Quantification of bindings of organometallic ruthenium complexes to GSTπ by mass spectrometry

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ARTICLE INFO

Article history:
Received 2 December 2014
Received in revised form 22 February 2015
Accepted 22 February 2015
Available online 2 March 2015

Keywords:
Mass spectrometry
Quantification
Organometallic ruthenium complexes
Glutathione-S-transferase
Binding stoichiometry

ABSTRACT

Electrospray ionization mass spectrometry (ESI-MS) has been widely used to identify binding sites of metal complexes to proteins. However, the MS quantification of the metal–protein coordination remains a challenge. We have recently demonstrated by ESI-MS analysis that organometallic ruthenium complexes [(η⁶-arene)Ru(en)Cl]+ (arene = p-cymene (1), biphenyl (2) or 9,10-dihydrophenanthrene (3); en = ethylenediamine) bound to human glutathione-S-transferase π (GSTπ) at Cys15 and Cys48 within the G-site, and Cys102 and Met92 on the interface of the GSTπ dimer, showing inhibitory potency against the enzyme (J. Inorg. Biochem., 128 (2013) 77–84). Herein, we developed a mass spectrometric method to quantify the binding stoichiometry of the three complexes to GSTπ. The differences in signal intensities of the heavy-labelled peptides produced by tryptic digestion of the ruthenated GSTπ complexes and the respective light-labelled peptides in the tryptic digest of equimolar GSTπ were used to calculate the binding stoichiometry at specific residues. The results indicated that the pre-complexation of GSTπ with its substrate GSH significantly reduced the bindings of the ruthenium complexes at Met92 and Cys102, but had little impact on the bindings at Cys15 and Cys48. As the inhibitory activities of the ruthenium complexes against GSTπ are similar to those against GSTπ in complexation with GSH, these results suggest that the inhibition of the ruthenium complexes on GSTπ is attributed to the ruthenation at Cys15 and Cys48. The present work provides not only insights into the understanding on the inhibitory mechanism of ruthenium complexes GSTπ, but also a general method for quantitative characterization of metal–protein interactions.

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1. Introduction

With the advantages of high sensitivity, low sample consumption and chemical specificity, electrospray ionization mass spectrometry (ESI-MS) has become one of the most powerful tools for characterization of interactions between metallodrugs or drug candidates and proteins [1–4], in particular for elucidation of binding sites of metals in the proteins [5–13]. However, the uncertain alteration on ionization efficiency of proteins/peptides due to metallation and the lack of suitable internal standards make it a challenge to quantify the bindings of metal complexes to a specific site (residue) of the targeted proteins by ESI-MS, though inductive coupling plasma mass spectrometry (ICP-MS) in combination with various chromatography separation techniques has been widely used to determine the binding stoichiometry of metallodrugs to whole proteins [1,2,14–17].

Organometallic ruthenium(II) complexes [(η⁶-arene)Ru(YZ)(X)]n+, where X is a halide and YZ is a chelating diamine such as ethylenediamine (en), have been demonstrated in vitro and in vivo anticancer activity, including cytotoxicity against cisplatin-resistant cell lines [18–20]. The cytotoxicity towards human ovarian cancer cell line A2780 of the chlorido ruthenium complexes [(η⁶–arene)Ru(en)Cl]n+ increases with the increase in the size of the arene ligands [18,19]. Despite DNA has been thought to be a potential target for these ruthenium(II) arene complexes [21,22], they have also been shown to be active for binding to cysteine and methionine [23], histidine [24] and the three types of residues in glutathione (GSH) [25], serum proteins albumin [7] and transferrin [5]. Importantly, the Ru-thiol coordination can induce the oxidation of thiolates to sulfenates or sulfinites [7,25], and subsequently stabilize the sulfenato ligands [26], which as free ligands are unstable and highly reactive [27,28]. Because the thiol groups in proteins often play a crucial role in regulation of protein functions, the bindings of ruthenium arene complexes to protein
thiols as well as the subsequently induced oxidation of the thiolate ligands may be involved in the action of these drug candidates.

