3 subcutaneously twice a day for 7 days or a hypothyroid state by giving 0.05% propylthiouracil for 2 weeks as drinking water. Body weights at death were reduced significantly in both hyperthyroid and hypothyroid rats compared with those in control rats (246 ± 8 g in control rats, 225 ± 12 g in hyperthyroid rats $P < .05$ *v* control; 208 ± 13 g in hypothyroid rats, mean ± SD, four rats in each group).

Statistical Analysis

Statistical analysis for differences in rat body weight, PDI protein contents, and isomerase activity was determined by ANOVA and Scheffe's F test using StatView 4.0 (Abacus Concepts, Berkeley, CA).

RESULTS

Tissue Distribution of PDI Protein Analyzed by Western Blotting

Proteins in rat liver and kidney homogenates were separated by SDS-PAGE and the immunoblots probed with a specific anti-PDI antibody. A main band with a molecular weight of 55 kd was demonstrated in each tissue. Control serum or unrelated antibody such as anti-T_3 receptor antibody[20] did not detect the 55-kd band (Fig 1A). On the basis of molecular size and specificity of immunodetection with anti-PDI antibody, the 55-kd band was considered to be a PDI protein. Occasionally, other bands were detected around the 55-kd band, especially when the membranes were exposed to the NBT/BCIP solution for a long time. Since these bands were not constantly detected, and it was difficult to determine their specificity, they were not included in the analysis.

To estimate the concentration of PDI proteins in various tissues, we first examined the linearity between the protein contents of the sample loaded on the gel and the intensity of the PDI protein band measured by a densitometer. Linearity was observed up to 40 μg protein per lane of the liver and 200 μg protein per lane of the kidney homogenate (data not shown). Protein samples of 20 μg of the liver homogenate and 100 μg of other tissue homogenates were analyzed by Western blotting. As Fig 1 shows, the intensity of the 55-kd band was the strongest in liver among tissues, followed by kidney and fat, which showed about one third and one fourth of the intensity in liver, respectively. PDI protein levels in heart and muscle were both about one tenth that in liver. In brain, a band with slightly faster electrophoretic mobility was sometimes, but not always, detected. The estimated molecular weight of this protein was approximately 52 kd. Although it was not certain whether this band was nonspecific or represented a PDI-related protein, the level of this protein with 52 kd in brain was, if estimated from some suitable densitograms, similar to that in heart and muscle. Table 1 lists the relative concentrations of PDI protein in various tissues.

Changes in PDI Protein Contents in Liver During Fasting and Refeeding

The alternation of the PDI protein level in liver during fasting and refeeding was studied. Fasting for 1 day decreased PDI proteins slightly, and 3-day fasting reduced them to 60% of control (Fig 2A and B). When rats were refed for 3 and 6 days after 3-day fasting, PDI proteins were completely recovered to the basal level.

PDI Isomerase Activity in Liver

To determine whether the changes in PDI protein level reflected enzyme activity, PDI isomerase activity was measured

