Coordination Chemistry Reviews 253 (2009) 1692–1707

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

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

Review

Thioredoxin reductase: A target for gold compounds acting as potential anticancer drugs

Alberto Bindoli [a,*], Maria Pia Rigobello [b], Guido Scutari [b], Chiara Gabbiani [c], Angela Casini [d], Luigi Messori [c,**]

[a] Istituto di Neuroscienze (CNR), Sezione di Padova, c/o Dipartimento di Chimica Biologica, Viale G. Colombo 3, 35121 Padova, Italy
[b] Dipartimento di Chimica Biologica, Università di Padova, Viale G. Colombo 3, 35121 Padova, Italy
[c] Laboratory of "Metals in Medicine" (METMED), Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Firenze, Italy
[d] Laboratory of Organometallic and Medicinal Chemistry, Ecole Polytechnique Fédérale de Lausanne, SB-ISIC LCOM, BCH 2404, CH-1015 Lausanne, Switzerland

Contents

ARTICLE INFO

Article history:
Received 3 December 2008
Accepted 25 February 2009
Available online 9 March 2009

Keywords:
Thioredoxin reductase
Gold compounds
Cancer

ABSTRACT

The thioredoxin system plays a key role in regulating the overall intracellular redox balance. It basically comprises the small redox protein thioredoxin (Trx), nicotinamide adenine dinucleotide phosphate, in its reduced form (NADPH), and thioredoxin reductase (TrxR), a large homodimeric selenzoenzyme controlling the redox state of thioredoxin. Details of the thioredoxin system are provided herein, particular emphasis being given to the protein chemistry of thioredoxin reductases. Several lines of evidence point out today that the thioredoxin system represents an effective "druggable" target for the development of new anticancer agents. Accordingly, a number of established anticancer agents were retrospectively found to be potent inhibitors of thioredoxin reductases and to induce severe oxidative stress. During the last decade a variety of gold compounds, either gold(I) or gold(III), were reported to manifest outstanding antitumor properties, forming a promising class of experimental anticancer agents. In turn, recent studies have revealed that several cytotoxic gold compounds, either gold(I) or gold(III), are potent TrxR inhibitors. Details of their mechanism of selenoenzyme inhibition are currently under investigation, in our laboratory, and some new results will be anticipated here; notably, preferential gold targeting of active site selenolate could be experimentally supported. Based on the numerous experimental evidences now available, both at the molecular and cellular level, we propose that the relevant cytotoxic actions produced by gold compounds are mainly the result of potent inhibition of thioredoxin reductase; the alterations

Abbreviations: Akt, protein kinase B; ASK1, apoptosis signal-regulating kinase 1; Bax, Bcl-2-associated X protein; BIAM, N-(biotinoyl)-N′-(iodoacetyl) ethylenediamine; DNCB, 1-chloro-2,4-dinitrobenzene; DsbA, periplasmic protein disulfide isomerase; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); ERK1/2, extracellular signal-regulated kinase 1/2; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GPx, glutathione peroxidase; Grx, glutaredoxin; HIF-1α, hypoxia-inducible factor-1α; MAP kinase, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; Prx, peroxiredoxin; PDI, protein disulfide isomerase; PTP-1B, protein tyrosine phosphatase-1B; PKA, protein kinase A; PKC, protein kinase C; ROS, reactive oxygen species; Trx, thioredoxin; TrxR, thioredoxin reductase.

* Corresponding author. Tel.: +39 049 8276138; fax: +39 049 8073310.
** Corresponding author. Tel.: +39 055 4573388; fax: +39 055 4573385.
E-mail addresses: bindoli@bio.unipd.it (A. Bindoli), luigi.messori@unifi.it (L. Messori).

0010-8545/$ – see front matter
doi:10.1016/j.ccr.2009.02.026

of mitochondrial functions, elicited by profound TrxR inhibition, would eventually lead to cell apoptosis. A general and unitary framework is thus offered to interpret the mode of action of cytotoxic gold compounds, according to which they should be primarily considered as antimitochondrial drugs. The peculiar properties of gold compounds highlighted in this review might be further exploited for the obtainment of newer and selective anticancer agents.

1. Gold compounds, new attractive metallodrugs for cancer treatment

The serendipitous discovery of the anticancer properties of cisplatin and its platinum(II) analogues and their wide clinical success in current cancer treatments promoted a great deal of interest in the area of metal-based antitumor agents [1–3]. The outstanding anticancer effects observed for platinum(II) compounds suggested that other metal-based compounds might be similarly useful as antitumor drugs while, hopefully, displaying different patterns of anticancer activities and selectivities [1]. Among the several classes of metal compounds that have been taken into consideration as potential anticancer agents starting from the 80's (*e.g.* ruthenium, palladium, titanium, copper, tin, and so on), a few investigations have focused on a variety of gold compounds in the oxidation states either +3 or +1 [4–6]. Notably, gold(I) compounds were quickly considered as possible antiproliferative agents soon after the discovery of cisplatin, as some of them were already in the clinics for the treatment of rheumatoid arthritis (Fig. 1A); in turn, gold(III) compounds looked pairwise very attractive for cancer treatment as the gold(III) centers are known to originate square planar complexes that are isoelectronic and isostructural to those of platinum(II) and might exhibit similar biological actions.

A conspicuous number of gold(I) and gold(III) compounds were thus assayed, during the 80's, either *in vitro* or *in vivo*, as antitumor agents (see Fig. 1 for the chemical structures of some representative gold compounds) but the results obtained at that time were rather disappointing [4–6]. Indeed, gold(I) compounds turned out to be fairly active *in vitro* but practically ineffective *in vivo*, most likely as a consequence of extensive binding to serum proteins and inactivation; conversely, gold(III) compounds, although highly cytotoxic *in vitro*, manifested, on the average, a poor chemical stability and, also, a rather pronounced systemic toxicity in animal models that heavily limited their further investigation or application [4–6]. Studies on gold compounds as experimental anticancer agents were therefore rapidly stopped and almost completely abandoned for many years.

Only around the mid 1990s, new classes of gold(III) compounds were synthesized and characterised that showed improved stability profiles and turned out to be more appropriate for pharmacological application; most of those novel compounds were assayed on simple *in vitro* or *in vivo* biological models of cancer, with quite promising results [4–6]. Representative compounds of this group are shown in Fig. 1B. They comprise classical gold(III) compounds with polydentate ligands (*e.g. AuTerpy*) [7], a few organogold(III) compounds (*e.g. DAMP* and *Aubipy*$_c$) [8,9], various gold(III) porphyrins [10–15], two major gold(III) dithiocarbamates [16–19], a series of dinuclear gold(III) compounds (*e.g. Auoxo6*) [20,21]. Pairwise, various gold(I) compounds, with innovative chemical and biological features, were prepared and characterised, in the same years, by Berners-Price and coworkers [22].

On the average, all these novel gold compounds turned out to be highly cytotoxic *in vitro* against representative human tumor cell lines, with IC_{50} values often falling in the low micromolar or even nanomolar range. For a few of them an appreciable activity *in vivo* was also established although the reported *in vivo* data were rather fragmentary and preliminary. Mechanistic studies showed that the majority of these novel cytotoxic gold compounds induce evident signs of apoptosis in treated human tumor cells but their actual modes of action were not univocally identified. However, as gold compounds manifested, on the whole, a far lower affinity for DNA than platinum(II) compounds, it was hypothesized that apoptotic cell death should be the result of *DNA-independent processes*. Accordingly, the new cytotoxic gold compounds were often found to overcome resistance to cisplatin confirming the occurrence of a substantially different mode of action. Pairwise, at variance with classical platinum(II) compounds, only very modest effects on the cell cycle were highlighted. These observations led researchers to postulate the existence of preferential protein targets for gold compounds, as it will be detailed below.

Precise mechanistic hypotheses were formulated for some classes of novel gold compounds. In the case of gold(III) dithiocarbamates, Fregona and coworkers found that they can induce cancer cell death through both apoptotic and non-apoptotic mechanisms [16–19]. In addition, gold(III) dithiocarbamates were shown to inhibit deeply thioredoxin reductase activity, to generate free radicals, to modify some mitochondrial functions, and to increase ERK1/2 phosphorylation [19]. Altogether, these observations led the authors to conclude that deregulation of the thioredoxin reductase/thioredoxin redox system is a major mechanism involved in their anticancer action. On the other hand, another study by the same authors revealed that gold(III) dithiocarbamates may also cause concomitant, strong inhibition of the proteasome system, another biochemical pathway that is known to trigger potently cell apoptosis [18].

Che and coworkers extensively investigated the cytotoxic mechanisms of gold(III) porphyrins. They showed that HONE1 cells exposed to gold(III) porphyrin-1a present clear signs of apoptosis after 24 h incubation. Functional proteomic studies revealed characteristic changes in the expression of several cytosolic proteins following gold(III) porphyrin-1a treatment [10–15]. The affected proteins mainly included enzymes participating in energy production processes and proteins involved in the cellular redox balance. Interestingly, upon *gold(III) porphyrin-1a* treatment, a quick attenuation of the mitochondrial membrane potential was evidenced with clear alterations of Bcl-2 family proteins, and release of both cytochrome *c* and apoptosis-inducing factor (AIF). Cytochrome *c*, on turn, activates both caspase-9 and caspase-3. Subsequent studies revealed that ROS (the "*reactive oxygen species*") play pairwise an important role in *gold(III) porphyrin-1a* induced apoptosis by regulating the mitochondrial membrane potential. In summary, these recent results clearly documented that *gold(III) porphyrin-1a* is able to induce apoptosis through both caspase-dependent and caspase-independent mitochondrial pathways; intracellular oxidation also contributes to gold(III) porphyrin 1a-induced apoptosis. Thus, *gold(III) porphyrin 1a* clearly emerged from these studies as a promising anticancer drug lead and a novel therapeutic agent directed towards mitochondria.

Barnard and Berners-Price, in recent review articles [23,24] formulated a rather general hypothesis for the mechanism of action of gold(I) compounds, implying their direct effect at the mitochondrial level. Two distinct classes of antitumor gold(I) phosphine complexes were taken into consideration having either linear two-coordinate (*e.g.* auranofin) or tetrahedral four-coordinate

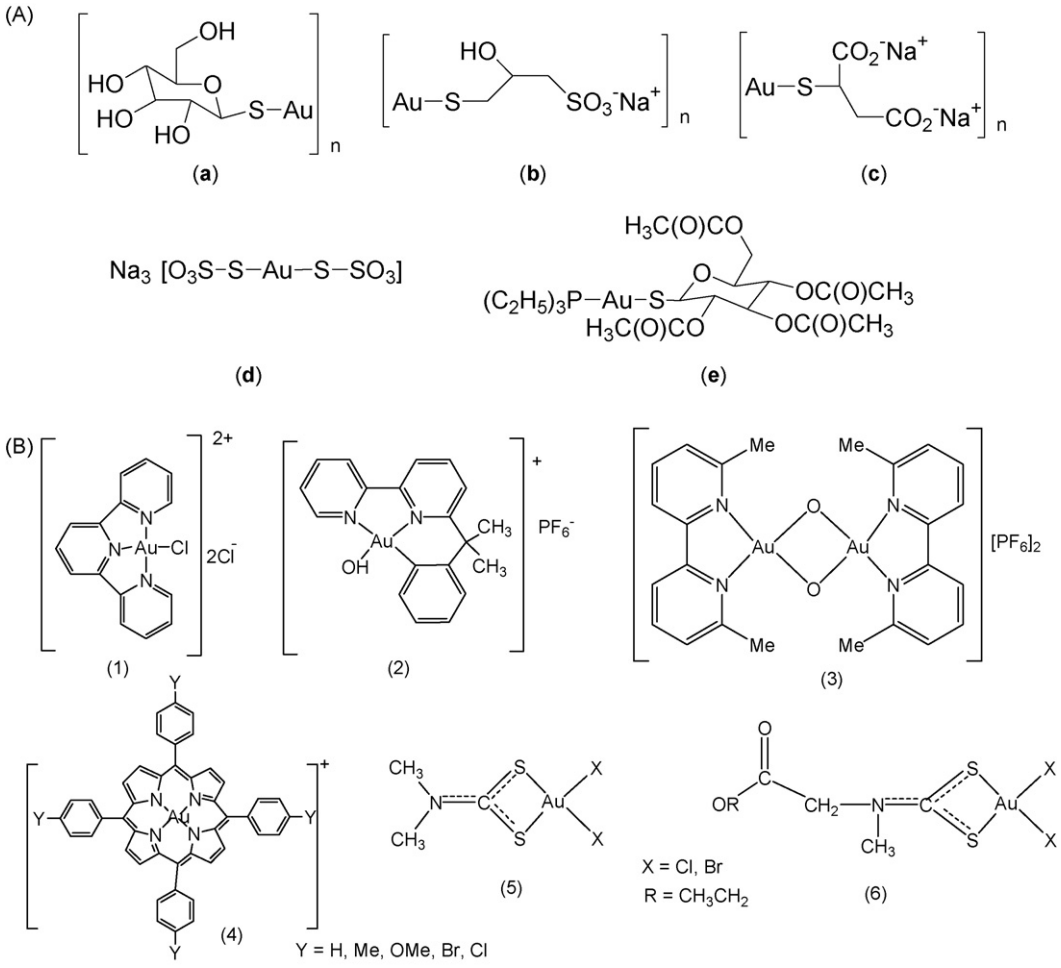

Fig. 1. (A) First gold(I) compounds for cancer treatment: solganol (a), allocrysin (b), myocrysin (c), sanocrysin (d) and auranofin (e). (B) Schematic drawing of $[Au(terpy)Cl]Cl_2$ (1), $[Au(bipy^{c}\text{-}H)(OH)][PF_6]$ (2), (III) *meso*-tetraarylporphyrins complexes (3), $Au(py^{dmb}\text{-}H)(AcO)_2]$ (4) (where py^{dmb} = 2-(1,1-dimethylbenzyl)-pyridine)) and of the gold(III) dithiocarbamate complexes containing N,N-dimethyldithiocarbamate (5) and ethylsarcosinedithiocarbamate (6) ligands.

geometries (*e.g.* $[Au(dppe)_2]^+$). Both classes of gold(I) compounds were able to target mitochondria, but different mechanisms appeared to be involved, owing to their different propensity to undergo ligand exchange reactions with biological ligands. The antiarthritic gold(I) phosphine drug, *auranofin*, was proposed to induce apoptosis *via* selective inhibition of the mitochondrial isoform of thioredoxin reductase. In turn, the antitumor activity of $[Au(dppe)_2]^+$, and of related tetrahedral gold(I) phosphine complexes, that do not undergo ligand exchange reactions as easily, was ascribed to their lipophilic cationic properties, in analogy to other delocalized lipophilic cations that accumulate in mitochondria.

