Journal of Inorganic Biochemistry 119 (2013) 38–42

Contents lists available at SciVerse ScienceDirect

Journal of Inorganic Biochemistry

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

A new target for gold(I) compounds: Glutathione-S-transferase inhibition by auranofin

Anastasia De Luca [a], Christian G. Hartinger [b], Paul J. Dyson [c,*], Mario Lo Bello [a], Angela Casini [d,**]

[a] Department of Biology, University of Rome Tor Vergata, Via della Ricerca Scientifica snc, Rome 00133, Italy
[b] Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[c] School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
[d] Pharmacokinetics, Toxicology and Targeting, Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 17 May 2012
Received in revised form 15 August 2012
Accepted 17 August 2012
Available online 24 August 2012

Keywords:
Gold complexes
GST enzymes
Cytotoxicity
Mass spectrometry

ABSTRACT

Nowadays, gold compounds occupy a relevant position constituting a promising class of experimental anticancer metallodrugs. Several research efforts have been devoted to the investigations of the pharmacological properties of gold(I) complexes bearing phosphine ligands, such as the antiarthritic drug auranofin, that has also been shown to produce anticancer effects *in vitro*. In spite of the numerous studies that appeared in the literature the biological mechanisms of action of auranofin and analogues are still controversial. Here, we report on the inhibition effects of glutathione S-transferase P1-1 (GST P1-1) exerted by auranofin. The compound was able to inhibit GST P1-1 with a calculated IC_{50} of 32.9 ± 0.5 μM. Interestingly, the inhibition of GST P1-1 and its cysteine mutants by the gold(I) compound is essentially the same, suggesting that probably the cysteine residues are not so essential for enzyme inactivation in contrast to other reported inhibitors. High-resolution electrospray ionisation Fourier transform ion cyclotron mass spectrometry (ESI FT-ICR MS) studies allowed characterising the binding of the compound with GST enzymes at a molecular level, confirming that similar gold binding sites may be present in the wild-type protein and its Cys mutants.

1. Introduction

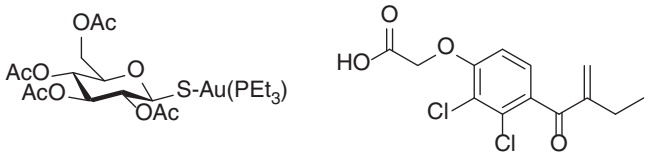
A number of injectable gold(I) thiolate drugs, in particular myochrysine, sanochrysine, allochrysine, and solganol have been widely employed in the clinic for the treatment of rheumatoid arthritis [1]. In 1979, the antiarthritic gold(I) phosphine compound [(2,3,4,6-tetra-O-acetyl-1-(thio-κS)-β-D-glucopyranosato)(triethylphosphine)gold(I)], auranofin (AUF), (Chart 1) was introduced in the clinic, with the significant advantage of oral administration [2,1]. Interestingly, early studies also indicated that AUF possesses *in vitro* anticancer activity similar to that of cisplatin [3,4], and subsequently many gold(I) compounds have been shown to manifest outstanding antiproliferative properties against selected human tumour cell lines sensitive and resistant to classical platinum drugs [5–8], among which thiolate Au(I) derivatives with phosphine ligands [9,10]. Moreover, some of these compounds performed remarkably well even in tumour models *in vivo*. Notably, the properties of the gold centre impart innovative pharmacological profiles to gold complexes and novel molecular mechanisms that allow them to overcome resistance pathways related to platinum-based chemotherapy. Indeed, for gold compounds, at variance with cisplatin, DNA is not the major pharmacological target, but inhibition of essential enzymes appears to be relevant [11,12]. As an example, some of us recently reported on the potent inhibition of the zinc finger protein PARP-1 by cytotoxic gold(I) and gold(III) complexes [13,14].

