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MINIREVIEW

Thioredoxin reductase as a pathophysiological factor and drug target

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Human cytosolic thioredoxin reductase (TrxR), a homodimeric protein containing 1 selenocysteine and 1 FAD per subunit of 55 kDa, catalyses the NADPH-dependent reduction of thioredoxin disulfide and of numerous other oxidized cell constituents. As a general reducing enzyme with little substrate specificity, it also contributes to redox homeostasis and is involved in prevention, intervention and repair of damage caused by H_2O_2-based oxidative stress.

Being a selenite-reducing enzyme as well as a selenol-containing enzyme, human TrxR plays a central role in selenium (patho)physiology. Both dietary selenium deficiency and selenium oversupplementation, a lifestyle phenomenon of our time, appear to interfere with the activity of TrxR. Selenocysteine 496 of human TrxR is a major target of the anti-rheumatic gold-containing drug auranofin, the formal K_i for the stoichiometric inhibition being 4 nM. The hypothesis that TrxR and extracellular thioredoxin play a pathophysiologic role in chronic diseases such as rheumatoid arthritis, Sjögren's syndrom, AIDS, and certain malignancies, is substantiated by biochemical, virological, and clinical evidence. Reduced thioredoxin acts as an autocrine growth factor in various tumour diseases, as a chemoattractant, and it synergises with interleukins 1 and 2. The effects of anti-tumour drugs such as carmustine and cisplatin can be explained in part by the inhibition of TrxR. Consistently, high levels of the enzyme can support drug resistance.

TrxRs from different organisms such as *Escherichia coli*, *Mycobacterium leprae*, *Plasmodium falciparum*, *Drosophila melanogaster*, and man show a surprising diversity in their chemical mechanism of thioredoxin reduction. This is the basis for attempts to develop specific TrxR inhibitors as drugs against bacterial infections like leprosy and parasitic diseases like amebiasis and malaria.

Keywords: antioxidant systems; aurothioglucose; carmustine; diselenide; drug resistance; Epstein–Barr virus; leprosy; malaria; rheumatoid arthritis; selenium metabolism.

Thioredoxin reductase (TrxR; EC 1.6.4.5; thioredoxin-S_2 + NADPH + H^+ ⇌ thioredoxin-$(SH)_2$ + $NADP^+$) belongs to a family of glutathione reductase-like homodimeric flavoenzymes [1]. Genetic and mechanistic aspects of TrxRs from different species are covered in more detail by other articles in this review series and elsewhere [1–5]. As shown in Fig. 1, the 35-kDa (subunit M_r) TrxRs occurring in prokaryotes but also in plants and fungi differ fundamentally from the 55- to 60-kDa TxRs that have been identified so far in mammals, *Caenorhabditis elegans*, *Drosophila melanogaster*, and in the malaria parasite *Plasmodium falciparum.* The high M_r TrxRs contain a C-terminal peripheral redox centre that communicates with the central redox-active catalytic site [6]. Whereas the peripheral redox centre of *P. falciparum* TrxR is represented by Cys535 and Cys540 [7] and by Cys489–Cys490 in *D. melanogaster* (S. M. Kanzok, H. Bauer, R. H. Schirmer & K. Becker, unpublished results), all known mammalian TrxRs possess a Cys-Sec sequence as the C-terminal redox pair [8–10]. Thus mammalian TrxR is a member of the small and exclusive class of selenoenzymes [11,12]. Here we report on the pathophysiological aspects and potential clinical implications of TrxR (Table 1).

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Abbreviations: ADF, adult T-cell leukemia derived factor (also termed thioredoxin); DTNB, 5,5′-dithiobis(2-nitrobenzoate); EBV, Epstein–Barr virus; ECEF, eosinophil cytotoxicity-enhancing factor; GSH, reduced glutathione; GSNO, *S*-nitrosoglutathione; GS-Se-SG, selenodiglutathione; HIV, human immunodeficiency virus; HTLV1, human T-lymphotropic virus 1; NK-lysin, natural killer cell-lysin; NO, nitric oxide; Trx, thioredoxin; TrxR, thioredoxin reductase.

Enzyme: Thioredoxin reductase (TrxR; EC 1.6.4.5)

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MEDICAL IMPLICATIONS OF THE BROAD SUBSTRATE SPECIFICITY OF HIGH M_R TRXR

TrxR is well known as the enzyme reducing the 12-kDa protein thioredoxin (Trx) which in turn provides reducing equivalents for the synthesis of deoxyribonucleotides via ribonucleotide reductase (Fig. 2). The *in vivo* activity of TrxR (> 1 U per mL cells) meets the demand for DNA building blocks, which is as high as 10 μmol per g new cells. This appears to apply also when the glutaredoxin system is nonfunctional as GSH-depleted mammalian cells in culture were found to grow as well as controls and showed no alterations in the deoxyribonucleotide pool [13]. Consequently, the thioredoxin system (TrxR and Trx) can be regarded as an essential constituent of the cell-proliferating machinery in mammalian cells.

Due to the unusually broad substrate specificity, high M_r TrxRs are involved in numerous metabolic pathways [3]. In addition, there are the Trx-mediated functions of TrxR. The

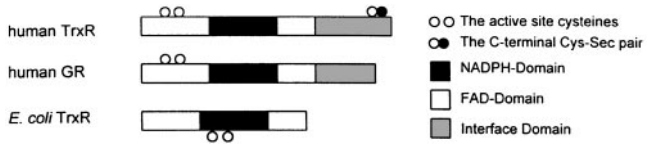

Fig. 1. Disulfide reductases. Scheme of the domain arrangement in the enzyme subunit. Human glutathione reductase (GR), a well-studied family member, is compared with human TrxR and *E. coli* TrxR. In high M_r TrxR, the C-terminal redox centre communicates with the active site cysteine pair of the other subunit (modifed from [5]).

functional diversity is illustrated by the following medically relevant examples.

