Interactions Between Proteins and Platinum-Containing Anti-Cancer Drugs

Cristina Bischin, Alexandru Lupan, Vicentiu Taciuc and Radu Silaghi-Dumitrescu*

Department of Chemistry and Chemical Engineering, "Babes-Bolyai" University, 11 Arany Janos str, Cluj-Napoca RO-400028, Romania

Abstract: Cisplatin and its congeners are well-known to exert their therapeutic effects on cancer *via* interaction with DNA in the cell nucleus. On the other hand, the undesirable side-effects of these drugs appear to also be linked, at least to some extent, to interaction of the platinum with proteins and peptides. For other classes of anticancer drugs, interaction with proteins is in fact the primary pathway whereby therapeutically-useful effects are achieved. Here, a review is given of the known instances of interaction of cisplatin and related compounds with proteins and biologically relevant peptides, with emphasis on structural and reactivity aspects.

Keywords: Cisplatin, cysteine, methionine, adduct, toxicity, resistance.

INTRODUCTION

One of the most important drugs employed in treating various types of cancers is cisplatin. Cisplatin is a yellow crystalline solid, slightly soluble in cold water and insoluble in most common solvents except *N,N*-dimethylformamide. Unlike most other drugs, which have an organic structure, cisplatin is a simple inorganic compound, [$H_6Cl_2N_2Pt$] (cf. Fig. **1**). It has two isomer forms, cis- and trans-, with the former being the active one (hence the name *cis*platin); in aqueous solution, cisplatin slowly changes to the *trans*-form which has a low pharmacological activity [1,2].

After administration, one of the chloride ligands at the cisplatin platinum center is displaced by water. Subsequent displacement of this water allows the platinum to coordinate to a basic site in DNA. After that, crosslinking of two DNA bases occurs *via* displacement of the other chloride ligand from platinum. The damaged DNA elicits DNA repair mechanisms, which in turn activate apoptosis when repair proves impossible [1-3].

Two other widely-used platinum derivatives feature organic ligands to the metal: oxaliplatin [$C_8H_{14}N_2O_4Pt$] and carboplatin [$C_6H_{14}N_2O_4Pt$] (cf. Fig. **1**). These two drugs show lower toxicity than cisplatin, allowing higher dosage during treatment; both have a kinetically slower leaving group, and are also less nephrotoxic than cisplatin. The limiting toxicity of oxaliplatin is peripheral sensory neuropathy, also seen with cisplatin. Because the drugs have different profiles, the targets and mechanisms of action for these drugs are different; thus, oxaliplatin can be used with the patients who have developed resistance to cisplatin. Compared to cisplatin, oxaliplatin features a dicarboxylic ligand replacing the chloride ligands; its molecule is much bigger and its reactivity is much lower than cisplatin. Carboplatin is better retained in the body, so that its effect is longer lasting. Its toxicity is lower than that of cisplatin [1,2].

The need for more effective drugs as well as the wide range of side-effects (such as nausea, progressive peripheral sensory neuropathy, fatigue, vomiting, alopecia, hematological suppression, renal damage) have, for several decades now, fuelled interest into understanding the complex mechanisms of interaction of cisplatin and related compounds with various biomolecules [3]. One notable observation in this respect has been that cisplatin-derived platinum can bind to a range of proteins, as demonstrated by elemental analyses, chromatography and mass spectrometry. Indeed, it has been estimated that less than 5% of the cisplatin that has entered a cell will be found bound to DNA; the rest will bind to proteins and small peptides [4]. As expected, cisplatin has a preference for binding thiol groups, with glutathione and metallothionein as important targets [5]. In fact, cisplatin induces a rise in the level of thiol groups in cells, which is at least in part responsible for the resistance developed against this drug. Thiol-blocking reagents favor cisplatin-DNA binding *in vivo* [6]. The thioether sulfur in methionine is also an important target, as witnessed among others by the fact that Pt-methionine adducts were detected in the urine of cisplatin-treated patients [7,8].

Using the nucleopeptide Met-d(TPG) containing a metionine moiety covalently linked to TpG dinucleotide [9] L-Met or homocysteine [10] it was demonstrated that the reaction with platinum complexes leads to platinum coordination to the sulfur atom which subsequently is substituted by the N^7 atom of guanine. Monofunctional Pt migration from S to N^7 can occur in a nucleopeptide under physiological condition. However the chelate containing a Pt-(S, N^7)bond is stable towards Pt-S dissociation and migration [9].

*Address correspondence to this author at the Department of Chemistry and Chemical Engineering, "Babes-Bolyai" University, 11 Arany Janos str, Cluj-Napoca RO-400028, Romania; Tel: +40264593833;
Fax: +40264590818; E-mail rsilaghi@chem.ubbcluj.ro

1389-5575/11 $58.00+.00

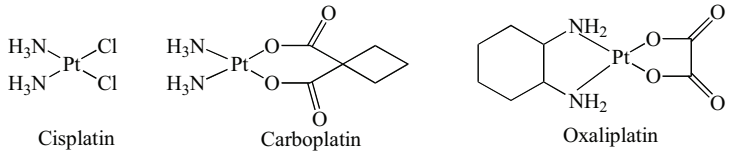

Fig. (1). Chemical formulae of the most common platinum-containing drugs for cancer therapy.

Glutathione (GSH)

Glutathione is a cysteine-containing tripeptide with multiple protective roles and with intracellular concentrations as high as 10 mM. Cisplatin binds to glutathione in several manners, all involving the sulfur. While administration of glutathione together with cisplatin was found to have beneficial effects in increasing the efficiency of anti-cancer treatment [11-13], it remains to be examined to what extent this increase in efficiency was due to the reduced toxicity due to platinum ligation by glutathione, or due to the antioxidant effect of this peptide, or, rather, due to formation of more active platinum species under the influence of glutathione [14]. It is also known that the mechanisms whereby cells develop resistance to cisplatin [15] do involve a rise in glutathione concentration as well as elimination of Pt-GSH adducts from the cell. Furthermore, a decrease in glutathione concentration at the kidney accompanies the nephrotoxic side-effects of this drug [16].

Metallothionein (MT)

Metallothionein is a low-molecular weight protein (60-70 aminoacids in mammals) featuring 20 cysteines and 9 methionines. With such a rich potential for metal ligation (which is of course not limited to the sulfur atoms), this protein can bind at least up to 7 metals at a time. Thus, MT may function to maintain normal metal homeostasis and regulate important cellular activities. Some evidence suggests that MT can also function as an antioxidant [17,18]. It has been known that intracellular oxidants may have a role in anticancer drug mediated programmed cell death. The observation that MT expression can be regulated by ambient oxygen levels led to speculation that MT may be an inducible antiapoptotic gene product [19]. While Zn is naturally bound to MT, this metal can easily be displaced by others, including platinum as evidenced by high-performance liquid chromatography (HPLC), atomic absorption spectroscopy and ultraviolet (UV) absorption spectroscopy [20]. In cells treated with cisplatin, MT expression is increased as part of the resistance response elicited by the drug [20-23].