Interestingly, all the above mentioned mechanistic studies and hypotheses suggest that mitochondria represent the most likely site for the biological action of gold compounds; in other words, gold-based cytotoxic drugs should be primarily considered as *selective antimitochondrial drugs*. There is indeed much recent evidence suggesting that mitochondria play a critical role in the regulation of apoptosis (programmed cell death), making them an attractive target for the design of new anticancer drugs. This issue is of particular interest as mitochondria are critically involved in the regulation of the intracellular redox state. In addition, mitochondria contain a specific thioredoxin reductase, a selenzoenzyme that seems to be an optimal target for gold compounds. Strong inhibition of mitochondrial thioredoxin reductase would eventually lead to altered mitochondrial functions and to initiation of the apoptotic process. All these tightly interwoven issues will be described in more detail in the following sections.

2. Cellular production of oxidant species

In the cell, oxidant species are naturally produced from several different sources. Enzymes such as xanthine oxidase [25], lipoxygenases [26], γ-glutamiltransferase [27], peroxisomal enzymes [28], as well as cytochromes P450 and b5 [25] are well-recognized sources of ROS. Other relevant enzymes are monoamine oxidases (MAO) [29,30] and polyamine oxidases [31]. Although all these sources can give rise to a significant contribution to cell oxidant formation, the current opinion considers NADPH oxidases and mitochondria as the most significant producers of ROS.

NADPH oxidase is a multienzyme complex present in phagocytic cells such as neutrophils and macrophages and responsible of a large oxygen uptake ("respiratory burst"), which essentially results in the formation of oxidants acting as host defence against invading microorganisms [32,33]. However, several homologues of $gp91^{phox}$ (Nox2), the catalytic subunit of NADPH oxidase, were found in many tissues indicating that NADPH oxidase activity is present also in non-phagocytic cells [34,35]. Consequently, the ability of NADPH oxidase to produce reactive oxygen species in a regulated manner is not restricted to phagocytic cells and similar enzymes appear involved in several different functions such as cell proliferation,

differentiation, migration and survival [33,36]. They act as a highly regulated multienzyme system that rapidly forms localized concentrations of hydrogen peroxide which, in turn, can also be easily degraded by the glutathione and thioredoxin-dependent peroxidases. Therefore, NADPH oxidases are endowed with all the features to serve in signal transduction pathways where the activation process takes place in a time scale of minutes [37].

Mitochondria, in addition to their major metabolic role of producing energy, display additional functions such as control of cellular calcium fluxes [38,39], induction of apoptosis mediated by the release of proapopototic factors [40], and production of reactive oxygen species [41–45]. Mitochondrial formation of superoxide was first observed many decades ago [42,43] and presently mitochondria are considered the most important cellular source of ROS. Superoxide anion originates from the autoxidation of some components of the respiratory chain and rapidly dismutes, upon the action of the manganese superoxide dismutase, to hydrogen peroxide that can diffuse without restraint through the mitochondrial membranes to the cytosol. In addition to autoxidation also a specialized enzyme ($p66^{Shc}$) appears able to take up electrons from reduced cytochrome *c* and produce hydrogen peroxide [46]. H_2O_2 produced by mitochondria is largely removed by the glutathione and thioredoxin systems leading to a steady state between formation and consumption of hydrogen peroxide (Fig. 2) [47–49]. This equilibrium might be modified by an increased production of ROS or by a decreased removal. The latter condition occurs after inhibition of thioredoxin or glutathione systems and the net amount of hydrogen peroxide released outside the mitochondrion is dictated by the difference between H_2O_2 production and removal.

The subcellular local concentration of ROS and their persistence over time play a critical role in eliciting the signalling response. The formation of ROS by mitochondria is not as finely tuned as that elicited by NADPH oxidase [50,51], yet mitochondrial ROS production, in addition to an involvement in the pathogenesis of degenerative diseases and aging, is also believed to participate in signalling [51–59]. However, most of the factors able to stimulate ROS production by mitochondria lead to cell growth inhibition or to cell death through the apoptotic or necrotic pathways [60–62]. Furthermore, several inhibitors of respiration stimulate ROS production and induce cell death [63]. As a result, the persistent production of ROS by mitochondria is more involved in apoptosis and/or cell cycle arrest than in cell proliferation at variance with NADPH oxidases, which give rise to a short-lived ROS production, resulting in a proliferation signal [64].

3. Cellular redox systems dependent on thiols

The cellular control of the thiol redox state is essentially exerted by the glutathione and thioredoxin systems. In fact, hydrogen peroxide, through the action of glutathione- or thioredoxin-dependent peroxidases is on a tight redox communication with the cellular thiols (Fig. 2). Therefore, glutathione and thioredoxin systems represent major pathways devoted to the control of the cellular redox state and are present both in the cytosol and mitochondria.

Glutathione is the most abundant non-protein thiol of the cell, present up to 10 mM concentration [65] and acting as an enzyme substrate and a detoxifying agent [65,66]. The glutathione system (Fig. 2) is formed by glutathione, glutathione reductase (GR) and NADPH. Reduced glutathione, is able to transfer its reducing equivalents to several enzymes including glutaredoxin that, similarly to thioredoxin, can interact with ribonucleotide reductase and a large number of other proteins involved in cell signalling and transcription control such as NF-κB, PTP-1B, PKA, PKC, Akt and ASK1 [67]. Glutaredoxin is present both in the cytosol and mitochondria. It is well known that glutathione can form mixed disulfides with protein thiols and in this way regulates their functions. Glutaredoxin exerts a critical role in the reversible formation of protein mixed disulfides as it is able to catalyze not only the scission of mixed disulfides involving protein thiols and glutathione, but also their formation [68,69] according to the cell redox conditions.

The thioredoxin system (Fig. 2) is constituted by thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH. The protein chemistry of thioredoxin reductase and its action mechanism are extensively described in the next section. Both cytosolic (Trx1) and mitochondrial (Trx2) thioredoxins contain the catalytic Cys-xx-Cys sequence (see below). Furthermore, Trx1 contains three additional cysteines that, after glutathionylation, S-nitrosation or oxidation, can contribute to the regulation of thioredoxin functions [75]. Proteins belonging to the thioredoxin superfamily are characterised by a structural motif known as "thioredoxin fold" and consisting of four central β-sheets surrounded by three α-helices [70]. In addition to thioredoxin, other oxidoreductases controlling the cellular redox state such as glutaredoxin, protein disulfide isomerase (PDI), glutathione *S*-transferase and *Escherichia coli* DsbA, contain the Trx fold

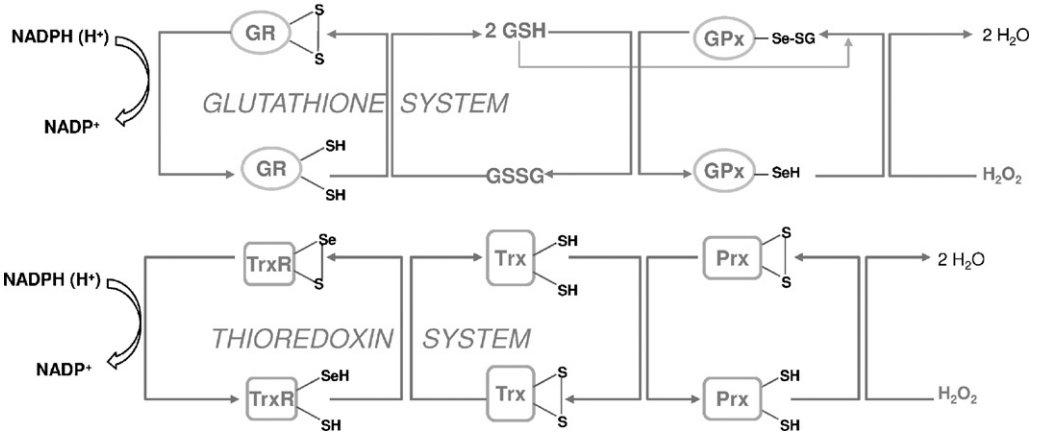

Fig. 2. Glutathione and thioredoxin pathways mediate the reduction of hydrogen peroxide. Mitochondrial respiratory substrates and the cytosolic pentose phosphate cycle reduce $NADP^+$ to NADPH that, in turn, feeds reducing equivalents to both thioredoxin and glutathione systems. In mitochondria, electrons are transferred from NADH to $NADP^+$ by the membrane-bound transhydrogenase. The thiol/disulfide redox systems finally transfer electrons to hydrogen peroxide that is reduced to water. Inhibition of either pathways markedly increases hydrogen peroxide concentration in the cell. Thioredoxin reductase and glutathione peroxidase are selenoenzymes. *Abbreviations*: $GR(SH)_2$, reduced glutathione reductase; GR(SS), oxidized glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GPx-SeH, reduced glutathione peroxidase; GPx-SeSG, oxidized glutathione peroxidase; $TrxR(SH)_2$, reduced thioredoxin reductase; TrxR(SS), oxidized thioredoxin reductase; $Trx(SH)_2$, reduced thioredoxin; Trx(SS), oxidized thioredoxin; $Prx(SH)_2$, reduced peroxiredoxin; Prx(SS), oxidized peroxiredoxin.

[76]. The modulation of the cell redox milieu depends on the presence of Cys-xx-Cys catalytic motif at the N-terminus of the protein [70,76]. The amino acids between the two cysteines appear critical in conferring the redox properties to the various components of the Trx superfamily [70]. Consequently, some proteins act as good reductants, while others behave as oxidants [70,76].

The thioredoxin system regulates crucial cell functions such as viability and proliferation [77–80]. In addition to Trx, its natural substrate, thioredoxin reductase is able to reduce a great deal of protein substrates and low molecular weight molecules. The first include protein disulfide isomerase and calcium binding proteins 1 and 2. Mitochondrial thioredoxin reductase (TrxR2) is also able to reduce mitochondrial glutaredoxin (Grx2). Small molecules acting as substrates of thioredoxin reductase comprise several disulfides (DTNB, lipoic acid), quinonoid compounds (vitamin K, alloxan) and diverse and chemically unrelated substrates such as ascorbate, S-nitrosoglutathione and lipid hydroperoxides [107 and references therein]. Finally, selenite and many selenium derivatives such as selenocysteine, selenodiglutathione, and ebselen are efficient substrates of thioredoxin reductase [193].

Thioredoxin, once reduced by thioredoxin reductase, supplies electrons to a number of enzymes such as ribonucleotide reductase [81], methionine sulfoxide reductase [82] and peroxiredoxin [83,84]; the latter rapidly regulates the level of cellular hydrogen peroxide [83]. Reduced thioredoxin-1 is able to bind to ASK1, and to inhibit its activity and hence acts as a negative effector of apoptosis. This inhibition is removed after oxidation of thioredoxin, which dissociates from ASK1 [85]. The redox state of thioredoxin also modulates the activity of several transcription factors possessing redox sensitive cysteines. For instance, NF-κB is inhibited in the cytosol by reduced thioredoxin, while, in the nucleus, reduced thioredoxin promotes its binding to DNA [86,87]. Other transcription factors such as the tumor suppressor p53 [88], the hypoxia-inducible factor 1α (HIF-1α) [89], the glucocorticoid receptor [90] and the AP-1 protein complex [91] are sensitive to the redox conditions of thioredoxin.

The thioredoxin and glutathione systems share several similarities and, in particular, both act in the removal of hydrogen peroxide, however, a relevant difference is represented by the intracellular concentrations of glutathione and thioredoxin, which are in the millimolar and micromolar range respectively [92]. In addition, the two systems appear to operate independently and hence they play multiple signalling roles [93,94]. On the other hand, it was recently shown [95] that, in mitochondria, thioredoxin reductase is able to reduce glutaredoxin indicating a link between the two pathways.

4. Protein chemistry of thioredoxin reductases

Thioredoxin reductases (EC 1.8.1.9) are homodimeric flavoproteins [96] that catalyze the NADPH-dependent reduction of thioredoxin, an ubiquitous 12-kDa protein which is the major protein disulfide reductase in cells [97]. Belonging to the pyridine nucleotide-disulfide oxidoreductase family such as glutathione reductase, lipoamide dehydrogenase and trypanothione reductase, TrxRs form homodimers and each subunit contains a redox-active disulfide bond and a bound FAD molecule. Two different kinds of TrxRs are known that show significantly different modes of catalysis. Low-M_r TrxRs ($M_r \approx 35$ kDa) are usually found in prokaryotes, archaea, plants, and lower eukaryotes, while high-M_r TrxRs ($M_r \approx 55$ kDa) are found in higher organisms. The first mammalian TrxR to be cloned, TrxR from human placenta, was found to have only 31% sequence identity with prokaryotic TrxRs, but to have 44% identity with eukaryotic and prokaryotic glutathione reductase [98]. Remarkably, thioredoxin reductases perform biological functions that are essential for life in higher organisms; as a matter of fact, targeted disruption of either TrxR1 or TrxR2 genes results in an embryonic lethal phenotype. In mice, TrxR1 null embryos are affected primarily by compromised cell proliferation whereas TrxR2 null embryos suffer from severe anaemia and improper heart development [99–102].

In mammals, three isozymes of high-M_r TrxRs have been identified: a cytosolic one (TrxR1) [103,70,71,72] a mitochondrial one (TrxR2) [73,104,74] and a third isozyme highly expressed in testis, thioredoxin glutathione reductase (TrxR3) [105,106] that can also reduce glutathione disulfide. Sequence alignment of these enzymes is reported in Fig. 3A. TrxR3 carries – in contrast to TrxR1 and TrxR2 – an N-terminally located glutaredoxin domain and is able to act as both TrxR and glutathione reductase. Mammalian TrxRs have a conserved -Cys-Val-Asn-Val-Gly-Cys- catalytic site, also found in human glutathione reductase, located in the FAD binding domain of the enzyme, and a NADPH binding site (Fig. 3B). In addition, mammalian TrxRs contain a selenocysteine residue at the C-terminal active site that is crucial for catalysis [107] and that is not found in GR or *E. coli* TrxR [108] (Fig. 3B). This redox center is located on a flexible arm, solvent-exposed and reactive towards electrophilic agents [109]; it thus constitutes an optimal target for the development of selective enzyme inhibitors. As reported in the previous section, beyond the reduction of the natural substrate thioredoxin, the Cys497-Sec498 redox pair reacts with a large variety of structurally diverse low-M_r substrates.