Selenite and selenodiglutathione. Selenite and selenodiglutathione (GS-Se-SG) are both considered major intermediates in selenium metabolism. Both are NADPH-dependently reduced by TrxR indicating a role of the enzyme in physiological selenium chemistry. The resulting selenide is, in phosphorylated form, the precursor for the selenol group of the proteinogenic amino acid selenocysteine (Sec) [12].

NK-lysin. NK-lysin, a 9-kDa pore-forming peptide secreted by natural killer cells, is efficiently reduced and inactivated by human TrxR. Thus it is likely that a membrane-associated TrxR of mammalian cells serves as a physiological protectant that confines the effects of highly toxic substances like NK-lysin to invading microorganisms. TrxR-containing tumour cells are, of course, also protected against NK-lysin [14].

Lipoic acid (lipoate disulfide), a physiological compound, is used pharmacologically in the treatment of diabetic patients with peripheral neuropathy and of persons with acute mushroom poisoning. The administered lipoate disulfide is reduced *in vivo* by TrxR to its metabolically active form. However, the clinical results with lipoic acid have not yet met expectations for this compound. One reason might be the high K_m value of 510 μM with human TrxR [15]. Thus, the concentrations of reduced lipoic acid reached *in vivo* might be too low for sufficiently high turnover. New lipoic acid derivatives with low K_m values are expected to overcome this obstacle and prove to be more effective.

Ascorbyl free radicals. Ascorbyl free radicals representing one-electron oxidized ascorbate are important indicators of oxidative stress. These radicals are recycled by thioredoxin reductase, the K_m being in the lower micromolar range. TrxR also contributes to the reduction of dehydroascorbate but this process is probably mediated by Trx. These results indicate that, in addition to protecting the cell from oxidative stress by maintaining Trx in its reduced state, TrxR may play a role in the recycling of ascorbate [16,17].

S-Nitrosoglutathione (GSNO), an important metabolite and transport form of nitric oxide (NO), is also a substrate of mammalian TrxR. However, the indirect reduction via Trx can maintain much higher GSNO fluxes than the direct reaction of GSNO with TrxR ($NADPH + GSNO + O_2 \rightarrow NADP^+ + GSH + NO + O_2^-$). The Trx-mediated reaction is not only faster but protects TrxR from the inactivating effects of the reaction products $NO + O_2^-$, which can readily combine to highly reactive peroxynitrite ($ONOO^-$) [18,19].

Trx-mediated reduction processes. The reactions of GSNO illustrate that one has to compare direct conversion of a given substrate by TrxR and indirect conversion by the thioredoxin redox cycle. In the latter case, the substrate rapidly reacts with $Trx(SH)_2$, and the resulting $TrxS_2$ is re-reduced by TrxR (Fig. 2).

Likewise, the high M_r TrxR/Trx system has been shown to detoxify harmful agents such as H_2O_2, alkylhydroperoxides and other oxidants and it is involved in prevention, intervention and the repair of protein damage by oxidative stress [1,3–5]. The hitherto neglected reduction of glutathione disulfide (Fig. 2) by the thioredoxin system is presently being studied in our group; GSSG can reach significant concentrations. In this context of protection from oxidative stress, it is noteworthy that the thioredoxin system can serve *in vitro* as an electron donor for

Table 1. Pathophysiologic conditions (P) and therapeutic interventions (T) with Trx/TrxR involvement.

Condition	Ref.	Comment
Diabetic neuropathy (T)	[15]	Reduction of lipoic acid by TrxR. The high K_m might be a reason for low effectiveness *in vivo*
Reperfusion arrhythmias of the heart (T)	[58]	Reduced Trx is more efficient in preventing ventricular arrhythmias than other proteins
Rheumatoid arthritis (P)	[42,44]	Extracellular concentration of Trx correlates well with degree of local inflammation. Therapeutic gold-compounds are effective inhibitors of TrxR *in vitro* and *in vivo*.
Sjögren's syndrome (P)	[41]	Correlation between production of EB-virions and Trx-synthesis in infected cells
Tumours (P)	[3–5,8,27,31]	The Trx system supports growth and antioxidative defense of tumour cells. On the other hand, it prevents normal cells from turning malignant by protecting them against certain mutagens.
HIV/AIDS (P)	[21,45,46]	Trx-level in blood plasma correlates with disease stage. Alterations in selenoprotein levels probably affect functions of the Trx-system
Protection of pathogens from respiratory burst metabolites (P)	[1,50,53,54]	Human tissues are protected by specific thioredoxin systems of their own from oxidative stress [3–5,57]
Drug resistance (P)	[1,3–5,51,54,56]	Cisplatin, mitomycin C, doxorubicin, and etoposide in mammalian tumours; metronidazole and isoniazid in pathogenic microorganisms