Transferrin (TF)

Human serum transferrin is found in blood at concentrations of ~35 μM and normally transports iron; it can, however, also bind other metal ions – platinum included [24]. Reactions of human serum transferrin and Pt(II) compounds have been investigated with [^{1}H,^{15}N] NMR using the signals of the Pt complexes themselves, and with [^{1}H, ^{13}C] for the ε-CH_3 groups of the protein methionine residues. Methionines 256 and 499 are the main residues that serve as ligands to Pt(II); Cys 34, aspartates and tyrosines have also been demonstrated to interact with platinum. Nanoelectrospray ionization quadrupole time-of-flight mass spectrometry (nano ESI-QTOF-MS) and size-exclusion high-performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC/ICPMS) were also employed for characterization of Pt drugs-holo-transferrin interactions [25]. Pt binding to TF at lower concentrations did not significantly change the structure of the protein (the complex formation in the case of oxaliplatin may occur through non-covalent binding). In fact, holo-Tf was proposed as a useful carrier for cisplatin delivery, as it allows prolonged liberation of the drug within the organism [26] (Fig. **2**).

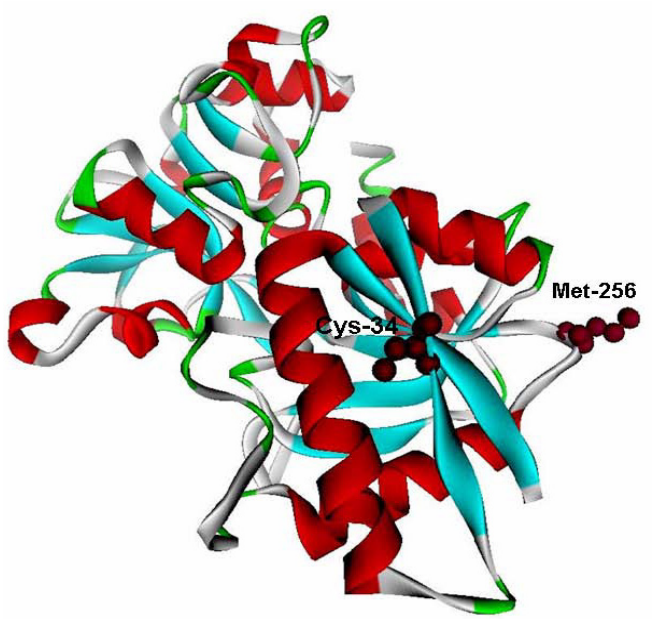

Fig. (2). Structure of transferrin, highlighting residues known to ligate platinum.

Ubiquitin

Ubiquitin is a small protein (8.5 kDa) involved in key cellular processes such as cell cycle control, DNA repair, and protein degradation. The N-terminal methionine and His68 are the two main targets for cisplatin and related compounds in ubiquitin, as evidenced by ESI-MS and NMR measurements. Platinum-modified ubiquitin interferes with proteolysis and ubiquitination processes. The agents that can affect the ubiquitin system might be useful for the treatment of tumors and the ubiquitin system could be a new target for cancer chemotherapy [27-30].

Thioredoxin (TRX) and Thioredoxin Reductase

Thioredoxin is a ubiquitous 13-kDa redox enzyme which uses electrons provided by NADPH *via* thioredoxin reductase. TRX protects against oxidative stress; increased TRX levels in certain types of tumors accompany cisplatin resis-

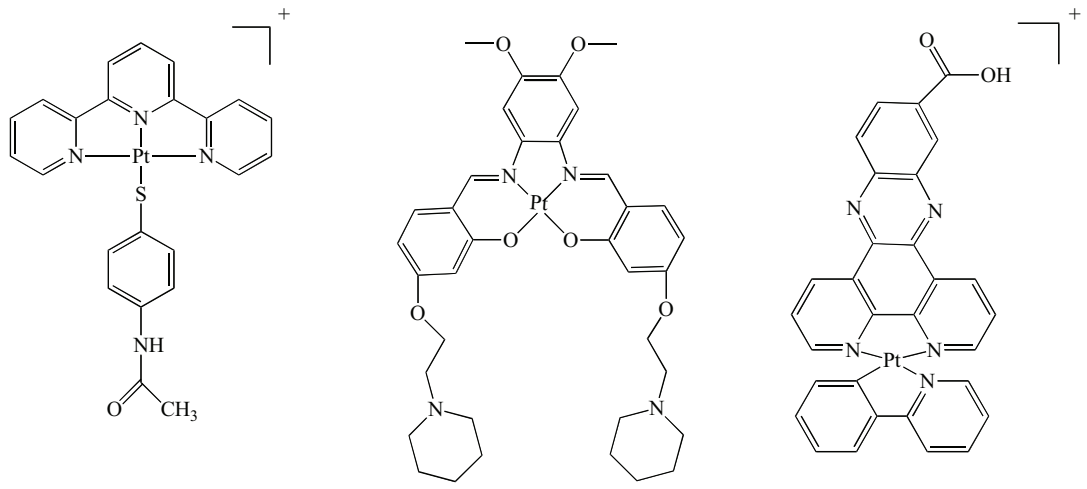

Fig. (3). Examples of metal-based drugs with inhibitory effects on proteins/enzymes.

tance phenomena. Cisplatin and oxaliplatin have been shown to in fact inhibit TRX directly. Thus, one hypothesis is that antioxidants such TRX may mediate resistance to cisplatin by blocking the pathway of apoptosis [31-33].

The Pt(II)-(2,2':6'2'-terpiridine) adduct shown in Fig. (**3**) binds to the selenocysteine active site in thioredioxin reductase, thereby acting as inhibitor [2].

DNA-Processing Enzymes

Topoisomerase II is inhibited by Pt(II)-(2,2':6'2'-terpiridine) *via* two mechanisms – interaction with DNA and direct binding to the enzyme, which eventually involves a thiol group [2].

Metalloproteinase

Matrix metalloproteinases play an important role in various physiological and pathological conditions such as tissue remodeling and cancer cell invasion and metastasis. A series of platinum (II) complexes (Fig. **3**) with two or three labile ligands were found to inhibit matrix metalloproteinase (MMP-3) and the adducts were characterized by ESI-MS, CD, and NMR-spectroscopy, with evidence for binding at His224 [2]. Also, the decrease of the MMP-2 level in cisplatin treatment might represent an additional mechanism by which cisplatin provides its antineoplastic effects [34].

Hemoglobin (Hb) and Myoglobin

The interaction of cisplatin, oxaliplatin and carboplatin with hemoglobin was also studied with nanoelectrospray ionization quadrupole time-of-flight mass spectrometry (nanoESI-QTOF-MS) and size-exclusion high performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC/ICPMS) [35]; heme release was a noted side effect of platinum binding. Furthermore, cisplatin–Hb complexes were shown to be formed at clinically relevant concentrations of cisplatin and Hb, and differences were found between cisplatin, oxaliplatin and carboplatin in terms of adduct formation both with pure hemoglobin and when incubating blood with these drugs. Cysteine and proline residues were shown to be involved in the Hb-platinum interactions [36-38]. Given the known importance of subtle conformational changes in cooperativity within Hb, and the importance of this cooperativity in controlling the affinity for molecular oxygen, it is conceivable that formation of Hb-Pt adducts would affect the oxygen-binding capacity of Hb, which would represent an undesirable side-effect *in vivo*.

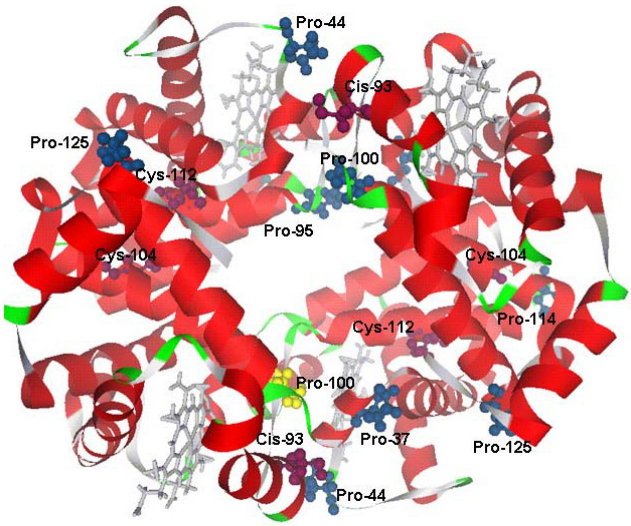

Fig. (4). Structure of hemoglobin highlighting the cysteine and proline residues involved in Pt binding.