High resolution crystal structures have been obtained for both TrxR1 and TrxR2 proteins. The first three-dimensional structure was solved by Sandalova et al. for a Sec498Cys rat TrxR1 mutant complexed with $NADP^+$ [110]. Crystal structure analysis reveals the overall fold of the enzyme, provides insight into the architecture of the active site, and allows a detailed comparison to other members of the pyridine nucleotide disulfide oxidoreductase family, *in primis* GR. The structure confirmed the obligatory "head-to-tail" arrangement of high-M_r TrxR, with the redox-active C-terminal tail of one subunit interacting with the active site of the opposing subunit. Notably, the rat TrxR1 monomer comprises a FAD binding domain (residues 1–163 and 297–367), a NADP(H) binding domain (residues 164–296), and the so called "interface domain" (residues 368–499) (Fig. 3B). The FAD and NADPH binding domains present similar folds; each domain comprises a larger five-stranded parallel β-sheet and an adjacent three-stranded β-meander. The other side of the parallel sheet is covered by several α-helices. The interface domain, containing an antiparallel five-stranded β-sheet flanked by helices, participates in the interactions between two protein subunits that are packed in a head-to-tail arrangement. The functionally relevant disulfide, formed by Cys59 and Cys64, is located on an helix (specifically helix α2) in the FAD domain, as in GR, and not in the NADP(H) binding domain as in prokaryotic TrxR [98]. Finally, the C-terminal active site containing the essential Cys497 and Sec498 couple is oriented at the interface between the two subunits. Authors suggested that, in accordance with previously reported molecular modelling studies [111], the mobile C-terminal tail containing the selenenylsulfide not only serves as a third redox-active group, but it also blocks oxidized glutathione from binding to the enzyme.

As stated above, the protein was found to be roughly similar to GR including the three domain structure of a monomer as well as FAD and NADPH binding domains. Interestingly, most residues directly interacting with glutathione disulfide (GSSG) in GR are conserved in rat TrxR although the enzyme does not turn over GSSG [110].

In 2005 the structure of mouse TrxR2 has been solved, that differs from rat TrxR1 in some active site residues [112]. Conformational changes were observed upon NADPH binding and an active

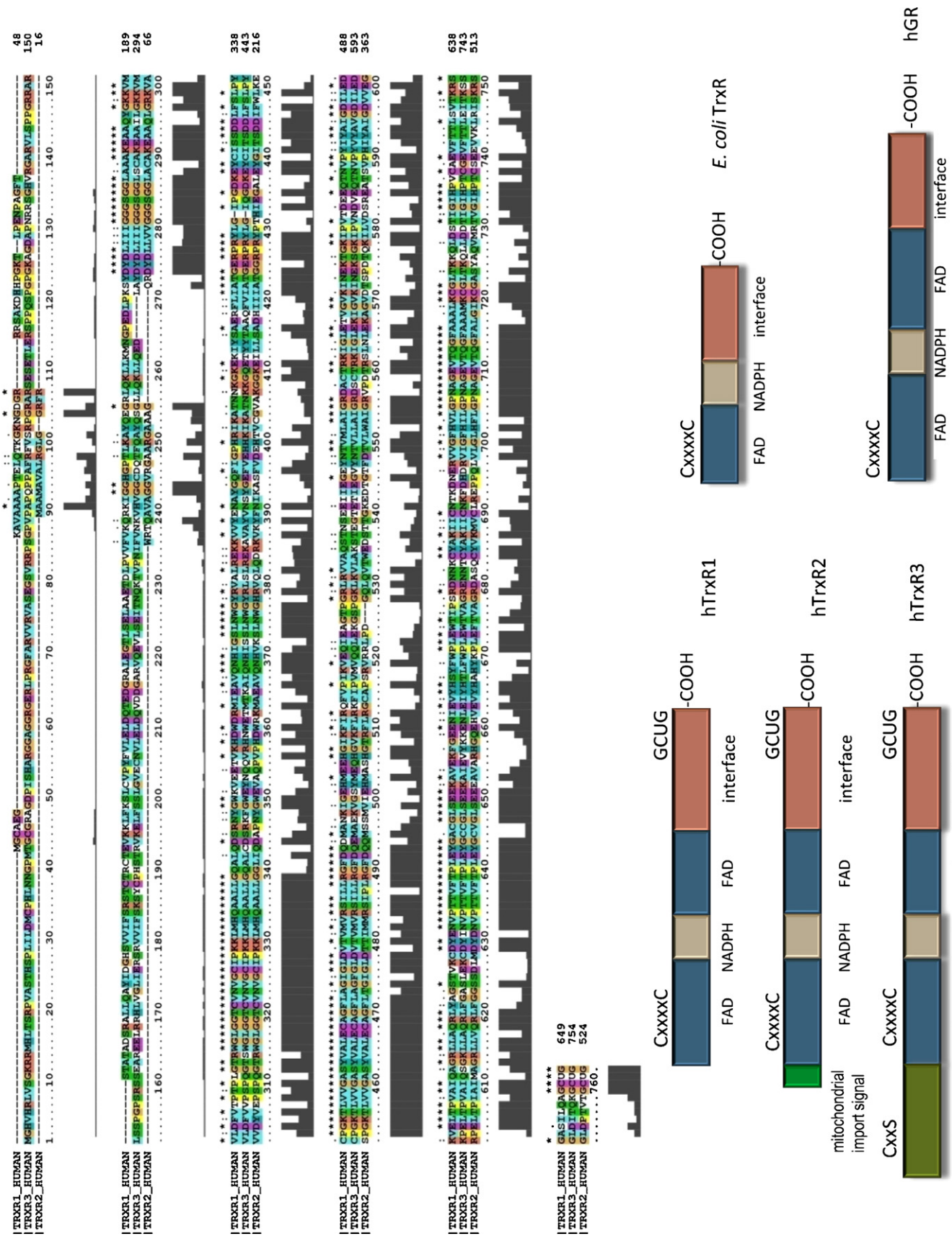

Fig. 3. Sequence alignment and domain organization of human thioredoxin reductases and homologus proteins. (A) The sequences of cytosolic (TrxR1; entry: Q16881) and mitochondrial (TrxR2; entry: Q9NNW7) thioredoxin reductases and of thioredoxin glutathione reductase (TrxR3; entry: Q86VQ6) were obtained from Swiss-Prot/TrEMBL protein sequence database and aligned with Clustal W 2.0.10 Multiple Sequence Alignment [197]. Selenocysteine (U) appears as the penultimate amino acid. (B) Domain organization of human TrxR1 (hTrxR1), TrxR2 (hTrxR2), TrxR3 (hTrxR3), *E. coli* TrxR and human glutathione reductase (hGR). The different domains are specified below each schematic representation, while in the upper part the motifs of the active centers are reported.

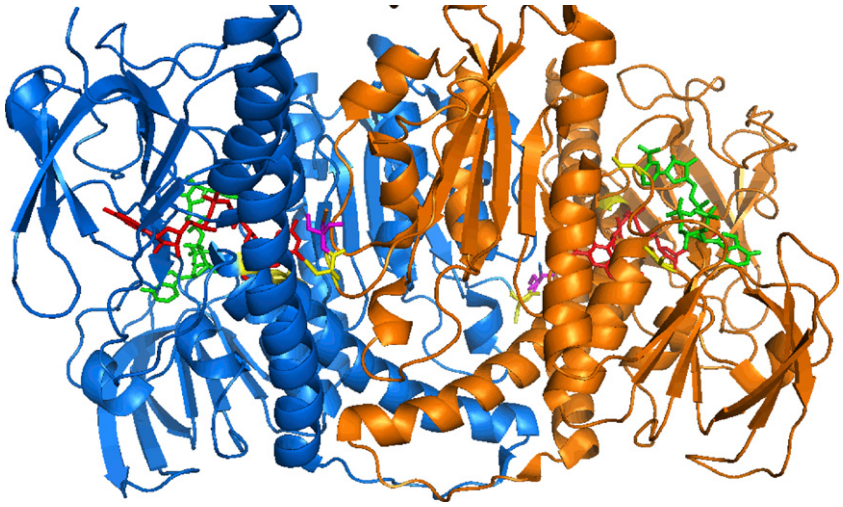

Fig. 4. Ribbon representation of the dimer of human TrxR (Sec → Cys) (PDB entry 2J3N). The two subunits are shown in blue or orange colours, respectively. Cys58/Cys63, Cys58′/Cys63′, Cys497 and Cys 497′ are shown in yellow, Cys498 and Cys498′ in magenta. Bound FAD (red) and $NADP^+$ (green) are shown as ball-and-stick models (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.).

site tyrosine residue (Tyr228) was found to be responsible for the optimal positioning of the nicotinamide moiety. Variations in the flexible C-terminal part of mouse TrxR2 suggested possible differences in activities between cytosolic and mitochondrial TrxRs.

The crystal structure of hTrxR1 (Sec → Cys) in complex with FAD and $NADP^+$ at a resolution of 2.8 Å has been reported by Becker and coworkers [113]. A schematic drawing of the three-dimensional structure of hTrxR1 is shown in Fig. 4. Notably, the overall fold of the protein is very similar to rat TrxR1 and mouse TrxR2. In particular, similarities have been found for the NADP(H) binding site while structural differences occur mainly at the C-terminal amino acid region, where the second redox center of large TrxR is located. Indeed, at least three different conformations (namely CI, CII and CIII) for the last six amino acid residues of hTrxR1 were identified by X-ray analysis that are consistent with the postulated catalytic cycle (see Section 5). These structural rearrangements most likely occur during redox cycling and cofactor binding and are fundamental to orient the C-terminus to the surface of TrxR where Trx is expected. Notably, the reported structure highlights the presence of two reduced surface-exposed cysteine residues (Cys458 and Cys458′ on the second monomer) in the interface domain. These residues have been previously hypothesized to constitute an additional redox center [114] and the X-ray data support this possibility.

The precise knowledge of the crystal structure of thioredoxin reductases is of extreme help for a full understanding of its complicate enzymatic mechanism. In addition, specific inhibitors may be designed and optimised that interact with protein regions critically involved in the catalytic function. It may be anticipated that the dithiol on helix α2, the selenol group at the C-terminal site, the intersubunit cavity and both the NADPH and FAD binding domain will represent optimal anchoring sites for the design of potent and selective enzyme inhibitors. Conversely, the probable binding sites and interaction modes for known inhibitors of thioredoxin reductase may be retrospectively identified.

5. The catalytic mechanism of thioredoxin reductases

The catalytic mechanism through which thioredoxin reductases perform their major biological function – *i.e. thioredoxin reduction* – is extremely complicate and still controversial. Overall, these homodimeric enzymes transfer reducing equivalents from pyridine nucleotides and disulfide/dithiol substrates and catalysis is brought about by FAD and a redox-active disulfide. However, reducing equivalents are transferred from an apolar flavin binding site to the protein substrate by different mechanisms in the two forms of TrxR, namely the higher M_r TrxRs (*e.g.* from higher eukaryotes and from *Plasmodium falciparum*) and the low-M_r enzymes (*e.g.* from *E. coli*) [115]. In particular, high-M_r thioredoxin reductase differs from low-M_r thioredoxin reductase and also from other pyridine nucleotide-disulfide oxidoreductases, including lipoamide dehydrogenase and glutathione reductase, in having a third redox-active group in addition to FAD and the redox-active disulfide adjacent to the flavin. This third redox-active group can be a second redox-active disulfide or a selenosulfide, depending on the species.

A first precise hypothesis for the catalytic mechanism of high-M_r TrxRs was formulated for *Drosophila melanogaster* TrxR (DmTrxR) (Fig. 5) [116]. At the time it was proposed, it was the most detailed mechanism available for high molecular weight TrxR and undoubtedly remains of great interest. The overall reaction can be separated into two half reactions as shown in Fig. 5. In the reductive half-reaction, the oxidized enzyme, Eox, is reduced by the first equiv of NADPH to form the 2-electron reduced enzyme (EH2); NADPH is positioned close to the isoalloxazine ring of FAD so that a $FADH^-$-$NADP^+$ charge-transfer complex is produced (EH2A). $FADH^-$ transfers reducing equivalents to the N-terminal redox-active disulfide (Cys57 and Cys62) and the thiolate-FAD charge-transfer complex involving Cys62 is produced (EH2B). The reducing equivalents interchange between the nascent N-terminal dithiol and the C-terminal disulfide (Cys489′ and Cys490′) (from EH2B to EH2D). Subsequently, a second NADPH molecule reduces EH2 to produce 4-electron reduced enzyme (EH4A). EH4A is converted to EH4B and $NADP^+$ dissociates (EH4C) to complete the reductive half-reaction. In the oxidative half-reaction the nascent C-terminal dithiol interchanges with the disulfide of Trx forming a mixed disulfide intermediate (MDS) to return the enzyme to the EH2 state. Thus, the enzyme, in the course of catalysis, cycles between EH2 and EH4 states.

The proposed catalytic mechanism implies that two dithiol-disulfide interchange reactions are involved in the overall process. Thiol groups with high pK_a are chemically scarcely reactive species. Thus, formation of a reactive thiolate anion is required to initiate the interchange reaction. However, the pK_a value of a thiol group in aqueous solution is approximately 8.3 [117], which is considerably above physiological pH. The protein milieu is needed to lower the pK_a value of the thiol to facilitate the reaction. In the case of the closely related enzyme glutathione reductase it has been shown

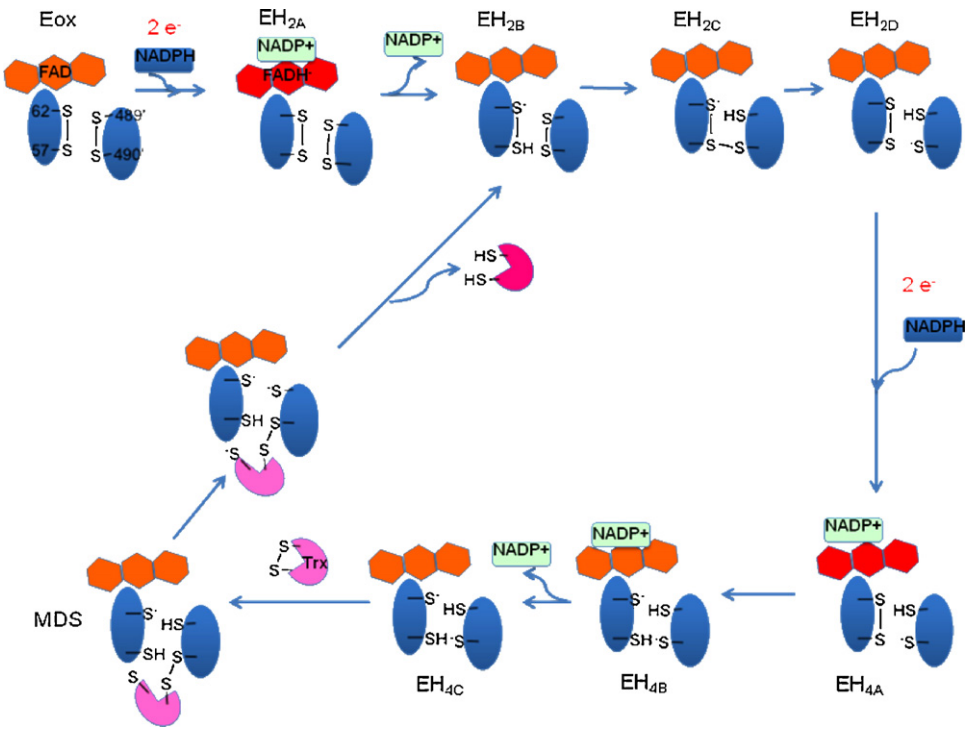

Fig. 5. Model of the catalytic mechanism of *Dm*TrxR for Trx reduction. The cycle comprehends a reductive half-reaction (from E_{ox} to EH_{4C}) and an oxidative half-reaction (MDS complexes). The flavin near the N-terminal redox-active site (Cys57 and Cys62) is provided by one subunit, and the C-terminal active site of the same reaction center by the other subunit (Cys489′ and Cys490′). Note that mammalian TrxR1 has a selenyl sulfide and not a disulfide like in *Dm*TrxR.

that the pK_a of the interchange thiol is low, due to interactions with a nearby His residue [118]. Mechanistic studies have postulated the same function for other His residues in DmTrxR (*e.g.* His464) [119], which seems to be positioned exactly as it is in GR. In addition, the alignment of amino acid sequences of high-M_r TrxRs shows that histidine and glutamate residues are conserved among the TrxRs from different species. Therefore, glutamate-histidine dyads are proposed to act as an acid–base catalyst of TrxRs and to play an essential role in the enzymatic reaction.

