Biochemical Pharmacology 83 (2012) 815–820

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Pyrroloquinoline quinone modulates the kinetic parameters of the mammalian selenoprotein thioredoxin reductase 1 and is an inhibitor of glutathione reductase

Jianqiang Xu, Elias S.J. Arnér *

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

ARTICLE INFO

Article history:
Received 24 November 2011
Accepted 20 December 2011
Available online 29 December 2011

Keywords:
Pyrroloquinoline quinone
Juglone
Redox cycling
Thioredoxin reductase
Selenoprotein
Glutathione reductase

ABSTRACT

Pyrroloquinoline quinone (PQQ) is a redox active cofactor for bacterial quinoproteins. Dietary PQQ also has prominent physiological effects in mammals although no mammalian quinoprotein has yet been conclusively identified. Here we found that PQQ has substantial effects on the redox active mammalian selenoprotein thioredoxin reductase 1 (TrxR1). PQQ efficiently inhibited the activity of TrxR1 with its main native substrate thioredoxin and acted as a low efficiency substrate in a Sec-dependent TrxR1-catalyzed reduction. Interestingly, PQQ also stimulated redox cycling of TrxR1 with another quinone substrate, juglone, as much as 13-fold (k_{cat}/K_m increased from 105 min^{-1} μM^{-1} to 1331 min^{-1} μM^{-1} for juglone in the presence of 50 μM PQQ, mainly through a lowered apparent K_m for juglone). Glutathione reductase was also inhibited by PQQ but in contrast to the effects of PQQ on TrxR1, its quinone reduction was not further stimulated. These results reveal that glutathione reductase and the mammalian selenoprotein TrxR1 are direct PQQ protein targets, although not being genuine quinoproteins. These findings may help explain several of the effects of PQQ seen in mammals.

1. Introduction

Dietary pyrroloquinoline quinone (PQQ) at low micromolar concentrations has potent physiological effects in rodent models by affecting, often in a protective manner, reproduction, early development, growth, immune function, effects of oxidative stress, neural and cardiac function [1]. PQQ was first discovered as an essential redox active cofactor of bacterial quinoproteins [2–5]. These prokaryotic enzymes bind PQQ through a unique "propeller" structure and use PQQ in redox reactions typically catalyzing alcohol or glucose oxidation [6,7]. As a prosthetic group, PQQ is capable of stably catalyzing continuous redox cycling (repeated oxidation and reduction cycles) and does so more efficiently than most other redox active cofactors [1]. However, as with all redox active compounds, pro-oxidant actions may also be promoted and PQQ can under certain conditions induce oxidative protein modifications [8]. PQQ could hence function as either antioxidant or a pro-oxidant, depending upon the overall redox milieu [8–10]. Claims of a mammalian genuine quinoprotein [11] were subsequently questioned [12,13] and conclusive evidence for the existence of a mammalian quinoprotein are lacking. Direct PQQ protein targets in mammals, helping to explain the physiological functions of this compound, are thus yet essentially unknown. However, using microarray analyses of the physiological responses to PQQ administration, the transcriptional response pattern was recently found to be compatible with that of an oxidative stress, with Nrf2 and thioredoxin (Trx) transcripts being among the most induced [14]. This suggested to us that thioredoxin reductase (TrxR1), a key antioxidant selenoprotein known to interact with selected quinone compounds [15,16], might be a PQQ target.

Mammalian TrxR1 is a selenoprotein flavoenzyme that possesses two redox active cysteines (Cys59-Cys64), FAD in an N-terminal domain, and a Sec-containing selenolthiol motif (Cys497-Sec498) at its C-terminus which is the proper active site reducing Trx [17–20]. TrxR1 also catalyzes reduction of the quinone substrate juglone (5-hydroxy-1,4-naphthoquinone; walnut toxin) in a reaction that depends mainly upon the N-terminal redox active motif of TrxR1 and is thus maintained also by Sec compromised species of the enzyme; juglone reduction products furthermore inhibit TrxR irreversibly for its normal reduction of Trx [16,21]. We therefore here wished to analyze whether PQQ could have any effects on TrxR1 resembling those of juglone. Instead, we found that PQQ has other effects on TrxR1, modifying the enzyme kinetic properties in a non-covalent reversible manner so that its native Trx1 reduction becomes inhibited while its activities as a juglone reductase become strongly stimulated. We also found that glutathione reductase (GR), a non-selenoprotein flavoenzyme closely related to TrxR1 [22], also becomes inhibited by PQQ, but GR did not display the marked alterations of its kinetic parameters as found with TrxR1 upon PQQ addition. To our knowledge, these findings are the first to identify direct mammalian enzyme targets for PQQ, the targeting of which are

* Corresponding author. Tel.: +46 8 5248 69 83; fax: +46 8 31 15 51.
E-mail address: Elias.Arner@ki.se (Elias S.J. Arnér).

0006-2952/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.12.028

well in line with several of the effects seen *in vivo* upon administration of dietary PQQ to animals.

2. Materials and methods

2.1. Materials

The BL21 (DE3) *gor*$^-$ host strain and pure recombinant human wild-type Trx1 were generously provided by Arne Holmgren, Karolinska Institutet, Stockholm, Sweden. 2′5′-ADP Sepharose™ 4B, Superdex™ 200 10/300 GL column and NAP™-5 Desalting column were purchased from GE Healthcare Life Sciences (Uppsala, Sweden). All other reagents or chemicals were obtained from Sigma-Aldrich Chemicals (Steinheim, Germany), unless stated otherwise. A 50 mM Tris–Cl buffer containing 2 mM EDTA (pH7.5) was used for protein purification and activity assays (TE buffer), if not stated otherwise.