Hb is present in high concentrations in blood and is particularly sensitive to changes in redox status, to the extent that under stress conditions such as physical effort or certain pathological conditions it engages in toxic reactions with oxidative stress agents- primarily peroxide – yielding free radicals and highly-oxidizing states at the iron (ferryl, Compound II) [39-42]. As such, it may be expected that Hb might be sensitive to the stress imposed by cisplatin in patients;

indeed, in a preliminary report we have shown that the autooxidation rate of hemoglobin is affected by cisplatin and related compounds [43]. Interestingly, upon incubation with whole blood a significant part of the total platinum was sequestered into the erythrocyte within the first 2 hours, and was not exchangeable into plasma thereafter [44].

Mass spectrometry was employed to show that myoglobin can bind up to two cisplatin-derived Pt ions, at residues His116, Ser117, Lys118, and His 119 [45] (Figs. **4**, **5**).

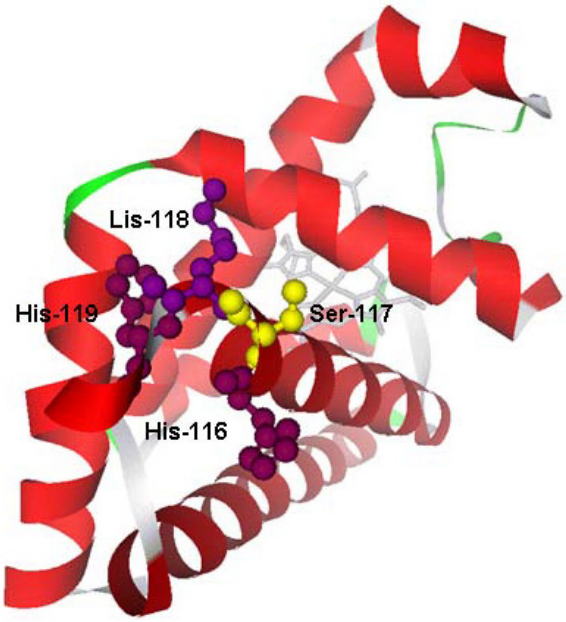

Fig. (5). Structure of myoglobin, highlighting the Pt binding site residues.

Serum Albumin

Albumin is the most abundant protein in blood serum (~0.6 mM). Since albumin has a free thiol as well as 17 disulfide bridges, one may expect efficient binding of platinum to this protein, too. Indeed, albumin is known to bind and transport a wide range of metabolites and exogenous substances (Fig. **6**).

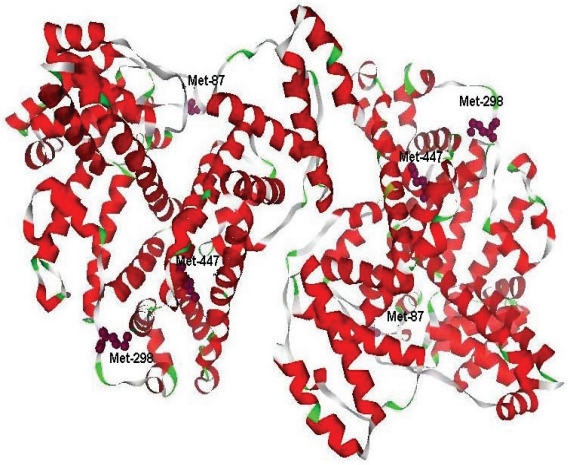

Fig. (6). Structure of human serum albumin, highlighting Met residues involved in Pt binding.

It was shown that ~one day after cisplatin administration, 65-98% of the total platinum was bound to blood proteins and especially to albumin. This binding does not limit the cytotoxicity of the platinum, but does limit its urinary excretion. In fact, administration of albumin together with cisplatin was found to limit nephrotoxicity [24]. Cisplatin causes distinct variations in protein conformation including considerable decrease of the helical structure and a change in binding affinity towards other molecules [46].

Capillary isoelectric focusing (CIEF) with whole column imaging detection (WCID) [47], fluorescence spectroscopy, FT-IR, circular dichroism (CD) [48,49], inductively coupled plasma mass spectrometry (ICPMS) [50] and capillary electrophoresis (CE) [51], ^{1}H and ^{15}N NMR spectroscopy [46] were employed in order to investigate the interaction of albumin with oxaliplatin and cisplatin.

Met298 is the most accessible binding site and the main target for cisplatin in albumin; Met87 and Met446 were also shown to bind platinum, while, contrary to expectations, Cys34 had a less important role. A particular feature of albumin is that cisplatin can induce inter-protein crosslinking *via* sulfur-platinum linkages [24,46,52,53]. It is important to note that results obtained on cisplatin cannot be extrapolated to its congeners: while oxaliplatin is slightly more reactive than cisplatin towards albumin [44], nedaplatin and lobaplatin (Fig. **7**) bind only very weakly to serum proteins [14,52-55].

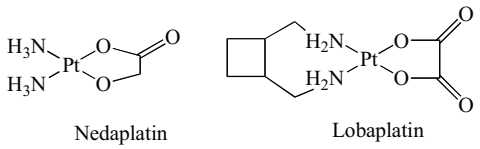

Fig. (7). Structures of the two platinum (II) complexes with less affinity towards serum proteins than cisplatin.

Cytochrome c

Cytochrome *c* serves as key component of the electron transport chain but is also involved, most likely *via* redox reactions linked to peroxides, in the apoptosis process and as such may be expected to be a sensitive target for exogenous compounds such as cisplatin [56-58] (Fig. **8**).

The interaction of platinum compounds with cytochrome *c* was studied with electrospray ionization mass spectrometry (ESI-MS), tandem mass spectrometry (MS/MS), Fourier transform mass spectrometry(FT-MS) and ^{1}H NMR [59-62]. Cisplatin and carboplatin-derived platinum were found to have a preference for the iron ligand Met65 and for Met80 [59-62]. Other targets for platinum (whether derived from cisplatin, carboplatin, or other compounds) in cytochrome *c* are Cys14, Cys17, His18, His26, and His33. Such binding does not limit the cytotoxic effects of the metal [60].

Copper-Binding Proteins

The copper transporter 1 (CTR1) is responsible for the uptake of Cu from extracellular space and has been shown to play a major role in the accumulation of platinum-based drugs. Cisplatin interacts with CTR1 both at ^{40}MXXM^{45} and at a site outside the N-terminal domain that produces the

conformational changes that trigger degradation. It was speculated that CTR1 may mediate the transport of cisplatin through a pore it forms in the plasma membrane, as well as *via* endocytosis [63]. Another two Cu transporters involved in cisplatin efflux are ATP7A and ATP7B [64,65].