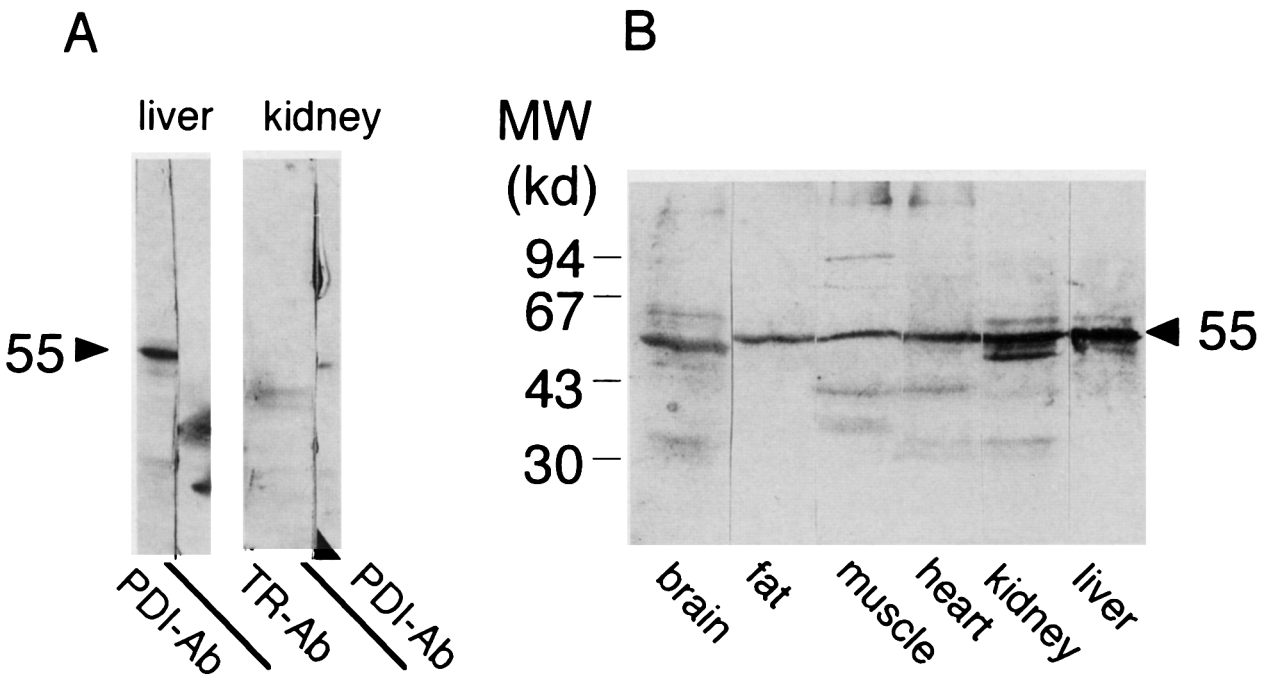

Fig 1. Tissue distribution of PDI proteins analyzed by Western blotting. (A) Homogenates of the liver (20 μg of protein/lane) and kidney (100 μg protein/lane) were separated by SDS-PAGE with 10% polyacrylamide gel, transferred to a nitrocellulose membrane, and immunodetected by anti–PDI antibody (PDI-Ab) or anti-nuclear T_3 receptor antibody (TR-Ab). The 55-kd band was detected by anti-PDI antibody. (B) Homogenates of the various tissues (20 μg of protein in liver, 100 μg of protein in other tissues) were immunoreacted with the anti-PDI antibody. The 55-kd bands were revealed in liver, kidney, muscle, heart, and fat. In brain, a band with a smaller molecular weight (≈52 kd) was sometimes, but not constantly, detected, which could not to be determined to be a PDI protein band. The immunoblot density was quantified by densitometry.

by the scrambled ribonuclease assay. Isomerase activity declined significantly after 24-hour fasting, and it decreased to 50% of control after 3-day fasting (Fig 3). Different from the PDI protein level, enzyme activity was not recovered by refeeding. It remained at a low level, even after 6-day refeeding.

Effects of Fasting and Refeeding on PDI Protein and Enzyme Activity in Kidney

The PDI protein in kidney slightly but not significantly increased at 24-hour fasting. However, kidney showed a 40% reduction of PDI protein compared with control values at 3-day fasting. Refeeding completely recovered it to control levels (Fig 4A). PDI isomerase activity in kidney also showed similar changes to that in liver. As shown in Fig 4B, enzyme activity was reduced significantly by 3-day fasting and was not recovered by refeeding.

Table 1. Relative PDI Protein Contents in Various Tissues

Tissue	%
Liver	100
Kidney	36.1 ± 2.2
Fat	26.1 ± 23.3
Heart	11.1 ± 3.41
Muscle	9.15 ± 3.82

NOTE. Intensities of the 55-kd PDI bands in the various tissues detected by Western blotting were quantified by densitometry and relatively expressed as percent of the intensity in liver. The experiment was repeated 5 times, and results are expressed as means ± SD.