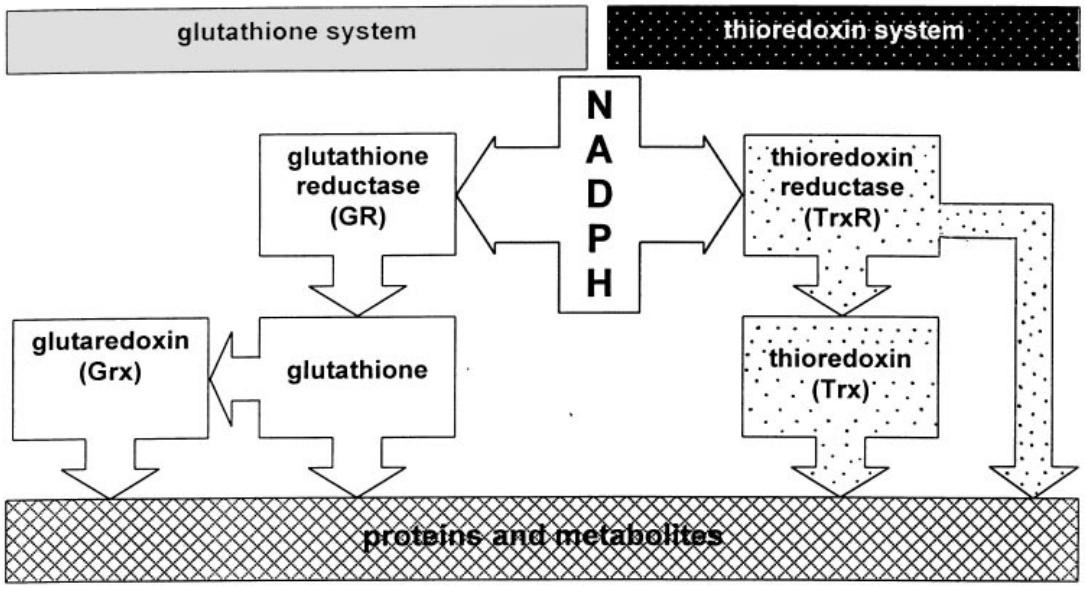

Fig. 2. The major intracellular disulfide-reducing systems. The glutathione system is shown on the left and the thioredoxin system on the right hand side. The arrows indicate the flow of reducing equivalents originating from NADPH.

the so called plasma glutathione peroxidase [20]. As the physiological concentration of Trx (but also GSH!) in blood plasma is low [21,22] and an efficient reduction of Trx via a membrane-associated TrxR remains to be established, the *in vivo* importance of this finding requires further studies.

The impressive variety of TrxR substrates, which includes proteins and low M_r molecules, gives rise to the question at which redox centre of TrxR these different compounds are reduced. Not only the size of a substrate but also its charge and polarity seem to play a role in this context. Our current concept of the catalytic mechanisms suggests that larger substrates react at the C-terminal redox active site whereas certain small compounds may also take the shortcut via the flavin and/or the internal catalytic cysteines. Data obtained from TrxR with a proteolytically cleaved C-terminus support this idea as the artificial substrate 5,5′-dithiobis(2-nitrobenzoate) (DTNB) is still reduced by the modified enzyme whereas Trx is not [23]. However, the small molecule Tris(2-carboxylethyl)phosphine also reacts exclusively at the C-terminal redox center [2]. These aspects are of practical importance as inhibitors blocking the selenol or another function at the C-terminal redox centre could still allow reduction of low M_r molecules at the central dithiol [2].

EFFECTS OF SELENIUM COMPOUNDS ON HUMAN TRXR

Selenium compounds are discussed as therapeutic agents for the treatment of diseases such as rheumatoid arthritis and malignant tumours. TrxRs play a key role because they are involved in selenium assimilation and because they are seleno-enzymes themselves. As selenium is a trace element, its concentration can be assumed to be a limiting factor in TrxR synthesis [4,12,24]. Initial attempts to produce active recombinant human TrxR failed because the problem of cotranslational selenocysteine formation in a heterologous system had not yet been recognized. The recombinant protein lacking two C-terminal residues was reported to be not only inactive but also structurally unstable and unable to bind FAD [25]. With a view to drug development, it will be interesting to consider compounds that can remove selenium from the mature form of TrxR [12] (see below).

On the level of cell cultures, often no clear correlation between TrxR activity and cell proliferation was found. This may be due to the fact that many normal cell lines have inadvertedly been selected for growth in selenium-deficient media [4]. Indeed, most biological studies on selenium compounds were conducted with malignant cells. Tumour-stimulating effects were observed at higher nanomolar concentrations whereas selenium had inhibitory effects on tumour growth at micromolar levels [4,12,24]. In terms of TrxR, the stimulatory effects are plausible because a larger selenium pool facilitates production of active TrxR which in turn is essential for DNA synthesis. Furthermore, cell cultures supplemented with ^{75}Se for weeks initially showed increased TrxR protein and TrxR activity levels but then the specific enzyme activity dropped. The inhibitory effects might be explained by the following hypothesis [12]. Methylselenenate, or other metabolites of Se-containing compounds given as supplements, covalently inhibit TrxR by forming a TrxR-Se-Se-CH_3 derivative. The inactive TrxR adducts (diselenide bridges exhibiting redox potentials more negative than −400 mV are very stable) would accumulate and account for the high TrxR protein level and also explain the low specific enzyme activity of the TrxR pool after selenium treatment.

Even though the hypothesis of TrxR inactivation by diselenide formation still needs further experimental support, it should make us aware of potential pitfalls when interpreting clinical observations. In addition, this concept may eventually provide a theoretical basis for selenium treatment in clinical conditions when TrxR activity is a major pathogenetic factor. These aspects may be illustrated by the following observation. In patients with adult T-cell leukemia, chemoresistance of the malignant cells against the cytostatic agent adriamycin was found to correlate with the level of reduced thioredoxin in blood plasma [26]. Chemosensitivity, however, could be restored by supplementing the medium with sodium selenite. This is consistent with, but does not prove, the interpretation that excess selenium inhibits thioredoxin reductase.

REDUCTION OF EXTRACELLULAR THIOREDOXIN

Among the numerous functions of thioredoxin in reduced form, its activity as an extracellular hormone-like factor is of special relevance in diagnostic and therapeutic medicine.

Indeed, human Trx was first purified from serum of leukemia patients and referred to as adult T-cell leukemia derived factor (ADF) [27]; only subsequently was the identity of ADF with Trx recognized. For healthy individuals, the reference range is 15–40 μg Trx per L blood plasma [22]. Currently, plasma Trx is not a standard clinical parameter but a number of pathological conditions under which the serum level of Trx is affected have already been identified. Extracellular Trx in its reduced state is an autocrine growth factor, especially for tumour cells [28], but an exception is known where Trx appears to have the opposite effect [29]. Furthermore, lymphocytes infected with the Epstein–Barr virus or human T-lymphotropic virus 1 (HTLV1) have been shown to synthesize and secrete thioredoxin [30]. The major biochemical questions are: Which cells, and under which conditions, secrete Trx? What is the mechanism of secretion? How is extracellular Trx kept in the reduced state, in particular, is there an extracellular membrane-associated thioredoxin reductase?