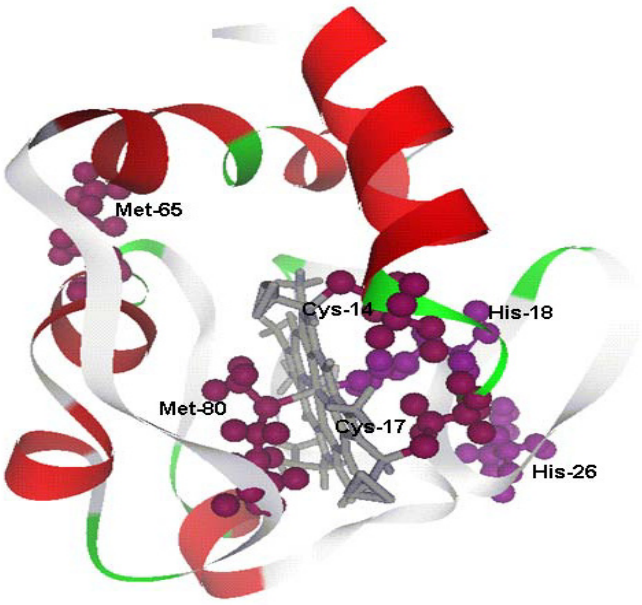

Fig. (8). Structure of cytochrome c, highlighting Met 65 and Met 80 residues known to bind cisplatin.

Other Membrane Platinum-Binding Proteins

MRP1 is an integral membrane multidrug resistance associated phosphoglyco-protein of the ATP-binding cassette superfamily. MRP1 confers resistance to several chemotherapeutic agents, including cisplatin. This phenomenon involves mislocalisation of MRP1 as well as accumulation of a partly or unglycosilated form of the protein, under conditions where the level of gene expression remains the same. An increase of the protein concentration in the cytoplasm as well as effects on endocytosis and membrane vesicle recycling and on other membrane proteins such as folate-binding protein (FBP) were also noted [66].

Cytoskeletal Platinum-Binding Proteins

Two carboplatin –binding proteins (actin and filamin) were shown to be down-regulated in cisplatin-resistant cells. Moreover, reduced expression of β-tubulin, keratin and dynamin 2 was also demonstrated. Because actin, filamin and dynamin all play important roles in the endocytosis machinery, it was proposed that the platinum-induced defects of the cytoskeletal system contribute to the reduced uptake of cisplatin and carboplatin [67].

Coordinative Binding of Platinum to Proteins in X-Ray Diffraction Structures

A number of crystal structures are now known where platinum is bound to proteins. Not all cases involve cisplatin and its congeners: the presence of platinum may at times be fortuitous. However, all instances are reviewed here, insofar as they are instructive for the manners in which Pt-containing biologically-active compounds may interact with proteins. Table **1** summarizes the cases where platinum is bound to proteins, as indexed in the Protein Data Bank. One general observation is that platinum appears to preferentially bind to sulfur-containing aminoacids (methionine, cysteine) and nitrogen-containing ones (especially histidine). According to the HSAB theory (hard-soft acid-base) the Pt^{2+} ion, with its large radius and relatively small charge, should be regarded as an essentially soft center, able to adjust the shape of its electron cloud in order to engage in interactions bearing significant covalent character; therefore, ideal ligands for such a metal center are the soft ones (such as the sulfur in methionine and cysteine) and to some extent the borderline ones (such as the nitrogen in the histidine imidazole ring) [68].

Davies *et al.* crystalised a *transposon Tn5* bound to a terpyridine Pt(II) ligand (pdb code 1B7E) [69]. The platinum interacts directly with the polypeptide chain *via* the sulfur atom of cysteine 402 (Pt-Cys, Pt-S 2.7 Å), while the accompanying pyridine moieties also interact with other residues (Glu394 and Trp424).

Gorman *et al.* have determined the crystal structure of the *human DNA repair endonuclease HAP1* co-crystallized with heavy metal atoms (pdb code 1BIX). The platinum interacts with Met271 (Pt-S 1.97 Å) and Lys 276 (Pt-N 2.91 Å) [70].

Pt(II) was also found in the crystal structure of the extracellular membrane-anchored *trehalose/maltose-binding protein (TMBP)* from the hyperthermophilic Archaeon *Thermococcus litoralis* (pdb code 1EU8) [71]. Here, the Pt ion interacts with the sulfur atom of Met327 (2.68 Å).

Schubot *et al.* [72] have presented the crystal structure (pdb code 1JJF) of *feruloyl esterase* involved in the cleavage of ferulic acid's bonds to arabinoxylan and pectin. Here, Pt(II) interacts with with Met173 (Pt-S 2.75 Å).

Another example of interaction with methionine is *shikimate kinase* (pdb code 1L4U) [73] (Pt-S 2.45 Å). A 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid molecule serves as additional ligand to the platinum ion.

The structural analysis of the *chaperone adaptor protein ClpS* in complex with the N-terminal domain of Clp (pdb code 1LZW) revealed the presence of platinum(II) ion interacting with Met103 (2.53 Å) [74].

The crystal structure of a truncated *human EGFR ectodomain* bound to TGFalpha (pdb code 1MOX) [75] reveals the presence of Pt bound to Met30 (Pt-S 2.3 Å).

Interaction with methionine (Pt-Met79 2.42 Å) was also reported for the first structure of a choline binding domain, from the toxin-releasing enzyme pneumococcal major *autolysin* (LytA) (pdb code 1NNW) [76].

The h*uman complement regulator CD55* is a key molecule protecting self-cells from complement-mediated lysis. X-ray diffraction (pdb code 1OK9) [77] shows a $PtCl_2$ moiety linked to a methionine (Met227, Pt-S 2.4Å) and to a histidine (His231, Pt-N 1.89 Å and 3.01 Å respectively).

The crystal structure of *pitrilysin* (pdb code 1Q2L), the prototype of insulin-degrading enzymes, reveals the presence

Table 1. Crystal Structure for Some Proteins and Platinum Binding Complexes Determined Up to Date

Pdb code	Protein name	Pt interaction
1AY2	*Neisseria gonorrhoeae* pilin	His
2PIL	*Neisseria gonorrhoeae* pilin	His
1B7E	Transposon Tn5	Cys
1BIX	human DNA repair endonuclease HAP1	Lys Met
1EU8	trehalose/maltose-binding protein from the hyperthermophilic Archaeon *Thermococcus litoralis*	Met
1GWB	Glycoprotein Ib (GPIb)	Thr
1RLI	*Bacillus subtilis* trp repressor binding protein	Gln, His, Lys, Met, Thr
1JJF	*Clostridium thermocellum* cellulosomal xylanase Z	Met
1L4U	*Mycobacterium tuberculosis* shikimate kinase	Met
1LZW	*Escherichia coli* adaptor protein ClpS	Met
1MOX	truncated human EGFR ectodomain bound to TGFalpha	Met
1NNW	*Pyrococcus furiosus* Pfu-1218608	Met
1OK9	human complement regulator CD55	His, Met
1Q2L	pitrilysin	Arg, His
1QJ8	*Escherichia coli* OmpX membrane protein	Met
1QVR	*Thermus thermophilus* ClpB chaperone	Arg, Gln, Lys, Met, Thr, Tyr
1QYY	N-terminal domain of human platelet receptor glycoprotein Ib-alpha	His
1RH7	resistin	Met
1RNL	*Escherichia coli* response regulator NarL	Met, Glu
1RR7	Mor protein of bacteriophage Mu	His, Met
1SEN	Endoplasmic reticulum protein Rp19	Cys, Lys
1SF9	*Bacillus subtilis* YfhH hypothetical protein	Met
1SJ7	talin rod spanning residues 482-789	Ser, Arg, Lys
1UAS	rice alpha-galactosidase complexed with D-galactose	Met
1W07	*Arabidopsis thaliana* Acyl-CoA oxidase 1	Met,Glu, His, Asn
1XC3	*Bacillus subtilis* putative fructokinase	Met
2BHO	*Yersinia enterocolitica* type III secretion chaperone SycT	His

(Table 1). Contd…..