2.2. Construction of TrxR1 mutants

The plasmid encoding wild type rat TrxR1 and the bacterial type SECIS element for Sec insertion was used as a template for following Phusion™ PCR mutagenesis as described [17] to construct two C189 mutants of rat TrxR1. The primers were as follows: C189S-f, 5′-gcggtctcccttacTCAccggggaagaccctagtg-3′; C189A-f, 5′-gcggtctcccttacGCTccggggaagaccctagtg-3′; C189-r 5′-gcggtctcgtaaggcaaggagaaaag-3′. Phusion® High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Waltham, USA) was utilized for the inverse PCRs, together with the addition of 20 pmol of each primer and 10 ng of template plasmid in a total volume of 50 μl. Reactions were initiated at 98 °C for 2 min, followed by 30 cycles of amplification (98 °C, 10 s; 60 °C, 15 s; 72 °C, 3 min) and extended at 72 °C for additional 10 min. The resulting PCR products were analyzed by 1% agarose electrophoresis and the targeted PCR band was cut and then purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany), digested with Eco *31I* and Dpn *I* (Thermo Fisher Scientific, Waltham, USA) at 37 °C for 30 min, re-purified with the PCR purification kit (Qiagen, Germany), and ligated using T4 DNA ligase (Thermo–Fisher) at 22 °C for 2 h. The DNA was transformed into *Escherichia coli* BL21 (DE3) gor$^-$ competent cells (Tet$^+$) and then DNA sequencing verified the mutations (GATC Biotech, Konstanz, Germany). The plasmids were then transformed into *E. coli* BL21(DE3) gor-strain followed by a co-transformation of the pSUABC plasmid [23] for further expression and purification.

2.3. TrxR1 expression and purification

Mammalian rat TrxR1 and its C189S and C189A mutants were expressed in the *E. coli* gor$^-$ BL21 (DE3) strains co-transformed with the pET-TRS$_{TER}$-derived plasmids and the pSUABC plasmid [23] according to the method of engineering a gene compatible with the bacterial selenoprotein synthesis machinery and using the '2.4/24/24' protocol as described previously [24] with the exception that a rich LB broth containing 10 g NaCl, 10 g peptone and 10 g yeast extract per liter was used. The UGA-truncated form of TrxR1 was expressed in BL21 (DE3) *gor*$^-$ as described previously [21]. After suspending the cell pellets with 50 mM TE buffer (pH7.5), 1 mg/ml lysozyme was added to lyse bacterial cells and then the samples were placed on ice for 30 min. Freeze and thaw for three times. After ultra-sonication on ice to cleave the cellular DNA, the samples were centrifuged at 23,000 × *g* for 30 min. The soluble supernatants were filtered through 0.20um sterile membrane before the 2′5′-ADP Sepharose™ (GE Healthcare Life Sciences, Uppsala, Sweden) column purification as described [17]. Flavoprotein concentration was determined by measuring the FAD absorbance at 463 nm ($\varepsilon_{FAD,\ 463\ nm}$ = 11.30 mM^{-1} cm^{-1}). Protein concentrations were measured using a Bio-Rad Protein Assay Kit (Hercules, USA) with BSA as standard.

2.4. Kinetic analysis of TrxR1 variants

Enzymatic assay of TrxR1 and its mutants was determined using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) as a model substrate, with formation of TNB monitored as the increase in absorbance at 412 nm ($\varepsilon_{TNB,\ 412\ nm}$ = 13.6 mM^{-1} cm^{-1}) [25]. In all other assays such as hTrx1-coupled insulin reduction, PQQ reduction, or juglone reduction, the oxidation of NADPH was monitored as the decrease in absorbance at 340 nm ($\varepsilon_{NADPH,\ 340\ nm}$ = 6.22 mM^{-1} cm^{-1}) [25]. The standard reaction mixture (550 μl) contained 0.2 mM NADPH and 5–136 nM enzyme in TE buffer (pH7.5) with the reaction performed at 25 ± 1 °C in an UV–visible Spectrophotometer (GE Healthcare, Uppsala, Sweden), always using the same reaction mixture lacking enzyme as reference. For each data point, the initial velocity was determined in triplicate over at least six different substrate concentrations. Control assays, lacking the substrate, were also routinely included as references. Kinetic constants were calculated with the GraphPad Prism® 5 software (GraphPad, Avenida de la Playa, La Jolla, USA) after direct plotting of the catalytic velocity *versus* substrate concentrations followed by Michaelis–Menten fit with nonlinear regression.

2.5. Kinetic analysis of GR

Enzymatic assay of yeast GR was determined by using glutathione disulfide (GSSG) or juglone as substrates, with oxidation of NADPH monitored over time as the decrease in absorbance at 340 nm ($\varepsilon_{NADPH,\ 340\ nm}$ = 6.22 mM^{-1} cm^{-1}). The standard reaction mixture (550 μl) contained 0.2 mM NADPH and 5–100 nM enzyme in TE buffer (pH7.5) with the reaction performed at 25 ± 1 °C in an UV–visible Spectrophotometer (GE Healthcare, Uppsala, Sweden), using the same reaction mixture lacking enzyme as reference. Kinetic constants were calculated with the GraphPad Prism® 5 software as described under Section 2.4.