In a proteomic study on platinum-resistant human ovarian cancer cell lines five proteins were identified to be differentially expressed compared to their parental cisplatin-resistant cells, including a glutathione S-transferase (GST) [15], which is overexpressed in several chemoresistant tumours [16]. GSTs (E.C. 2.5.1.18) are a group of enzymes involved in cellular detoxification processes from toxic compounds of both endogenous and xenobiotic origin [17]. They exert their function *via* the conjugation of reduced glutathione (GSH) to the electrophilic centre of noxious compounds, representing the first step of the mercapturic acid detoxification pathway [18]. GSTs may be divided in three major families: cytosolic, mitochondrial and microsomal GSTs [19]. Among the cytosolic GSTs, attention has been focused on GST P1-1 due to its emerging role in cancer and the acquisition of drug resistance [20]. In cancer cell lines the extent of GST P1-1 expression may be correlated to the efficacy of anticancer drugs [21].

Several studies are aimed to the synthesis of new GST P1-1 inhibitors designed to inactivate the detoxification effect of the enzyme and promoting a higher efficacy of the anticancer drugs in the tumour that had acquired resistance to chemotherapeutic treatments [22,23,16,24–26]. Some of these molecules show considerable promise and are currently

* Corresponding author. Fax: +41 21 693 98 85.
** Corresponding author. Fax: +31 50 363 3274.
E-mail addresses: paul.dyson@epfl.ch (P.J. Dyson), a.casini@rug.ch (A. Casini).

0162-0134/$ – see front matter © 2012 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.jinorgbio.2012.08.006



auranofin (AUF) ethacrynic acid (EA)

Chart 1. Structures of auranofin (AUF) and ethacrynic acid (EA).

undergoing clinical trials in combination regimens for the treatment of specific forms of advanced cancers [27]. Within this frame, the *trans*-Pt^{IV} carboxylate complex containing ethacrynate ligands, ethacraplatin, designed as an anticancer metallodrug targeting cytosolic GST enzymes, was reported to cause potent and selective inhibition of GST P1-1 [28].

The lack of cross-resistance of auranofin with cisplatin with respect to cancer cells is intriguing, and we hypothesised that it might be due to direct enzyme inhibition of GST. Consequently, we evaluated the enzyme inhibitory activity of AUF and characterised the interactions between the compound and GST at a molecular level by high-resolution electrospray ionisation Fourier transform ion cyclotron mass spectrometry (ESI FT-ICR MS).

2. Results and discussion

The active site in the various classes of GST enzymes is constituted by the G site, the site of GSH binding, and the H site, for the binding of electrophilic substrates such as 1-chloro-2,4-dinitrobenzene (CDNB) [29–31]. GST P1-1 possesses two solvent accessible cysteine residues that affect catalytic activity when modified. Cys47 is located near the G-site and is critical for maintaining the conformation and stability of the G-site. Cys101 is located at the dimer interface and can form a disulfide bridge with Cys47, requiring a large scale conformational change of the active site, and leading to inactivation of the enzyme [32]. These two cysteine residues can also interact with metal ions [33,34]. Therefore, GST P1-1 and different mutants were prepared containing single or double mutation at Cys47 and Cys101, and their inhibition by AUF was evaluated spectrophotometrically (see Experimental section for details). The inhibition curves and IC_{50} values determined for the wild-type protein and the cysteine mutants C47S, C101S and C47S/C101S are reported in Fig. 1 and Table 1, respectively. A similar approach was used to study the efficacy of ethacrynic acid (EA, Chart 1), a known GST inhibitor undergoing clinical evaluation [17], and organometallic ruthenium-based inhibitors of GST P1-1 [35,22]. In the case of AUF the inhibition of GST P1-1 and its cysteine mutants is essentially the same, suggesting that

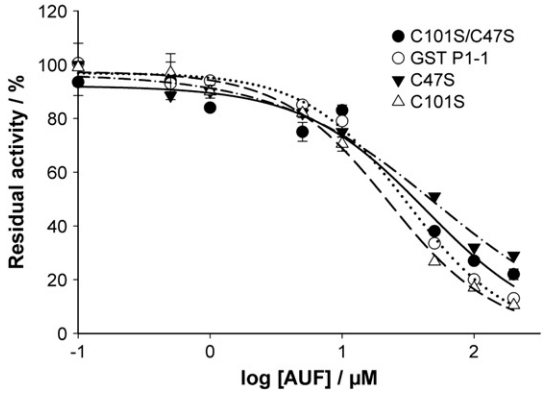


Fig. 1. Inhibition curves of AUF on GST P1-1 and the C101S and C47S mutants. The activities of the wild-type enzyme GST P1-1, and its cysteine mutants, C101S, C47S and C101S/C47S were assayed in the presence of increasing concentrations of AUF.