Pdb code	Protein name	Pt interaction
2CH8	Epstein-Barr virus oncogene BARF1	Met, His, Glu
2DQA	*Tapes japonica* lysozyme	Met
2I6Z	Ciplatin and hen egg white lysozyme adduct	His
2IS9	DCN-1 protein	Met
2HR8	*Pyrococcus horikoshii* OT3 PH0725	Met, Glu
2HUV	*Pyrococcus horikoshii* OT3 PH0725	His
2HUX	*Pyrococcus horikoshii* OT3 PH0725	His, Met
2PL1	PhoP regulator	His
2X2B	*Bacillus subtilis* FapR protein	Met
2Z8Z	platinum-bound S445C mutant of *Pseudomonas sp*. MIS38 lipase	Cys
2ZZB	human thioredoxin reductase 1	Cys
3IWL, 3IWX	cisplatin bound to a human copper chaperone	Cys
3M7K	PacI-DNA enzyme complex	Met

of several platinum ions establishing the following interactions involving four different platinum ions: Pt(1) with the His522 imidazole at 2.32 Å and with Arg886 at 3.18 Å, Pt(2) with the same Arg at 3.4 Å, Pt(3) with both nitrogen atoms of the His465 at 3.21 Å and 3.36 Å, Pt(4) with one nitrogen of His114 at 2.41 Å and Pt(5) with one nitrogen of His426 at 2.3 Å and with Arg304 at 3.16 Å.

Vogt and Schulz [78] reported the crystal structure of the *outer membrane protein X (OmpX)* from *Escherichia coli*. The coordination spheres of two bound platinum ions are described, the first bound to the two sulfur atoms of Met21 and Met18 at 2.39 Å and 2.58 Å, respectively, and the second to Met118 at 1.94 Å. Both Pt complexes are planar quadratic with chloride atoms in *trans* positions.

The structure of *Thermus thermophilus* ClpB (pdb code 1QVR) [79] showed a significant number of platinum ions: Pt(1) interacting with Met619 at 2.36 Å, Pt(2) with Lys73 at 3.07 Å and Met24 at 2 Å, Pt(4) with Met234 at 2.55 Å, Pt(5) with Met628 at 2.5 Å and with the two oxygen atoms of Thr625 at 2.1 Å and 2.46 Å respectively, Pt(6) with Met394 at 2.48 Å, Pt(7) with the two nitrogen atoms of Arg393 at 1.92 Å and 3.46 Å and with the amidic nitrogen and oxygen atoms of Gln184 at 2.37 Å and 3.46 Å respectively, and Pt(8) with Met624 at 2.2 Å and with the oxygen atom of Tyr627 at 2.88 Å.

Varughese et.al. studied the platinum-induced space-group transformation in crystals of the *platelet glycoprotein Ib* alpha N-terminal domain (pdb code 1QYY) [80]. A platinum atom binds to His86 (Pt-N 2.31 Å) and a second one to His95 (Pt-N 2.47 Å).

Resistin is a member of the resistin-like molecule (RELM) hormone family. It is secreted selectively from adipocytes and induces liver-specific antagonism of insulin action, thus providing a potential molecular link between obesity and diabetes. Crystal structures of resistin and RELMß (pdb code 1RH7) [81] reveal a hexameric structure wherein Pt-S (Met42) distances vary from 2.31 to 3.07 Å.

The crystal structure analysis of the *nitrate/nitrite response regulator protein NarL* (pdb code 1RNL) [82] reveals the presence of three platinum ions bound to Met28 and three others bound to Met175 with one of them interacting also with Glu127 at 3.1 Å.

Two platinum(II) ions were reported in the crystal structure of the *phage-encoded activator protein Mor* of bacteriophage Mu, a member of the Mor/C family of transcription activators (pdb code 1RR7) [83]. The first one is bound to His63 (2.11 Å) and the second to Met116 (2.33 Å).

The crystal structure of *Bacillus subtilis* hypothetical protein YfhH (pdb code 1SF9) shows the presence of one platinum ion and one chloride ion. Interactions are with Met25 and Met69 at 2.43 and 3.49 Å respectively.

The interaction between the cytoskeletal proteins *talin* and vinculin plays a key role in integrin-mediated cell adhesion and migration. The crystal structures of two domains from the talin rod spanning residues 482-789 (pdb code

1SJ7) [84] revealed a number of platinum-protein interactions involving Ser630 (3.29 Å), Arg624 (1.5 Å) Lys540 (2.84 Å).

A platinum ion is also encountered in the crystal structure of rice *alpha-galactosidase* complexed with D-galactose (pdb code 1UAS) [85], showing interactions with Met140 at 2.34 Å and with Met176 at 3.46 Å.

Pedersen and Henriksen [86] reported the structure of *peroxisomal acyl-CoA oxidase* from *Arabidopsis thaliana* (1W07) which plays an essential role in lipid metabolism by catalyzing the conversion of acyl-CoA into trans-2-enoyl-CoA during fatty acid beta-oxidation. Three platinum ions are reported: one bound to Met256 at 2.51 Å, one bound to Glu2 at 2.76 Å, to His6 at 2.27 Å and to Asn624 at 3.45 Å, and a third platinum interacting with Met281 at 2.85 Å and His374 at 2.89 Å.

Cuff *et al.* determined the structure of a putative *fructokinase* from *Bacillus subtilis*. The two Pt(II) ions are bound to Met33 (1.88 Å) and Met94 (2.11 Å) respectively (pdb code 1XC3).

Locher *et al.* [87] solved the crystal structure (2BHO) of the *Yersinia enterocolitica* type III s*ecretion chaperone SycT,* a system that injects effector proteins into host cells. Here, a platinum ion binds to His42 (2.25 Å).

The Epstein-Barr virus is a human gamma-herpes virus that persistently infects more than 90% of the human population and is associated with numerous epithelial cancers, principally undifferentiated nasopharyngeal carcinoma and gastric carcinoma. The BARF1 gene is expressed in a high proportion of these cancers. An oncogenic, mitogenic and immortalizing activity of the BARF1 protein has been shown. The structure of the *secreted BARF1 glycoprotein* expressed in a human cell line (pdb code 2CH8) [88] reveals a dimeric organization with several platinum ions present, bound either to Met and His or to Met and Glu (e.g., Pt(2): Met160 at 1.26 Å, His196 at 1.96 Å; Pt(4) Met176 at 1.51 Å and Glu178 at 2.72 Å).

The crystal structure of *Tapes japonica lysozyme* with a substrate analogue (pdb code 2DQA) [89] reveals the presence of a Pt-S Met bond (with Met29, at 2.61 Å). Additionally, the interactions of cisplatin and its analogues, transplatin, carboplatin and oxaliplatin, with hen egg white *lysozyme* were analyzed using ESI mass spectrometry, and the resulting metallodrug–protein adducts identified; the X-ray crystal structure of the cisplatin lysozyme derivative [90], solved at 1.9 Å resolution (pdb code 2I6Z), reveals selective platination of imidazole N (Pt-N 2.12 Å).

Covalent modification by Nedd8 (neddylation) stimulates the ubiquitin-protein isopeptide ligase (E3) activities of *cullins*. DCN-1, an evolutionarily conserved protein, promotes neddylation of cullins. The 1.9 Å resolution structure of yeast DCN-1 (pdb code 2IS9) [91] contains a platinum(II) ion bound to Met230 (Pt-S 2.06 Å).