3. Results

3.1. PQQ is a weak substrate of Sec-containing TrxR1 and inhibits its Trx reductase activity

Analyzing PQQ interactions with recombinant rat TrxR1 we first noted that PQQ was a weak substrate of the enzyme (Fig. 1A, dashed bars). In the presence of Trx and the Trx disulfide substrate insulin, PQQ slightly inhibited total NADPH oxidation and thus appeared as an alternative and inhibiting subversive substrate (Fig. 1A, open bars). However, analyzing the Trx-mediated insulin reduction in the same experiment we found that this was very efficiently inhibited by PQQ, as illustrated by the lack of free thiol formation as a result of insulin reduction (Fig. 1B). Separate experiments showed that any effects of PQQ on TrxR1 were lost upon gel filtration, which recovered TrxR1 with intact native properties, thus illustrating that PQQ did not irreversibly alter the enzymatic properties of TrxR1 (data not shown).

The PQQ reduction by TrxR1 required an intact Sec residue, as illustrated by a complete lack of reaction using a Sec-deficient two-amino acid truncated TrxR1 variant (Fig. 2A). Other mutants of TrxR1 with Cys189, the only surface-exposed non-active site Cys residue in the enzyme [26], changed to Ser or Ala showed comparable activities with PQQ as the native enzyme (Fig. 2A).

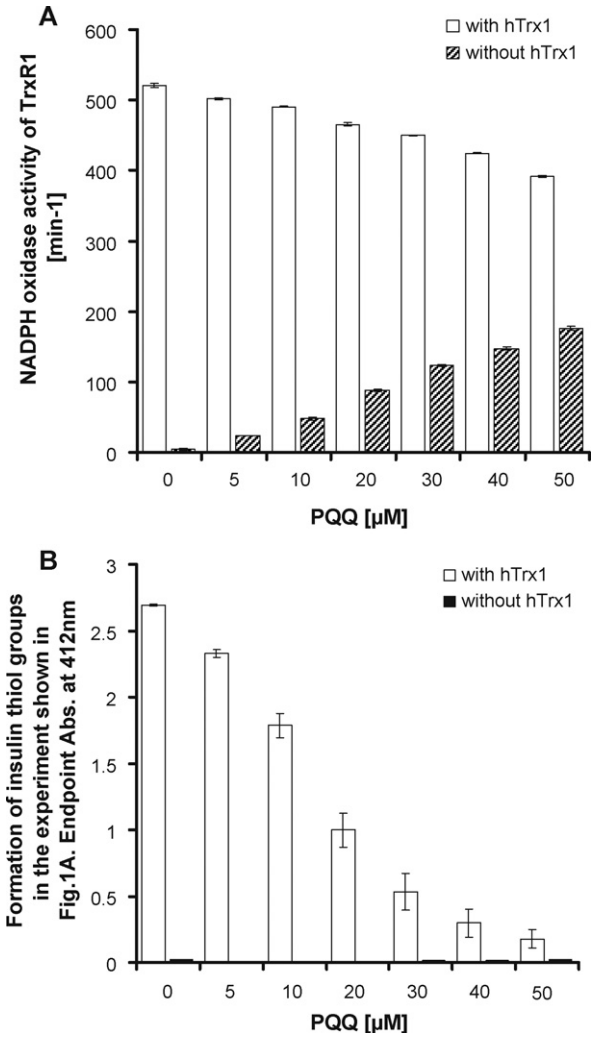

Fig. 1. PQQ inhibits mammalian TrxR1 activity in an assay for Trx-mediated insulin reduction and is a low efficiency direct substrate of the enzyme. In (A), the TrxR1 activity in Trx-coupled insulin reduction was determined by measuring the NADPH consumption at 340 nm using recombinant rat TrxR1 (136 nM), recombinant human Trx1 (10 μM) and insulin as disulfide substrate (160 μM), in the presence of PQQ addition (0–50 μM) as indicated in the figure (open bars). As a comparison, the NADPH consumption in the absence of Trx1 was also measured, reflecting direct PQQ reduction (filled bars). In (B), the formation of free insulin thiol groups formed in the experiment shown in panel (A) was measured by the addition of DTNB (2 mM) in GuHCl (8 M) after 10 min of reaction and determination of absorbance at 412 nm. The results are shown as mean ± S.D. from three independent experiments.