In mammalian TrxRs the second C-terminal disulfide found in DmTrxR is replaced by a selenyl sulfide. Surprisingly, the catalytic competence of these orthologous enzymes is similar, whereas direct Sec-to-Cys substitution of mammalian TrxR yields almost inactive enzyme [120,121]. Moreover, in the *D. melanogaster* type enzyme it has been shown that the presence of the selenium atom is not required to catalyze the reduction of the disulfide bond of Trx [122].

Overall, the role of Sec498 in mammalian TrxRs has been analyzed in detail and found to be consistent with the one identified for DmTrxR [116]. The catalytic reaction starts by reduction of the selenenylsulfide to the selenolate anion. The selenolate anion (-Se^-) attacks the disulfide of Trx. The resulting enzyme-Trx-mixed selenenylsulfide is attacked by Cys497 to regenerate the selenenylsulfide, which will be reduced by the active-site thiolate from the other subunit again. During the reaction, the active-site dithiol maintains the selenol in the reduced state. As the selenolate anion is both a better nucleophilic and a better leaving group than the thiolate anion, this explains why the reduction rate of Trx by the Sec498 → Cys mutant TrxR is much slower, and the dithiol should have a higher redox potential.

The three different C-terminal conformations found in hTrxR1 structure (see Section 4) are consistent with the mechanistic studies using redox titrations and fast kinetics. As it is known that TrxRs' catalytic mechanism cycle between the two electrons (EH_2) and four electrons (EH_4) reduced states (see Fig. 5) the structural data well reflect the first part of this cycle, namely the reductive half-reaction. According to this hypothesis conformation CI represents the E_{ox} species of TrxR with a C-terminal buried disulfide; conformation CII represents one of the possible EH_2-species with a still buried C-terminus; and finally conformation CIII with the C-terminus exposed to the surface could correspond to one of the enzyme species able to start the oxidative half-reaction with Trx.

To explain the comparable activities of Sec-containing TrxRs relatively to the Cys-containing enzymes (such as DmTrxR) it is fundamental to consider how the Cys-Cys or Cys-Sec dyad becomes reduced by the N-terminal disulfide redox center. It has been proposed that an 8-membered ring is formed by the dyad in the catalytic cycle as shown in Fig. 6 [123]. The reduction of the 8-membered ring by the vicinal disulfide induces a "ring-opening" step in which the attacking nucleophile (X = thiol or selenol) initiates attack on the disulfide bond of Trx.

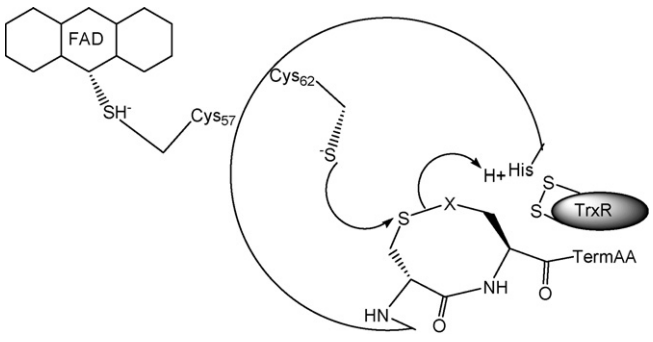

Fig. 6. Schematic model of the 8-membered ring intermediate showing the position of the C-terminal peptide of TrxR1 as it is undergoing ring-opening (reproduced from [124]). TermAA is the last amino acid of the tetrapeptide and X is the leaving group residue (either Cys or Sec). In mammalian TrxRs that contain Se as leaving group, protonation would be unnecessary.

Recently reported studies claim that differences in the conformation of the eight-membered ring between the two classes of enzymes can explain the need for utilization of Sec by the mammalian TrxR [124]. According to this study the ring is fundamental in the Cys-containing TrxRs since its structure allows both reduction of the Cys-Sec dyad and protonation of the leaving group sulfur atom (X). Instead in mammalian TrxR the ring conformation seems to be less important and Sec is required for the lower pK_a of the selenol relative to a thiol, which obviates its need to be protonated upon S–Se bond scission and permits physical separation of the selenol.

Overall, an accurate description of the enzymatic mechanism allows highlighting those protein residues that are essential for catalysis and might be considered for the development of new inhibitors.

6. TrxRs as a target for anticancer agents: established inhibitors of thioredoxin reductases

A few observations revealed that cancer cells often overexpress both thioredoxin and thioredoxin reductase [125–129] implying that the thioredoxin system may have a crucial role in tumor onset and progression. Accordingly, both thioredoxin and thioredoxin reductase might be considered as suitable targets for the development of new anticancer agents [22,130–135]. The view that thioredoxin reductase constitutes an effective druggable cancer target has received significant experimental support and validation. Indeed, Yoo et al. [136], by transfecting lung cancer cells with siRNA specifically directed against TrxR1, found that these cells largely reverse their tumor morphology and growth properties returning similar to normal cells. Moreover, some established antitumor agents used in clinic several years ago were retrospectively found to act as potent inhibitors of thioredoxin reductase as it will be shown in the next section.

It follows that targeting thioredoxin reductase may be an appropriate strategy in modern cancer drug research. Interestingly, TrxR contains a very accessible selenocysteine on its flexible C-terminal arm; thus the several electrophilic compounds that are capable of modifying – selectively and irreversibly – this active site residue might be well considered as potential enzyme inhibitors and candidate anticancer agents.

It is now well established that thioredoxin reductases can be easily and efficiently inhibited by a large array of compounds ranging from natural products to synthetic organic compounds (dinitrohalobenzenes, quinines, etc.), from simple metal ions (Ca, Zn, Mn, Cd, Tl) to structurally elaborated metal coordination compounds (Au, Pt, Ru, Gd). Several inhibitors of thioredoxin reductase, either of natural origin or synthetic, have been developed during the last decade and are of potential interest as anticancer agents. The role of TrxR inhibitors with reference to cancer therapy was recently reviewed by Urig and Becker [135]; a list of representative thioredoxin reductase inhibitors with their respective IC_{50} values is reported in Table 1.

It is apparent that most of the above compounds will directly target the active site selenolate as this chemical group is characterised by an appreciably high reactivity. In the case of a few organic compounds, *e.g. carmustine and curcumin*, that are known to be efficient alkylating agents, inactivation of the selenolate group *via alkylation* has been proposed and documented. However, for other bulkier organic TrxR inhibitors such as quinoids, anthracyclines and naphthoquinones, a direct interaction at the FAD binding site is also conceivable.

Inorganic compounds will preferentially target the active site selenolate function. Thus, arsenic and organochalcogenides are believed to form direct As–Se and Te–Se bonds [194,195].

Table 1
TrxR inhibitors (see ref. [135]).

Substance class	TrxR inhibition potency; in vitro data
Nitrosureas	$IC_{50} \sim 50$ μM for BCNU
Platinum(II) complexes	$IC_{50} \sim 15$ μM for cisplatin (*cis*-diammine-dichloroPt(II))
Phosphole complexes	$IC_{50} \sim 2$ nM for Pt-phosphole
Organochalcogenides	$IC_{50} \sim 0.26$ μM for a diorganyl telluride
Polyphenol flavonoids	$IC_{50} \sim 3.6$ μM for curcumin
Texaphyrins (MGD)	$IC_{50} \sim 6$ μM for MGD (Motexafin gadolinium)
Quinoid componds	$IC_{50} \sim 6.3$ μM for 9,10 phenanthrene quinone
Naphthoquinones spyroketal derivatives	$IC_{50} \sim 0.35$ μM for palmarumycin

Pairwise, in the case of "soft" metal ions such as platinum(II) and gold(I), direct metal coordination to the selenolate function is thought to represent the primary mechanism for enzyme inhibition. However, rather surprisingly, a few ruthenium compounds were found not to alter the selenolate function but to bind to active site His residues [152,153]; in the case of gold(III) complexes oxidative mechanisms seem to be prevailing over simple metal coordination as it will be shown later. A similar situation might hold in the case of motexafin gadolinium [196].

Only in few cases the mechanism of enzyme inhibition has been described in detail. Curcumin was reported to act both as an inhibitor of TrxR and as an antitumor agent. Curcumin is able to alkylate both cysteine and selenocysteine at the catalytic active site of thioredoxin reductase, converting the enzyme into an oxidase with a high production of ROS [137]. This inhibitory mechanism appears similar to that proposed for the immunomodulatory agent dinitrochlorobenzene (DNCB) [138] that covalently binds to cysteine and selenocysteine of TrxR active site. The resulting nitroradical anions can transfer electrons to oxygen producing superoxide [138,139]. In turn, thioredoxin shifts towards its oxidized form and is released from the cell contributing to the immunomodulating properties of these compounds [139,140]. Also, curcumin analogs were studied as inhibitors of thioredoxin reductase and some of them turned out to be more effective than curcumin itself [141]. An inhibitory mechanism similar to that of curcumin appears also to occur with several flavonoids [142].

A large number of quinones of synthetic or natural origin act both as inhibitors or electron acceptors of thioredoxin reductase [143]. Consequently, they are able to stimulate a large production of superoxide anion and hydrogen peroxide that, in turn, might contribute to the overall cytotoxic effect. Among the quinonoid compounds the naphthoquinone spiroketal fungal metabolite palmarumycin CP1 and their analogs appears to act as antitumor agents possibly reacting with the active site selenocysteine moiety of thioredoxin reductase [144].

Oxidative stress induces alterations of all cell components and unsaturated lipids are particularly subjected to the challenge exerted by ROS as they are easily peroxidized. Lipid peroxidation is an unavoidable process in normal cells, but largely increases upon oxidative stress and leads to the breakdown of the fatty acids chains forming a great deal of products including highly reactive unsaturated aldehydes. 4-Hydroxy-2-nonenal (HNE) deriving from $n-6$ unsaturated fatty acids readily reacts with many cell components bearing amino and sulfhydryl groups. HNE inactivates both TrxR and Trx provided they are in their reduced form [145]. Therefore, both cysteine and selenocysteine of TrxR are possibly modified. In the case of Trx, the formation of covalent adducts between Trx and HNE was confirmed by mass spectrometry [145]. Acrolein, another α, β-unsaturated aldehyde present as an environmental pollutant, but also produced by cellular metabolism and lipid peroxidation processes is also a potent inhibitor of thioredoxin reductase [146].

Low concentrations of arsenic trioxide (As_2O_3) were shown to be effective in the treatment of acute promyelocytic leukaemia. In addition, this compound is able to induce growth inhibition and apoptosis in several tumor cell lines accompanied by production of ROS. At the molecular level, As_2O_3 readily interacts with glutathione and protein thiols. It was early recognized that arsenite [147] and, in general, arsenical compounds, are potent inhibitors of thioredoxin reductase. More recently, binding of arsenic to the selenolate group of TrxR1 was documented, through detailed mass spectrometry experiments, in TrxR1 samples treated with arsenic trioxide [67]. The mechanism by which motexafin gadolinium, a texaphyrin, causes enzyme inhibition is more controversial. It may be hypothesized that the bulky texaphyrin complex may block the access of NADPH to its binding site or that the enzyme is damaged by redox processes [148].

Finally, as mentioned above, metal complexes of platinum and ruthenium were shown to be good inhibitors of thioredoxin reductase. Cisplatin (*cis*-diamminedichloroplatinum(II)) was able to irreversibly inhibit thioredoxin reductase activity suggesting a covalent interaction with the thiol/selenol active site [149]. A similar effect on TrxR was also observed with other platinum complexes such as terpyridine platinum(II) [150]. After the successful utilization of platinum complexes in cancer chemotherapy other metal complexes were considered and, in particular, ruthenium complexes were shown to present antitumor activity mainly against metastatic cancer [151]. Interestingly, several ruthenium(III) complexes were shown to markedly inhibit, although at different levels, the cytosolic isoform of thioredoxin reductase, while they are scarcely effective towards the mitochondrial isoform [152,153]. This effect is similar to that of calcium ions that potently inhibit TrxR1, but are weak inhibitors of the mitochondrial isoform [154], indicating that the redox signalling stimulated by these complexes occurs primarily in the cytosolic compartment. The numerous studies appeared so far concerning the interactions of gold compounds with thioredoxin reductase will be considered in detail in the next paragraph.

7. Gold compounds as TrxR inhibitors

Since the very first studies on the protein chemistry of thioredoxin reductases and the discovery of an essential selenocysteine residue in its active site, it was soon apparent that gold compounds (in particular gold(I) compounds) might behave as potent enzyme inhibitors. This hypothesis was initially grounded on the simple chemical consideration that gold compounds, especially those in the oxidation state +1, are "soft Lewis acids" and "thiol reactive species" with the ability to target, potently and selectively, thiol and selenol groups of proteins.

The first studies showing the inhibitory effects of gold complexes such as aurothioglucose on crude preparations of thioredoxin reductase were performed by Hill et al. [155].

The inhibitory properties of gold compounds towards thioredoxin reductase were then characterised in more detail by Gromer et al., and appeared in 1998 [156]; these authors found that the enzyme, in its physiological, NADPH-reduced form, is strongly inhibited by aurothioglucose and auranofin, two representative thiolate and phosphine gold(I) complexes widely used in the treatment of rheumatoid arthritis. For auranofin, an IC_{50} value of 4 nM was measured; in contrast, the gold(III) compound tetrachloroaurate turned out to be a far weaker inhibitor. At 1000-fold higher concentrations, *i.e.* at micromolar levels, the mentioned gold(I) drugs also inhibited human glutathione reductase and the selenoenzyme glutathione peroxidase.

