The Mammalian Cytosolic Selenoenzyme Thioredoxin Reductase Reduces Ubiquinone

A NOVEL MECHANISM FOR DEFENSE AGAINST OXIDATIVE STRESS*

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The selenoprotein thioredoxin reductase (TrxR1) is an essential antioxidant enzyme known to reduce many compounds in addition to thioredoxin, its principle protein substrate. Here we found that TrxR1 reduced ubiquinone-10 and thereby regenerated the antioxidant ubiquinol-10 (Q10), which is important for protection against lipid and protein peroxidation. The reduction was time- and dose-dependent, with an apparent Km of 22 µM and a maximal rate of about 12 nmol of reduced Q10 per milligram of TrxR1 per minute. TrxR1 reduced ubiquinone maximally at a physiological pH of 7.5 at similar rates using either NADPH or NADH as cofactors. The reduction of Q10 by mammalian TrxR1 was selenium dependent as revealed by comparison with Escherichia coli TrxR or selenium-deprived mutant and truncated mammalian TrxR forms. In addition, the rate of reduction of ubiquinone was significantly higher in homogenates from human embryo kidney 293 cells stably overexpressing thioredoxin reductase and was induced along with increasing cytosolic TrxR activity after the addition of selenium to the culture medium. These data demonstrate that the selenoenzyme thioredoxin reductase is an important selenium-dependent ubiquinone reductase and can explain how selenium and ubiquinone, by a combined action, may protect the cell from oxidative damage.

Thioredoxin reductase is part of a family of pyridine nucleotide-oxidoreductases to which glutathione reductase, lipoprotein dehydrogenase, and mercuric ion reductase also belong (1). Mammalian cytosolic thioredoxin reductase (TrxR1) is a multifunctional homodimeric selenoenzyme with a FAD, a functional disulfide/dithiol, and a penultimate C-terminal selenocysteine residue in each subunit of 58 kDa (2, 3). This selenocysteine residue is essential for catalytic activity and, together with the adjacent cysteine, forms a selenenylsulfide as the active site that is kept reduced by the conserved sequence Cys-Val-Asn-Val-Gly-Cys at the N-terminal of the other subunit (4, 5). TrxR1 has an exceptionally broad substrate specificity, not only reducing thioredoxins from a variety of species but many low molecular compounds as well; it is also a key enzyme in selenium metabolism, reducing selenium compounds to the active form for its own synthesis and for the synthesis of all other selenoproteins (6, 7). TrxR1 is a central enzyme for protection against oxidative stress (8), both directly and linked to antioxidant functions of thioredoxin and peroxidases (9). The role of the enzyme is also implicated in a variety of physiological and pathophysiological processes (7, 9).

Directly, TrxR1 may function as a peroxidase, reducing organic hydroperoxides including lipid hydroperoxides, in particular 15-S-HPETE, which is associated with atherosclerosis and lipid peroxidation (10). It may also reduce hydrogen peroxide, a reaction exclusively linked to the selenocysteine residue (4), and also ascorbate (11, 12) and lipoic acid (13).

Ubiquinone is a widely distributed, redox-active quinoid compound originally discovered as an essential part of the mitochondrial respiratory chain in mammals (14). The number of isoprenoid units of the side chain of ubiquinone is specific in different species and is reflected in the nomenclature, e.g. in rat ubiquinone-9 (Q9) and in human ubiquinone-10 (Q10) with 9 and 10 isoprenoid units, respectively. In humans, the reduced form of this molecule, ubiquinol-10, is the only endogenously synthesized lipid-soluble antioxidant. The antioxidant function is predominately protection against lipid and protein peroxidation (15, 16). The actions in peroxidation are mainly to stop the initiation of the reaction by reducing the perferryl radical and to terminate the propagation phase by the regeneration of vitamin E necessary for the reduction of lipid peroxyl radicals (16). Although the antioxidant function requires that ubiquinol be continuously regenerated enzymatically, the non-mitochondrial enzymatic systems involved are characterized only to a limited extent.