Recently, with the use of ESI-MS combined with tryptic digestion, we have demonstrated that the ruthenium arene complexes [(η⁶-arene)Ru(en)Cl]⁺ (arene = p-cymene (p-cym, 1), biphenyl (bip, 2) or dihydrophenanthrene (dhpa, 3), en = ethylenediamine) [19,29] (Fig. 1a) could inhibit the activity of human glutathione-S-transferase π (GSTπ) by binding to Cys15 and Cys48 residues within the G-site of GSTπ, and Met92 and Cys102 residues on the interface of the GSTπ dimers [10]. The binding pattern of the (arene)Ru(en) complexes to GSTπ is different from that of the (EA-arene)Ru(pta) complex (EA = ethacrynic acid; pta = 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane), which has been shown to bind preferentially to Cys102 (Cys101 in the literature) of the enzyme [30]. Furthermore, the (arene)Ru(en)-thiol coordination was found to be able to induce the oxidation of thiolates, affording stable sulfenato adducts [10] as did the bindings of ruthenium arene complexes to GSH [25,31]. The inhibitory efficiency towards GSTπ depended on type of the arene ligands in the three ruthenium dimers [10].

![Fig. 1.](image)

Fig. 1. (a) Chemical structures of the organometallic ruthenium complexes studied in this work; (b) Diagrammatic scheme for the determination of binding stoichiometry of ruthenium arene complexes to a cysteine (R=H) or methionine (R=CH₃) residue in a targeted protein. When the ruthenium complexes bind to cysteinyl thiols (−SR= −SH), the thiolato adducts will present in −S[Ru]⁺ form. [Ru]=[(η⁶-arene)Ru(en)Cl]⁺, [Ru]⁺=[(η⁶-arene)Ru(en)]²⁺. It is notable that the scheme demonstrates only the mono-alkylation labelling at the terminal amine group of a tryptic peptide.
complexes, decreasing in the order of $3 > 1 > 2$ [10]. However, lacking quantitative data on the Ru–S coordination, the exact mechanism of the inhibition remains unclear. In the present work, therefore, we developed a mass spectrometric strategy as shown in Fig. 1b to quantify indirectly the binding stoichiometry of ruthenium arene complexes 1, 2 and 3 to specific residues in GSTπ, aiming to provide insights into further understanding on the inhibition mechanism of the ruthenium arene complexes against the enzyme.

2. Experimental

2.1. Materials

$[\eta^5-(p$-cymene$)\text{Ru(en)}\text{Cl}FP_6 (1)[FP_6]], \ [\eta^5-(\text{biphenyl})\text{Ru(en)}\text{Cl}FP_6 (2)[FP_6]]$ and $[\eta^5-(9,10$-dihydrophenanthrene$)\text{Ru(en)}\text{Cl}FP_6 (3)[FP_6]]$ were synthesized following the procedures described in the literature [19, 29]. The N-$h_3$-acetoxysuccinimide and N-$d_3$-acetoxysuccinimide were purchased from Sigma-Aldrich, imidazole from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China), tryptone and yeast extract from Thermo Fisher Scientific, and trypsin (Golden grade) from Promega. Microcon centrifugal ultra-filtration units with a 3 kDa molecular weight cut-off were purchased from Millipore. Aqueous solutions were prepared using MilliQ water (MilliQ Reagent Water System). The GSTπ was expressed in *Escherichia coli* BL21 cells by transformation of pET28a-GSTπ Plasmid as our previous work described [10].

| Table 1 | Ruthenation ratio (%) represented in mean ± SD (n = 3) of GSTπ and GSTπ–GSH by ruthenium complexes 1, 2 and 3 at Cys15, Cys48, Met92 and Cys102 as determined by ESI-MS analysis.
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<td>15 ± 1</td>
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<td>2</td>
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2.2. Preparation of GSNTr-(arene)Ru(en) complexes

