Short Communication

Thioredoxin Reductase 1 Is Important for Selenoprotein Biosynthesis in HeLa Cells

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Abstract

Selenium, an essential trace element, is co-translationally incorporated into selenoproteins as the 21st amino acid selenocysteine. Selenite serves as an inorganic selenium source for selenoprotein biosynthesis through reduction to selenide, which is converted to selenophosphate, the essential selenium donor in selenocysteyl-tRNA$^{[Ser]Sec}$ synthesis. However, the pathways for selenite reduction in mammalian cells have not yet been clarified. Based on metabolic labeling with [^{75}Se]selenite and RNA silencing studies, we here present evidence that thioredoxin reductase 1, but not thioredoxin, is crucial for selenite utilization to form selenoproteins in HeLa cells. We suggest that thioredoxin reductase 1 plays a role as a selenite-reducing enzyme *in vivo*.

Keywords : thioredoxin reductase, selenoprotein, RNAi, selenium, HeLa cell

Introduction

Selenium is an essential trace element that exerts its biological function predominantly as a catalytically active selenocysteine residue (Sec) in selenoproteins. Sec is co-translationally incorporated into a nascent polypeptide chain, as directed by the UGA codon, tRNA$^{[Ser]Sec}$, *cis*-acting RNA elements, and *trans*-acting protein factors [1]. The reactive selenium donor selenophosphate, which is required for the synthesis of Sec-tRNA$^{[Ser]Sec}$, is produced from selenide and ATP by the action of selenophosphate synthetase (SPS) *in vitro* [2]. Widely accepted models of selenide synthesis in mammalian cells include either degradation of L-selenocysteine via selenocysteine lyase [3] or stepwise reduction of selenite with glutathione (GSH) [4] or thioredoxin (Trx) [5].

Mammalian TrxRs are homodimeric flavoenzymes with a catalytic dithiol/disulfide and a Sec residue in the penultimate position [6]. TrxR using NADPH and Trx as substrates functions as a general protein disulfide-reducing system [7]. Trx is a 12-kDa ubiquitous protein with a redox active dithiol/disulfide in the active site. Three mammalian TrxRs have been characterized, namely, the cytosolic TrxR1, the mitochondrial TrxR2 [8], and the mouse testis TrxR3/TGR [9]. TrxR1 displays very broad substrate specificity, reducing not only Trx but also several other oxidized proteins and low-molecular-weight compounds, including selenium compounds, hydroperoxides, and ubiquinone [10]. Holmgren's group [5,11] found that incubation of selenite with

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calf thymus TrxR under aerobic conditions resulted in excessive oxidation of NADPH over stoichiometry, which was stimulated by the addition of Trx. Thus, they suggested that TrxR is responsible for selenide generation from selenite [5,11]. However, little evidence has been presented for selenide production by the TrxR system or its physiological role in selenoprotein biosynthesis. The present study was undertaken to clarify selenite reduction systems primarily participating in the biosynthesis of selenoproteins in mammalian cells.

Materials and Methods

Materials—The anti-TrxR1 antibody, from Lab Frontier (Seoul, Korea); the anti-Trx antibody, from Redox Bioscience (Kyoto, Japan); the anti-GAPDH antibody, from Ambion (Austin, TX); and horseradish peroxidase-conjugated secondary antibodies, from Bio-Rad (Hercules, CA). [^{75}Se]Selenite (850 mCi/mg) was obtained from the University of Missouri Research Reactor Center (Columbia, MO). siRNA targeted to human TrxR1 (sense strand: 5'- UGAUAGAAGCUGUACAGAATT-3') was purchased from Samchully Pharm. Co. (Seoul, Korea); siRNA targeted to Trx (catalog no. S 100753606), from Qiagen (Valencia, CA); Silencer negative control #1 siRNA, from Ambion. Rainbow [^{14}C] methylated protein markers were obtained from GE Healthcare Biosciences (Uppsala, Sweden). All other chemicals and reagents were commercial products of the highest grade available.

Cell Culture and siRNA Transfection—The HeLa cell line used in this study was obtained from the American Type Culture Collection (Manassas, VA) and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37℃ in 5% CO_2 under a humidified atmosphere. HeLa cells were transfected with siRNA oligo duplexes for Trx or TrxR1 at a final concentration of 40 nM using Lipofectamine RNAiMAX according to the manufacturer's protocol.

^{75}Se Labeling of Cells—Cells cultured for 72 h after siRNA transfection as described above were labeled with 15 nM [^{75}Se]selenite (1.25 μCi/ml-medium) for 11 h. Labeled cells were harvested, washed with PBS three times, and lysed in 100 μl of a 25 mM Tris-HCl buffer (pH 8.3) containing 192 mM glycine and 0.1% SDS. A 10-μl portion of the lysates was analyzed on a 4-20% gradient gel on SDS-polyacrylamide gel electrophoresis (PAGE) and stained with Coomassie Brilliant Blue (CBB). The gels were dried and exposed to a Storage Phosphor Screen (GE Healthcare Biosciences). The signal from the radiolabeled ^{75}Se-selenoprotein was detected using a Typhoon 9400 (GE Healthcare Biosciences).

Western Blot Analysis—Proteins solubilized with the M-PER reagent containing 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride were separated by 12.5% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Immunoreactive proteins were detected with the ECL Plus chemiluminescent detection system (GE Healthcare, UK).