In subsequent years, tight linkages were highlighted between the thioredoxin system, its inhibition by gold compounds, the mitochondrial membrane permeability transition, and the triggering of apoptosis [74,157]. Auranofin was shown to be a potent inducer of the swelling of freshly isolated rat liver mitochondria, that is highly suggestive of a transition in the permeability status of the mitochondrial membranes [157]. Reversion of the effect of auranofin on mitochondrial permeability by cyclosporin A supported a mechanism involving the mitochondrial permeability transition pore complex. Notably, permeability changes occurred at auranofin concentrations corresponding to the selective inhibition of mitochondrial thioredoxin reductase, with few effects on the mitochondrial electron transport chain or on glutathione reductase. This hypothesis was reinforced by the concomitant studies by McKeage et al. [158,159]

Starting from 2004, the attention was also extended to a variety of gold(III) compounds for which relevant TrxR inhibitory properties could be unambiguously established. In particular, the effects of four novel gold(III) complexes, *i.e.* ([Au(2,2′-diethylendiamine)Cl]Cl_2 [(Au(2-(1,1-dimethylbenzyl)-pyridine)$(CH(3)COO)_2$], [Au(6-(1,1-dimethylbenzyl)-2,2′-bipyridine)(OH)](PF_6) [Au(bipy(dmb)-H)(2,6-xylidine)](PF_6)) on mitochondrial thioredoxin reductase and on mitochondrial functions were examined in depth in comparison to those of a few representative gold(I) complexes (auranofin, triethylphosphine gold and aurothiomalate) [160]. Both gold(I) and gold(III) complexes resulted to be extremely efficient inhibitors of thioredoxin reductase with IC_{50} values ranging from 0.020 to 1.42 μM. At variance, a few other metal ions and metal complexes not containing gold (*e.g.* cadmium(II) ions, etc.) were significantly less effective. It was concluded that gold compounds are highly specific inhibitors of mitochondrial thioredoxin reductase; the observed potent inhibition is believed to heavily influence other mitochondrial functions such as overall membrane permeability properties.

In a subsequent paper, Coronnello et al. [161] showed that a few other cytotoxic organogold(III) compounds, namely [Au($bipy^{dmb}$-H)(OH)][PF_6] (1), Au($bipy^{dmb}$-H)(2,6-xylidine-H)][PF_6] (2) (in which $bipy^{dmb}$ = 6-(1,1-dimethylbenzyl)-2,2′-bipyridine), and [Au(py^{dmb}-H)$(AcO)_2$] (3) (in which py^{dmb} = 2-(1,1-dimethylbenzyl)-pyridine), may cause selective and deep inhibition of thioredoxin reductases. The tested compounds also produced significant antiproliferative effects on the A2780 ovarian carcinoma cell line and promoted apoptosis to a greater extent than platinum drugs while causing only modest cell cycle modifications. These findings further reinforced the view that the mitochondrial pathways are primarily involved in the gold-dependent proapoptotic effects, most likely in relation to selective inhibition of thioredoxin reductase [161].

During the last 3 years a few other studies have appeared concerning inhibition of thioredoxin reductase by gold(III) compounds [162]. Engman and Powis, looking for new anticancer agents, evaluated a representative ensemble of gold(III) compounds – with none, one, two or three carbon–gold bonds, respectively – for their capacity to inhibit TrxR and the growth of MCF-7 cancer cells *in vitro* [162]. Compounds with up to two carbon–gold bonds often resulted to be potent inhibitors of TrxR with IC_{50} values as low as 2 nM when measuring the direct reduction of added DTNB. A far lower inhibiting potency was instead detected using the thioredoxin-dependent insulin reduction assay. However, the inhibitory concentrations of these organogold(III) compounds did not correlate with the ability to kill cells. Out of the several tested organometallics, only a single compound, bearing two carbon–gold bonds, was able to inhibit colony formation by MCF-7 breast cancer cells, at low micromolar concentrations (IC_{50} = 1.6 μM). However, rather disappointingly, that compound did not reveal any significant antitumor activity against MCF-7 breast cancer and HT-29 colon cancer xenografts in scid mice.

Fregona et al. prepared a few innovative gold(III) compounds with a dithiocarbamate carrier ligand and analyzed their respective biological profiles (see Section 1 and references therein). Notably, these compounds were reported to induce extensive cancer cell death through both apoptotic and non-apoptotic mechanisms. It was found that they are able to inhibit thioredoxin reductase activity, to generate free radicals, to modify mitochondrial functions, and to increase ERK1/2 phosphorylation [19]. A working model was proposed suggesting that deregulation of the thioredoxin reductase/thioredoxin redox system is a major mechanism involved in the anticancer activity of these gold(III)–dithiocarbamato complexes [19].

Conversely, a few other studies were directed to delve more deeply into the effects of gold(I) compounds as possible inhibitors of thioredoxin reductase.

Omata et al. exposed rat TrxR1 to auranofin, gold sodium thiomalate, sodium aurothiosulfate, triphenyl phosphine gold chloride, or gold acetate, and measured TrxR activity *ex vivo* [163]. All above gold compounds inhibited TrxR1 at IC_{50} concentrations ranging from 5 to 4000 nM. Gold(I)-phosphine compounds (triphenyl phosphine gold chloride and auranofin) were the most potent inhibitors of TrxR. All TrxR1 inhibitory concentrations were sublethal to mitochondrial activity in both THP1 and OSC2 cells. They thus concluded that diverse types of gold compounds may be effective inhibitors of TrxR1 at concentrations that do not suppress cellular mitochondrial function; inhibition may be optimized to some degree by altering the ligand configuration of the compounds.

On turn, Urig et al. [164] reported the effects of a novel gold(I) phosphole on human glutathione reductase (hGR), on thioredoxin reductase (hTrxR), and on DNA, as well as its growth-inhibitory action on tumor cells. Overall important effects of gold phosphole on the above mentioned biomolecules were highlighted. Remarkably, studies on TrxR mutants indirectly confirmed the Sec residue as the prime target of the investigated gold compound. Indeed, the inhibition of the Sec → Cys mutant was orders of magnitude less strong than that of the wild-type TrxR.

Rackam et al. [165] recently showed that water soluble gold(I) compounds, with the gold(I) center bis-chelated with the ligand 1,3-bis(di-2-pyridylphosphino)propane importantly lower thioredoxin reductase activity and irreversibly modify thioredoxin, leading to cell apoptosis.

In a very recent paper, Hickey et al. [166] described some new interesting gold(I) compounds. Specifically, a family of lipophilic, cationic gold(I) complexes of N-heterocyclic carbenes (NHCs) was designed as new mitochondria-targeted antitumor agents that combine both selective mitochondrial accumulation and selective thioredoxin reductase inhibition properties within a single molecule. Two-step ligand exchange reactions with cysteine (Cys) and selenocysteine (Sec) were described with release of the NHC ligands. At physiological pH the rate constants for the reactions with Sec are 20–80-fold higher than those with Cys. Notably, gold(I) carbenes were shown to be selectively toxic to two highly tumorigenic breast cancer cell lines but not to normal breast cells; the degree of selectivity and potency were optimized by a modification of the substituent. The lead compound is shown to accumulate in mitochondria of cancer cells, to cause cell death through a mitochondrial apoptotic pathway and to inhibit the activity of thioredoxin reductase (TrxR) but not the closely related and Se-free enzyme glutathione reductase.

Overall, we can state that several gold compounds have now been evaluated as potential inhibitors of thioredoxin reductase. Table 2 reports a list of representative gold compounds for which strong inhibitory properties towards thioredoxin reductase were established.

It is evident that most reported gold compounds cause profound enzyme inhibition with IC_{50} values typically ranging from nanomo-

Table 2
Inhibitory effect (IC_{50}) of some gold (I/III) compounds on different thioredoxin reductase isoforms.

Compound	Human cytosolic TrxR1	Rat cytosolic TrxR1	Rat mitochondrial TrxR2
Au(I)			
Auranofin	0.0200[#]	0.0007	0.0020[‡]
Au (triethylphosphine)Cl		0.0012	0.0058[‡]
Aurothiomalate		0.0050	0.0280[‡]
Aurothioglucose	0.0650[#]		
Aurothiosulfate		0.0500[§]	
Au(triphenylphosphine)Cl		0.1000[§]	
(Dimethylsulfide)AuCl			0.5840[¥]
Au phenyl(di(2-pyridyl) phosphole)Cl	0.0008[&]		
Au(III)			
Tetrachloroaurate	0.0058[*]	0.0120	0.1000
$Au(OAc)_3$		4.000[§]	
[Au(2,2′-diethylendiamine)]Cl_2	0.2000[*]		
[Au(2,2′-diethylentriamine)Cl]Cl_2		0.0028	0.4200[‡]
(Au (py^{dmb}-H)$(OAc)_2$		0.0147	1.4200[‡]
[Au(bipy ^{dmb}-H))(OH)](PF_6)		0.0043	0.2800[‡]
[Au($bipy^{dmb}$-H)(2,6-xylidine)] (PF_6)		0.0041	0.2100[‡]
Au(2,2′-bipyridine)Cl_2	0.0120[*]		
Au(2-phenylpyridine)Cl_3	0.0300[*]		
Au(2-phenylpyridine)Cl_2	0.0360[*]		
Au(damp)Cl_2	0.1800[*]		
Au(damp)$(OAc)_2$	0.0300[*]		
Au(damp)(phenyl)Cl	0.0022[*]		
Au(dimethyl)(damp)	1.8000[*]		
Au(trimethyl)(triphenylphosphine)	0.6800[*]		
Au(DMDT)Cl_2		0.0057[◦]	0.0247[◦]
Au(DMDT)Br_2		0.0077[◦]	0.0284[◦]
Au(ESDT)Cl_2		0.0170[◦]	0.0346[◦]
Au(ESDT)Br_2		0.0139[◦]	0.0359[◦]

IC_{50} values are μM; TrxR activity was measured with the DTNB reduction method. Abbreviations: py^{dmb} = (2-(1,1-dimethylbenzyl)-pyridine); $bipy^{dmb}$ = 6-(1,1-dimethylbenzyl)-2,2′-bipyridine; damp = 2-[(dimethylamino) methyl]-phenyl; DMDT = N,N- dimethyl dithiocarbamato; ESDT = ethyl sarcosine dithiocarbamato. The reported data originate from the following references: [162][*]; [19][◦]; [163][§]; [156][#]; [135][&]; [160][‡] [157][¥]. The other values are unpublished data.

lar to micromolar. From inspection of Table 2 it also emerges that gold(I) compounds, on the average, are far more effective than gold(III) compounds and that the cytosolic isozyme is usually more sensitive to gold compounds than the mitochondrial one.

Some specific insights concerning the actual molecular mechanism of the various gold compounds may be achieved. The correlation of the measured inhibitory potency with the "soft character" supports the view that the selenol group is very likely the primary binding site for gold compounds. This idea is reinforced by the drop in the IC_{50} values noted for a gold phosphole on passing from the native selenoenzyme to its Sec-to-Cys mutant. Moreover, it is evident that gold compounds, in order to be effective, must possess at least one labile ligand whose release may allow formation of a direct gold–selenium bond. Accordingly, the coordinatively saturated and scarcely reactive gold(III) complex *Aucyclam* is a very poor thioredoxin reductase inhibitor. Gold(III) compounds, less soft in nature, display a lower inhibitory potency than gold(I) compounds but still appreciable. It is conceivable that gold(III) compounds may act through a different mechanism than metal coordination to active site selenol, causing for instance oxidative damage to the enzyme.

In spite of the rather numerous studies appeared so far on TrxR inhibition by gold compounds, the interaction mechanisms have not been explored satisfactorily at the molecular level. Only very recently we have started investigating those interactions by specific biophysical and biochemical methods. Some preliminary results will be described in the next paragraph.

8. Mechanistic insights into gold-induced TrxR inhibition from mass spectrometry and biochemical studies

Although the effects of numerous gold compounds on thioredoxin reductase activity were widely investigated during the last decade and potent enzyme inhibition highlighted in several cases, the actual mechanisms of interaction of gold compounds with this enzyme have been poorly explored at the molecular level. It was just assumed that the "soft", catalytically relevant, selenolate group should constitute the common anchoring site for gold-based inhibitors; this assumption was grounded on the concept that "soft" gold ions, especially in the oxidation state +1, are able to form strong bonds with "soft" Lewis donors. Notably, important although indirect, support to this hypothesis was recently provided by Deponte et al. [167] who reported that gold phospholes inhibit native thioredoxin reductase orders of magnitude stronger than a mutant where the C-terminal selenol group is replaced by a cysteine.

This substantial lack of molecular information prompted us to analyze in more detail the interactions taking place between gold compounds and thioredoxin reductase with the aid of a few specific biophysical and biochemical tools. In particular, we have exploited advanced mass spectrometry methods to explore the reactions of representative gold compounds with thioredoxin reductase; in turn, a specific biochemical assay, the BIAM assay, already applied to thioredoxin reductase [137] was found to be valuable in assessing the occurrence of binding of gold compounds to the active site selenol group and to other protein thiols. Some remarkable results emerging from our studies will be briefly described below.

8.1. Mass spectrometry results

Today's mass spectrometry methods may allow a direct monitoring of the TrxR enzyme in spite of its relatively large size (~110 kDa for the homodimeric protein). A series of MALDI (Matrix-Assisted Laser Desorption Ionization) TOF (Time-of-Flight) spectra were thus recorded on TrxR1 samples, pre-reduced with NADPH, either alone or incubated with a stoichiometric excess of various gold complexes, for 1 h at 37 °C; a similar approach had been previously adopted by Deponte et al., when studying the reaction of gold phospholes with human glutathione reductase [167]. Representative MALDI spectra for TrxR1 and for its auranofin adduct are shown in Fig. 7. Interestingly, treatment of TrxR1 with gold compounds results into significant mass increases, whose extent clearly depends on the applied gold-to-protein molar ratios. For example, in the case of the *auranofin*-treated sample, the main peak is located at ~56,600 Da, while the peak of untreated TrxR1 falls at ~55,500 Da, suggesting protein binding of ~4 $AuPEt_3^+$ fragments. These observations provide direct evidence for extensive protein metallation and point out that a certain number of sites, beyond Sec, are available for gold binding on the protein surface, most likely Cys, Met and His residues. A similar situation, *i.e.* the presence of multiple gold binding sites on the protein surface, was recently hypothesized for the reaction of human serum albumin with antiarthritic gold(I) compounds [168] and cytotoxic gold(III) compounds [169].