3.2. PQQ stimulates juglone reduction by TrxR1 while inhibiting its Trx reduction

Because PQQ diverted the activity of TrxR1 away from Trx1-coupled insulin reduction (Fig. 1) and because juglone is an efficient direct quinone substrate of TrxR1 not dependent upon its Sec residue [27,28], we next analyzed the effects of PQQ on TrxR1 activity using a substrate mixture of juglone and Trx1 together with insulin. This revealed that with all four TrxR1 preparations analyzed here, the overall enzyme activities with this mixture of substrates were stimulated by the addition of 10–50 μM PQQ, as judged by total NADPH oxidation (Fig. 2B). When subsequently analyzing the insulin reduction that had occurred in those samples this was, as expected, virtually absent with the Sec deficient truncated enzyme that cannot reduce Trx. With the wild type enzyme or the C189A and C189S variants, however, comparable Trx-linked insulin reduction activities were seen in the absence of PQQ, but these disulfide reductase activities of all variants were

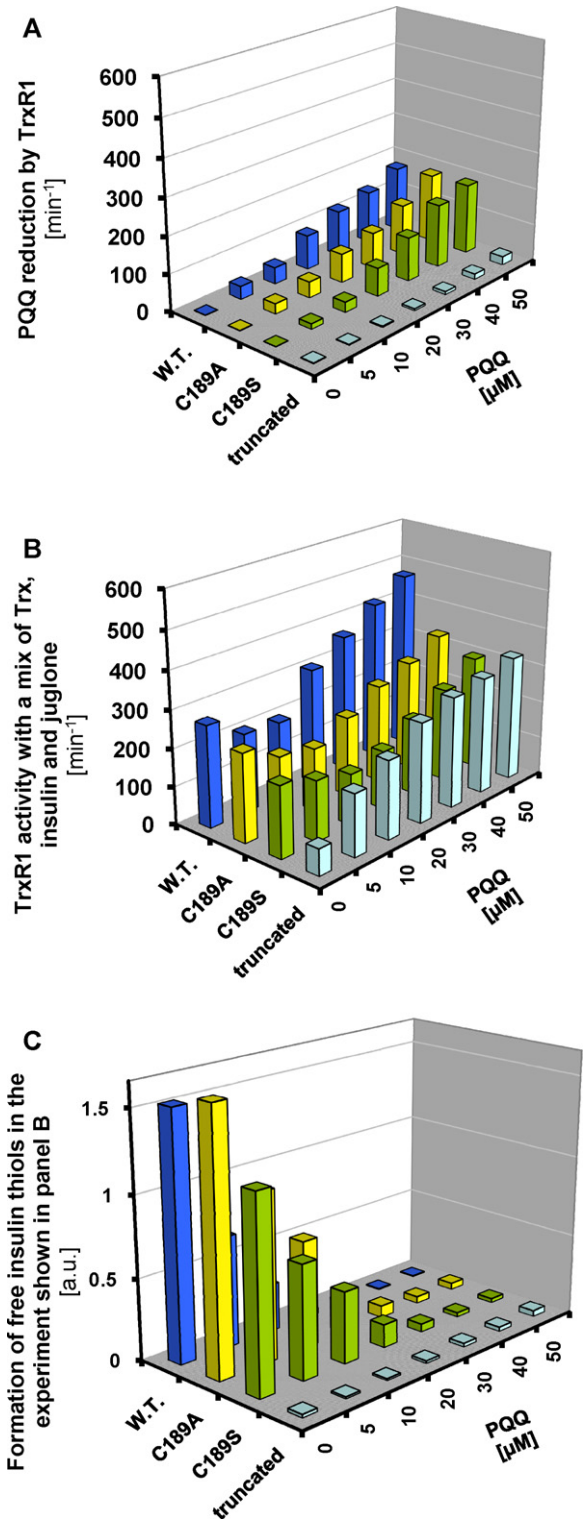

Fig. 2. PQQ switches mammalian TrxR1 from a Trx reducase to a juglone reductase. In (A), PQQ reduction by TrxR1 was monitored by following NADPH consumption by reduced absorbance at 340 nm using 200 μM NADPH and 136 nM of the TrxR variants and addition of PQQ as indicated in the figure. In (B), the effects of PQQ on NADPH consumption in the Trx-coupled insulin reduction assay combined with the juglone reduction assay were studied in the same reaction mixture system. Total NADPH consumption was followed at 340 nm using the TrxR1 variants (136 nM) with addition of Trx1 (10 μM), insulin (160 μM), juglone (3 μM), NADPH (200 μM) and PQQ at the concentrations indicated in the figure. In (C), the formation of free insulin thiol groups after 10 min reaction of the experiment shown in panel (B) was determined as explained in Fig. 1B. All reactions were performed at room temperature (25 ± 1 °C). The experiment was performed once. For the effects of PQQ on the kinetic parameters in juglone reduction by TrxR1, see Table 1.

Table 1
Effects on kinetic parameters in juglone reduction by TrxR1 and GR upon addition of PQQ.

	TrxR1[a]			GR[b]		
	[PQQ, μM]					
	0	10	50	0	10	50
Juglone[c]						
k_{cat} (min^{-1})	797 ± 29	590 ± 13	453 ± 10	638 ± 21	599 ± 14	524 ± 11
K_m (μM)	7.57 ± 0.89	2.77 ± 0.24	0.34 ± 0.06	19.0 ± 2.3	17.7 ± 1.5	13.9 ± 1.2
k_{cat}/K_m (min^{-1} μM^{-1})	105	213	1331	34	34	38
NADPH[d]						
k_{cat} (min^{-1})	655 ± 15	645 ± 15	641 ± 14	769 ± 18	797 ± 23	647 ± 35
K_m (μM)	17.4 ± 1.8	19.2 ± 2.1	20.6 ± 2.1	33.6 ± 2.7	39.9 ± 3.6	38.5 ± 6.6
k_{cat}/K_m (min^{-1} μM^{-1})	38	34	31	23	20	17

