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Review

Metabolism of selenium compounds catalyzed by the mammalian selenoprotein thioredoxin reductase

Jun Lu, Carsten Berndt, Arne Holmgren *

Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE 171 77 Stockholm, Sweden

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ABSTRACT

The mammalian thioredoxin reductases (TrxR) are selenoproteins with a catalytic selenocysteine residue which in the oxidized enzyme forms a selenenylsulfide and in the reduced enzyme is present as a selenolthiol. Selenium compounds such as selenite, selenodiglutathione and selenocystine are substrates for the enzyme with low K_m-values and the enzyme is implicated in reductive assimilation of selenium by generating selenide for selenoprotein synthesis. Redox cycling of reduced metabolites of these selenium compounds including selenide with oxygen via TrxR and reduced thioredoxin (Trx) will oxidize NADPH and produce reactive oxygen species inducing cell death at high concentrations explaining selenite toxicity. There is no free pool of selenocysteine since this would be toxic in an oxygen environment by redox cycling via thioredoxin systems. The importance of selenium compounds and TrxR in cancer and cardiovascular diseases both for prevention and treatment is discussed. A selenazol drug like ebselen is a direct substrate for mammalian TrxR and dithiol Trx and ebselen selenol is readily reoxidized by hydrogen peroxide and lipid hydroperoxides, acting as an anti-oxidant and anti-inflammatory drug.

1. Introduction

Mammalian thioredoxin reductase and thioredoxin were purified and characterized during 1970 to 1982 based on knowledge about the corresponding proteins from *Escherichia coli* [1,2]. *E. coli* Trx consists of 108 amino acid residues with an active site CGPC motif and was discovered as a dithiol hydrogen donor for ribonucleotide reductase which is critical for DNA synthesis. By using the noble art of enzyme purification from whole organs, homogeneous Trx from calf liver [3] was isolated and used in assays with bovine thymus TrxR [4]. In these studies it was discovered that the combination of Trx and TrxR with NADPH in fact was identical to an enzyme called protein disulfide reductase, and catalyzed disulfide reduction in model proteins like insulin [4]. This simplified assay system, which enabled determination of either Trx or TrxR activity independent of ribonucleotide reductase by using an excess of the other component was critical for successful isolation of pure mammalian TrxR. The early studies [4] revealed that mammalian Trx had extra thiol groups and required reducing conditions to be purified and moreover TrxR had very different properties from the well known *E. coli* enzyme [1]. Finally, homogeneous rat liver TrxR and Trx were obtained by purification and characterized [5]. The mammalian TrxR had a higher molecular weight and a broad substrate specificity and was shown to reduce alloxan, menadione, oxidized lipoamide or DTNB. The K_m-value for rat liver or calf liver Trx was 2.5 μM, whereas the K_m-value for *E. coli* Trx was considerably higher (35 μM) [5]. The enzyme contained FAD and was relatively labile, but there was no obvious evidence that this would be a selenoenzyme, which is knowledge of today [6].

It is well known that Trx is a ubiquitous redox active 12 kDa protein from archaea to human. Trx is a highly structured protein and the core structure (Trx fold) consists of five β-strands surrounded by four α-helices [1]. Human and mammalian Trx contain three structural cysteine residues in addition to the conserved CGPC active site. Trx has a very close link to selenoproteins. Many selenoproteins possess the Trx fold structure with a CXXU active site motif [7,8]. Trx is a potential electron donor for oxidized SECIS binding protein 2 (SBP2) and may thus be involved in regulating the selenoprotein synthesis via controlling the SBP2 redox state and trafficking [9].