TUMOURS

With respect to a malignant disease, the clinician is concerned with three major aspects: prevention, early detection and effective treatment. The thioredoxin system plays a role in all three aspects.

Oxidative stress is considered a key factor for DNA damage. Therefore it is clear that the antioxidant thioredoxin system is regarded as a tumour-preventing system. This applies not only to the detoxification of reactive oxygen metabolites but also to signalling processes. Recent results suggest that the selenol group of TrxR may function as a primary sensor for mutagenic H_2O_2 and initiate a signal cascade leading to the transcription of genes encoding antioxidative proteins [31]. Furthermore, transcription factors such as NF-κB, NF-Y, and the tumour suppressor protein p53 are redox-dependent in their activity [32]; in the case of NF-κB, a link to the Trx system has already been established [3–5].

Studies on human p53 in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* cells indicate that yeast TrxR is crucial for the growth arrest activity of p53 [33]. Whether TrxR in mammalian cells has a similar effect on p53 is currently not known. In this context, it should be stressed that yeasts, like bacteria, possess a low M_r thioredoxin reductase. In the past, several studies in cancer research have employed *Escherichia coli* TrxR. The results of a number of these studies with *E. coli* TrxR could not be confirmed when human TrxR was used ([34] and Table 2).

As discussed above, secreted Trx (ADF) plays a role as an autocrine growth factor in various tumour diseases. The Trx concentration can be quantitated in blood plasma or in other extracellular spaces. Such analyses may become important for the detection of tumours and the monitoring of tumour diseases. Furthermore, as published in a case report [35], autoantibodies to TrxR were found in a patient with ovarian cancer, suggesting their potential value as tumour markers.

Once a tumour has become established, the effects of Trx and TrxR are no longer solely beneficial for the patient. Tumour proliferation is crucially dependent on a constant deoxyribonucleotide supply which in turn depends on an active Trx/TrxR system [13]. Furthermore, this system provides reduced extracellular thioredoxin as a growth factor and it protects the tumour cells from NK-lysin, tumour necrosis factor-α, and from the respiratory burst of immune cells [14,36]. It is therefore not surprising that tumour cells have been observed to express several fold increased TrxR levels [4] and that a number of potent anti-neoplastic agents such as carmustine, fotemustine [6,37,38] and cisplatin [39] are effective inhibitors of mammalian TrxRs (Table 2). When considering the tumour-promoting effects of TrxR, it is clear that this selenoenzyme is a major drug target. The clinically used inhibitors of TrxR react only with the reduced form of the enzyme [2,6]; the oxidized form of the enzyme is not inactivated. In this context, it is noteworthy that TrxRs are relatively unstable in the presence of NADPH, that is under the reducing conditions in the cytosol [40]. These findings have two implications for the development of novel thioredoxin reductase inhibitors. Firstly, the NADPH-reduced

Table 2. Clinically applied drugs with postulated effects on thioredoxin reductases. Enzyme species e = *E. coli*; *r* = rat; m = mouse; h = human.

TrxR Inhibitor	Studied Enzyme	Reference	Comment
Carmustine (BCNU) and other nitrosoureas	h-TrxR, m-TrxR e-TrxR	[6,37,38]	Very effective irreversible TrxR inhibitors; however, not selective, since mechanistically related enzymes are also inactivated, and DNA is alkylated
Cisplatin	h-TrxR	[39]	Consistently, high Trx-levels appear to be involved in cisplatin resistance
Anthracyclines	r-TrxR	[4]	The inhibitory effects published for the rat enzyme were not reproducible with the human enzyme in our hands
Azelaic acid (dicarboxylic acids)	e-TrxR, h-TrxR	[5]	The original studies were partly conducted with the *E. coli* enzyme. We found no inhibition of human hTrxR
13-cis-Retinoic acid	e-TrxR, h-TrxR	[5,59]	Results obtained with the *E. coli* enzyme were mixed with data from human enzyme. *All-trans* retinoic acid increases TrxR levels when applied with interferon
1-Chloro-2,4-dinitrobenzene	h-TrxR	[4,5,23]	Experimental drug in dermatology with promises for clinical use
Auranofin and aurothioglucose	h-TrxR	[42]	Selective tight-binding inhibitors, the formal K_i for auranofin being 4 nM

enzyme species should be defined as the prime chemotherapeutic target and secondly it should be taken into account that drugs interacting with the reduced enzyme may contribute to its denaturation and subsequent degradation. Such inhibitors can act at substoichiometric concentrations [40]. As indicated above, agents which remove selenium from the enzyme might have a similar effect even in the absence of NADPH.

RHEUMATOID ARTHRITIS AND SJÖGREN'S SYNDROME

Sjögren's syndrome, a chronic systemic inflammatory disorder associated with Epstein–Barr virus (EBV) infection, is characterized by lymphocyte infiltration of the mucosal and other tissues. The patients suffer from dryness of the eyes, mouth and other mucous membranes as well as from rheumatic symptoms. This syndrome shares features with rheumatoid arthritis, a very common chronic disease characterized by symmetrical inflammation of peripheral joints; indeed the two conditions can appear together. In the inflamed tissues of patients with Sjögren's syndrome, a strong correlation between virion production and Trx synthesis was observed [41]. The hypothesis that the Trx system is involved in the pathophysiology of chronic diseases is supported by the fact that the activity of interleukin-1, an inflammation mediator in rheumatoid arthritis, is enhanced by reduced Trx, which itself is an effective growth factor in lymphatic tissues [30]. Recently, reduced *E. coli* Trx was found to be 1000-fold more effective in the activation of interleukin-1β-converting enzyme than dithiothreitol (D. A. Giegel & C. H. Williams, Jr, personal communication).

Another indication is that organic gold compounds such as auranofin and aurothioglucose are widely and effectively used in the treatment of rheumatoid arthritis. Because these compounds were known to react with selenol-containing residues, we studied them as inhibitors of thioredoxin reductase and found that auranofin inhibits the enzyme in stoichiometric amounts, the formal K_i being ≈ 4 nM [42].