In the crystal structure of the protein *PH0725* from *Pyrococcus horikoshii* OT3 (pdb code 2HR8) three platinum ions are found: the first one binds to Met1 by Pt-N 2.56 Å and Pt-S 2.0 Å while also interacting to Glu77 (Pt-O 3.27 Å), the second one to Met174 (2.16 Å) and the third one to Met220 (2.54 Å). Another structure, 2HUX, contains only one platinum ion, bound to His126 (2.46 Å) and to Met243 (2.02 Å).

The crystal structure of the receiver domain of the response regulator *PhoP* from *Escherichia coli* (pdb code 2PL1) contains three platinum ions, of which one is bound to His15 (2.29 Å) and another one to His25 (2.21 Å) [92].

The crystal structure of the yeast *SLA1 SH3 domain* shows a platinum-protein bond involving Met12 (2.2 Å) (pdb code 2V1Q).

Two platinum ions are present in the structure of malonyl-ACP(acyl carrier protein) from *Bacillus subtilis* (2X2B) [93]; one of them interacts with Met1 (Pt-N 2.99 Å).

The crystal structure of a family I.3 *lipase* from Pseudomonas sp. MIS38 in a closed conformation was determined at 1.5 Å resolution (pdb code 2Z8Z) [94]. Platinum binds to Cys445 (2.29 Å).

A metal-protein bond (Pt-Cys498) of 2.23 Å was reported (pdb code 2ZZB) between a terpyridine-Pt complex and thioredoxin reductase [95].

Boal and Rosenzweig [96] determined crystal structures of cisplatin bound to Atox1, a *copper trafficking protein* involved in the cellular resistance to cisplatin (pdb codes 3IWX and 3IWL). Platinum coordination involves the conserved CXXC copper-binding motif. In 3IWL platinum ion is bound to Cys15 (2.35 Å) and to Cys12 (Pt-S 2.29 Å and Pt-N 2.27 Å). In the case of 3IWX Pt binds to two Cys residues (distances 2.1 and 2.3 Å).

The *HNH restriction endonuclease PacI*, which induces a remarkable DNA deformation, binds platinum to Met23 and Met28 of an α helix (pdb code 3M7K).

To conclude, cisplatin and related compounds are known to bind to several classes of proteins, with roles as diverse as structural, antioxidant, electron transfer, small molecule or ion transport, or DNA processing. Structural features of these binding phenomena were reviewed here, as well as instances where such binding was shown to result in altered function/structure of the respective proteins. These interactions are likely to be worth considering among the mechanisms whereby platinum-containing drugs induce toxic side-effects in humans. Further systematic experimental work aimed at delineating the mechanisms of interaction between proteins and platinum-containing drugs is expected to provide valuable information not only on the degree to which these interactions are directly involved in toxic side-effects, but also on structural features which may be modified in order to limit interaction with those proteins where the effect of platinum is most critical.

ACKNOWLEDGEMENTS

The work shown here has been supported by the Romanian Ministry for Education and Research (grants PCCE 140/2008 and 312/2008). A Ph.D. scholarship from Contract POSDRU/88/1.5/S/60185 – "Innovative doctoral studies in a knowledge based society" is gratefully acknowledged by C.B.

REFERENCES

[1] Rosenberg, B. Noble metal complexes in cancer chemotherapy. *Adv. Exp. Med. Biol*., **1977**, *91*, 129-50.

[2] Che, C.-M.; Siu, F.M. Metal complexes in medicine with a focus on enzyme inhibition. *Curr. Opin. Chem. Biol*., **2010**, *14*, 255-261.

[3] Cossa, G.; Gatti, L.; Zunino, F.; Perego, P. Strategies to improve the efficacy of platinum compounds. *Curr. Med. Chem*., **2009**, *16*(19), 2355-65.

[4] Cullen, K.J.; Yang, Z.; Schumaker, L.; Guo, Z. Mitocondria as a critical target of the chemotherapeutic agent cisplatin in head and neck cancer. *J. Bioenerg. Biomembr*., **2007**, *39*, 43-50.

[5] Hagrman, D.; Goodisman, J.; A-K., S. Kinetic study of the reaction of cisplatin with glutathion. *J. Pharmacol. Exp.Ther*., **2004**, *308*(2), 658-666.

[6] Dabrowiak, J.K.; Goodisman, J.; Soid, A.-K. Kinetic study of the reaction of cisplatin with thiols. *Drug. Metab. Rev.*, **2002**, *30*(12), 1378-1384.

[7] Nagai, N.; Okuda, R.; Kinochita, M.; Ogata, H. Decomposition kinetics of cisplatin in human biological fluids. *J. Pharm. Pharmacol*., **1996**, *48*, 918-924.

[8] Riley, C.M.; Sternson, L.A.; Repta, A.J.; Slyter, S.A. Monitoring the reactions of cisplatin with nucleotides and methionine by reversed-phase high-performance liquid chromatography using cationic and anionic pairing ions. *Anal. Biochem*., **1983**, *130*, 203-214.

[9] Teuben, J.-M.; van Boom, S.S.G.E.; Reedijk, J. Intamolecular migration of co-ordinated platinum from a sulfur to N7 in the nucleopeptide Met-d(TpG)(5'-O-methioninate-N-ylcarbonyltymidine 2'-deoxiguanosine momophosphate. *J. Chem. Soc. Dalton Trans*., **1997**, 3979-3980.

[10] Chen, Y.; Guo, Z.; Mudoch, P.S.; Zang, E.; Sadler, P.J. Interconversion between S- and N-bound L/methionie adducts of Pt(dien)2+ (dien= diethylenetriamine) *via* dien ring-opened intermediates. *J. Chem. Soc., Dalton Trans*., **1998**, 1503-1508.

[11] Anderson, M.E.; Naganuma, A.; Meister, A. Protection against cisplatin toxicity by administration of glutathione. *FASEB J*., **1990**, *4*, 3251-3255.

[12] Casciniu, S.; Cordella, L.; Del Ferro, E.; Fronzoni, M.; Catalano, G. Neuroprotective effect of reduced glutathione on cisplatin based chemotherapy in advanced gastric cancer: a randomized double-blind placebo-controlled trial. *J. Clin. Oncol*., **1995**, *13*, 26-32.

[13] Jenderny, S.; Lin, H.; Garrett, T.; Tew, K.D.; Townsend, D.M. Protective effects of a glutathione disulfide mimetic (NOV-002) against cisplatin induced kidney toxicity. *Biomed. Pharmacother*., **2010**, *64*, 73-76.

[14] Petrovic, D.; Stojimirovic, B.; Petrovic, B.; Bugarcic, Z.M.; Bugarcic, Z.D. Studies of interactions between platinum (II) complexes and some biologically relevant molecules. *Bioorg. Med. Chem*., **2007**, *15*, 4203-4211.

[15] Komiya, S.; Gebhardt, M.C.; Mangham, D.C.; Inoue, A. Role of glutathione in cisplatin resistance in osteosarcoma cell lines. *J. Orthop. Res*., **1998**, *16*, 15-22.

[16] Prasad, S.B.; Rosangkima, G.; Khynriam, D. Cisplatin-induced toxicological effects in relation to the endogenous tissue glutathione level in tumor bearing mice. *Asian J. Exp. Sci*., **2006**, *20*(1), 55-68.

[17] Schwartz, M.A.; Lazo, J.S.; Yallowich, J.C.; Reynolds, I.; Kagan, V.E.; Tyurin, V.; Kim, Y.A.; Watkins, S.C.; Pitt, B.R. Cytoplasmic metallothionein overexpression protects NIH 3T3 cells from tert-butyl hydroperoxide toxicity. *J. Biol. Chem*., **1994**, *269*, 15238-43.