Results and Discussion

Since the thioredoxin system comprising Trx, TrxR, and NADPH acts as a major redox regulation tool in mammalian cells [10], we examined whether it participates in the utilization of selenite as a selenium source for selenoprotein biosynthesis. An siRNA was designed to target human TrxR1 and tested for its ability to reduce TrxR1 expression in HeLa cells. Western blot analysis revealed that HeLa cells transfected with the TrxR1-siRNA exhibited 59% reduction in TrxR1 protein levels, as compared with the control cells transfected with a non-silencing siRNA (Fig. 1*A*). The TrxR1-depleted cells were cultivated for 11 h in the presence of [^{75}Se]selenite and analyzed by SDS-PAGE. As shown in Fig. 1*C*, no significant difference was found between the CBB-stained proteins in the TrxR1-depleted cells and those in the non-silencing siRNA-treated cells. However, an autoradiogram of the same PAGE gel revealed that the incorporation of the ^{75}Se label into selenoproteins was markedly decreased by the TrxR1 depletion (Fig. 1*C*). We then examined HeLa cells transfected with Trx-siRNA, which showed 99% reduction in the Trx concentration as compared with the control, in which a non-silencing siRNA was used (Fig. 1*B*). It is noteworthy that the depletion of TrxR1 only slightly affected the amount of Trx. The Trx-depleted cells were labeled with [^{75}Se]selenite, and the incorporation of the ^{75}Se label into selenoproteins by the cells was analyzed (Fig. 1*C*). Little difference was found between the Trx-depleted cells and the non-silencing control cells. These results indicate that TrxR1, but not Trx, plays a crucial role in the metabolism of selenite for the production of selenoproteins in mammalian cells.

Kumer et al. [5] demonstrated a direct reduction of selenite by TrxR with NADPH *in vitro.* Based on the finding that 3 mol of NADPH is oxidized per mol of selenite by calf thymus TrxR, they suggested that selenide is probably formed in the reaction. Our RNAi studies have shown that TrxR1, but not Trx, plays a important role in

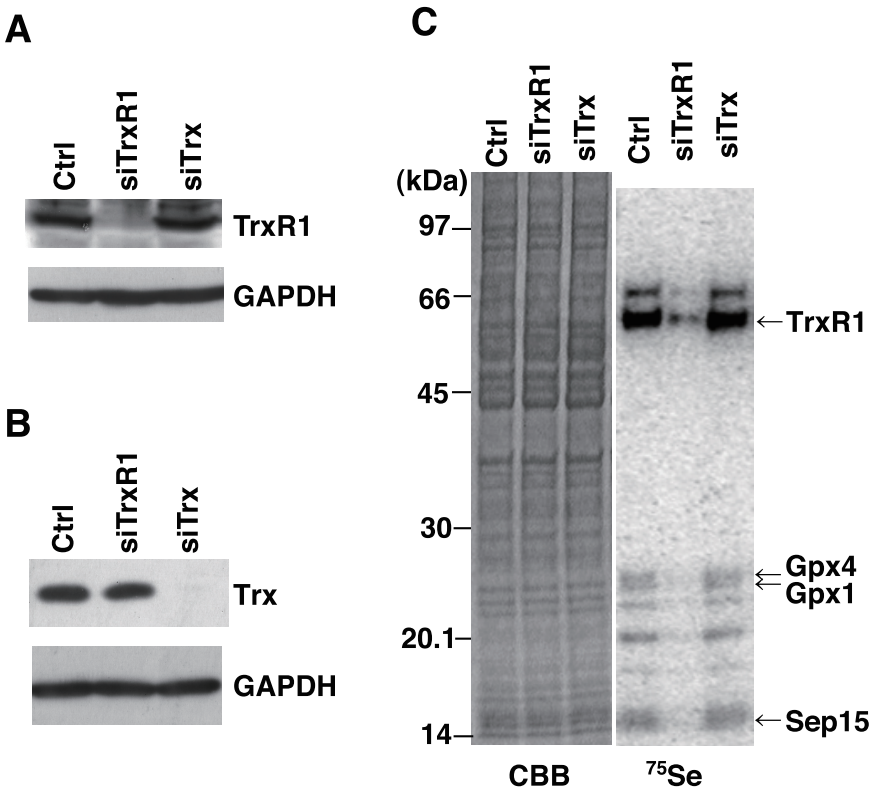

Fig. 1 Reduction of TrxR1 (A) or Trx (B) expression induced by siRNA targeting and effect of siRNA-mediated depletion of TrxR1 or Trx on the biosynthesis of ^{75}Se-labelled selenoproteins (C). HeLa cells cultured on 30-mm dishes were transfected with negative control siRNA (Ctrl), TrxR1 siRNA (siTrxR1), or Trx siRNA (siTrx). A and B, at 72 h after transfection, a 10-µl portion of total homogenate proteins was separated by 12.5% SDS-PAGE, and the expression of TrxR1 (upper panel in A), Trx (upper panel in B), and GAPDH (lower panels in A and B) was detected by Western blotting with specific antibodies. C, at 72 h after siRNA transfection, cells were labeled with [^{75}Se]selenite (1.25 µCi/ml-medium) for 11 h. A 10 µl-portion of cell lysates was separated on 4-20% gradient gel by SDS-PAGE. Gels were stained with Coomassie Blue (right panel) and subjected to PhosphorImager analysis for ^{75}Se-selenoproteins (left panel). The sizes of ^{14}C-methylated molecular-weight markers (in kDa) are shown at left. The positions of TrxR1, Gpx4, Gpx1, and Sep15 were assigned based on their calculated molecular masses.

supplying selenium derived from selenite to selenoprotein biosynthesis in HeLa cells. Our results establish an interesting relationship between TrxR1 and the selenium metabolism in mammalian cells : the selenoprotein TrxR 1 is responsible for the generation of selenide required for its own synthesis.

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