Values in the table represent mean ± S.D. derived from measurements performed in triplicate.
[a] Kinetic parameters were determined at room temperature (25 ± 1 °C) following the consumption of NADPH at 340 nm using wild type recombinant rat TrxR1 (136 nM) with a specific activity of 24 U/mg.
[b] Kinetic parameters were determined at room temperature (25 ± 1 °C) following the consumption of NADPH at 340 nm using yeast GR (100 nM).
[c] For the juglone reduction kinetic parameters with either TrxR1 or GR, a fixed concentration of 200 μM NADPH was used.
[d] For the NADPH oxidation kinetic parameter, a fixed concentration of either 100 μM or 250 μM juglone were used with TrxR1 or GR, respectively.

severely inhibited upon addition of increasing concentrations of PQQ (Fig. 2C). These results thereby suggested to us that the increase in total NADPH oxidase activity of TrxR1 with this mixture of substrates upon addition of PQQ (Fig. 2B) must have been due to a switch from Trx reduction to a reduction of PQQ and juglone. When comparing the magnitude of activity in this assay with that seen upon addition of solely PQQ it was evident that the total turnover with the substrate mixture (Fig. 2B) was higher than that seen in the presence of only PQQ (Fig. 2A). Also, the decrease in total turnover seen upon addition of low concentrations of PQQ (0–10 μM) to the wild type, C189A or C189S variants but not to the truncated enzyme (Fig. 2B) should be due to inhibition of the Trx reduction catalyzed by the Sec-containing enzyme variants by PQQ, while their activities with either PQQ or juglone as substrates, at those conditions, were still low and thus comparable to that seen with the truncated enzyme. Therefore, addition of PQQ seemed to have modified the substrate preference of wild type TrxR1 from Trx to juglone. An alternative explanation could have been that PQQ rapidly re-oxidized Trx or insulin and therefore increased the overall activity, but this was less likely because in the absence of juglone addition to Trx, insulin and PQQ, the overall NADPH oxidation was slightly inhibited by PQQ and not stimulated, as shown above (Fig. 1A, open bars).

3.3. PQQ lowers the apparent K_m of TrxR1 for juglone and is also an efficient inhibitor of GR

To further probe the effects of PQQ on juglone reduction by TrxR1, we determined the kinetic parameters of TrxR1 for juglone as well as NADPH upon addition of either 10 μM or 50 μM PQQ as compared to the native enzyme (Table 1). We also compared those effects on TrxR1 to the corresponding effects of PQQ on the flavoenzyme GR, closely related to TrxR1 although it is not a selenoprotein (Tables 1 and 2). As shown earlier [27,28], TrxR1 reduces juglone more efficiently than GR in the absence of PQQ. We found, surprisingly, that upon addition of 50 μM PQQ this the catalytic efficiency of TrxR1 increased about 13-fold in reduction of juglone, from k_{cat}/K_m = 105 min^{-1} μM^{-1} in the absence of PQQ to k_{cat}/K_m = 1331 min^{-1} μM^{-1} upon addition of PQQ. This effect was mainly because of a lowered apparent K_m of TrxR1 for juglone, from K_m = 7.6 μM in the absence of PQQ to K_m = 0.34 μM in the presence of PQQ. This strong effect was not seen with GR although this enzyme also showed a tendency to a lowered K_m for juglone, but the k_{cat} was proportionally lowered and the catalytic efficiency thus remained virtually the same in the absence or presence of PQQ (Table 1). When determining the kinetic parameters for NADPH, addition of PQQ had no major impact on any of the two enzymes, using the juglone reduction assay (Table 1). However, PQQ was a clear inhibitor of GR in glutathione disulfide (GSSG) reduction, in this case with the catalytic efficiency reduced approximately 2-fold and 5-fold with regards to GSSG and NADPH, respectively, using only 5 μM PQQ (Table 2). The kinetics in the PQQ inhibition were complex and involved effects on both k_{cat} and K_m.

4. Discussion

In this study, we found that PQQ modulates the catalytic parameters of TrxR1, promoting a switch from Trx reduction to an

Table 2
Effects on kinetic parameters in GR-catalyzed GSSG reduction by PQQ.

	GR[a]				
	[PQQ, μM]				
	0.0	0.5	1.0	2.0	5.0
GSSG[b]					
k_{cat} (min^{-1})	$13.3 \times 10^3 \pm 0.26 \times 10^3$	$8.23 \times 10^3 \pm 0.20 \times 10^3$	$6.51 \times 10^3 \pm 0.18 \times 10^3$	$5.07 \times 10^3 \pm 0.14 \times 10^3$	$3.89 \times 10^3 \pm 0.12 \times 10^3$
K_m (μM)	41.4 ± 3.4	35.8 ± 3.8	29.5 ± 3.4	28.1 ± 3.8	22.8 ± 3.5
k_{cat}/K_m (min^{-1} μM^{-1})	322	230	220	180	170
NADPH[c]					
k_{cat} (min^{-1})	$13.5 \times 10^3 \pm 0.40 \times 10^3$	$11.0 \times 10^3 \pm 0.62 \times 10^3$	$9.43 \times 10^3 \pm 0.58 \times 10^3$	$6.77 \times 10^3 \pm 0.30 \times 10^3$	$5.18 \times 10^3 \pm 0.25 \times 10^3$
K_m (μM)	33.5 ± 3.8	62.8 ± 10	88.2 ± 14	70.2 ± 8.9	58.3 ± 8.6
k_{cat}/K_m (min^{-1} μM^{-1})	402	176	107	96	89

Values in the table represent mean ± S.D. derived from measurements performed in triplicate.
[a] Kinetic parameters were determined at room temperature (25 ± 1 °C) following the consumption of NADPH at 340 nm using yeast GR (10 nM) with NADPH dependent GSSG reduction.
[b] In the determination of kinetic parameters in GSSG reduction a fixed concentration of 200 μM NADPH was used.
[c] In the determination of kinetic parameters in NADPH oxidation a fixed concentration of 1 mM GSSG was used.