Effects of Thyroid Hormone Status on PDI

The effects of thyroid hormone on PDI proteins and enzyme activities were studied in liver and kidney. As shown in the Fig 5, neither PDI protein contents nor PDI isomerase activities were changed significantly by hyperthyroidism or hypothyroidism in either tissue.

DISCUSSION

The enzyme activity of protein disulfide isomerase is widely detectable in various tissues. Brockway et al[13] reported that the highest activity of PDI was found in liver and pancreas, in which the proteins with disulfide bonds were actively synthesized and secreted, whereas kidney, heart, muscle, and brain had one-fifth to one-twentieth isomerase activity in liver. The expression of PDI-mRNA was reported to be generally consistent with the distribution of isomerase activity. However, in white adipose tissue, it has been demonstrated that no enzyme activity was detected despite the very high PDI-mRNA level.[21] Immunohistochemical study showed that PDI protein is abundant in liver, pituitary, thyroid, and pancreas, but was not detected in kidney, muscle, and brain.[12] In the present study, we quantitatively measured PDI protein contents in various tissues with Western blot analysis. PDI protein was most abundant in liver, followed by kidney, fat tissues, heart, and muscle. The findings were generally consistent with those of PDI activity reported by Brockway et al.[13] The discrepancy of PDI protein levels in kidney and muscle between the immunohistochemical study previously reported[12] and our present Western blot study