Similar to our previous work [10], recombinant GSNTr (16 μM) dialyzed against 20 mM phosphate buffer (pH 7.4) was incubated with 50-fold excess of complex 1, 2 or 3 at 310 K for 2 h, the unbound ruthenium complexes were removed by centrifugal ultra-filtration 3 times with phosphate buffer, affording ruthenated GSNTr complexes, namely GSNTr+1, GSNTr+2 and GSNTr+3. To prepare ruthenated GSNTr in complexation with GSH, 100 μM GSH was added to the 20 mM phosphate buffer used for the dialysis of recombinant GSNTr and the preparation of ruthenated GSNTr complexes which are designated as GSNTr–GSH+1, GSNTr–GSH+2, GSNTr–GSH+3, respectively.

2.3. Tryptic digestion and isotopic labelling of GSNTr and ruthenated GSNTr complexes

An aliquot (50 μL) of GSNTr, GSNTr–GSH or each ruthenated GSNTr complex (56 μM in GSNTr) was mixed with 2 μg trypsin, giving a substrate/enzyme ratio of ca. 40:1, and incubated at 310 K for 14 h for digestion [7,10]. Then, the trypptic digests of GSNTr or GSNTr–GSH and the ruthenated GSNTr complexes individually reacted with N-acetoxy-h3-succinimide (h3-NAS) and N-acetoxy-d3-succinimide (d3-NAS) at a GSNTr/NAS ratio of 1:50 at room temperature for 5 h [32], producing light-labelled trypptic peptides of GSNTr or GSNTr–GSH and heavy-labelled trypptic complexes of each ruthenated GSNTr complex, respectively, for subsequent MS analysis.

2.4. HPLC–ESI-MS

Positive-ion electrospray ionization mass spectra were obtained on a Micromass Q-TOF mass spectrometer (Waters) coupled to a Waters CapLC system. The trypptic digests of GSNTr and ruthenated GSNTr adducts were separated on a Symmetry-C18 column (1.0 × 50 mm, 100 Å, 3.5 μm, Waters). Mobile phases were A: 95% H2O containing 4.9% acetonitrile and 0.1% formic acid, and B: 95% acetonitrile containing 4.9% H2O and 0.1% formic acid. The peptides were eluted with a 50 min linear gradient from 1% to 55% B at a rate of 30 μL min⁻¹. The eluents were directly infused into the mass spectrometer through the ESI probe. The spray voltage of the mass spectrometer was 3.30 kV and the cone voltage 35 V. The desolvation temperature was 413 K and source gas with a voltage 35 V. The spray voltage of the mass spectrometer was 3.30 kV and the cone voltage 35 V.

2.5. Enzyme inhibition assays

The enzyme inhibition assays of GSNTr in pre-complexation with GSH by ruthenium complexes were described in our previous report [10]. In brief, in order to characterize the inhibition potency of the ruthenium complexes against purified GSNTr or GSNTr in complexation with GSH, 16 μM of recombinant GSNTr dialyzed against 20 mM phosphate buffer (pH 7.4) without or with 100 μM GSH was incubated with complex 1, 2 or 3, the majority of which was present in the aqua form [23,25,33], at a molar ratio of 1:50 in 20 mM phosphate buffer containing 0 or 100 μM GSH (pH 7.4) at 310 K for 2 h, affording respective ruthenated GSNTr or GSNTr–GSH complex. Then, an aliquot (2 μL/well) of GSNTr or ruthenated GSNTr was added to phosphate buffer (199 μL/well) containing 1 mM CDNB and 1 mM GSH. The final concentration of GSNTr or each ruthenated GSNTr complex was 160 nM in the reaction mixtures in a 96-well plate. The absorbance at 340 nm of the reaction mixtures was immediately recorded at 20 s intervals on a SpectraMax MS Reader (Molecular Devices, USA). All experiments were performed in eight repeats.