The aim of the present study was to characterize the reduction of ubiquinone-10 by thioredoxin reductase. Additionally, we investigated whether the reaction was specific for the mammalian enzyme and hence selenium-dependent, using the smaller non-selenium-containing Escherichia coli enzyme as...
well as mutant human TrxR and recombinant truncated rat TrxR lacking the last two Sec-Gly residues. We have also probed the reaction in a cellular context using transfected cell lines overexpressing thioredoxin reductase, which were grown with and without the addition of selenite to the culture medium. Based on the results presented here, we conclude that TrxR1 is a major cellular Q10 reductant.

EXPERIMENTAL PROCEDURES

Chemicals—Tris-malate, Tris-HCl, EDTA, bovine serum albumin, ubiquinone-10, ubiquinone-6, HEPES, glycerol, NADH, and NADPH were obtained from Sigma. Methanol, petroleum ether (b.p. 40–60 °C), 2-propanol (analytical grade), n-hexane (analytical grade), sucrose, CuSO4·5H2O, NaK-tartrate tetrahydrate, NaOH, Na2CO3, NaCl, Folin-Ciocalteu’s phenol reagent, sodium deoxycholate, and ZnCl2 were purchased from Merck (Stockholm, Sweden). The pET-24d(+) vector (Novagen). The pET-34a/hTrxR1a yields a mutant hTrxR1 (called hTrxR5) with a translated C-terminal extension sequence of Ser-Gly-Leu-Ala-Asn-Gly-Thr-Arg-Pro-Val-Ala-Ala-His in place of the C-terminal amino acids Ala-Gly-Cys-Sec-Gly. The plasmid was transformed into the E. coli strain BL21(DE3). 0.5 liters of the bacteria was grown to an A600 of 0.5, and then isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to a final concentration of 0.2 mM to induce the overproduction of the protein. The cells were harvested after 20 h at 25 °C by centrifugation. The pellet was resuspended in 50 mM HEPES-NaOH, pH 7.5, 0.5 mM NaCl, 1% (v/v) Triton X-100 and 1% sarcosyl (w/v), and lysis of the cells was performed by the addition of 300 ml of lysozyme (50 mg/ml) and incubation on ice for 30 min with stirring. Then, 300 ml of each of 1 mM MgCl2, 0.1 mM MnCl2, 1 mg/ml DNase, and 1 mg/ml RNase were added to the lysate, which was further incubated for 45 min. The lysate was subsequently centrifuged, applied to a 2-ml 2',5'-ADP-Sepharose column equilibrated with 10 ml Tris-HCl, pH 8.0 buffer with 1 mM EDTA and purified as described (20).

Recombinant rat TrxR1 containing selenocysteine was produced in E. coli as described previously (20), and the two-amino acid truncated form was produced in the same bacterial system by excluding the selenocysteine insertion sequence element, as described (20). Activity of rat TrxR1 was normalized for the selenocysteine-containing fraction, i.e. 20% in the enzyme having 8 units/mg (20).