[18] Rojas, P.; Rios, C. Metalollothionein inducers protect against 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine neurotoxicity in mice. *Neurochem. Res.*, **1997**, *22*, 17-22.

[19] Murphy, B.J.; Laderoute, K.R.; Chin, R.J.; Suttherland, R.M. Metallothionein II A is up-regulated by hipoxya in human A431 squamous carcinoma cells. *Cancer. Res.*, **1994**, *54*, 5808-10.

[20] Hagrman, D.; Goodisman, J.; Dabrowiak, J., C.; Abdul-Kader, S. Kinetic study on the reaction of cisplatin with metallothionein. *Drug Metab. Dispos*., **2003**, *31*(7), 916-923.

[21] Kondo, Y.; Woo, E.S.; Michalska, A.E.; Andy Cho, K.H.; Lazo, J.S. Metallothionein null cells have increased sensitivity to anticancer drugs. *Cancer Res.*, **1995**, *55*, 2021.

[22] Kasahara, K.; Y., F.; Nishio, K.; Ohmori, T.; Sugimoto, Y.; Komiya, K.; T., M.; Saijo, N. Metallotionein content correlates with the sensitivity of human small cell lung cancer cell lines to cisplatin. *Cancer Res.*, **1991**, *51*, 3237-3242.

[23] Saga, Y.; Hashimoto, H.; Yachiku, S.; T., I.; Tokumitsu, M. Reversal of acquired cisplatin resistance by modulation of metallothionein in transplanted murine tumors. *Int. J. Urol*., **2004**, *11*(6), 407-15.

[24] Wang, X.; Guo, Z. The role of sulfur in Platinum Anticancer Chemotherapy. *Anti Cancer Agents Med. Chem.*, **2007**, *7*, 19-34.

[25] Zhao, Y.Y.; Mandal, R.; Li, X.F. Intact human holo-transferrin interaction with oxaliplatin. *Rapid. Commun. Mass. Spectrom*., **2005**, *19*, 1956-1962.

[26] Hoshino, T.; Misaki, M.; Yamamoto, M.; Schmitz, H.; Y, O.; Toguchi, H. Receptor-binding, *in vitro* cytotoxicity, and *in vivo* distribution of transferring-bound cis-platinum (II) of differing molar ratios. *J. Control. Release*, **1995**, 75-81.

[27] Berners-Price, S.J.; Ronconi, L.; P.J., S. Insight into the mechanism of action of platinum anticancer drugs from multinuclear NMR spectroscopy. *Prog. Nucl.Magn. Reson.*, **2006**, *49*, 65-98.

[28] Hartinger, C.G.; Ang, W.H.; Casini, A.; Messori, L.; Keppler, B.K.; Dyson, J. Mass Spectrometric Analysis of Ubiquitin-Platinum Interacations of Leading Anticancer Drugs: MALDY versus ESI; . *J. Anal. Atomic Spectrosc.*, **2007**, *22*(8), 960-967.

[29] Isoe, T.; Naito, M.; A., S.; Hirai, R.; Tsuruo, T. Inhibition of different steps of the ubiquitin sysem by cisplatin and aclarubicin. *Biochim. Biophys. Acta*, **1992**, *1117*(2), 131-135.

[30] Niture, S.K.; C.S., V.; Srivenugopal, K.S. Anticancer platinum drugs inhibit ubiquitin (ub)-dependent ptrotelysis through S-glutatyionilasion of ub-E1 and proteasome enzymes. *Proc. Am. Assoc. Cancer Res.*, **2006**, 47.

[31] Yamada, M.; Tomida, A.; Yoshikawa, H.; Taketani, Y.; Tsuruo, T. Overexpression of thioredoxin does not confer resistance to cisplatin in transfected human ovarian and colon cancer cell lines. *Cancer Chemother. Pharmacol.*, **1997**, *40*, 31-37.

[32] Yokomizo, A. Cellular levels of thioredoxin associated with drug sensitivity to cisplatin, mitomizcin c, doxorubicin, and etoposide. *Cancer Res.*, **1995**, *55*, 4293-4296.

[33] Witte, A.B.; K., A.; E., J.; H., E.; Arner, E.S.J. Inhibition of thioredoxin reductase but not glutathion reductase by the major classes of alkylating and platinum-containg anticancer compounds. *Free. Radic. Biol. Med.*, **2005**, *39*, 696-703.

[34] Chintala, S.K.; Ali-Osman, F.; Mohanam, S.; Rayford, A.; Go, Y.; Gokaslan, Z.L.; Gagercas, E.; Venkaiah, B.; Sawaya, R.; Nicolson, G.L.; Rao, J.S. Effects of cisplatin and BCNU on MMP-2 levels in human glioblastoma cell lines *in vitro*. *Clin. Exp. Metastasis*, **1997**, *15*, 361-7.

[35] Mandal, R.; Sawyer, M.B.; Li, X.F. Mass spectrometry study of hemoglobin-oxaliplatin complexes in colorectal cancer patients and potential association with chemotherapeutic responses. *Rapid Commun. Mass Spectrom.*, **2006**, *20*, 2533- 2538.

[36] Mandal, R.; Teixeira, C.; Li, X.-F. Studies of cisplatin and hemoglobin interaction using nanospray mass spectrometry and liquid chromatography with inductively-coupled plasma mass spectrometry. *Analyst*, **2003**, *128*, 629-634.

[37] Mandal, R.; R., K.; Li, X.-F. Interaction of oxaliplatin, cisplatin and carboplatin with Hemoglobin and the resulting release of a hem group. *Chem. Res. Toxicol*., **2004**, *17*, 1391-1397.

[38] Peng, J.; Mandal, R.; Sawyer, M.; Li, X.-F. Characterization of intact hemoglobin and oxaliplatin interaction by nanoelectrospray ionization tandem mass spectrometry. *Clin. Chem*., **2005**, *51*(12), 2274-2281.

[39] Silaghi-Dumitrescu, R.; Reeder, B.J.; Nicholls, P.; Cooper, C.E.; Wilson, M.T. Ferryl haem protonation gates peroxidatic reactivity in globins. *Biochem. J*., **2007**, *403*(3), 391-5.

[40] Vollaard, N.B.; Shearman, J.P.; Cooper, C.E. Exercise-induced oxidative stress:myths, realities and physiological relevance. *Sports Med*., **2005**, *35*(12), 1045-62.

[41] Vollaard, N.B.; Reeder, B.J.; Shearman, J.P.; Menu, P.; Wilson, M.T.; Cooper, C.E. A new sensitive assay reveals that hemoglobin is oxidatively modified *in vivo*. *Free Radic. Biol. Med.*, **2005**, *39*(9), 1216-28.

[42] Reeder, B.J.; Svistunenko, D.A.; Cooper, C.E.; Wilson, M.T. The radical and redox chemistry of myoglobin and hemoglobin: from *in vitro* studies to human pathology. *Antioxid. Redox. Signal*., **2004**, *6*(6), 954-966.

[43] Taciuc, V.; Bischin, C.; Silaghi-Dumitrescu, R. *A novel mechanism for platinum-based drugs: cisplatin and related compounds as pro-oxidants in blood*; Silaghi-Dumitrescu, R. and Garban, G., Ed.; Cluj University Press: Cluj-Napoca, Romania, 2009, pp 130-134.