Recent *in vivo* data further underline the potential involvement of TrxR in the pathogenesis of rheumatoid arthritis. Firstly, as tested in a mouse model, aurothioglucose also inhibits TrxR *in vivo* [43] and secondly, significantly increased levels of Trx and TrxR were found in synovial fluid and tissue, but not in blood plasma, of patients suffering from rheumatoid arthritis. This was not the case in individuals with other joint diseases such as gout or osteoarthritis [44]. In rheumatoid arthritis, the synovial Trx-levels correlated with the local severity of inflammation. These studies support the idea of considering the Trx-system not only as a drug target for the treatment but also as potential clinical parameter for diagnosis and therapeutic management as local alterations normally precede systemic symptoms.

The biochemical and pharmacological studies strongly suggest that auranofin and aurothioglucose react with Sec496 of human thioredoxin reductase [5,42]. As discussed above, selenium compounds are likely to form diselenide bridges with this residue, thereby inactivating TrxR. Consequently, we plan to reconsider selenium compounds for the treatment of rheumatoid arthritis. It should be emphasized that the underlying assumption is no longer the supply of the trace element for selenium dependent-reactions; on the contrary, it is the inhibition of an unduly active selenoenzyme by selenium compounds [12]. As selenium has reached the status of a lifestyle element, we feel obliged to state at this point that the therapeutic index of selenium compounds is very low and that the injudicious self-administration often leads to intoxication.

AIDS

Apart from EBV infections and rheumatoid arthritis, the thioredoxin system has been intensely studied in HIV infections. Alterations in seleno(protein) concentrations and increased plasma levels of Trx correlating with the stage of the disease have been observed with HIV-infected patients. An attractive hypothesis is that certain reactive oxygen metabolites displace selenium from selenoenzymes like TrxR; this would explain the decreased activity of selenoenzymes and the increase of low M_r selenium compounds [45]. Thioredoxin in AIDS is not only important as a marker for the stage of the disease. Extracellularly active Trx appears to inhibit the production of HIV in macrophages whereas a degradation product of Trx, the eosinophil cytotoxicity-enhancing factor, actually promotes HIV production [46]. Thus further studies on the Trx system and on selenium metabolism in HIV infections are urgently required to improve therapeutic interventions.

THIOREDOXIN REDUCTASES OF PARASITES AND INFECTIOUS BACTERIA

On a global scale of medical problems, infectious diseases still play a most important role. A number of diseases such as AIDS have emerged over the last decades and old scourges like malaria and tuberculosis represent ever increasing problems.

Most protozoan parasites are fast replicating organisms and can, in that respect, be compared with tumour cells. The parasite is therefore challenged by the same problems as a fast replicating tumour: it needs an adequate supply of deoxyribonucleotides for rapid DNA synthesis and protection from reactive oxygen metabolites generated by the host's immune system. The thioredoxin system can fulfil both tasks and therefore seems to be an essential part of the parasite's metabolism.

P. falciparum, the major human malaria parasite, possesses a high M_r TrxR (Fig. 1). Because in mammalian cells several TrxRs have been identified, it is likely that *P. falciparum* may also contain more than one TrxR. Interestingly, the mammalian and the parasite enzymes have different peripheral redox centers [1]. This difference may be exploited for drug design against malaria. Currently, a high throughput screening programme is being performed to identify parasite-specific inhibitors as tools for studies on the exact role of TrxR for the survival of the parasites. On the basis of preliminary results, it is likely that the thioredoxin redox cycle represents a major and efficient antioxidant system of the parasites (see also [2,7,47,48]). Recently, two other members of this redox system have been identified in *P. falciparum*, a thioredoxin and a thioredoxin-dependent peroxidase; this enzyme is able to detoxify hydrogen peroxide and alkyl hydroperoxides *in vitro* (Z. Krnajski, T. W. Gilberger, R. D. Walter & S. Müller, and S. Rahlfs & K. Becker, unpublished data). As another approach to functional studies of the Trx redox cycle, we have initiated the targeted disruption of the thioredoxin reductase gene of *P. falciparum* and are currently analysing the transfected parasites (T. W. Gilberger, Z. Krnajski, R. D. Walter, A. Cowman & S. Müller, unpublished data).

The role of the thioredoxin system has also been investigated in other protozoan parasites such as *Entamoeba histolytica* [49,50]. *E. histolytica*, a facultative anaerobe, can invade host tissues; there it is confronted with the human immune response and needs to deal with reactive oxygen species. A parasitic superoxide dismutase detoxifies superoxide anions released from activated macrophages. Hydrogen peroxide generated in

this reaction is subsequently reduced by the combined action of a thioredoxin reductase-like protein and a thioredoxin peroxidase-like protein. Recently it has been shown that these antioxidant enzymes are also responsible for the development of resistance to metronidazole [51], a drug used for more than 25 years in the treatment of anaerobic and microaerophilic pathogens. However, the exact involvement of the thioredoxin reductase-like enzyme in this process remains to be elucidated.

Mycobacterium leprae, the causative agent of human leprosy, resides in human macrophages and thus has to cope with the oxidative burst originating within their host cells or nearby monocytes. Because mycobacteria are prokaryotes, it is not surprising that they possess a low M_r TrxR. However, this reductase and its substrate Trx are transcribed from a single gene and form a 'fusion' protein [52,53]. This arrangement requires unique reaction mechanisms for the proteins to fulfil their different redox functions in the cell. It has been postulated that the hybrid protein represents an efficient detoxifying and escape system for the bacteria that enables them to survive under the extreme conditions prevailing in an activated host macrophage. Reports on *Mycobacterium tuberculosis* link the thioredoxin redox cycle with resistance to the tuberculostatic drug isoniazid [2,54].