8.2. BIAM assay

Independent and complementary information on the occurring protein metallation processes was then achieved through application of a specific biochemical assay, based on the thiol-tagging reagent, BIAM (biotin-conjugate iodoacetamide) [137]. BIAM alkylates selectively TrxR in dependence of pH; at pH 6.0 only selenocysteines (and low pK_a cysteines) are alkylated, while both cysteines and selenocysteines are modified at pH 8.5 [137]. In our experiments, TrxR1 was first reacted with each metal complex; afterward, sample aliquots were transferred to test tubes containing BIAM, either at pH 6.0 or 8.5, and the mixtures subjected to SDS-PAGE. Proteins labelled with BIAM were detected with horseradish peroxidase-conjugated streptavidin. Notably, with the

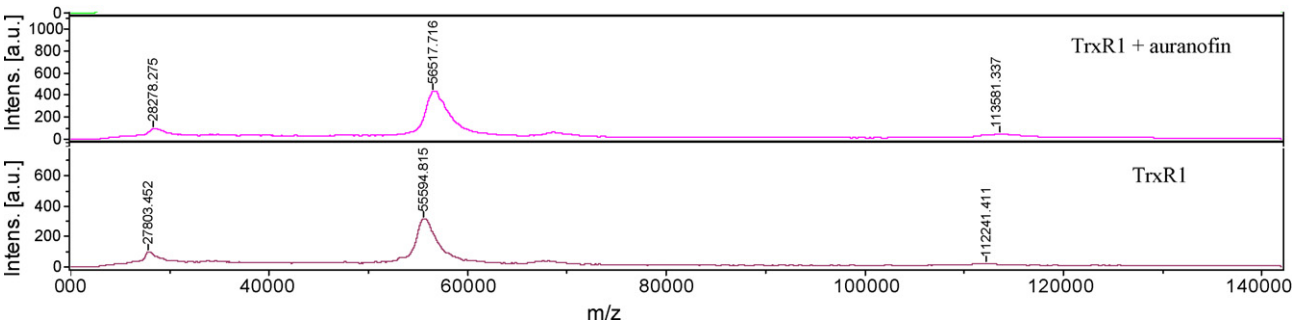

Fig. 7. MALDI MS spectra of intact rat TrxR1, pre-reduced with NADPH, alone or after 1 h incubation with auranofin, at 10:1 gold to protein molar ratio, and removal of unbound gold.

gold(I) compound, aurothiomalate (Fig. 1) a pattern similar to that already observed for curcumin modification of TrxR1 [137] was apparent with blotting band intensity of treated samples, at pH 6.0, far weaker than the control. This result is highly suggestive of selective gold(I) binding to active site selenocysteine [170]. At variance, in the case of gold(III) complexes, the results of the BIAM experiment are indicative of a lower degree of selectivity. Indeed, most applied gold(III) compounds turned out to modify most cysteine residues and the selenocysteine of TrxR1 though at different extents. In any case, upon increasing gold(III) concentration up to its highest value (100 μM), the intensity of the BIAM bands markedly weakened and eventually disappeared. These results strongly suggest that gold(III) complexes produce a progressive and indiscriminate oxidation of selenol and thiol groups, thus blocking BIAM binding. In other words, for gold(III) complexes, oxidation of thiol/selenol groups seems to prevail over metal coordination. Again, *Aucyclam* (cyclam: 1,4,8,11-tetraazacyclotetradecane) turned out to be nearly uneffective in contrasting BIAM modification given its low oxidising character and its poor binding properties.

In conclusion, both advanced MS methods and a specific biochemical assay (the BIAM assay) were exploited to unveil the molecular mechanisms through which cytotoxic gold compounds inhibit and modify thioredoxin reductase. MS results allowed a direct monitoring of the whole enzyme and provided clear evidence for its extensive metallation ("auration") at a variety of sites. In turn, BIAM assays showed that a number of cysteines and the active site selenocysteine are greatly altered by gold compounds. Preferential modification of active site selenocysteine was clearly supported for gold(I) compounds. At variance, progressive oxidative damage of the thiol and selenol residues was highlighted in the case of gold(III) compounds.

9. The mode of action of gold anticancer compounds based on biochemical and cellular studies

A large body of literature indicates that gold complexes induce apoptosis in cancer cells [19,161,165,171–173] and even in cells that have acquired resistance to specific drugs [173,174]. In cancer cells, although many different targets are potentially available for gold complexes, the observed inhibition of mitochondrial and cytosolic proteins thioredoxin reductases seems to play a major role. The most important consequence of the inhibition of cell thioredoxin reductase is a marked alteration of the balance between production and removal of hydrogen peroxide in favour of the first due to inactivation of one of the key systems devoted to hydrogen peroxide reduction. This effect is particularly evident in mitochondria where, as mentioned above, the rate of production of mitochondrial hydrogen peroxide depends not only on the alteration of the electron flow along the respiratory chain but also on an efficient removal of this oxidant by glutathione and thioredoxin systems. Consequently, after inhibition of thioredoxin reductase, a large concentration of hydrogen peroxide builds up inside mitochondria with a concomitant thiol redox shift primarily involving thioredoxin [175]. In fact, the inhibition of thioredoxin reductase in the presence of a large amount of hydrogen peroxide leads to a peroxiredoxin-mediated complete oxidation of thioredoxin. On turn, oxidized thioredoxin cannot be reduced back as reducing equivalents coming from NADPH are no longer available (Fig. 8). Similarly, previous studies in *E. coli* showed that impairment of glutathione or thioredoxin systems leads to formation of intracellular disulfide bridges [176]. It has been also shown that the inactivation of the gene for thioredoxin reductase causes the formation of disulfide bonds in the cytosol that do not simply depend on the lack of reductase activity but also on the direct oxidizing action exerted by the oxidized

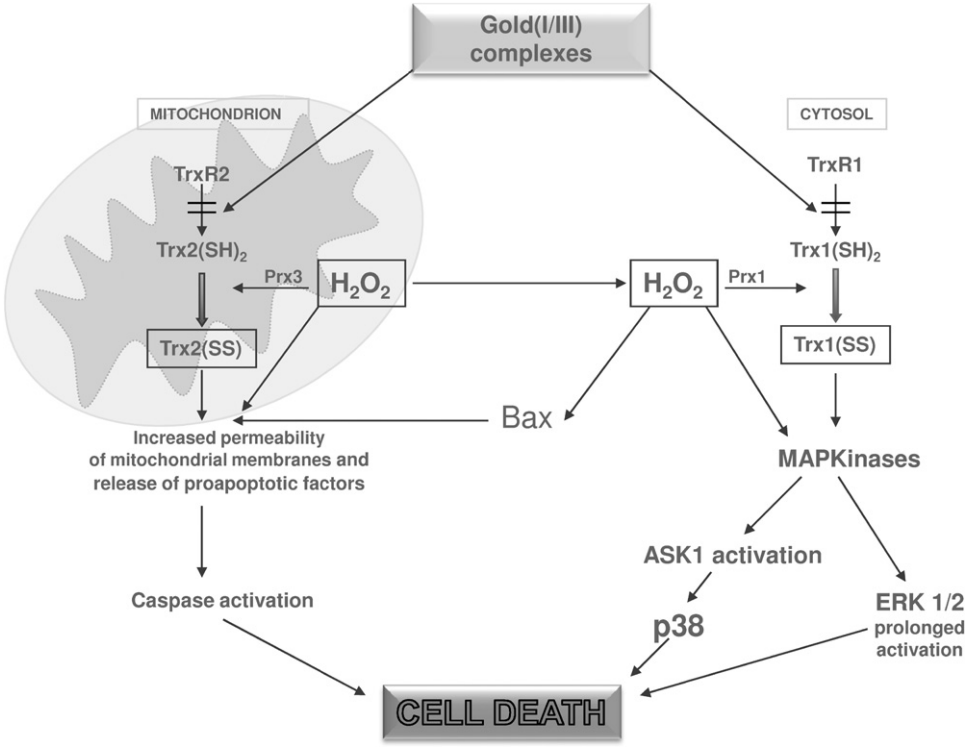

Fig. 8. Model depicting the mechanism of action of cell death induction by gold(I/III) compounds. The mitochondrial respiratory chain produces superoxide anion that dismutes to hydrogen peroxide and oxidizes thioredoxin in a reaction mediated by peroxiredoxin. Thioredoxin reductase, inhibited by gold(I/III) complexes, is unable to reduce back oxidized thioredoxin that accumulates together with hydrogen peroxide and both act on several different intramitochondrial targets leading to the opening of the mitochondrial permeability transition pore and/or to an increase of the permeability of the outer membrane. Hydrogen peroxide is then released to the cytosol where causes oxidation of Trx1, that, similarly, to mitochondrial thioredoxin (Trx2), cannot be reduced back by the gold(I/III)-inhibited thioredoxin reductase. Oxidized thioredoxin stimulates the MAP kinases pathways leading to cell death.

form of thioredoxin [177]. It was recently reported that mitochondrial peroxiredoxin (Prx3) is selectively oxidized after treatment of cancer cells with doses of auranofin that cause apoptosis and rapidly accumulate inside the mitochondrion [178]. Oxidized peroxiredoxin appears responsible of the permeabilization of the outer mitochondrial membrane and of the consequent apoptotic events controlled by the Bcl2 family of proteins [178]. Consequently, in mitochondria, the increased levels of hydrogen peroxide, associated to the oxidation of thioredoxin and peroxiredoxin, alter the permeability condition of the mitochondrial membranes, resulting in the opening of the mitochondrial permeability transition pore [23,157,179] and/or in the permeabilization of the outer membrane [178,180] (Fig. 8). Cancer cells in comparison to normal cells are well known to be resistant to permeability transition [40] and, therefore, the induction of this condition, leading to cell death, is considered a therapeutic objective in tumors [40]. All these effects were observed in isolated mitochondria, where auranofin was seen to induce swelling and loss of membrane potential indicating the occurrence of mitochondrial membrane permeability transition in a process dependent on calcium ions and prevented by cyclosporin and uncouplers [157,179]. This capability of the gold(I) compound auranofin to induce permeability transition is also shared by several gold(III) complexes [19]. Concomitant with membrane permeability transition gold(I) and gold(III) complexes induce a large formation of hydrogen peroxide [19,181], a slight decrease of total thiol groups [179] but not of glutathione [180], and a marked release of cytochrome *c* [179]. The release of the latter occurs also in the presence of cyclosporin indicating its independence on mitochondrial permeability transition. This lack of effect by cyclosporin was observed not only in isolated mitochondria but also in whole cells [180]. Recently it was found that cell apoptosis induced by auranofin is completely inhibited by the overexpression of Bcl-2 or by deficiency of Bax/Bak indicating that auranofin-induced apoptosis is essentially regulated by the Bcl-2 family instead of the mitochondrial membrane permeability transition pore [178]. Previously, it was also shown that gold(III)–methylsarcosine dithiocarbamate complexes are able to downregulate Bcl-2 and upregulate Bax [182]. Therefore, mitochondrial permeability transition appears more important in driving the cell towards necrosis instead of apoptosis [183]. The production of hydrogen peroxide occurring in cells treated with gold complexes does not lead to an extensive lipid peroxidation indicating that a marked oxidative stress is not involved in the observed process of cell death [180]. Therefore, mitochondria play a critical role in cell apoptosis both by releasing proapoptotic factors and by producing a sustained and long lasting increment in H_2O_2 production. Due to the gold-dependent inhibition of mitochondrial thioredoxin reductase, a lack of removal of hydrogen peroxide occurs inside mitochondria. Consequently, the concentration of this oxidant increases and, as it can freely cross the mitochondrial membranes, a large concentration of H_2O_2 is found in the cytosol. Like in the mitochondrial matrix, also in this subcellular compartment hydrogen peroxide cannot be removed as cytosolic thioredoxin reductase is likewise inhibited by gold(I/III) complexes (Fig. 8). In the cytosol, hydrogen peroxide activates various signalling pathways further stimulating apoptosis. The MAP kinases pathways are particularly involved in this process [19,171]. As reported above, reduced thioredoxin, after binding to the apoptosis signal-regulating kinase (ASK1), prevents apoptosis [184] but this inhibitory effect is removed by the oxidation of Trx [184]. Auranofin was shown to activate the p38 mitogen-activated protein kinase leading to apoptosis accompanied by ROS generation, cytochrome *c* release and caspases activation [171]. In HeLa cells, gold(III)-ethylsarcosine dithiocarbamato complex determines a rapid increase of the phosphorylation level of ERK1/2 [19] in a reaction completely inhibited by treatment with the thiol compound N-acetyl cysteine (NAC) [19]. Activation of ERK1/2 pathway is generally considered to play a role in cell survival, however, there is increasing evidence that ERK1/2 can also stimulate apoptosis [185–187]. Several studies indicate that ERK1/2 pathway can be activated by ROS [185,188–190]. Furthermore, it was shown that low-potassium induced apoptosis in cultured cerebellar granule neurons occurring after phosphorylation of ERK1/2 was dependent on the activation of the ASK1-p38 MAP kinase pathway [191]. Consequently, both the cytosolic increase of hydrogen peroxide and thioredoxin oxidation induced by the gold-dependent thioredoxin reductase inhibition stimulate ERK1/2 phosphorylation. It has to be remembered that the apoptotic stimulus depends also on the duration of MAP kinases activation as transient activation leads to proliferation, while a prolonged activation acts in the opposite way causing cell death [192]. Gold(III) dimethyldithiocarbamate complex was shown to inhibit the proteasome system [18] hence explaining the observed persistence of phosphorylated ERK1/2 [19]. In general, gold complexes are scarcely selective as they exert a comparable cytotoxicity towards both cancer cells and normal cells. However, some newly synthesized gold(I) derivatives appear to selectively induce cancer cell death [165]. Among these is the bis-chelated Au(I) complex of the ligand 1,3-bis(di-2-pyridylphosphino)propane able to induce apoptosis in breast cancer cells but not in normal cells [165]. This effect appears mediated by the inhibition of both thioredoxin and thioredoxin reductase particularly in the cancer cells [165]. Similarly, Au(I) N-heterocyclic carbene compounds are selectively toxic to breast cancer cell lines but not to normal cells [166]. The proper choice of ligands to the Au(I) center based on a fine-tuning of their steric and electronic features can confer to these complexes the specific properties enabling them to selectively exert a toxic effects solely to the cancer but not to the normal cells.

10. Conclusions

Gold compounds form today an important class of cytotoxic agents of potential interest as anticancer agents. There is much attention in disclosing the effective cellular and molecular mechanisms through which these compounds induce their important antiproliferative effects. A number of recent investigations suggested that the redox metabolism and mitochondria are the likely cellular targets for several metal-based inorganic compounds, capable of inducing oxidative stress. Apparently, the selenoprotein thioredoxin reductase plays a crucial role in this complicate network of biological actions and processes as it is, on one hand, a major and general receptor for gold compounds capable of targeting protein selenol and thiol groups; on the other hand, its inhibition may trigger severe mitochondrial deregulation eventually leading to cell apoptosis. In this review the recent relevant findings and achievements concerning the role of gold compounds as potent inhibitors of thioredoxin reductase have been surveyed. Particular emphasis has been given to recent biophysical studies concerning the protein chemistry of thioredoxin reductases and the description of their interactions with a variety of gold compounds, at the molecular level. Our working hypothesis, that is detailed in the previous section and summarised in Fig. 8, provides a rather satisfactory and unitary framework to explain the mechanism of action of novel anticancer gold compounds, though in the presence of a very complicate biochemical and cellular scenario and of significant differences among the various investigated metallodrugs. It follows that anticancer gold compounds might be primarily considered as "*antimitochondrial agents*". According to our interpretation the role of thioredoxin reductase as an effective druggable target for the development of novel anticancer agents is highlighted and further reinforced.

Acknowledgments

This work was supported by grants from the National Research Council of Italy (CNR), and University of Padova (Progetto di Ateneo CPDA065113/06). We wish to thank Dr. Luca Cesaro for providing the sequence alignment figure. A.C. thanks the Swiss National Science Foundation for financial support (AMBIZIONE project no. PZ00P2_121933).