2.6. Molecular modelling

The molecular modelling was performed using Sybyl X 1.1 (Tripos Inc.), running on Dual-core Intel(R) E5300 CPU 2.60 GHz, RAM Memory 2 GB under the Windows XP system. The initial crystal structure of the GSNTr dimer (PDB code 3GSS) in complexation with GSH and ethacrynic acid (EA) [34] was collected from Protein Data Bank. After removing water molecules and the ligands (GSH and EA), all the hydrogen atoms were added to define the correct configuration and tautomeric states, and the atom type was defined as Kollman All. After adding the charge, the modelled structure was energy-minimized using Powell energy minimization algorithm with Kollman All force field, distance dependent dielectric function and current charges with the constraint energy gradient 0.05 kcal mol⁻¹ generating molecular model of GSNTr. The structures of ruthenium complexes 1, 2 and 3 built by the Sybyl program were then individually docked onto Cys15 or Cys48 via replacement of Cl by S, and the initial cooordinative bond length of Ru–S was set to 2.40 Å [7]. The resulting models were energy-minimized again using the same method described above to give respective molecular models of ruthenated GSNTr complexes. Finally, the models of GSNTr and each ruthenated GSNTr complex were overlapped to measure the shift distance of the α-carbon atoms or the centre of five-/six-membered rings in the significant residues around the binding sites.

3. Results and discussion

To achieve the quantification of the bindings of the three ruthenium complexes to GSNTr, the GSNTr–{(arene)Ru(en)} complexes were firstly prepared following the procedure described in the experimental section. Then, non-bound GSNTr and the ruthenated GSNTr complexes, designated as GSNTr+1, GSNTr+2 and GSNTr+3, were individually digested by trypsin, and isotopically labelled by h3-NAS and d3-NAS, respectively. After that, the heavy-labelled trypptic peptides of each ruthenated GSNTr complex were mixed with light-labelled trypptic peptides produced by equal molar GSNTr, and the resulting peptide mixtures were then separated by a C18 reversed-phase column, followed by online ESI-MS analysis. The average change in the signal intensities (peak heights) of isotopic peaks of each pair of heavy/light peptides represents the ruthenation ratio of the respective peptide (Fig. 1b).

We have previously demonstrated by means of bottom-up MS analysis that the tested ruthenium complexes bind to GSNTr at Cys15 (1 and 3) in peptide P2 (C15AALR19), Cys48 (1, 2 and 3) in P5 (A40SCLYCQPK35), Met92 (2 and 3) in P8 (D92QEAALVDVMNHDVRLEP31) and Cys102 (1) in P9 (vide infra) (C102KYVSLNTYNEAGK116) [10]. Being consistent with the MS identification, as shown in Fig. S1 in the Supplementary information, there were no changes in the peak intensities between the pairs of light- and heavy-labelled trypptic peptides P7 (T55GGLGYCK59) and P11 (L148SAR157), of which both do not contain binding sites for the ruthenium arene complexes, produced by the digestion of GSNTr and equimolar GSNTr+1, GSNTr+2 or GSNTr+3 complex.

Due to bindings of the ruthenium complexes, however, the mass spectra (Fig. 2a) showed that the signal intensities of heavy-labelled peptides P2 and P5, which contain the ruthenation sites Cys15 and Cys48, respectively, arising from the digestion of GSNTr+1 complex decreased by 16% (P2) and 14% (P5) in comparison to those of the respective light-labelled peptides in the digest of equimolar GSNTr. This indicates that 1 mol of GSNTr attached 0.16 and 0.14 mol ruthenium