Construction of Transfected Stable Overexpressing Human Embryo Kidney Cell Lines (HEK293)—It is well known that transfection for overexpression of selenoproteins is difficult, and hTrxR1 is no exception (21–24). To our surprise, we could nonetheless establish stable cell lines overexpressing hTrxR1 using the following protocol. A detailed molecular characterization of these cells is in progress.3 The primers hTrxR1-F1 (5'-GAATTCCACCACCATTGACCGACCCCTGAAGATCTTC-3') and hTrxR1-R1–3' and hTrxR1-R1–3'-UTR (5'-CCATTTCCGAATGCGCACAATGATGAGGACGT-3') were used to amplify, by PCR, the hTrxR1 cDNA from a human adrenal cDNA library and the introduction of EcoRI restriction sites for subsequent cloning. The construct includes the selenocysteine insertion sequence (SECIS element) and the first three of the six AU-rich elements located in the 3'-UTR (21) and was cloned into the pGEMT easy vector (Promega) and sequenced. Stable cell lines overexpressing hTrxR1 were subsequently generated using the pIRES vector system (Clontech). The pGEMT easy-hTrxR1 mRNA was cleaved with EcoRI and ligated into the pIREs vector. 10 μg of the pIRES/hTrxR1 construct was then used to transfect HEK293 cells using polyethylenimine (PEI) (Aldrich). 0.5 μg of 0.1 μM PEI was added to 0.1 μg/μl DNA in water. The mixture was vortexed and incubated for 10 min at room temperature and then added to the cells. The cells were allowed to grow for 2 days without supplementation of sodium selenite and were then passaged. Resistant colonies were selected with 1 mg/ml (HEK-TrxR11) or 1.5 mg/ml (HEK-TrxR15) G418 (Calbiochem). Control cells were prepared by transfecting HEK293 cells with the empty pIRES vector and then selected as above (HEK-ires). Several resistant colonies were selected, expanded, expanded, and overexpression of activity measurements and Western blot analysis. Reselection and Maintenance of Cells—The HEK293 cells were grown until confluence at 37 °C and 5% CO2 in Dulbecco’s modified Eagle’s medium (containing 1 mg/ml glucose) and F-12 nutrient mixture (ratio 1:1) supplemented with 10% fetal calf serum (Invitrogen). The medium was changed every other day. The cells were resellected every 4 weeks with one passage of medium as above containing 1 mg/ml G418. No supplementation of sodium selenite was used in the medium during the maintenance of the cells.

Culturing Cells for Ubiquinone-10 Reduction Experiments—Each experiment was started with 600 ml of 107–108 cells per culture dish (100 mm, Sarstedt). The cells were left to adhere for 6 h before adding sodium selenite, where indicated, in different concentrations. 10–12 cell culture dishes were used per concentration. After 72 h the cells were harvested, and homogenates and cytosol were prepared. For the preparation of cytosol, cells were sonicated in 50 mM phosphate buffer, pH 7.0, and centrifuged at 105,000 × g for 60 min at 4 °C. The protein concentrations were measured as described (25).

RESULTS

Characterization of the Reduction of Ubiquinone-10 by Purified Bovine TrxR1, Rat TrxR1, E. coli TrxR, Human Mutant TrxR, and Truncated Rat TrxR—The reduction of

3 N. Pavlidou, D. Dardimopoulos, T. Nordman, J. M. Olsson, M. Björnstedt, E. S. J. Arner, and G. Spyrou, manuscript in preparation.
ubiquinone-10 by commercially available bovine thioredoxin reductase was characterized (Fig. 1). There was a linear relation between the reduction of Q10 and the amount of enzyme added to the reaction mixture (Fig. 1a) as well as to the time of incubation in the presence of NADPH as cofactor (Fig. 1b). The half-maximal rate of reduction was achieved at a substrate concentration of 22 $\mu$M (apparent $K_m$) (Fig. 1c). The maximal rate of reduction in this assay was $110^{11}$ nmol of Q10 reduced per milligram of selenoprotein TrxR1 per minute, and similar results were obtained by recombinant rat selenocysteine-containing TrxR1 but not with TrxR forms lacking selenocysteine (Fig. 2). Zinc, which is known to be a potent inhibitor of thiol oxidoreductases (26) with a number of known substrates, also inhibited the reduction of Q10 by TrxR1; at a concentration of 50 $\mu$M zinc the activity was decreased to 50%, and at 100 $\mu$M the activity was only $-15\%$ (Fig. 1d). Furthermore, the pH dependence of the reaction was measured in the interval 5–8.5, and the reaction had a sharp physiological pH optimum of $7.5$ (Fig. 1e).