Originally, Trx was closely linked to ribonucleotide reductase, which is known to be an S-phase enzyme expressed particularly in replicating cell or fast growing tumours. Therefore, it became important to understand the distribution of Trx in organisms and this was done by studies on the general activity of Trx and TrxR in animals. The specificity of protein disulfide reduction was studied [10] and it was observed that only some disulfides were substrates of Trx in the presence of NADPH and TrxR, but a possibility of regulating activity via Trx dependent disulfide reduction was obvious. Later, the immunohistochemical localization of Trx and TrxR was determined in adult rats and it was observed that Trx and TrxR occurred in neurons, liver

Abbreviations: TrxR, thioredoxin reductase; Trx, thioredoxin; Sec(U), selenocysteine; GPx, glutathione peroxidase; GSH, glutathione; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTT, dithiothreitol; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; SBP2, SECIS binding protein 2; ROS, reactive oxygen species; Ebselen, 2-phenyl-1,2-benzisoselenazol-3(2H)-one

* Corresponding author. Tel.: +46 8 52487686; fax: +46 8 7284716.
E-mail address: arne.holmgren@ki.se (A. Holmgren).

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and many secretory cells, which had no relation to cell growth [11]. For instance, axoplasmic transport of Trx and TrxR occurred in the rat sciatic nerve [12]. Furthermore, immunohistochemically Trx and TrxR were located in both mouse exocrine and endocrine pancreatic cells including the betacells, the source of insulin. These studies formed a background for ideas on the role of Trx and TrxR in redox control, but also redox regulation.

TrxR belongs to the pyridine nucleotide-disulfide oxidoreductase family and is a dimeric flavoenzyme. Two distinct classes of TrxRs exist in nature, a low molecular mass enzyme (about 70 kDa) in prokaryotes, archaea, lower eukaryotes and an enzyme (110 kDa or higher) in higher eukaryotes [13]. There are big structural and mechanistic differences between two classes [13–15]. The low molecular mass enzyme has high substrate specificity [13,14] and the catalytic process of this enzyme involves a large conformational change [16]. Mammalian TrxR is a higher molecular mass TrxR and has a broad substrate specificity. The catalysis process of mammalian TrxR is proposed not involve a big conformational change [15]. Most interestingly, the structure of mammalian TrxR is similar to that of glutathione reductase. It contains an identical N-terminal disulfide/dithiol and a 16 residue C-terminal tail with the conserved active site GCUG [6,15]. Here we will focus to review the metabolism of selenium compounds catalyzed by the mammalian TrxR.

2. Selenium compounds

Selenium was treated as a toxic substance following its discovery by the Swedish chemist Jöns Jacob Berzelius in 1818 but was found to be essential for animals and human health by Schwarz and Foltz in 1957 [17]. Selenium deficiency has been proposed to predispose to the development of pathologic conditions including higher risk for cancer and infection, male infertility, etc [8,18]. The main source of selenium for animals is from the diet, including selenomethionine (SeMet), selenocysteine (Sec), selenate, and selenite, etc. The biological effects of selenium compounds are dependent on their concentration, chemical form and metabolism, in which TrxR together with Trx play critical roles.

Experiments on selenium compounds and mammalian TrxR were originally inspired by published studies on selenium supplementation and the growth of Ehrlich ascites tumours [19]. It was shown that inorganic forms of selenium such as sodium selenite, sodium selenate or selenium dioxide completely prevented growth *in vivo* in Ehrlich ascites tumours in rats injected with 500,000 live tumour cells, while all the animals receiving identical doses of selenomethionine developed tumours [19]. The idea of selenite acting as an inhibitor of TrxR turned out to be wrong since selenite was an excellent substrate for the enzyme [20]. Further studies showed that selenite is a substrate for reduction by both Trx and NADPH-TrxR and that the reaction results in a large non-stoichiometric oxidation of NADPH in the presence of oxygen [21]. Selenite (10 μM) plus calf thymus TrxR (50 nM) at pH 7.5 oxidized 100 μM NADPH in 30 min. In contrast, TrxR from *E. coli* showed no direct reaction with selenite, but addition of *E. coli* Trx (3 μM) also resulted in a non-stoichiometric oxidation of NADPH, demonstrating oxidation of Trx dithiols to a disulfide by selenite. Anaerobic experiments [21] demonstrated that 1 mol of selenite oxidized 3 mol of NADPH after the reaction stopped; admission of air then resulted in continued consumption of NADPH consistent with of oxidation of selenium intermediates. The overall reaction anaerobically can be described by Eq. (1):

$$SeO_3^{2-} + 3NADPH + 3H^+ \xrightarrow{\text{calf TrxR}} Se^{2-} + 3NADP^+ + 3H_2O \quad (1)$$

This reaction will generate selenide, which is known to be highly sensitive to auto-oxidation [22].