References

[1] Chem. Rev. 99 (9) (1999) (special issue).
[2] P.C. Bruijnincx, P.J. Sadler, Curr. Opin. Chem. Biol. 12 (2008) 197.
[3] C.X. Zhang, S.J. Lippard, Curr. Opin. Chem. Biol. 7 (2003) 481.
[4] L. Messori, G. Marcon, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems, vol. 42, Marcel Dekker Inc., New York, 2004.
[5] E.R.T. Tiekink, Crit. Rev. Oncol. Hematol. 42 (2002) 225.
[6] C. Gabbiani, A. Casini, L. Messori, Gold Bull. 40 (2007) 73.
[7] L. Messori, F. Abbate, G. Marcon, P. Orioli, M. Fontani, E. Mini, T. Mazzei, S. Carotti, T. O'Connell, P. Zanello, J. Med. Chem. 43 (2000) 3541.
[8] R.G. Buckley, A.M. Elsome, S.P. Fricker, G.R. Henderson, B.R. Theobald, R.V. Parish, B.P. Howe, L.R. Kelland, J. Med. Chem. 39 (1996) 5208.
[9] G. Marcon, S. Carotti, M. Coronnello, L. Messori, E. Mini, P. Orioli, T. Mazzei, M.A. Cinellu, G. Minghetti, J. Med. Chem. 45 (2002) 1672.
[10] C.K.-L. Li, R.W.-Y. Sun, S.C.-F. Kui, N. Zhu, C.-M. Che, Chem. Eur. J. 12 (2006) 5253.
[11] C.T. Lum, Z.F. Yang, H.Y. Li, R.W.-Y. Sun, S.T. Fan, R.T.P. Poon, M.C.M. Lin, C.-M. Che, H.F. Kung, Int. J. Cancer 118 (2006) 1527.
[12] Y. Wang, Q.-H. He, R.W.-Y. Sun, C.-M. Che, J.-F. Chiu, Cancer Res. 65 (2005) 11553.
[13] Y. Wang, Q.-Y. He, C.-M. Che, J.-F. Chiu, Proteomics 6 (2006) 131.
[14] R.W.-Y. Sun, W.-Y. Yu, H. Sun, C.-M. Che, ChemBioChem 5 (2004) 1293.
[15] C.-M. Che, R.W.-Y. Sun, W.-Y. Yu, C.-B. Ko, N. Zhu, H. Sun, Chem. Commun. (2003) 1718.
[16] L. Ronconi, L. Giovagnini, C. Marzano, F. Bettìo, R. Graziani, G. Pilloni, D. Fregona, Inorg. Chem. 44 (2005) 1867.
[17] L. Ronconi, C. Marzano, P. Zanello, M. Corsini, G. Miolo, C. Maccà, A. Trevisan, D. Fregona, J. Med. Chem. 49 (2006) 1648.
[18] V. Mlacic, D. Chen, L. Ronconi, K.R. Landis-Piwowar, D. Fregona, Q.P. Dou, Cancer Res. 66 (2006) 10478.
[19] D. Saggioro, M.P. Rigobello, L. Paloschi, A. Folda, S.A. Moggach, S. Parsons, L. Ronconi, D. Fregona, A. Bindoli, Chem. Biol. 14 (2007) 1128.
[20] C. Gabbiani, A. Casini, L. Messori, A. Guerri, M.A. Cinellu, G. Minghetti, M. Corsini, C. Rosani, P. Zanello, M. Inorg. Chem. 47 (2008) 2368.
[21] A. Casini, M.A. Cinellu, G. Minghetti, C. Gabbiani, M. Coronnello, E. Mini, L. Messori, J. Med. Chem. 49 (2006) 5524.
[22] A.S. Humphreys, A. Filipovska, S.J. Berners-Price, G.A. Koutsantonis, B.W. Skelton, A.H. White, Dalton Trans. 21 (2007) 4943.
[23] P.J. Barnard, S.J. Berners-Price, Coord. Chem. Rev. 251 (2007) 1889.
[24] S.J. Berners-Price, A. Filipovska, Aust. J. Chem. 61 (2008) 661.
[25] A.R. Cross, O.T.G. Jones, Biochim. Biophys. Acta 1057 (1991) 281.
[26] V.B. O'Donnell, A. Azzi, Biochem. J. 318 (1996) 805.
[27] A. Paolicchi, S. Dominici, L. Pieri, E. Maellaro, A. Pompella, Biochem. Pharmacol. 64 (2002) 1027.
[28] M. Schrader, H.D. Fahimi, Biochim. Biophys. Acta 1763 (2006) 1755.
[29] G. Sandri, E. Panfili, L. Ernster, Biochim. Biophys. Acta 1035 (1990) 300.
[30] N. Pizzinat, N. Copin, C. Vindis, A. Parini, C. Cambon, Naunyn-Schmiedeberg's Arch. Pharmacol. 359 (1999) 428.
[31] N. Seiler, Neurochem. Res. 25 (2000) 471.
[32] A.R. Cross, A.W. Segal, Biochim. Biophys. Acta 1657 (2004) 1.
[33] J.D. Lambeth, Nat. Rev. Immunol. 4 (2004) 181.
[34] M. Geiszt, T.L. Leto, J. Biol. Chem. 279 (2004) 51715.
[35] J.D. Lambeth, T. Kawahara, B. Diebold, Free Radic. Biol. Med. 43 (2007) 319.
[36] K. Bedard, K.H. Krause, Physiol. Rev. 87 (2007) 245.
[37] H. Sauer, M. Wartenberg, J. Hescheler, Cell. Physiol. Biochem. 11 (2001) 173.
[38] P.S. Brookes, Y. Yoon, J.L. Robotham, M.W. Anders, S.-S. Sheu, Am. J. Physiol. Cell. Ph. 287 (2004) C817.
[39] T.E. Gunter, D.I. Yule, K.K. Gunter, R.A. Eliseev, J.D. Salter, FEBS Lett. 567 (2004) 96.
[40] G. Kroemer, L. Galluzzi, C. Brenner, Physiol. Rev. 87 (2007) 99.
[41] E. Cadenas, K.J.A. Davies, Free Radic. Biol. Med. 29 (2000) 222.
[42] H.J. Forman, J.A. Kennedy, Biochem. Biophys. Res. Commun. 60 (1974) 1044.
[43] G. Loschen, A. Azzi, C. Richter, L. Flohé, FEBS Lett. 42 (1974) 68.
[44] G. Loschen, L. Flohé, B. Chance, FEBS Lett. 18 (1971) 261.
[45] S. Raha, B.H. Robinson, Trends Biochem. Sci. 25 (2000) 502.
[46] M. Giorgio, M. Trinei, E. Migliaccio, P.G. Pelicci, Nat. Rev. Mol. Cell. Biol. 8 (2007) 722.
[47] A.Y. Andreyev, Y.E. Kushnareva, A.A. Starkov, Biochemistry (Moscow) 70 (2005) 200.
[48] M.P. Rigobello, A. Folda, G. Scutari, A. Bindoli, Arch. Biochem. Biophys. 441 (2005) 112.
[49] F. Zoccarato, L. Cavallini, A. Alexandre, J. Biol. Chem. 279 (2004) 4166.
[50] W. Dröge, Physiol. Rev. 82 (2002) 47.
[51] D.X. Zhang, D.D. Gutterman, Am. J. Physiol. Heart Circ. Physiol. 292 (2007) H2023.
[52] P.S. Brookes, A.-L. Levonen, S. Shiva, P. Sarti, V.M. Darley-Usmar, Free Radic. Biol. Med. 33 (2002) 755.
[53] E. Cadenas, Mol. Aspects Med. 25 (2004) 17.
[54] L.A. Dada, N.S. Chandel, K.M. Ridge, C. Pedemonte, A.M. Bertorello, J.I. Sznajder, J. Clin. Invest. 111 (2003) 1057.
[55] T. Finkel, FEBS Lett. 476 (2000) 52.
[56] K.H. Kim, A.M. Rodriguez, P.M. Carrico, J.A. Melendez, Antioxid. Redox Signal. 3 (2001) 361.
[57] S. Nemoto, K. Takeda, Z.-X. Yu, V.J. Ferrans, T. Finkel, Mol. Cell. Biol. 20 (2000) 7311.
[58] A.C. Ranganathan, K.K. Nelson, A.M. Rodriguez, K.-H. Kim, G.B. Tower, J.L. Rutter, C.E. Brinckeroff, T.-T. Huang, C.J. Epstein, J.J. Jeffrey, J.A. Melendez, J. Biol. Chem. 276 (2001) 14264.
[59] E. Werner, Z. Werb, J. Cell. Biol. 158 (2002) 357.
[60] N. Li, K. Ragheb, G. Lawler, J. Sturgis, B. Rajwa, J.A. Melendez, J.P. Robinson, J. Biol. Chem. 278 (2003) 8516.
[61] G. Pani, B. Bedogni, R. Colavitti, R. Anzevino, S. Borrello, T. Galeotti, IUBMB Life 52 (2001) 7.
[62] A. Ramachandran, D. Moellering, Y.M. Go, S. Shiva, A.L. Levonen, H. Jo, R.P. Patel, S. Parthasarathy, V.M. Darley-Usmar, Biol. Chem. 383 (2002) 693.
[63] S. Orrenius, V. Gogvadze, B. Zhivotovsky, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 143.
[64] A. Bindoli, J.M. Fukuto, H.J. Forman, Antioxid. Redox Signal. 10 (2008) 1549.
[65] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, fourth ed., Oxford University Press, Oxford, UK, 2007.
[66] D.A. Dickinson, H.J. Forman, Biochem. Pharmacol. 64 (2002) 1019.
[67] J. Lu, E.-H. Chew, A. Holmgren, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 12288.
[68] S.M. Beer, E.R. Taylor, S.E. Brown, C.C. Dahm, N.J. Costa, M.J. Runswick, M.P. Murphy, J. Biol. Chem. 279 (2004) 47939.
[69] C. Berndt, C.H. Lillig, A. Holmgren, Am. J. Physiol. Heart Circ. Physiol. 292 (2007) H1227.
[70] L.V. Papp, J. Lu, A. Holmgren, K.K. Khanna, Antioxid. Redox Signal. 9 (2007) 775.
[71] V.N. Gladyshev, K.T. Jeang, T.C. Stadtman, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 6146.
[72] L. Zhong, E.S. Arnér, J. Ljung, F. Aslund, A. Holmgren, J. Biol. Chem. 273 (1998) 8581.
[73] S.-R. Lee, J.-R. Kim, K.-S. Kwon, H.W. Yoon, R.L. Levine, A. Ginsburg, S.G. Rhee, J. Biol. Chem. 274 (1999) 4722.
[74] M.P. Rigobello, M.P. Callegaro, E. Barzon, M. Benetti, A. Bindoli, Free Radic. Biol. Med. 24 (1998) 370.
[75] J. Haendeler, Antioxid. Redox Signal. 8 (2006) 1723.
[76] C.W. Gruber, M. Čemažar, B. Heras, J.L. Martin, D.J. Craik, Trends Biochem. Sci. 31 (2006) 455.
[77] E.S. Arnér, A. Holmgren, Eur. J. Biochem. 267 (2000) 6102.
[78] C.H. Lillig, A. Holmgren, Antioxid. Redox Signal. 9 (2007) 25.
[79] G. Powis, M. Briehl, J. Oblong, Pharmacol. Ther. 68 (1995) 149.
[80] K.F. Tonissen, G. Di Trapani, Mol. Nutr. Food Res. 53 (2009) 87.
[81] T.C. Laurent, E.C. Moore, P. Reichard, J. Biol. Chem. 239 (1964) 3436.
[82] R.L. Levine, J. Moskovitz, E.R. Stadtman, IUBMB Life 50 (2000) 301.
[83] B. Hofmann, H.J. Hecht, L. Flohé, Biol. Chem. 383 (2002) 347.
[84] S.G. Rhee, H.Y. Chae, K. Kim, Free Radic. Biol. Med. 38 (2005) 1543.
[85] M. Saitoh, H. Nishitoh, M. Fujii, K. Takeda, K. Tobiume, Y. Sawada, M. Kawabata, K. Miyazono, H. Ichijo, EMBO J. 17 (1998) 2596.
[86] Y. Kabe, K. Ando, S. Hirao, M. Yoshida, H. Handa, Antioxid. Redox Signal. 7 (2005) 395.
[87] J.R. Matthews, N. Wakasugi, J.L. Virelizier, J. Yodoi, R.T. Hay, Nucleic Acid Res. 20 (1992) 3821.
[88] R. Brigelius-Flohé, Free Radic. Biol. Med. 27 (1999) 951.
[89] S.J. Welsh, W.T. Bellamy, M.M. Briehl, G. Powis, Cancer Res. 62 (2002) 5089.
[90] Y. Makino, N. Yoshikawa, K. Okamoto, K. Hirota, J. Yodoi, I. Makino, H. Tanaka, J. Biol. Chem. 274 (1999) 3182.
[91] K. Hirota, M. Matsui, S. Iwata, A. Nishiyama, K. Mori, J. Yodoi, Proc. Natl. Acad Sci. U.S.A. 94 (1997) 3633.
[92] A. Holmgren, M. Luthman, Biochemistry 17 (1978) 4071.
[93] P. Patwari, R.T. Lee, Am. J. Pathol. 170 (2007) 805.
[94] E.W. Trotter, C.M. Grant, EMBO Rep. 4 (2003) 184.
[95] C. Johansson, C.H. Lillig, A. Holmgren, J. Biol. Chem. 279 (2004) 7537.
[96] C.H. Williams Jr., in: F. Müller (Ed.), Chemistry and Biochemistry of Flavoenzymes III, CRC, Boca Raton, FL, 1992.
[97] M. Matsui, M. Oshima, H. Oshima, K. Takaku, T. Maruyama, J. Yodoi, M.M. Taketo, Dev. Biol. 178 (1996) 179.
[98] P.Y. Gasdaska, J.R. Gasdaska, S. Cochran, G. Powis, FEBS Lett. 373 (1995) 5.
[99] C. Jakupoglu, G.K. Przemeck, M. Schneider, S.G. Moreno, N. Mayr, A.K. Hatzopoulos, M.H. de Angelis, W. Wurst, G.W. Bornkamm, M. Brielmeier, M. Conrad, Mol. Cell. Biol. 25 (2005) 1980.
[100] A.A. Bondareva, M.R. Capecchi, S.V. Iverson, Y. Li, N.I. Lopez, O. Lucas, G.F. Merrill, J.R. Prigge, A.M. Siders, M. Wakamiya, S.L. Wallin, E.E. Schmidt, Free Radic. Biol. Med. 43 (2007) 911.
[101] L. Nonn, R.R. Williams, R.P. Erickson, G. Powis, Mol. Cell. Biol. 23 (2003) 916.
[102] M. Conrad, C. Jakupoglu, S.G. Moreno, S. Lippl, A. Banjac, M. Schneider, H. Beck, A.K. Hatzopoulos, U. Just, F. Sinowatz, W. Schmahl, K.R. Chien, W. Wurst, G.W. Bornkamm, M. Brielmeier, Mol. Cell. Biol. 24 (2004) 9414.
[103] T. Tamura, T.C. Stadtman, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 93, 1006.