Fig. 2 shows that there was no difference in the rate of Q10 reduction when NADH was used instead of NADPH as the electron donor. This finding was surprising, because no other known reaction catalyzed by mammalian TrxR1 uses NADH as cofactor with similar rates as NADPH; for example, using lipoamide as substrate for TrxR1, NADH instead of NADPH gives only $5\%$ activity, and with lipoic acid NADH cannot function at all as a reductant (13). Thioredoxin reductase from E. coli did not essentially reduce Q10. The reduction of Q10 was specific for mammalian selenocysteine-containing TrxR1, as shown in Fig. 2. A major difference between E. coli and mammalian TrxR1 is the presence of a redox-active selenolthiol motif formed by a cysteine and a selenocysteine residue in the C-terminal of the mammalian enzyme (2). To further study the importance of the selenium moiety of mammalian TrxR1 for Q10 reduction, mutant human TrxR (hTrxR5) in which the penultimate C-terminal selenocysteine residue was substituted for alanine along with additional amino acids (see “Experimental Procedures”) as well as a truncated recombinant rat TrxR lacking the last two amino acids, including the selenocysteine residue (4, 20), were also investigated. These forms of the mammalian enzyme did not essentially reduce Q10, suggesting

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3 L. Xia, T. Nordman, J. M. Olsson, and M. Björnstedt, unpublished observations.
that the presence of selenocysteine was essential for TrxR1-mediated reduction of Q10 (Fig. 2).

The effects of lowering concentrations of NADPH and NADH on the reduction of Q10 by the recombinant selenocysteine-containing rat TrxR1 showed that the similar rates of reduction using both cofactors were also maintained at lower concentrations (Fig. 3). Thus, the reduction of Q10 differs in this respect compared with the reduction of other known substrates, by TrxR1.

Reduction of Ubiquinone-10 in Homogenates from Stable Human Kidney Cell Lines Overexpressing TrxR1—The identification of TrxR1 as an enzyme regenerating ubiquinol-10 was confirmed by analysis of the relative Q10 reduction rates in homogenates from two selected stable cell lines overexpressing thioredoxin reductase, HEK-TrxR11 and HEK-TrxR15 (Fig. 4).

DISCUSSION

This paper demonstrates an important novel function for the selenoenzyme TrxR1 as an ubiquinone-10 reductase implicating a vital function in the protection against oxidative stress. At physiological conditions, the reduction of ubiquinone-10 by mammalian TrxR1 was shown to be the most efficient described to date. Our data using cells overexpressing TrxR1 strongly support the belief that TrxR1 is an important enzyme in the extramitochondrial ubiquinone-10/ubiquinol-10 redox cycle.

The molecular basis for cellular non-mitochondrial Q10 reduction has previously been known only partly. However, the characteristics of the reaction by TrxR1 as found here are nearly identical to the kinetic parameters found in the cytosol from normal rat liver and rat liver nodules. For example, in the cytosol >90% of the reductase activity was found in the protein fraction with a molecular mass above 100 kDa (TrxR1; 116 kDa) and the reaction had a pH optimum of 7.5. Also, the reduction of Q10 in rat liver cytosol was inhibited by the addition of zinc to the reaction mixture. The observation that the rate of Q10 reduction was similar with both NADH and NADPH as cofactors using purified enzyme preparations as well as cytosolic protein is surprising and intriguing. Possibly, Q10 slightly affects the conformation of the TrxR1 enzyme so that NADH becomes better accepted as an electron donor or, alternatively and more likely, the relatively low turnover compared with other substrates such as thioredoxin or DTNB, which is probably determined by the rate of reduction of Q10 by the selenolthiol motif, obscures the differences in rates between NADH and NADPH in the first reductive half-reaction. Nonetheless, the increased activity in TrxR1 and the corresponding increase in Q10 reductase activity after the addition of selenite to the transfected cells implicate a direct relation between the increase in Q10 reductase activity and the increased activity of TrxR1.