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1 Pi (i = 1, 2, 3 ... or 14) represents the order number of tryptic peptides of GSNTr identified by bottom-up mass spectrometry. For details, see Table S1 in the Supplementary information.
fragment \([(\eta^6-\text{p-cym})\text{Ru(en)}]\) at Cys15 and Cys48, respectively (Table 1). The binding of complex 1 to Cys102 in GSTπ has been previously shown to prevent Lys103 from proteolysis by trypsin, leading to the presence of ruthenated peptide P9’ \((\text{C102KYVSLIYTNYEAGK}_{116})\) in the tryptic digest of GSTπ + 1 complex [10]. As a consequence, we postulate that the intensity of tryptic peptide P9 \((\text{Y104VSLIYTNYEAGK}_{116})\) formed by digestion of ruthenated GSTπ by 1 would be lowered by the binding of complex 1 to Cys102. Indeed, the peak intensity of heavy-labelled P9 was lower than that of the light-labelled P9 in the tryptic digest of GSTπ + 1 complex (Fig. 2a), and the ruthenation ratio of Cys102 was calculated to be 7% (Table 1).

Notably, the mono-alkylated labelling of the peptide P8 containing Met92 made only 3 Da difference in the mass of the light-/heavy-labelled peptides, leading to overlap between isotopic peaks of the doubly-charged light/heavy peptide P8 (Fig. 3a). Thus, a correction was performed on the basis of the theoretical isotopic ratio of doubly-charged P8 (Fig. 3b). After this correction, the average intensity ratio of the first three isotopic peaks of heavy/light P8 was 0.84 (Fig. 3c), indicating that 1 mol GSTπ bound 0.16 mol \([(\eta^6-\text{p-cym})\text{Ru(en)}]\) at Met92. It is worthy of pointing out that our previous MS identification did not observe ruthenated P8 in the digest of GSTπ + 1 complex formed by the reaction of complex 1 with GSTπ dialyzed against phosphate buffer containing 100 μM GSH [10]. The possible reason for failure identifying ruthenated P8 by complex 1 may be attributed to the competition of GSH for ruthenium binding (vide infra).

The bindings of complexes 2 and 3 to GSTπ were analogically quantified using the method described above. The mass spectral data are shown in Figs. S2–S5, and the calculated ruthenation ratios of GSTπ at different sites are listed in Table 1. These results indicate that the bindings of complexes 2 and 3 to Cys102 are negligibility, in agreement with our previous work where no binding of complexes 2 and 3 to Cys102 was observed by MS analysis of the tryptic digests of GSTπ + 2 and GSTπ + 3 complexes [10], while the reactions of complexes 2 and 3 with GSTπ lead to ruthenation of Cys15 and Cys48 up to 12% and 25%, and 15% and 9%, respectively (Table 1). Notably, no binding of complex

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**Fig. 3.** The raw (a, d) and centred (b, e) mass spectra for the doubly-charged ions of light/heavy labelled peptide P8 \((\text{D83QQEAALVDMVNDGVEPLR}_{101})\) arising from the 1:1 mixtures of (a, b) the light-labelled tryptic digest of purified GSTπ and the heavy-labelled tryptic digest of GSTπ + 1 complex, and (d, e) the light-labelled tryptic digest of GSTπ–GSH and the heavy-labelled tryptic digest of GSTπ–GSH + 1 complex, which show the signal overlap between the light/heavy labelled peptide pair; (c, f) mass spectra showing the first three isotopic peaks of the light/heavy labelled P8 after correction of the overlap as shown in (b) and (e), respectively.
2 to GSTπ at Cys15 in GSTπ–GSH complex was observed by previous MS identification [10]. Again, the absence of ruthenated P2 containing Cys15 by complex 2 in the tryptic digest of GSTπ+2 complex may be owe to the competition of GSH for binding to complex 2 such that the abundance of ruthenated P2 was too low to be detectable (vide infra).

Taken together, among the three complexes, complexes 1 and 3 have higher affinity to Cys15 than complex 2, while complex 2 is more active to Cys48 than complexes 1 and 3, and complex 3 is the most active binder to Met92 (Table 1).