As stated above, bacterial and mammalian TrxRs differ significantly from each other. The relatively new insights into mechanistic and structural differences between these TrxR types [2] strongly suggest that bacterial TrxRs be considered as targets of novel antibiotic drugs. *E. histolytica* and possibly other parasitic protozoa also possess a bacteria-like TrxR. As indicated by the examples of metronidazole and isoniazid resistance, TrxR inhibitors might also help to solve problems of resistance against existing antimicrobial drugs.

OUTLOOK

The examples given in this article demonstrate that the thioredoxin redox cycle is involved in a whole range of pathophysiological conditions, and that it is an important target of chemotherapeutic intervention. Most results require confirmation. A boost for systematic studies is expected from the availability of TrxRs and Trxs from mammals and pathogenic microorganisms. Efficient purification schemes for the authentic or recombinant proteins have recently been developed [1–8,55]. A number of pharmacological substances, some of which have been clinically applied (Table 2), have been identified as TrxR inhibitors. Given the efficacy of inhibitors like auranofin and nitrosoureas, the search for new, more specific and less toxic compounds is well justified.

The role of the selenol group for the function of mammalian TrxR and also for stability of the enzyme must be further investigated. In addition, the conditions under which the selenium can be released from the mature protein *in vivo* are poorly understood. In the same vein, inactivation of TrxR by oxidation or complexing of the selenium moiety by organometallic compounds needs further study. Of high theoretical and possible practical interest is TrxR inactivation by diselenide formation between the enzyme and another selenium compound.

E. coli type TrxR should be discarded as a model in research on human diseases such as tumours or rheumatoid arthritis. However, in analogy to the successful example of dihydrofolate reductases, the difference between bacterial and mammalian TrxRs may be exploited for the development of novel antibiotics. Similarly, TrxRs of several protozoan parasites will be considered as potential drug targets because they differ structurally and mechanistically from their mammalian counterpart.

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REFERENCES

1. Poole, L.B., Reynolds, C.M., Wood, Z.A., Karplus, P.A., Ellis, H.R. & Li Calzi, M. (2000) AhpF and other NADH:peroxiredoxin oxireductases, homologues of low M_r thioredoxin reductase. *Eur. J. Biochem.* **267**, 6126–6133.
2. Williams, C.H. Jr, Arscott, L.D., Müller, S., Lennon, B.W., Ludwig, M.L., Wang, P.-F., Veine, D.M., Becker, K. & Schirmer, R.H. (2000) Thioredoxin reductase. Two modes of catalysis have evolved. *Eur. J. Biochem.* **267**, 6110–6117.
3. Arnér, E.S.J. & Holmgren, A. (2000) Physiological functions of thioredoxin and thioredoxin reductase. *Eur. J. Biochem.* **267**, 6102–6111.
4. Mustachich, D. & Powis, G. (2000) Thioredoxin reductase. *Biochem. J.* **346**, 1–8.
5. Gromer, S., Schirmer, R.H. & Becker, K. (1999) News and views on thioredoxin reductase. *Redox Report* **4**, 221–228.
6. Arscott, L.D., Gromer, S., Schirmer, R.H., Becker, K. & Williams, C.H. Jr (1997) The mechanism of thioredoxin reductase from human placenta is similar to the mechanisms of lipoamide dehydrogenase and glutathione reductase and is distinct from the mechanism of thioredoxin reductase from *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **94**, 3621–3626.
7. Gilberger, T.W., Bergmann, B., Walter, R.D. & Müller, S. (1998) The role of the C-terminus for catalysis of the large thioredoxin reductase from *Plasmodium falciparum*. *FEBS Lett.* **425**, 407–410.
8. Tamura, T. & Stadtman, T.C. (1996) A new selenoprotein from human lung adenocarcinoma cells: purification, properties, and thioredoxin reductase activity. *Proc. Natl Acad. Sci. USA* **93**, 1006–1011.
9. Gladyshev, V.N., Jeang, K.T. & Stadtman, T.C. (1996) Selenocysteine, identified as the penultimate C-terminal residue in human T-cell thioredoxin reductase, corresponds to TGA in the human placental gene. *Proc. Natl Acad. Sci. USA* **93**, 6146–6151.
10. Lee, S.R., Kim, J.R., Kwon, K.S., Yoon, H.W., Levine, R.L., Ginsburg, A. & Rhee, S.G. (1999) Molecular cloning and characterization of a mitochondrial selenocysteine-containing thioredoxin reductase from rat liver. *J. Biol. Chem.* **274**, 4722–4734.
11. Stadtman, T.C. (1996) Selenocysteine. *Annu. Rev. Biochem.* **65**, 83–100.
12. Ganther, H.E. (1999) Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. *Carcinogenesis* **20**, 1657–1666.
13. Spyrou, G. & Holmgren, A. (1996) Deoxyribonucleoside triphosphate pools and growth of glutathione-depleted 3T6 mouse fibroblasts. *Biochem. Biophys. Res. Commun.* **220**, 42–46.
14. Andersson, M., Holmgren, A. & Spyrou, G. (1996) NK-lysin, a disulfide-containing effector peptide of T-lymphocytes, is reduced and inactivated by human thioredoxin reductase. Implication for a protective mechanism against NK-lysin cytotoxicity. *J. Biol. Chem.* **271**, 10116–10120.
15. Arnér, E.S., Nordberg, J. & Holmgren, A. (1996) Efficient reduction of lipoamide and lipoic acid by mammalian thioredoxin reductase. *Biochem. Biophys. Res. Commun.* **225**, 268–274.
16. May, J.M., Mendiratta, S., Hill, K.E. & Burk, R.F. (1997) Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. *J. Biol. Chem.* **272**, 22607–22610.
17. May, J.M., Cobb, C.E., Mendiratta, S., Hill, K.E. & Burk, R.F. (1998) Reduction of the ascorbyl free radical to ascorbate by thioredoxin reductase. *J. Biol. Chem.* **273**, 23039–23045.
18. Nikitovic, D. & Holmgren, A. (1996) *S*-Nitrosoglutathione is cleaved by the thioredoxin system with liberation of glutathione and redox regulating nitric oxide. *J. Biol. Chem.* **271**, 19180–19185.
19. Becker, K., Savvides, S.N., Keese, M., Schirmer, R.H. & Karplus, P.A.