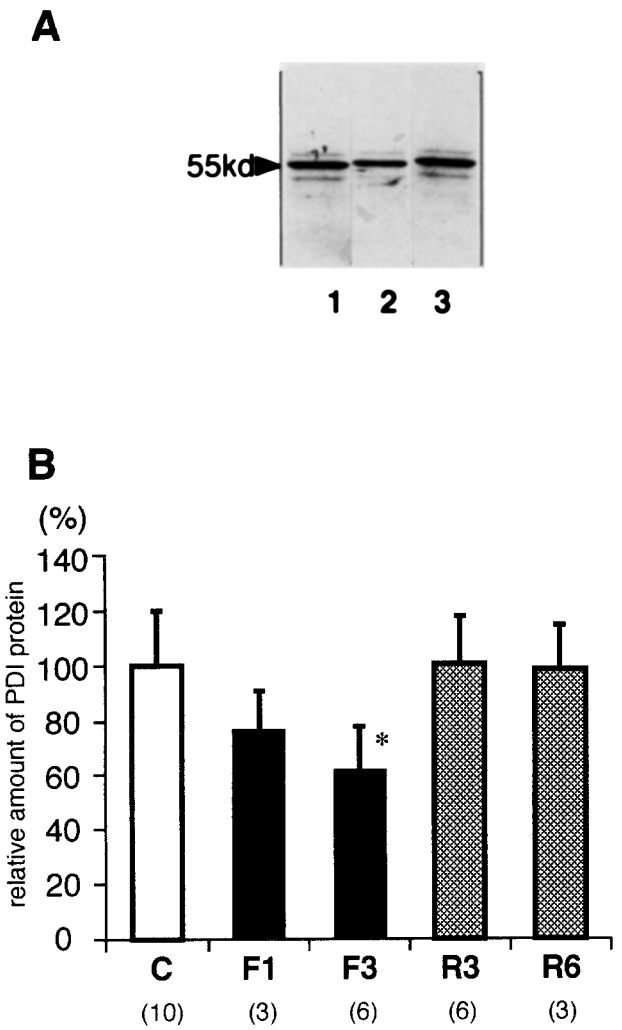

Fig 2. Change in PDI protein level in liver during fasting and refeeding. (A) Western blotting. Lane 1, control rats fed on stock diet and water ad libitum. Lane 2, rats fasted for 3 days. Lane 3, rats refed for 3 days after a 3-day fasting. Homogenates (20 μg of protein/lane) were immunoreacted with the anti-PDI antibody. (B) Change in PDI protein level in liver. Density of the 55-kd bands was quantified by densitometry. Hepatic PDI proteins in rats of 1-day-fasted (F1), 3-day-fasted (F3), 3-day-refed (R3), and 6-day-refed (R6) groups (see Methods) were relatively estimated, and PDI proteins in control rats (C) were expressed as 100%. Numbers in parentheses are rats in each group. Results are means ± SD. *$P < .05$ *v* control and 6-day-refed group.

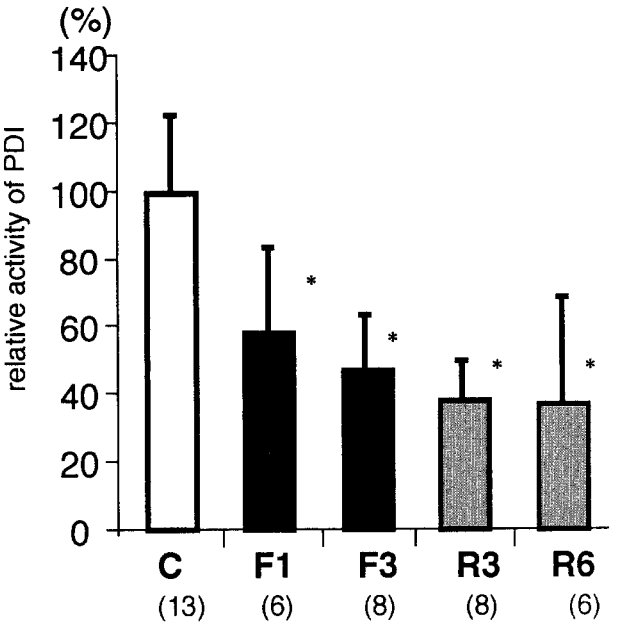

Fig 3. Change in PDI isomerase activity in liver during fasting and refeeding. PDI activity is measured by the scrambled ribonuclease assay as described in the Methods. Experimental rat groups are described in the legend to Fig 2. PDI isomerase activity in each group was relatively estimated and activity in the control group was expressed as 100%. Results are means ± SD. Numbers in parentheses are rats in each group. *$P < .05$ *v* control.

may be due to the difference in the anti-PDI antibodies used and also differences in the analytical methods. Regarding PDI in adipose tissue, in which a discrepancy between mRNA and enzyme activity levels has been reported, we confirmed the relatively plentiful existence of PDI protein in adipose tissues.

In rat brain, where PDI-mRNA was demonstrated,[22] we failed to observe the same PDI 55-kd band as detected in other tissues. Instead, a protein band with a smaller molecular weight was sometimes detected. The level of this protein seemed to be similar to that in heart and muscle, but we could not conclude that this band represented PDI protein.

The physiological alteration of the enzyme activity and the protein content of PDI has been poorly studied. We found in this study that 3-day fasting decreased PDI activity and protein levels in liver and kidney. Varandani[6] reported that hepatic PDI activity measured as glutathione-insulin transhydrogenase significantly decreased in starved or alloxan-induced diabetic rats. Varandani suggested that the low insulin levels in the manipulated animals might contribute to decreased in PDI activity. However, recent studies have shown that insulin negatively regulates the PDI gene at the transcriptional level.[23] Thus, it remains unclear how insulin affects PDI.