Table 1
IC_{50} values calculated for AUF inhibition of wild type GST P1-1 and its cysteine mutants. Values for EA are taken from Ref. [35].

Protein	IC_{50} (μM) (±SD)	
	AUF	EA
GST P1-1	32.9 ± 0.5	12.0
C47S	23.2 ± 0.4	42.9
C101S	26.8 ± 0.7	19.4
C47S/C101S	24.6 ± 0.5	–

probably the cysteine residues are not preferentially targeted by AUF in contrast to other inhibitors [35]. Moreover, the extent of inhibition induced by AUF is inferior to that of EA (32.9 ± 0.5 *vs.* 12.0 for wild type GST P1-1) [35]. EA is known to bind irreversibly to Cys47 *via* a Michael addition over time [17], which also indicates a different mode of binding with respect to gold complex.

Competitive inhibition studies analysing GST P1-1 activity while varying the substrate concentration (GSH or CDNB) in the presence of fixed AUF concentrations were undertaken. The obtained results (see Supplementary information available, Figs. S1–S2) demonstrate that AUF acts as a competitive inhibitor towards CDNB and as a non-competitive inhibitor towards GSH. This behaviour affects the kinetic parameters of the enzyme (Table 2) increasing the apparent K_m^{CDNB} by *ca.* 3-fold, and reducing *ca.* 7-fold the catalytic efficiency of the enzyme (k_{cat}/K_m^{CDNB}). The K_i values are also comparable to the ones previously reported for the ruthenium complexes mentioned above [22]. From these studies it could be assumed that the complex binds at the catalytic H-site, in a similar fashion to EA and the ruthenium-based GST inhibitors.

Further analysis on the metallation properties of AUF on GST P1-1 was performed by incubating GST P1-1 and the mutants with AUF at 37 °C at 1:2 protein:metal ratio, and monitoring the activity over a period of 30 min (Fig. 2). GST P1-1 reached 50% inactivation after 7 min of incubation, whereas the C47S mutant reached the same level of inactivation after only 1 min. The C101S mutant and the doubly mutated C47S/C101S enzyme are more resistant to inactivation and were reaching only up to 30% inactivation after 3 and 5 min, respectively. These data suggest that the main metallation target of AUF is C101, resulting in a higher inactivation when it is the only cysteine present, as in the case of the C47S mutant. Moreover, in the absence of both C47 and C101, as simulated by the double mutant C47S/C101S, the drug appears not being able to find other metallation sites effective for potent enzyme inhibition.

To ascertain the nature of the interaction between AUF and the enzyme at a molecular level, the various GST enzymes incubated with AUF were analysed by high-resolution ESI-FT-ICR-MS. This experimental approach is similar to that previously reported by some of us, in which the reactivity of representative metallodrugs with proteins was probed without using any chromatographic separation prior to analysis [36,37]. Incubation of GST P1-1 (molecular weight 23,357 Da) with AUF in 1:2 (metal:protein) stoichiometric ratio for 1 h at 37 °C leads to the formation of GST adducts. Fig. 3 shows the ESI-FT-ICR-mass spectra of GST P1-1 recorded before (top spectrum) and 30 min after addition of AUF (bottom spectrum), focusing on the *m/z* range containing the 21+ ions. Notably, the protein spectrum shows two major peaks at approximately *m/z* 1106 and 1113 corresponding to GST P1-1 and its isoform deprived of a Met residue [GST P1-1 − Met] [38], respectively. After 1 h incubation, in addition to the peaks of the unreacted protein, new signals appear corresponding to gold adducts. Fragments in which only the triethylphosphine (PEt_3) ligand has been retained by gold, *i.e.* [GST–Au(PEt_3)], are observed bound to the two protein isoforms as detailed in Table 3. Both mono- and bis-adducts of GST with these metallo-fragments are formed, indicating that at least two gold