(1998) Enzyme inactivation through sulfhydryl oxidation by physiologic NO- carriers. *Nat. Struct. Biol.* **5**, 267–271.

20. Bjornstedt, M., Xue, J., Huang, W., Akesson, B. & Holmgren, A. (1994) The thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase. *J. Biol. Chem.* **269**, 29382–29384.
21. Nakamura, H., De Rosa, S., Roederer, M., Anderson, M.T., Dubs, J.G., Yodoi, J., Holmgren, A. & Herzenberg, L.A. (1996) Elevation of plasma thioredoxin levels in HIV-infected individuals. *Int. Immunol.* **8**, 603–611.
22. Nakamura, H., Vaage, J., Valen, G., Padilla, C.A., Bjornstedt, M. & Holmgren, A. (1998) Measurements of plasma glutaredoxin and thioredoxin in healthy volunteers and during open-heart surgery. *Free Radic. Biol. Med.* **24**, 1176–1186.
23. Zhong, L., Arnér, E.S., Ljung, J., Åslund, F. & Holmgren, A. (1998) 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.* **273**, 8581–8591.
24. Medina, D. & Oborn, C.J. (1984) Selenium inhibition of DNA synthesis in mouse mammary epithelial cell line YN-4. *Cancer Res.* **44**, 1176–1186.
25. Gasdaska, P.Y., Gasdaska, J.R., Cochran, S. & Powis, G. (1995) Cloning and sequencing of a human thioredoxin reductase. *FEBS Lett.* **373**, 5–9.
26. Wang, J., Kobayashi, M., Sakurada, K., Imamura, M., Moriuchi, T. & Hosokawa, M. (1997) Possible roles of an adult T-cell leukemia (ATL)-derived factor/thioredoxin in the drug resistance of ATL to adriamycin. *Blood* **89**, 2480–2487.
27. Gasdaska, P.Y., Oblong, J.E., Cotgreave, I.A. & Powis, G. (1994) The predicted amino acid sequence of human thioredoxin is identical to that of the autocrine growth factor human adult T-cell derived factor (ADF): thioredoxin mRNA is elevated in some human tumors. *Biochim. Biophys. Acta* **1218**, 292–296.
28. Powis, G., Oblong, J.E., Gasdaska, P.Y., Berggren, M., Hill, S.R. & Kirkpatrick, D.L. (1994) The thioredoxin/thioredoxin reductase redox system and control of cell growth. *Oncol. Res.* **6**, 539–544.
29. Rubartelli, A., Bonifaci, N. & Sitia, R. (1995) High rates of thioredoxin secretion correlate with growth arrest in hepatoma cells. *Cancer Res.* **55**, 675–680.
30. Wakasugi, N., Tagaya, Y., Wakasugi, H., Mitsui, A., Maeda, M., Yodoi, J. & Tursz, T. (1990) Adult T-cell leukemia-derived factor/thioredoxin, produced by both human T-lymphotropic virus type I- and Epstein-Barr virus-transformed lymphocytes, acts as an autocrine growth factor and synergizes with interleukin 1 and interleukin 2. *Proc. Natl Acad. Sci. USA* **87**, 8282–8286.
31. Sun, Q.A., Wu, Y., Zappacosta, F., Jeang, K.T., Lee, B.J., Hatfield, D.L. & Gladyshev, V.N. (1999) Redox regulation of cell signaling by selenocysteine in mammalian thioredoxin reductases. *J. Biol. Chem.* **274**, 24522–24530.
32. Sun, Y. & Oberley, L.W. (1996) Redox regulation of transcriptional activators. *Free Radic. Biol. Med.* **21**, 335–348.
33. Casso, D. & Beach, D. (1996) A mutation in a thioredoxin reductase homolog suppresses p53-induced growth inhibition in the fission yeast *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* **252**, 518–529.
34. Schallreuter, K.U., Pittelkow, M.R. & Wood, J.M. (1989) EF-hands calcium binding regulates the thioredoxin reductase/thioredoxin electron transfer in human keratinocytes. *Biochem. Biophys. Res. Commun.* **162**, 1311–1316.
35. Muro, Y., Ogawa, Y., Kato, Y. & Hagiwara, M. (1998) Autoantibody to thioredoxin reductase in an ovarian cancer patient. *Biochem. Biophys. Res. Commun.* **242**, 267–271.
36. Matsuda, M., Masutani, H., Nakamura, H., Miyajima, S., Yamauchi, A., Yonehara, S., Uchida, A., Irimajiri, K., Horiuchi, A. & Yodoi, J. (1991) Protective activity of adult T cell leukemia-derived factor (ADF) against tumor necrosis factor-dependent cytotoxicity on U937 cells. *J. Immunol.* **147**, 3837–3841.
37. Gromer, S., Schirmer, R.H. & Becker, K. (1997) The 58 kDa mouse selenoprotein is a BCNU-sensitive thioredoxin reductase. *FEBS Lett.* **412**, 318–320.
38. Schallreuter, K.U., Gleason, F.K. & Wood, J.M. (1990) The mechanism of action of the nitrosourea anti-tumor drugs on thioredoxin reductase, glutathione reductase and ribonucleotide reductase. *Biochim. Biophys. Acta* **1054**, 14–20.
39. Sasada, T., Nakamura, H., Ueda, S., Sato, N., Kitaoka, Y., Gon, Y., Takabayashi, A., Spyrou, G., Holmgren, A. & Yodoi, J. (1999) Possible involvement of thioredoxin reductase as well as thioredoxin in cellular sensitivity to cis-diamminedichloroplatinum (II). *Free Radic. Biol. Med.* **27**, 504–514.
40. Schirmer, M., Scheiwein, M., Gromer, S., Becker, K. & Schirmer, R.H. (1999) Disulfide reductases are destabilized by physiologic concentrations of NADPH. *Flavins Flavoproteins* **13**, 857–861.
41. Saito, I., Shimuta, M., Terauchi, K., Tsubota, K., Yodoi, J. & Miyasaka, N. (1996) Increased expression of human thioredoxin/adult T cell leukemia-derived factor in Sjogren's syndrome. *Arthritis Rheum.* **39**, 773–782.
42. Gromer, S., Arscott, L.D., Williams, C.H. Jr, Schirmer, R.H. & Becker, K. (1998) Human placenta thioredoxin reductase. Isolation of the selenoenzyme, steady state kinetics, and inhibition by therapeutic gold compounds. *J. Biol. Chem.* **273**, 20096–20101.
43. Smith, A.D., Guidry, C.A., Morris, V.C. & Levander, O.A. (1999) Aurothioglucose inhibits murine thioredoxin reductase activity *in vivo*. *J. Nutr.* **129**, 194–198.
44. Maurice, M.M., Nakamura, H., Gringhuis, S., Okamoto, T., Yoshida, S., Kullmann, F., Lechner, S., van der Voort, E.A., Leow, A., Versendaal, J., Muller-Ladner, U., Yodoi, J., Tak, P.P., Breedveld, F.C. & Verweij, C.L. (1999) Expression of the thioredoxin-thioredoxin reductase system in the inflamed joints of patients with rheumatoid arthritis. *Arthritis Rheum.* **42**, 2430–2439.
45. Gladyshev, V.N., Stadtman, T.C., Hatfield, D.L. & Jeang, K.T. (1999) Levels of major selenoproteins in T cells decrease during HIV infection and low molecular mass selenium compounds increase. *Proc. Natl Acad. Sci. USA* **96**, 835–839.
46. Newman, G.W., Balcewicz-Sablinska, M.K., Guarnaccia, J.R., Remold, H.G. & Silberstein, D.S. (1994) Opposing regulatory effects of thioredoxin and eosinophil cytotoxicity-enhancing factor on the development of human immunodeficiency virus 1. *J. Exp. Med.* **180**, 359–363.
47. Müller, S., Gilberger, T.W., Färber, P.M., Becker, K., Schirmer, R.H. & Walter, R.D. (1996) Recombinant putative glutathione reductase of *Plasmodium falciparum* exhibits thioredoxin reductase activity. *Mol. Biochem. Parasitol.* **80**, 215–219.
48. Wang, P.F., Arscott, L.D., Gilberger, T.W., Müller, S. & Williams, C.H. Jr (1999) Thioredoxin reductase from *Plasmodium falciparum*: evidence for interaction between the C-terminal cysteine residues and the active site disulfide-dithiol. *Biochemistry.* **38**, 3187–3196.
49. Bruchhaus, I., Richter, S. & Tannich, E. (1997) Characterization of two *E. histolytica* proteins that inactivate reactive oxygen species. *Arch. Med. Res.* **28**, 91–92.
50. Bruchhaus, I., Richter, S. & Tannich, E. (1998) Recombinant expression and biochemical characterization of an NADPH: flavin oxidoreductase from *Entamoeba histolytica*. *Biochem. J.* **330**, 1217–1221.
51. Wassmann, C., Hellberg, A., Tannich, E. & Bruchhaus, I. (1999) Metronidazole resistance in the protozoan parasite *Entamoeba histolytica* is associated with increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase. *J. Biol. Chem.* **274**, 26051–26056.
52. Wang, P.F., Marcinkeviciene, J., Williams, C.H. Jr & Blanchard, J.S. (1998) Thioredoxin reductase-thioredoxin fusion enzyme from *Mycobacterium leprae*: comparison with the separately expressed thioredoxin reductase. *Biochemistry* **37**, 16378–16389.
53. Wieles, B., van Noort, J., Drijfhout, J.W., Offringa, R., Holmgren, A. & Ottenhoff, T.H. (1995) Purification and functional analysis of the *Mycobacterium leprae* thioredoxin/thioredoxin reductase hybrid protein. *J. Biol. Chem.* **270**, 25604–25606.
54. Sherman, D.R., Mdluli, K., Hickey, M.J., Barry, C.E.R. & Stover, C.K.