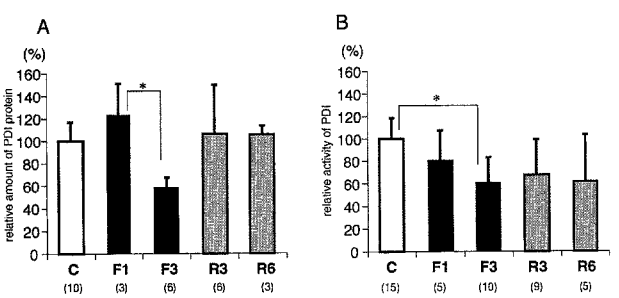

Fig 4. Effects of fasting and refeeding on PDI protein and isomerase activity in kidney. (A) Change in PDI protein level in kidney. Rat kidney homogenates (100 μg of protein/lane) were analyzed. (B) Change in the PDI isomerase activity in kidney. Isomerase activity was measured by scrambled ribonuclease assay using 600 μg protein in kidney homogenate. PDI protein contents and isomerase activity in kidney of control rats (C) were expressed as 100%, and those in other groups were relatively estimated. Results are means ± SD. Numbers in parentheses are rats in each group. Experimental rat groups are described in the legend to Fig 2. *$P < .05$.

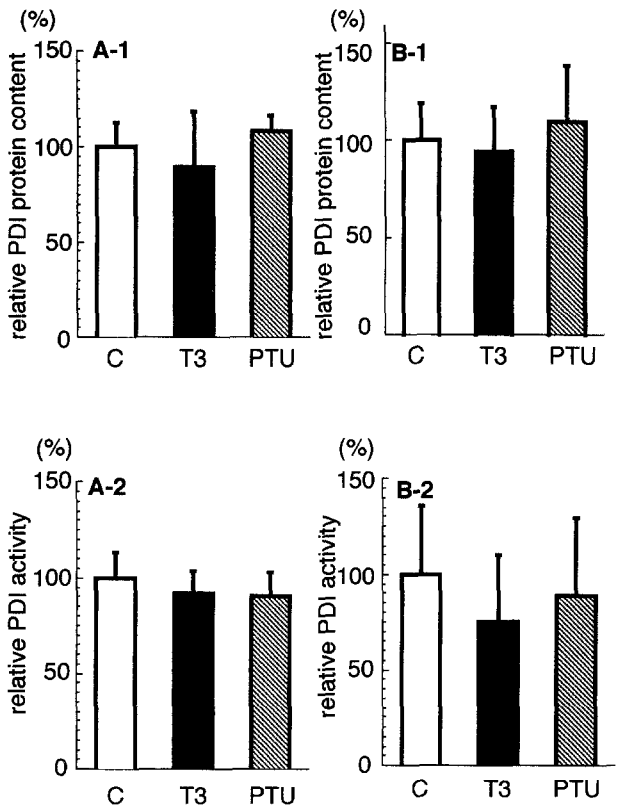

Fig 5. Effects of thyroid function on PDI protein and isomerase activity. PDI protein and isomerase activity in control rats (C) were expressed as 100%, and those in hyperthyroid (T_3) and hypothyroid rats (PTU) were relatively estimated. Results are expressed as means ± SD. Number of the rats in each group = 4. (A-1) Change of PDI protein in liver. (A-2) Change of PDI isomerase activity in liver measured by scrambled ribonuclease assay using 100 μg protein of homogenate. (B-1) Change of PDI protein contents in kidney. (B-2) Change of PDI isomerase activity in kidney measured by scrambled ribonuclease assay using 100 μg protein in the homogenate.

Previously, we examined the alteration of the rat nuclear T_3 receptors (NT_3R) measured in starvation or T_3 administration by the ligand-binding assay and found that the change in the NT_3R levels was less prominent in kidney than in liver.[24] Our later study using the Western blot analysis demonstrated that starvation decreased the NT_3R protein itself.[25] In the present study, no difference was found in the PDI change between liver and kidney, either in isomerase activity or in PDI protein contents.