The GSTπ enzyme in vivo is in complexation with GSH [35] which may compete with GSTπ for binding to ruthenium via Ru–S coordination [25]. Therefore, we also determined the ruthenation ratios of GSTπ in complexation with GSH by three ruthenium arene complexes. To maintain GSTπ in complex form with GSH, 100 μM GSH was added to the phosphate buffer during the dialysis of the recombinant GSTπ and to the reaction mixtures for preparation of ruthenated GSTπ–GSH complexes. Then, the same quantification analysis as described above was performed to determine the ruthenation ratios of the four tryptic peptides which contain the Ru-binding sites. The MS results are depicted in Figs. 4a–d, and Figs. S2–S5, and the calculated ruthenation ratios listed in Table 1. The results indicate that the ruthenium coordination at Met92 and Cys102 (for 1) significantly reduced, but the bindings of the ruthenium complexes at Cys15 and Cys48 had no pronounced change due to the pre-complexation of GSTπ with GSH. Because the three ruthenium complexes exhibited a similar inhibitory activity against GSTπ in complexation with GSH to that against purified recombinant GSTπ (Figs. 4 and Fig. S6), the MS quantification results suggest that the bindings of complexes 1, 2 and 3 at Cys15 and Cys48 are crucial to their inhibition potency towards GSTπ, and that the ruthenation of Met92 and Cys102 contributes little to the inhibition activity.

It is notable that complex 2 exhibited higher binding affinity to Cys48 within the G-site of the enzyme, but lower inhibitory activity towards GSTπ than complexes 1 and 3 (Table 1, Fig. 2, and Figs. S2, S4 and S6). To address the inconsistence of the binding affinity to the crucial residue (Cys48) and the inhibitory activity against GSTπ of the ruthenium complexes, we constructed the molecular models of the ruthenated GSTπ complexes with [(η^6-arene)Ru(en)] moieties binding to Cys15 or Cys48 using the Sybyl X 1.1 program. As shown in Figs. 5 and

Fig. 4. Residual activity for the first minute of enzymatic reactions of 160 nM GSTπ and GSTπ–GSH complex modified by complex 1, 2 or 3, designated as GSTπ+1, GSTπ+2 and GSTπ+3, and GSTπ–GSH+1, GSTπ–GSH+2 and GSTπ–GSH+3, respective. These results depicted in mean ± SD (n = 8) indicate that the pre-complexation of GSTπ with GSH has no pronounced effect on the inhibitory potency of the three ruthenium arene complexes against the enzyme.
S7, the bindings of the three complexes to Cys15 and Cys48 remarkably alter the conformation of the G-site, which functions to anchor the substrate GSH of the enzyme [35,36], and all residues around the ruthenation sites are more or less shifted relative to their original position in non-bound GSTn. Based on the constructed molecular models, the shift distances of the Cα atoms or the centres of the five/six-membered rings in the residues nearby the binding sites were measured and are listed in Table 2. The larger the shifts, the more the distortion of the residue caused by ruthenation and the more the influence on the interactions between GSTn and its substrate.

It has been reported that the N-terminal domain of GSTn has a ββαβα/αββ topology termed thioredoxin-like domain, and the helix α2, which consists of residues 36–52 plays a key role in the catalytic process of GSTn [36]. Following loop 2 in the N-terminal domain, the residue Cys48, which is highly conserved in GSTn of mammals, is involved in an electrostatic interaction with Lys45 so that the GSTn substrate bound to the enzyme is locked in a proper place [36]. The residues Trp39, Lys45, Gln52 and Leu53 form a “wall” to “catch” GSH via H-bond interaction, and Arg14, Gln52 and Gln65 are also involved in interaction with GSH [36,37]. In addition, the Tyr8 residue which serves as a hydrogen donor to form H-bond with GSH also plays an important role in the catalytic process of GSTn [36,37]. Despite the EA moiety released from the (EA-arene)Ru(pta) complex was showed to bind to the H-site of GSTn, it could lead to changes in the orientation of GSH-binding related residues such as Tyr8, Arg14, Gln52 and Leu53 [30].