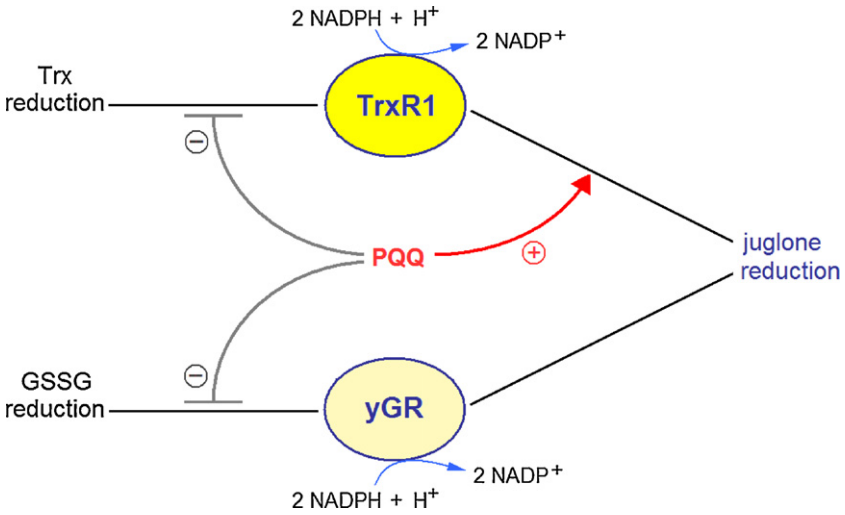

Fig. 3. Scheme of the effects of PQQ on GR and mammalian TrxR1. As schematically illustrated in this scheme, we here found that PQQ has prominent effects on both TrxR1 and GR, where TrxR1 displays an inhibited Trx reduction with concomitant increase in catalytic efficiency for juglone reduction, upon the addition of PQQ. With GR, on the other hand, PQQ inhibits the GSSG reduction as catalyzed by the enzyme but has no major effects on its juglone reduction. For more details, please see text.

increased quinone reductase activity, using either PQQ itself or the quinone compound juglone as substrate. We also found that GR was inhibited by PQQ, but the quinone reductase activity of GR was, in contrast to that seen with TrxR1, not further stimulated by addition of PQQ. These direct effects of PQQ on TrxR1 and GR, as schematically summarized in Fig. 3, can help to explain several of the known physiological effects of PQQ, which shall briefly be discussed as follows.

PQQ stimulates mitochondrial biosynthesis in mouse hepatocytes through the activation of CREB (cAMP response element-binding protein) and PGC-1α (peroxisome proliferators-activated receptor-γ coactivator-1α) [29]. Consistent with activation of the PGC-1α pathway, PQQ exposure also gave Nrf-1 and Nrf-2 activation, with increased Tfam, TFB1 M and TFB2 M mRNA expression [29]. Because TrxR1 is intimately linked to Nrf-2, with activation of Nrf-2 seen upon TrxR1 inactivation [30,31], it can hence be proposed that a direct targeting of TrxR1 by PQQ could be a molecular mechanism mediating the Nrf2-dependent cellular effects of PQQ in mammals. This notion is further strengthened by the fact that Trx and Trx-related transcripts were among the most affected genes in microarray studies of the cellular impact by dietary supplementation of PQQ in rat models [14].

In addition to effects on PGC-1 and Nrf1/2, PQQ can also activate Ras-related signaling transduction in mouse fibroblasts and stimulate STAT (signal transducers and activators of transcription) factors, of importance for cell growth, proliferation, differentiation and survival [32]. PQQ was also reported to affect the oxidation state of DJ-1, a protein responsive to oxidative stress and protecting from neuronal death [33]. It is thus clear that PQQ affects several pathways of cell signaling and apoptotic cell death, cell proliferation, mitochondria and oxidative metabolism, but the molecular mechanisms for these effects are not known [1]. Since the Trx and GSH systems are the two major redox systems in cells [34,35], the direct targeting of both TrxR1 and GR by PQQ may help explain the plethora of physiological effects displayed by PQQ. Still, for each cellular pathway where PQQ has an impact, the involvement of either TrxR1 or GR must clearly be specifically studied in order to determine any direct importance of PQQ targeting these enzymes for any specific effect. There are, however, several findings of TrxR1 being targeted by other small molecules that may be taken into context when studying the effects of PQQ.