Interestingly, different from the PDI protein level, isomerase activity was not normalized even at the sixth day after refeeding. Another hepatic T_3-binding protein, type I 5′-deiodinase, was also shown to decrease the enzyme activity during fasting, but the activity recovered gradually to the control level by the sixth day postrefeeding.[26] Declined activity of hepatic glutathione-insulin transhydrogenase was also normalized by 48-hour refeeding.[6] What is the mechanism of dissociation between PDI protein level and enzyme activity level during refeeding? Although it is known that the measurement of PDI isomerase activity is strongly dependent on the composition of the glutathione/glutathione disulfide redox buffer,[27] and that the concentration of the nonprotein sulfhydryl groups in liver homogenates is lowered by starvation,[28] it is unlikely that the decreased isomerase activity was due to the lowered nonprotein sulfhydryl groups in the assay mixture, since the addition of even 10 μmol/L DTT to the assay buffer could not restore PDI activity (data not shown). Alternatively, it is known that PDI activity is inhibited when some proteins bind to the PDI protein.[29] Indeed, isomerase activity declines to half when PDI binds to the α-subunits of prolyl-4-hydroxylase as its β-subunits.[3] If a portion of the PDI protein that increased during refeeding functions as a subunit of prolyl-4-hydroxylase or MTP, the increase in PDI protein may not reflect isomerase activity.

Starvation markedly affects thyroid functions. In food-deprived rats, serum T_3 and thyroxine (T_4) levels are reduced concomitantly and thyroid-stimulating hormone (TSH) is unchanged or decreased. These changes are rapidly reversed with refeeding.[30] The alteration of the PDI level induced by fasting and refeeding was not due to the change in thyroid hormone status. Hyperthyroidism and hypothyroidism did not significantly change the PDI protein level and isomerase activity. Although PDI is a T_3-binding protein,[10] the physiological meaning of T_3 binding in PDI remains to be clarified.

REFERENCES

1. Paver JL, Freedman RB, Parkhouse RME: Induction of protein disulfide-isomerase during lymphocyte activation and maturation. Biochem Soc Transact 16:56, 1988

2. Bulleid NJ, Freedman RB: Detective co-translational formation of disulfide bonds in protein disulfide-isomerase-deficient microsomes. Nature 335:649-651, 1988

3. Koivu J, Myllylä R, Helaakoski T, et al: A single polypeptide acts both as the β subunit of prolyl 4-hydroxylase and as a protein disulfide-isomerase. J Biol Chem 262:6447-6449, 1987

4. Vuori K, Pihlajaniemi T, Myllylä R, et al: Site-directed mutagenesis of human protein disulfide isomerase: Effect on the assembly, activity and endplasmic reticulum retention of human prolyl 4-hydroxylase in *Spodoptera frugiperda* insect cells. EMBO J 11:4213-4217, 1992

5. Wetterau JR, Coombs KA, McLean LR, et al: Protein disulfide isomerase appears to maintain the catalytically active structure of the microsomal triglycaride transfer protein. Biochemistry 30:9728-9735, 1991

6. Varandani PT: Insulin degradation. Diabetes 23:117-125, 1974

7. Segal J, Ingbar SH: Specific binding sites for triiodothyronine in the plasma membrane of rat thymocytes. J Clin Invest 70:919-926, 1982

8. Horiuchi R, Johnson ML, Willingham MC, et al: Affinity labeling of the plasma membrane 3,3′,5-triiodo-L-thyronine receptor in GH_3 cells. Proc Natl Acad Sci USA 79:5527-5531, 1982

9. Cheng S-y, Gong Q-H, Parkison C, et al: The nucleotide sequence of a human cellular thyroid hormone binding protein present in endoplasmic reticulum. J Biol Chem 262:11221-11227, 1987

10. Horiuchi R, Yamauchi K, Hayashi H, et al: Purification and characterization of 55-kDa protein with 3,5,3′-triiodo-L-thyronine-binding activity and protein disulfide-isomerase activity from beef liver membrane. Eur J Biochem 183:529-538, 1989