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4 L. Xia, M. Björnstedt, T. Nordman, L. Björkhem-Bergman, L. Eriksson, and J. M. Olsson, submitted for publication.
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The thioredoxin system may regulate the activity of several receptors and transcription factors through the reduction of conserved cysteine residues leading to conformational changes with resulting activation/deactivation of gene expression (7, 8). This process is known as thiol redox control and may be especially important during situations of oxidative stress. For instance, reduced Trx that mirrors the activity of TrxR1 prevents apoptosis by inhibition of ASK-1 (32). In addition, several important antioxidant reactions have been directly described for mammalian TrxR1, e.g. the reduction of hydrogen peroxide (4), lipid hydroperoxides (10), ascorbate (11), lipoic acid, and lipoamide (13). The results reported in this paper provide additional evidence that TrxR1 plays an important role in the cellular defense against oxidative stress, because reduction of Q10 could prevent and terminate peroxidation.

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The results that showed that the E. coli TrxR1, mutant mammalian TrxR, and truncated rat TrxR forms lacking the penultimate C-terminal selenocysteine residue failed to reduce Q10 demonstrates the specificity for the mammalian enzyme and an essential function for selenocysteine in this reaction. This is in agreement with previous reports of a vital role for selenocysteine in the reduction of other substrates and the participation of a unique selenothiol/selenenylsulfide motif in the catalytic cycle (2). The background activities detected with the E. coli TrxR and the selenium-deficient recombinant mammalian enzymes were in the same order of magnitude as the direct chemical reaction between FAD and ubiquinone described previously (30). Because all TrxRs contain a FAD in each subunit, we suggest that the background reaction was mainly caused by such a chemical reaction between protein-bound FAD and Q10. However, a slow reaction between Q10 and the thiols of the N-terminal domain part of the active center may not be excluded either (3).

Selenium in low to moderate concentrations is known to induce the expression of several selenoenzymes, including thioroxin reductases and glutathione peroxidases (33). Several reports have shown a relation between the levels of ubiquinone and selenium (34, 35). The molecular basis for this relation has not, however, been established. Our data now provide an explanation; TrxR1-mediated reduction of Q10 is dependent on the catalytic activity of the mammalian enzyme and selenium. We therefore conclude that the cytosolic cellular selenoenzyme TrxR1 is a link between the function of the antioxidant compound ubiquinol-10 and the trace element selenium.