Previously reported polyphenolic compounds targeting TrxR1, including curcumin [36], myricetin or quercetin [37], are, like PQQ, weak substrates of mammalian TrxR1. However, they also strongly and, in contrast to PQQ, typically inhibit the enzyme irreversibly for its Trx reduction. Certain quinone compounds, reduced by TrxR1 to auto-oxidizing hydroquinones, may also inactive TrxR1 while at the same time confer pro-oxidant properties to the derivatized enzyme [28]. In this context, PQQ was found here to have a novel property, namely stimulating the TrxR1-mediated reduction of juglone, another quinone compound, while inhibiting the reduction of Trx, in a reversible manner. Previous findings that PQQ could catalyze general oxidation of thiols in proteins such as GST P1-1 [8], the redox modulatory site of the NMDA (N-methyl-D-asparate) receptor [38,39], or many proteins in general [40] can potentially be part of the explanation for its effects on TrxR1 and GR, but the mechanisms for the increased catalytic efficiency of TrxR1 for juglone are not clear and should be studied further.

To conclude, we here found that PQQ in micromolar concentrations stimulated the juglone reduction by mammalian TrxR1 while efficiently inhibiting its physiological reduction of Trx. The NADPH oxidase activity of TrxR1 using PQQ as a unique substrate in the absence of juglone was a Sec-dependent process, while the increase of juglone reduction was seen also with Sec-compromised species of TrxR1. The non-selenoprotein TrxR1 homologue GR was also inhibited by PQQ, but could in contrast to TrxR1 not reduce PQQ directly and displayed no PQQ-mediated increase in juglone reduction. We conclude that the direct effects of PQQ on TrxR1 and GR may help explain the physiological effects of PQQ in mammals.

Acknowledgements

This study was supported by grants from the Swedish Cancer Society, the Swedish Research Council (Medicine) and Karolinska Institutet.

References

[1] Rucker R, Chowanadisai W, Nakano M. Potential physiological importance of pyrroloquinoline quinone. Altern Med Rev 2009;14:268–77.
[2] Duine JA, Frank J, van Zeeland JK. Glucose dehydrogenase from Acinetobacter calcoaceticus: a 'quinoprotein'. FEBS Lett 1979;108:443–6.
[3] Killgore J, Smidt C, Duich L, Romero-Chapman N, Tinker D, Reiser K, et al. Nutritional importance of pyrroloquinoline quinone. Science 1989;245:850–2.
[4] Anthony C, Zatman LJ. The microbial oxidation of methanol. The prosthetic group of the alcohol dehydrogenase of Pseudomonas sp. M27: a new oxidoreductase prosthetic group. Biochem J 1967;104:960–9.
[5] Anthony C. Quinoprotein-catalysed reactions. Biochem J 1996;320:697–711.