(1999) AhpC, oxidative stress and drug resistance in *Mycobacterium tuberculosis*. *Biofactors* **10**, 211–217.

55. Arnér, E.S.J., Sarioglu, H., Lottspeich, F., Holmgren, A. & Böck, A. (1999) 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.* **292**, 1003–1016.
56. Yokomizo, A., Ono, M., Nanri, H., Makino, Y., Ohga, T., Wada, M., Okamoto, T., Yodoi, J., Kuwano, M. & Kohno, K. (1995) Cellular levels of thioredoxin associated with drug sensitivity to cisplatin, mitomycin C, doxorubicin, and etoposide. *Cancer Res.* **55**, 4293–4296.
57. Rafferty, T.S., McKenzie, R.C., Hunter, J.A., Howie, A.F., Arthur, J.R., Nicol, F. & Beckett, G.J. (1998) Differential expression of selenoproteins by human skin cells and protection by selenium from UVB-radiation-induced cell death. *Biochem. J.* **332**, 231–236.
58. Aota, M., Matsuda, K., Isowa, N., Wada, H., Yodoi, J. & Ban, T. (1996) Protection against reperfusion-induced arrhythmias by human thioredoxin. *J. Cardiovasc. Pharmacol.* **27**, 727–732.
59. Hofmann, E.R., Boyanapalli, M., Lindner, D.J., Weihua, X., Hassel, B.A., Jagus, R., Gutierrez, P.L., Kalvakolanu, D.V. & Hofman, E.R. (1998) Thioredoxin reductase mediates cell death effects of the combination of beta interferon and retinoic acid. *Mol. Cell. Biol.* **18**, 6493–6504.