[104] P.Y. Gasdaska, M.M. Berggren, M.J. Berry, G. Powis, FEBS Lett. 442 (1999) 105.
[105] A. Miranda-Vizuete, C.M. Sadek, A. Jimenez, M. Krause, P. Sutovsky, R. Oko, Antioxid. Redox Signal. 6 (2004) 25.
[106] Q.A. Sun, D. Su, S.V. Novoselov, B.A. Carlson, D.L. Hatfield, V.N. Gladyshev, Biochemistry 44 (2005) 14528.
[107] D. Mustacich, G. Powis, Biochem. J. 346 (2000) 1.
[108] B.W. Lennon, C.H. Williams Jr., Biochemistry 36 (1997) 9464.
[109] S. Gromer, J. Wissing, D. Behne, K. Ashman, R.H. Schirmer, L. Flohé, K. Becker, Biochem. J. 332 (1998) 591.
[110] T. Sandalova, L. Zhong, Y. Lindqvist, A. Holmgren, G. Schneider, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 9533.
[111] Q.A. Sun, L. Kirnarsky, S. Sherman, V.N. Gladyshev, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 3673.
[112] E.I. Biterova, A.A. Turanov, V.N. Gladyshev, J.J. Barycki, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 15018.
[113] K. Fritz-Wolf, S. Urig, K. Becker, J. Mol. Biol. 370 (2007) 116.
[114] L. Johansson, L.D. Arscott, D.P. Ballou, C.H. Williams Jr., E.S. Arner, Free Radic. Biol. Med. 41 (2006) 649.
[115] L.D. Arscott, S. Gromer, R.H. Schirmer, K. Becker, C.H. Williams Jr., Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 3621.
[116] H. Bauer, V. Massey, L.D. Arscott, R.H. Schirmer, D.P. Ballou, C.H. Williams Jr., J. Biol. Chem. 278 (2003) 33020.
[117] H.F. Gilbert, Adv. Enzymol. Relat. Areas Mol. Biol. 63 (1990) 69.
[118] L.D. Arscott, C. Thorpe, C.H. Williams Jr., Biochemistry 20 (1981) 1513.
[119] S. Gromer, L. Johansson, H. Bauer, L.D. Arscott, S. Rauch, D.P. Ballou, C.H. Williams Jr., R.H. Schirmer, E.S. Arnér, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 12618.
[120] L. Zhong, E.S. Arnér, A. Holmgren, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 5854.
[121] L. Zhong, A. Holmgren, J. Biol. Chem. 275 (2000) 18121.
[122] S.M. Kanzok, A. Fechner, H. Bauer, J.K. Ulschmid, H.M. Müller, J. Botella-Munoz, S. Schneuwly, R. Schirmer, K. Becker, Science 291 (2001) 643.
[123] B.E. Eckenroth, M.A. Rould, R.J. Hondal, S.J. Everse, Biochemistry 46 (2007) 4694.
[124] B.M. Lacey, B.E. Eckenroth, S. Flemer Jr., R.J. Hondal, Biochemistry 47 (2008) 12810.
[125] M. Berggren, A. Gallegos, J.R. Gasdaska, P.Y. Gasdaska, J. Warneke, G. Powis, Anticancer Res. 16 (1996) 3459.
[126] P. Gasdaska, J.E. Oblong, I.A. Cotgreave, G. Powis, Biochim. Biophys. Acta 1218 (1994) 292.
[127] H. Nakamura, Y. Masutani, A. Tagaya, T. Yamauchi, Y. Inamoto, S. Nanbu, K. Fuji, J. Ozawa, Yodoi, Cancer 69 (1992) 2091.
[128] H. Nakamura, J. Bai, Y. Nishinaka, S. Ueda, T. Sasada, G. Ohshio, M. Imamura, A. Takabayashi, Y. Yamaoka, J. Yodoi, Cancer Detect. Prev. 24 (2000) 53.
[129] A.K. Rundlöf, E.S.J. Arnér, Antioxid. Redox Signal. 6 (2004) 41.
[130] E.S. Arnér, A. Holmgren, Semin. Cancer Biol. 16 (2006) 420.
[131] P.B. Cassidy, K. Edes, C.C. Nelson, K. Parsawar, F.A. Fitzpatrick, P.J. Moos, Carcinogenesis 27 (2006) 2538.
[132] S. Gromer, S. Urig, K. Becker, Med. Res. Rev. 24 (2004) 40.
[133] J.D. Pennington, T.J.C. Wang, P. Nguyen, L. Sun, K. Bisht, D.D. Smart, D. Gius, Drug Resist. Updat. 8 (2005) 322.
[134] G. Powis, D.L. Kirkpatrick, Curr. Opin. Pharmacol. 7 (2007) 392.
[135] S. Urig, K. Becker, Semin. Cancer Biol. 16 (2006) 452.
[136] M.-H. Yoo, X.-M. Xu, B.A. Carlson, V.N. Gladyshev, D.L. Hatfield, J. Biol. Chem. 281 (2006) 13005.
[137] J. Fang, J. Lu, A. Holmgren, J. Biol. Chem. 280 (2005) 25284.
[138] J. Nordberg, L. Zhong, A. Holmgren, E.S.J. Arnér, J. Biol. Chem. 273 (1998) 10835.
[139] E.S. Arnér, Biofactors 10 (1999) 219.
[140] N. Cenas, S. Prast, H. Nivinskas, J. Sarlauskas, E.S. Arnér, J. Biol. Chem. 281 (2006) 5593.
[141] Z. Liu, Z.-Y. Du, Z.-S. Huang, K.-S. Lee, L.-Q. Gu, Biosci. Biotechnol. Biochem. 72 (2008) 1.
[142] J. Lu, L.V. Papp, J. Fang, S. Rodriguez-Nieto, B. Zhivotovsky, A. Holmgren, Cancer Res. 66 (2006) 4410.
[143] N. Cenas, H. Nivinskas, Z. Anusievicius, J. Sarlauskas, F. Lederer, E.S. Arnér, J. Biol. Chem. 279 (2004) 2583.
[144] G. Powis, P. Wipf, S.M. Lynch, A. Birmingham, D.L. Kirkpatrick, Mol. Cancer Ther. 5 (2006) 630.
[145] J. Fang, A. Holmgren, J. Am. Chem. Soc. 128 (2006) 1879.
[146] Y.S. Park, N. Taniguchi, Ann. N.Y. Acad. Sci. 1126 (2008) 185.
[147] M. Luthman, A. Holmgren, Biochemistry 21 (1982) 6628.
[148] S.I. Hashemy, J.S. Ungerstedt, F.Z. Avval, A. Holmgren, J. Biol. Chem. 281 (2006) 10691.
[149] E.S.J. Arnér, H. Nakamura, T. Sasada, J. Yodoi, A. Holmgren, G. Spyrou, Free Radic. Biol. Med. 31 (2001) 1170.
[150] K. Becker, C. Herold-Mende, J.J. Park, G. Lowe, R.H. Schirmer, J. Med. Chem. 44 (2001) 2784.
[151] M.J. Clarke, Coord. Chem. Rev. 236 (2003) 209.
[152] P. Mura, M. Camalli, A. Bindoli, F. Sorrentino, A. Casini, C. Gabbiani, M. Corsini, P. Zanello, M.P. Rigobello, L. Messori, J. Med. Chem. 50 (2007) 5871.
[153] A. Casini, C. Gabbiani, F. Sorrentino, M.P. Rigobello, A. Bindoli, T.J. Geldbach, A. Marrone, N. Re, C.G. Hartinger, P.J. Dyson, L. Messori, J. Med. Chem. 51 (2008) 6773.
[154] M.P. Rigobello, F. Vianello, A. Folda, C. Roman, G. Scutari, A. Bindoli, Biochem. Biophys. Res. Commun. 343 (2006) 873.
[155] K.E. Hill, G.W. McCollum, M.E. Boeglin, R.F. Burk, Biochem. Biophys. Res. Commun. 234 (1997) 293.
[156] S. Gromer, L.D. Arscott, C.H. Williams Jr., R.H. Schirmer, K. Becker, J. Biol. Chem. 273 (1998) 20096.
[157] M.P. Rigobello, G. Scutari, R. Boscolo, A. Bindoli, Br. J. Pharmacol. 136 (2002) 1162.
[158] M.J. McKeage, L. Maharaj, S.J. Berners-Price, Coord. Chem. Rev. 232 (2002) 127.
[159] M.J. McKeage, Br. J. Pharmacol. 136 (2002) 1081.
[160] M.P. Rigobello, L. Messori, G. Marcon, M. Agostina Cinellu, M. Bragadin, A. Folda, G. Scutari, A. Bindoli, J. Inorg. Biochem. 98 (2004) 1634.
[161] M. Coronnello, E. Mini, B. Caciagli, M.A. Cinellu, A. Bindoli, C. Gabbiani, L. Messori, J. Med. Chem. 48 (2005) 6761.
[162] L. Engman, M. McNaughton, M. Gajewska, S. Kumar, A. Birmingham, G. Powis, Anti-Cancer Drugs 17 (2006) 539.
[163] Y. Omata, M. Folan, M. Shaw, R.L. Messer, P.E. Lockwood, D. Hobbs, S. Bouilaguet, H. Sano, J.B. Lewis, J.C. Wataha, Toxicol. In Vitro 20 (2006) 882.
[164] S. Urig, K. Fritz-Wolf, R. Réau, C. Herold-Mende, K. Tóth, E. Davioud-Charvet, K. Becker, Angew. Chem. Int. Ed. 45 (2006) 1881.
[165] O. Rackham, S.J. Nichols, P.J. Leedman, S.J. Berners-Price, A. Filipovska, Biochem. Pharmacol. 74 (2007) 992.
[166] J.L. Hickey, R.A. Ruhayel, P.J. Barnard, M.V. Baker, S.J. Berners-Price, A. Filipovska, J. Am. Chem. Soc. 130 (2008) 12570.
[167] M. Deponte, S. Urig, L.D. Arscott, K. Fritz-Wolf, R. Réau, C. Herold-Mende, S. Koncarevic, M. Meyer, E. Davioud-Charvet, D.P. Ballou, C.H. Williams Jr., K. Becker, J. Biol. Chem. 280 (2005) 20628.
[168] J. Talib, J.L. Beck, S.F. Ralph, J. Biol. Inorg. Chem. 11 (2006) 559.
[169] G. Marcon, L. Messori, P. Orioli, M.A. Cinellu, G. Minghetti, Eur. J. Biochem. 270 (2003) 4655.
[170] No BIAM results are reported for auranofin due to a severe interference of the phophine ligand with this kind of biochemical assay.
[171] S.J. Park, I.-S. Kim, Br. J. Pharmacol. 146 (2005) 506.
[172] F. Caruso, R. Villa, M. Rossi, C. Pettinari, F. Paduano, M. Pennati, M.G. Daidone, N. Zaffaroni, Biochem. Pharmacol. 73 (2006) 773.
[173] C. Marzano, V. Gandin, A. Folda, G. Scutari, A. Bindoli, M.P. Rigobello, Free Radic. Biol. Med. 42 (2007) 872.
[174] J.J. Liu, P. Galettis, A. Farr, L. Maharaj, H. Samarasinha, A.C. McGechan, B.C. Baguley, R.J. Bowen, S.J. Berners-Price, M.J. McKeage, J. Inorg. Biochem. 102 (2008) 303.
[175] Y.M. Go, D.P. Jones, Biochim. Biophys. Acta 1780 (2008) 1273.
[176] P.H. Bessette, F. Åslund, J. Beckwith, G. Georgiou, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 13703.
[177] E.J. Stewart, F. Åslund, J. Beckwith, EMBO J. 17 (1998) 5543.
[178] A.G. Cox, K.K. Brown, E.S.J. Arnér, M.B. Hampton, Biochem. Pharmacol. 76 (2008) 1097.
[179] M.P. Rigobello, G. Scutari, A. Folda, A. Bindoli, Biochem. Pharmacol. 67 (2004) 689.
[180] M.P. Rigobello, A. Folda, B. Dani, R. Menabò, G. Scutari, A. Bindoli, Eur. J. Pharmacol. 582 (2008) 26.
[181] M.P. Rigobello, A. Folda, M.C. Baldoin, G. Scutari, A. Bindoli, Free Radic. Res. 39 (2005) 687.
[182] D. Aldinucci, D. Lorenzon, L. Stefani, L. Giovagnini, A. Colombatti, D. Fregona, Anticancer Drugs 8 (2007) 323.
[183] Y. Tsujimoto, S. Shimizu, Apoptosis 12 (2007) 835.
[184] M. Saitoh, H. Nishito, M. Fujii, EMBO J. 17 (1998) 2596.
[185] J.L. Martindale, N.J. Holbrook, J. Cell. Physiol. 192 (2002) 1.
[186] J.A. McCubrey, M.M. LaHair, R.A. Franklin, Antioxid. Redox Signal. 8 (2006) 1775.
[187] S. Zhuang, R.G. Schnellmann, J. Pharmacol. Exp. Ther. 319 (2006) 991.
[188] M.-R. Kim, H.-S. Chang, B.-H. Kim, S. Kim, S.-H. Baek, J.-H. Kim, S.-R. Lee, J.-R. Kim, Biochem. Biophys. Res. Commun. 304 (2003) 119.
[189] N.R. Bhat, P. Zhang, J. Neurochem. 72 (1999) 112.
[190] B.G. Park, C.I. Yoo, H.T. Kim, C.H. Kwon, Y.K. Kim, Toxicology 215 (2005) 115.
[191] S. Yamagishi, T. Matsumoto, T. Numakawa, D. Yokomaku, N. Adachi, H. Hatanaka, M. Yamada, K. Shimoke, T. Ikeuchi, Brain Res. 1038 (2005) 223.
[192] S. Sakon, X. Xue, M. Takekawa, T. Sasazuki, T. Okazaki, Y. Kojima, J.H. Piao, H. Yagita, K. Okumura, T. Doi, H. Nakano, EMBO J. 22 (2003) 3898.
[193] L. Zhong, R. Zhao, A. Holmgren, Toxicol. Appl. Pharmacol. 207 (2005) 103.
[194] L. Engman, I. Cotgreave, M. Angulo, C.W. Taylor, G.D. Paine-Murrieta, G. Powis, Anticancer Res. 17 (1997) 4599.
[195] L. Engman, N. Al-Maharik, M. McNaughton, A. Birmingham, G. Powis, Bioorg. Med. Chem. 11 (2003) 5091.
[196] D. Magda, R.A. Miller, Semin. Cancer Biol. 16 (2006) 466.
[197] M.A. Larkin, G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, D.G. Higgins, Bioinformatics 23 (2007) 2947.