REFERENCES

1. Williams, C. H., Jr. (1992) in Chemistry and Biochemistry of Flavoenzyme (Müller, F., ed) Vol. 3, pp. 121–211, CRC Press, Inc., Boca Raton, FL
2. Zhong, L., Arner, E. S. J., and Holmgren, A. (2000) Proc. Natl. Acad. Sci.
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U. S. A. 97, 5854–5859
3. Sandalova, T., Zhong, L., Lindqvist, Y., Holmgren, A., and Schneider, G. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 9533–9538
4. Zhong, L., and Holmgren, A. (2000) J. Biol. Chem. 275, 18121–18128
5. Lee, S. R., Bar-Noy, S., Kwon, J., Levine, R. L., Stadtman, T. C., and Rhee, S. G. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 2521–2526
6. Holmgren, A., Arner, E. S. J., Ashlund, F., Björnstedt, M., Zhong, L., Ljung, J., Nakamura, H., and Nikitovic, D. (1998) in Oxidative Stress in Cancer, Aids, and Neurodegenerative Diseases (Montagnier, L., Olivier, R., and Pasquier, C., eds) pp. 229–46, Marcel Dekker, Inc., Paris
7. Arner, E. S. J., and Holmgren, A. (2000) Eur. J. Biochem. 267, 6102–6109
8. Nordberg, J., and Arner, E. S. J. (2000) Free Radic. Biol. Med. 31, 1287–1312
9. Becker, K., Gromer, S., Schirmer, R. H., and Muller, S. (2000) Eur. J. Biochem. 267, 6118–6125
10. Björnstedt, M., Hemberg, M., Kumar, S., Xue, J., and Holmgren, A. (1995) J. Biol. Chem. 270, 11761–11764
11. May, J. M., Mendiratta, S., Hill, K. E., and Burk, R. F. (1997) J. Biol. Chem. 272, 22897–22901
12. Li, X., Hill, K. E., Burk, R. F., and May, J. M. (2001) FEBS Lett. 508, 489–492
13. Arner, E. S. J., Nordberg, J., and Holmgren, A. (1996) Biochem. Biophys. Res. Commun. 225, 268–274
14. Ernst, L., Lee, I. Y., Norling, B., and Persson, B. (1969) Eur. J. Biochem. 9, 299–310
15. Frei, B., Kim, M. C., and Ames, B. N. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4879–4883
16. Ernst, L. (1993) in Active Oxygens, Lipid Peroxides, and Antioxidants. (Yagi, K., ed) pp. 1–36, CRC Press, Inc., Boca Raton, FL
17. Olsson, J. M., Xia, L., Eriksson, L. C., and Björnstedt, M. (1999) FEBS Lett. 448, 190–192
18. Stål, P., Olsson, J., Svedhå, P., Hultcrantz, R., Harms-Ringdahl, M., and Eriksson, L. C. (1997) J. Hepatol. 27, 562–571
19. Holmgren, A., and Björnstedt, M. (1995) Methods Enzymol. 252, 199–208
20. Arner, E. S. J., Sarigoula, H., Lotspeich, F., Holmgren, A., and Bock, A. (1999) J. Mol. Biol. 292, 1003–1016
21. Gasdaska, J. R., Harney, J. W., Gasdaska, P. Y., Powis, G., and Berry, M. J. (1999) J. Biol. Chem. 274, 25379–25385
22. Gasdaska, P. Y., Berggren, M. M., Berry, M. J., and Powis, G. (1999) FEBS Lett. 442, 105–111
23. Hofmann, R. R., Boyanapalli, M., Lindner, D. J., Weihsa, X., Hassel, B. A., Jagar, R., Gutierrez, P. L., Kalvakolanu, D. V., and Hofman, E. R. (1998) Mol. Cell. Biol. 18, 6493–6504
24. Ma, X., Hu, J., Lindner, D. J., and Kalvakolanu, D. V. (2002) J. Biol. Chem. 277, 22460–22468
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–276
26. Gazaryan, I. G., Krasnikov, B. F., Ashby, G. A., Thorneley, R. N. Kristal, B. S., and Brown, A. M. (2002) J. Biol. Chem. 277, 10964–10972
27. Fujii, Fujii, T., Fujii, J., and Taniguchi, N. (1999) Biochem. J. 340, 239–244
28. Björnstedt, L., Tellebrhun, H., Kesen, E., Olofsen, J. M., Eriksson, L. C., and Björnstedt, M. (2001) J. Hepatol. 35, 259–264
29. Olsson, J. M., Eriksson, L. C., and Dalinder, G. (1991) Cancer Res. 51, 3774–3780
30. Xia, L., Björnstedt, M., Nordman, T., Eriksson, L. C., and Olsson, J. M. (2001) Eur. J. Biochem. 268, 1468–1490
31. Beyer, R. E., Segura-Aguilar, J., Di Bernardi, S., Cavazzoni, M., Fato, R., Fiorentini, D., Galli, M. C., Setti, M., Landi, L., and Lenaz, G. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2528–2532
32. Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., Sawada, Y., Kawabata, M., Miyazono, K., and Ichijo, H. (1998) EMBO J. 17, 2596–2606
33. Gladyshev, V. N., and Kryukov, G. V. (2001) Biofactors 14, 87–92
34. Vadhanavik, S., and Ganther, H. E. (1993) Biochem. Biophys. Res. Commun. 190, 921–926
35. Vadhanavik, S., and Ganther, H. E. (1994) Mol. Aspects Med. 15, S103–S107