[6] Ghosh M, Anthony C, Harlos K, Goodwin MG, Blake C. The refined structure of the quinoprotein methanol dehydrogenase from Methylobacterium extorquens at 1.94 Å. Structure 1995;3:177–87.
[7] Anthony C, Ghosh M. The structure and function of the PQQ-containing quinoprotein dehydrogenases. Prog Biophys Mol Biol 1998;69:1–21.
[8] Ishii T, Akagawa M, Naito Y, Handa O, Takagi T, Mori T, et al. Pro-oxidant action of pyrroloquinoline quinone: characterization of protein oxidative modifications. Biosci Biotechnol Biochem 2010;74:663–6.
[9] Anthony C. Pyrroloquinoline quinone (PQQ) and quinoprotein enzymes. Antioxid Redox Signal 2001;3:757–74.
[10] He K, Nukada H, Urakami T, Murphy MP. Antioxidant and pro-oxidant properties of pyrroloquinoline quinone (PQQ): implications for its function in biological systems. Biochem Pharmacol 2003;65:67–74.
[11] Kasahara T, Kato T. Nutritional biochemistry: a new redox-cofactor vitamin for mammals. Nature 2003;422:832.
[12] Felton LM, Anthony C. Biochemistry: role of PQQ as a mammalian enzyme cofactor? Nature 2005;433:E10 (discussion E11–E12).
[13] Rucker R, Storms D, Sheets A, Tchaparian E, Fascetti A. Biochemistry: is pyrroloquinoline quinone a vitamin? Nature 2005;433:E10–1 (discussion E11–E12).
[14] Tchaparian E, Marshal L, Cutler G, Bauerly K, Chowanadisai W, Satre M, et al. Identification of transcriptional networks responding to pyrroloquinoline quinone dietary supplementation and their influence on thioredoxin expression, and the JAK/STAT and MAPK pathways. Biochem J 2010;429:515–26.
[15] Arnér ESJ. Focus on mammalian thioredoxin reductases – important selenoproteins with versatile functions. Biochim Biophys Acta 2009;1790:495–526.
[16] Cenas N, Nivinskas H, Anusevicius Z, Sarlauskas J, Lederer F, Arnér ESJ. Interactions of quinones with thioredoxin reductase – a challenge to the antioxidant role of the mammalian selenoprotein. J Biol Chem 2004;279:2583–92.
[17] Cheng Q, Antholine WE, Myers JM, Kalyanaraman B, Arnér ES, Myers CR. The selenium-independent inherent pro-oxidant NADPH oxidase activity of mammalian thioredoxin reductase and its selenium-dependent direct peroxidase activities. J Biol Chem 2010;285:21708–23.
[18] Zhong L, Arnér ES, Holmgren A. Structure and mechanism of mammalian thioredoxin reductase: the active site is a redox-active selenolthiol/selenenylsulfide formed from the conserved cysteine-selenocysteine sequence. Proc Natl Acad Sci U S A 2000;97:5854–9.
[19] Gromer S, Johansson L, Bauer H, Arscott LD, Rauch S, Ballou DP, et al. Active sites of thioredoxin reductases: why selenoproteins. Proc Natl Acad Sci U S A 2003;100:12618–23.
[20] Lu J, Holmgren A, Selenoproteins. J Biol Chem 2009;284:723–7.
[21] Anestål K, Prast-Nielsen S, Cenas N, Arnér ESJ. Cell death by SecTRAPs – thioredoxin reductase as a prooxidant killer of cells. PLoS ONE 2008;3:e1846.
[22] Zhong L, Arnér ESJ, Ljung J, Åslund F, Holmgren A. Rat and calf thioredoxin reductase are homologous to glutathione reductase with a carboxyl-terminal elongation containing a conserved catalytically active penultimate selenocysteine residue. J Biol Chem 1998;273:8581–91.
[23] Arnér ES, Sarioglu H, Lottspeich F, Holmgren A, Bock A. High-level expression in Escherichia coli of selenocysteine-containing rat thioredoxin reductase utilizing gene fusions with engineered bacterial-type SECIS elements and co-expression with the selA, selB and selC genes. J Mol Biol 1999;292:1003–16.
[24] Rengby O, Johansson L, Carlson LA, Serini E, Vlamis-Gardikas A, Karsnas P, et al. Assessment of production conditions for efficient use of *Escherichia coli* in high-yield heterologous recombinant selenoprotein synthesis. Appl Environ Microbiol 2004;70:5159–67.
[25] Arnér ES, Holmgren A. Measurement of thioredoxin and thioredoxin reductase. Curr Protoc Toxicol 2001. doi: 10.1002/0471140856.tx0704s05. Chapter 7:Unit 7.4.
[26] Cheng Q, Sandalova T, Lindqvist Y, Arnér ES. Crystal structure and catalysis of the selenoprotein thioredoxin reductase 1. J Biol Chem 2009;284:3998–4008.
[27] Cenas N, Prast S, Nivinskas H, Sarlauskas J, Arnér ES. Interactions of nitroaromatic compounds with the mammalian selenoprotein thioredoxin reductase and the relation to induction of apoptosis in human cancer cells. J Biol Chem 2006;281:5593–603.
[28] Cenas N, Nivinskas H, Anusevicius Z, Sarlauskas J, Lederer F, Arnér ES. Interactions of quinones with thioredoxin reductase: a challenge to the antioxidant role of the mammalian selenoprotein. J Biol Chem 2004;279:2583–92.
[29] Chowanadisai W, Bauerly KA, Tchaparian E, Wong A, Cortopassi GA, Rucker RB. Pyrroloquinoline quinone stimulates mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1alpha expression. J Biol Chem 2010;285:142–52.
[30] Suvorova ES, Lucas O, Weisend CM, Rollins MF, Merrill GF, Capecchi MR, et al. Cytoprotective Nrf2 pathway is induced in chronically txnrd 1-deficient hepatocytes. PLoS One 2009;4:e6158.
[31] Fourquet S, Guerois R, Biard D, Toledano MB. Activation of NRF2 by nitrosative agents and H_2O_2 involves KEAP1 disulfide formation. J Biol Chem 2010;285:8463–71.
[32] Kumazawa T, Hiwasa T, Takiguchi M, Suzuki O, Sato K. Activation of Ras signaling pathways by pyrroloquinoline quinone in NIH3T3 mouse fibroblasts. Int J Mol Med 2007;19:765–70.
[33] Nunome K, Miyazaki S, Nakano M, Iguchi-Ariga S, Ariga H. Pyrroloquinoline quinone prevents oxidative stress-induced neuronal death probably through changes in oxidative status of DJ-1. Biol Pharm Bull 2008;31:1321–6.
[34] Holmgren A, Lu J. Thioredoxin and thioredoxin reductase: current research with special reference to human disease. Biochem Biophys Res Commun 2010;396:120–4.
[35] Lillig CH, Holmgren A. Thioredoxin and related molecules – from biology to health and disease. Antioxid Redox Signal 2007;9:25–47.
[36] Fang J, Lu J, Holmgren A. Thioredoxin reductase is irreversibly modified by curcumin: a novel molecular mechanism for its anticancer activity. J Biol Chem 2005;280:25284–90.
[37] Lu J, Papp LV, Fang J, Rodriguez-Nieto S, Zhivotovsky B, Holmgren A. Inhibition of Mammalian thioredoxin reductase by some flavonoids: implications for myricetin and quercetin anticancer activity. Cancer Res 2006;66:4410–8.
[38] Aizenman E, Hartnett KA, Zhong C, Gallop PM, Rosenberg PA. Interaction of the putative essential nutrient pyrroloquinoline quinone with the N-methyl-D-aspartate receptor redox modulatory site. J Neurosci 1992;12:2362–9.
[39] Aizenman E, Jensen FE, Gallop PM, Rosenberg PA, Tang LH. Further evidence that pyrroloquinoline quinone interacts with the N-methyl-D-aspartate receptor redox site in rat cortical neurons in vitro. Neurosci Lett 1994;168:189–92.
[40] Park J, Churchich JE. Pyrroloquinoline quinone (coenzyme PQQ) and the oxidation of SH residues in proteins. Biofactors 1992;3:257–60.