11. Yamauchi K, Yamamoto T, Hayashi H, et al: Sequence of membrane-associated thyroid hormone binding protein from bovine liver: Its identity with protein disulfide isomerase. Biochem Biophys Res Commun 146:1485-1492, 1987

12. Willingham MC, Rutherford AV, Cheng S-Y: Immunohisochemical localization of a thyroid hormone-binding protein (p55) in human tissues. J Histochem Cytochem 34:1043-1046, 1987

13. Brockway BE, Foster SJ, Freedman RB, et al: The distribution of protein disulfide-isomerase activity in mammalian tissue. Biochem Soc Transact 10:115-116, 1982

14. Edman JC, Ellis L, Blacher RW, et al: Sequence of protein disulfide isomerase and implications of its relationship to thioredoxin. Nature 317:267-270, 1985

15. Myllylä R, Koivu J, Pihlajaniemi T, et al: Protein disulfide-isomerase activity in various cells synthesizing collagen. Eur J Biochem 134:7-11, 1983

16. Dorner AJ, Wasley LC, Raney P, et al: The stress response in chinese hamster ovary cells. J Biol Chem 265:22029-22034, 1990

17. Bradford M: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-250, 1976

18. Tagami T, Nakamura H, Sasaki S, et al: Estimation of the protein content of thyroid hormone receptor $\alpha 1$ and $\beta 1$ in rat tissues by Western blotting. Endocrinology 132:275-279, 1993

19. Hillson DA, Lambert N, Freedman RB: Formation and isomerization of disulfide bonds in proteins: Protein disulfide-isomerase. Methods Enzymol 107:281-294, 1984

20. Nakamura H, Tagami T, Masuda K, et al: Recognition of rat liver and kidney nuclear T_3 receptors by an antibody against c-*erb* A peptide. Biochem Biophys Res Commun 160:148-153, 1990

21. Klaus S, Casteilla L, Hentz E, et al: The mRNA of protein disulfide isomerase and its homologue the thyroid hormone binding protein is strongly expressed in adipose tissue. Mol Cell Endocrinol 73:105-110, 1990

22. Mira E, Castaño JG: Regulation of protein disulfide isomerasegene expression in brain and liver during rat development. Biochem Biophys Res Commun 170:421-425, 1990

23. Nieto A, Mira E, Castaño JG: Transcriptional regulation of rat liver protein disulfide-isomerase gene by insulin and in diabetes. Biochem J 267:317-323, 1990

24. Nakamura H, Yokota T, Imura H: Different alterations of nuclear triiodothyronine receptor capacity in liver and kidney induced by starvation and triiodothyronine administration. Acta Endocrinol 108:206-210, 1985

25. Tagami T, Nakamura H, Sasaki S, et al: Starvation-induced decrease in the maximal binding capacity for triiodothyronine of the thyroid hormone receptor is due to a decrease in the receptor protein. Metabolism 45:970-973, 1996

26. Kaplan MM, Utiger RD: Iodothyronine metabolism in rat liver homogenates. J Clin Invest 61:459-471, 1978

27. Lyles MM, Gilbert HF: Catalysis of the oxidative folding of ribonuclease A by protein disulfide isomerase: Dependence of the rate on the composition of the redox buffer. Biochemistry 30:613-619, 1991

28. Harris ARC, Fang S-L, Hinerfeld L, et al: The role of sulfhydryl groups on the impaired hepatic 3,3′,5-triiodothyronine generation from thyroxine in the hypothyroid, starved, fetal, and neonatal rodent. J Clin Invest 63:516-524, 1979

29. Morjana NA, Gilbert HF: Effect of protein and peptide inhibitors on the activity of protein disulfide isomerase. Biochemistry 30:4985-4990, 1991

30. Rondeel JMM, Heide R, Greef WJ, et al: Effect of starvation and subsequent refeeding on thyroid function and release of hypothalamic thyrotropin-releasing hormone. Neuroendocrinology 56:348-353, 1992