Our molecular modelling shows here (Figs. 5 and S7 and Table 2) that the bindings of the ruthenium complexes to Cys15 and Cys48 led to the shift of all the significant residues mentioned above. For example, the bindings of ruthenium complexes 1, 2 and 3 to Cys15 as well as the subsequent Ru-induced oxidation of Cys15 [10] made the centre of the aromatic ring of Tyr8 shift from its original position by 1.32, 0.89 and 2.26 Å, respectively. Due to the coordination of complexes 1, 2 and 3 to Cys48 and the Ru-induced oxidation of the thiolato ligand, the Cα of Gln52 shifts from its original position by 1.46, 0.86 and 1.44 Å, respectively. Also, significant shifts are observed for the Cα of other important residues, including Arg14 and Leu53, and the five-membered ring of Pro54 following the binding of the ruthenium complexes to Cys15 and Cys48. Importantly, the molecular modelling indicates that compared with complexes 1 and 2, complex 3 binding to Cys15 and Cys48 leads to the largest movements of the GSH-binding related residues such as Tyr8, Gln52 and Leu53 (Table 2). Thus, it is reasonable that among the three complexes, complex 3 exhibited the highest inhibitory potency against GSTn, though it is less active than complex 2 for binding to Cys48.

4. Conclusion

In order to further understand the inhibition mechanism of ruthenium arene complexes [η6-arene]Ru(en)Cl+(arene = p-cymene (1), biphenyl (2) or 9,10-dihydroanthracene (3)) against GSTn, a MS-based method has been developed to determine the binding stoichiometry of the complexes to individual amino acid residues in combination of isotopic labelling of peptides arising from the tryptic digests of non-bound GSTn and ruthenated GSTn complexes. The intensity ratios of the heavy-labelled tryptic peptides, which contain the ruthenation sites Cys15, Cys48, Met92 and Cys102 as revealed by our previous studies, of ruthenated GSTn complexes to the respective light-labelled tryptic peptides arising from digestion of equimolar recombinant GSTn were used to calculate the ruthenation ratios of specific residues. Combined with the enzyme inhibition assays and molecular modelling, the MS quantification results indicated that the bindings of the three complexes Cys15 and Cys48 within the G-site of GSTn as well as the Ru-induced oxidation of the thiolato ligands most contribute to the inhibition activity of the complexes against the enzyme, but the coordination of the ruthenium complexes to Met92 and Cys102 on the interface of GSTn dimer has little impact on the enzyme activity. Furthermore, among the three complexes, complex 3 binding to Cys15 and Cys48 significantly alters the conformation of the G-site, leading to the largest movements of the GSH-binding related residues, e.g. Tyr8, Arg14, Gln52 and Leu53. As a consequence, complex 3 showed the highest inhibitory activity against GSTn, though its binding affinity to Cys48 is lower than that of complex 2. These findings not only provide insights into the inhibition mechanism of the ruthenium arene complexes on GSTn, but also imply that quantitative characterization of interactions between metal inhibitors and enzymes can deliver more helpful information for the rational design of highly active enzyme inhibitors. We anticipate that after the appropriate modification, the MS-based method could be widely used for quantification of various metal-protein interactions.

Acknowledgements

We thank NSFC (Grant Nos. 21135006, 21272901, 21275148 and 21321003), the 973 Program of MOST (2013CB831805) for the support, and Professor Peter Sadler and Dr. Abraha Habtemariam for the stimulated discussion and the gifted ruthenium dpba complex.

Appendix A. Supplementary data

MS and molecular modelling data (Table S1, Figures S1–S7). This material is available free of charge via the Internet at http://dx.doi.org/10.1016/j.inorgbio.2015.02.015.

References


Table 2

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<td>0.26</td>
<td>0.34</td>
</tr>
<tr>
<td>Σ𝛿δs</td>
<td>6.83</td>
<td>5.89</td>
</tr>
</tbody>
</table>

a Not applicable.
b The total shift (Å) of all residues listed in the Table.