Selenite and reduced glutathione (GSH) react to form selenodiglutathione (GS-Se-SG) according to the Eq. (2):

$$2H^+ + 4GSH + SeO_3^{2-} \rightarrow GSSG + GS - Se - SG + 3H_2O \quad (2)$$

This has been proposed to be the major form of entry of selenium compounds into metabolism [23,24]. Ganther showed that GS-Se-SG is a substrate for glutathione reductase resulting in reduction to GS-Se$^-$ and GSH with stoichiometric amounts of NADPH consumption [25]. In addition, GS-Se-SG has been suggested to be one of the most effectively selenium compounds in inhibiting growth of neoplastic cells and perhaps also protein synthesis [23,24]. GS-Se-SG was isolated and shown to be an excellent substrate for NADPH and TrxR of calf thymus or reduced Trx. As with selenite there was a fast initial reaction followed by large continued oxidation of NADPH. This redox cycling reaction was oxygen dependent as seen for selenite. The reaction between GS-Se-SG and glutathione reductase was fast at the beginning, but the continued oxidation was very slow [24]. The mechanism for the reaction is suggested to be according to Eq. (3):

$$GS - Se - SG + NADPH \rightarrow GS - Se^- + GSH + NADP^+ \quad (3)$$

The continued reaction and its oxygen dependence are explained by a reaction according to Eq. (4):

$$HSe^- + R{-}(SH)_2 + (O) \rightarrow HSe^- + R{-}S_2 + H_2O \quad (4)$$

The HSe$^-$ component in the reaction is explained by reaction 5:

$$GSSe^- + GSH \rightarrow HSe^- + GSSG \quad (5)$$

At the time these results were obtained it was not known that mammalian TrxR is a selenoenzyme with a selenolthiol active site [23,24]. Therefore, the reactivity of either the dithiol active site of Trx or the selenolthiol of TrxR in the reaction with HSe$^-$ has to be taken into account. Note that GSSG is not a substrate for TrxR from mammalian cells [5], but is a substrate for reduced Trx [1]. Thus, inside mammalian cells the combination of Trx, TrxR and glutathione reductase takes care of both reduction of HSe$^-$ and GSSG.

Of special importance is the dramatically enhanced reactivity of glutathione by insertion of a selenium atom into the molecule to form GS-Se-SG. This was illustrated by the remarkable reactivity of GS-Se-SG [23,24]. GSSG is not a substrate for the mammalian TrxR, whereas GS-Se-SG is an excellent substrate. Even more dramatic is the oxidation of Trx by GSSeSG compared to GSSG. Oxidation of reduced Trx by any compound can be followed by excitation of fluorescence at 280 nm and recording fluorescence at 350 nm. The three-fold decrease of fluorescence upon oxidation of Trx enables easy determination of rate constants [26]. GS-Se-SG reacted much faster than insulin, which is known to have a high reactivity of more than 5×10^4 M^{-1} s^{-1}. In contrast, GSSG reacts with a rate constant of about 570 M^{-1} s^{-1}.

Overall, the mechanism of TrxR and Trx from mammalian cells may explain how selenium compounds enter metabolism in cells, but also how toxicity arises by continued NADPH oxidation via redox cycling of auto-oxidizable compounds. The latter also helps to explain why there is no free pool of selenocysteine in cells since this would burn NADPH and generate ROS. Selenocysteine is a substrate for mammalian TrxR with a K_m-value of 6 μM and a high turnover rate V_{max} similar to that of Trx, the natural substrate [27].

Selenate cannot be reduced by TrxR with or without Trx [23,24,27]. It is known however that both selenate and selenite can provide selenium for biological processes in mammalian cells and they inhibit human lymphocyte growth via different mechanisms [28]. The studies of human 3Bl6 and Bl41 lymphocytes showed that 10 μM selenite produced growth inhibitory effects similar to that of 250 μM selenate. Selenite inhibited cells in S-phase in an irreversible fashion, where selenate induced reversible growth inhibition in the G2 phase. At this