[44] Pendyal, L.; Creaven, P.J. *In vitro* citotoxicity, protein binding reed blood cell partitioning and biotransformation of oxaliplatin. *Cancer. Res.*, **1993**, *53*, 5970-6.

[45] Zao, T.; King, F.L. A mass spectrometric comparision of the interactions of cisplatin and transplatin with myoglobin. *J. Inorg. Biochem.*, **2010**, *104*, 186-192.

[46] Ivanov, A.I.; Christodoulou, J.; Parkinson, J.A.; Barnham, K.J.; A., T.; Woodrow, J.; Sadler, P.J. Cisplatin binding sites on human albumin. *J. Biol. Chem.*, **1998**, *273*(24), 14721-14730.

[47] Lemna, T.; Pawliszyn, J. Human serum albumin interaction with oxaliplatin studied by caplillary isoelectric focusing with the whole column imaging detection. *J. Pharm. Biomed. Anal.*, **2009**, *50*, 570-575.

[48] Yue, Y.; Chen, X.; Qin, J.; Yao, X. Spectroscopic investigation on the binding of antineoplastic drug oxaliplatin to human serum albumin and molecular modeling. *Colloids Surfaces B*, **2009**, *69*, 51-57.

[49] Trynda-Lemiesz, L.; Luczkowski, M. Human serum albumin: spectroscopies studies of the paclitaxel binding and proximity relationship with cisplatin and adriamycin. *J. Inorg. Biochem.*, **2004**, *98*, 1851-1856.

[50] Timerbaev, A.R.; Aleksenko, S.S.; Polec-Pawlak, K.; Ruzik, R.; Semenova, O.; Hartinger, C.G.; Oszwalddowski, S.; Galanski, M.; Jarosz, M.; Keppler, B.K. Platinum metallodrug-protein binding studies by capillary electrophoresis-inductively coupled plasma mass spectrometry: characterization of interaction between Pt(II) complexes and human serum albumin. *Electrophoresis*, **2004**, *25*, 1988-1995.

[51] Aleksenco, S.S.; Hartinger, C.G.; Semenova, O.; Meelich, K.; Timerbaev, A.R.; Keppler, B.K. Characterization of interaction between human serum albumin and tumor-inhibiting amino alcohol platinum(II) complexes using capillary electrophoresis. *J. Cromatogr.*, **2007**, *1155*, 218-221.

[52] Kratz, F. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. *J. Control. Release.*, **2008**, *132*, 171-183.

[53] Oettl, K.; Stauber, R.E. Physiological and pathological changes in the redox state of HSA. *Br. J. Pharm.*, **2007**, *151*, 580-590.

[54] Yoshiyama, Y.; Yamaguchi, K.; Matsuo, K.; Kurokawa, M.; Kanke, M. The effect of fosfomycin on nedaplatin-induced neprothoxicity in rats. *J. Infect. Chemother.*, **2005**, *11*, 14-17.

[55] Mross, K.; Meyberg, F.; Fiebig, H.H.; Hamm, K.; Hieber, U.; Aulenbacher, P.; Hossfeld, D.K. Pharmacokinetic and pharmacodynamic study with lobaplatin (D-19466), a new platinum complex, after bolus administration. *Onkologie*, **1992**, *15*, 139-146.

[56] Kagan, V.E.; Borisenko, G.G.; Tyurina, Y.Y.; Tyurin, V.A.; Jiang, J.; Potapovich, A.I.; Kini, V.; Amoscato, A.A.; Fujii, Y. Oxidative lipidomics of apoptosis: redox catalytic interactions of cytochrome c with cardiolipin and phosphatidylserine. *Free Radic. Biol. Med.*, **2004**, *37*(12), 1963-85.

[57] Kagan, V.E.; Tyurin, V.A.; Jiang, J.; Tyurina, Y.Y.; Ritov, V.B.; Amoscato, A.A.; Osipov, A.N.; Belikova, N.A.; Kapralov, A.A.; Kini, V.; Vlasova, II; Zhao, Q.; Zou, M.; Di, P.; Svistunenko, D.A.; Kurnikov, I.V.; Borisenko, G.G. Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. *Nat. Chem. Biol.*, **2005**, *1*(4), 223-32.

[58] Kagan, V.E.; Bayir, H.A.; Belikova, N.A.; Kapralov, O.; Tyurina, Y.Y.; Tyurin, V.A.; Jiang, J.; Stoyanovsky, D.A.; Wipf, P.; Kochanek, P.M.; Greenberger, J.S.; Pitt, B.; Shvedova, A.A.; Borisenko, G. Cytochrome c/cardiolipin relations in mitochondria: a kiss of death. *Free Radic. Biol. Med.*, **2009**, *46*(11), 1439-53.

[59] Zhao, T.; King, F.L. Direct determination of the primary binding site of cisplatin on cytochrome c by mass spectrometry. *J.Am. Soc. Mass. Spectrom.*, **2009**, *20*, 1141-1149.

[60] Gabbiani, C.; Casini, A.; Mastrobuoni, G.; Kirshenbaum, N.; Moshel, O.; Pieraccini, G.; Moneti, G.; Messori, L.; Gibson, D. Peculiar mechanistic and structural features of the carboplatin-cytochrome c system revealed by ESI-MS analysis. *J. Biol. Inorg. Chem.*, **2008**, *13*(5), 755-64.

[61] Yang, G.; Miao, R.; Chen, J.; Mey, Y.; Tang, H.; Hong, J.; Guo, Z.; Longgen, Z. Determination of binding sites in carboplatin-bound cytochrome c using electrospray ionization mass spectrometry and tandem mass spectrometry. *J. Mass. Spectrom.*, **2005**, *40*, 1005-1016.

[62] Boswell, A.P.; Moore, G.R.; Williams, R.J.P. The binding of platinum complexes to tuna cytochrome c. *Biochem. J.*, **1982**, *201*, 523-526.

[63] Larson, A.C.; Adams, P.L.; Jandial, D.D.; Blair, B.G.; Safaei, R.; Howel, S.B. The role of the N-terminus of mammalian copper transporter 1 in the cellular accumulation of cisplatin. *Biochem. Pharmacol.*, **2010**, *80*, 448-454.

[64] Dolgova, N.V.; Olson, D.; Lutsenko, S.; Dmitriev, O.Y. The soluble metal-binding domain of the copper transporter ATP7B binds and detoxifies cisplatin. *Biochem. J.*, **2009**, *419*(1), 51-6.

[65] Arnesano, F.; Natile, G. "Platinum on the road": Interactions of antitumoral cisplatin with proteins. *Pure Appl. Chem.*, **2008**, *80*, 2715-2725.

[66] Liang, X.-J.; Shen, D.-W.; Garfield, S.; Gottesman, M.M. Mislicalisation of Membrane Proteins Associated with Multidrug Resistance in Cisplatin-resistant Cancer Cell Lines. *Cancer Res.*, **2003**, *63*, 5909-5916.

[67] Shen, D.-W.; Liag., X.-J.; Gawinowicz., M.A.; Gottesman., M.M. Identification of cytoskeletal [14C] Carboplatin-Binding Proteins Reveals Reduced Expression and Disorganization of Actin and Filamin in Cisplatin-Resistant Cell Lines. *Mol. Pharmacol.*, **2004**, *66*, 789-793.

[68] Holm, R.H.; Kennepohl, P.; Solomon, E.I. Structural and Functional Aspects of Metal Sites in Biology. *Chem. Rev.*, **1996**, *96*(7), 2239-2314.

[69] Davies, D.R.; Braam, L.M.; Reznikoff, W.S.; Rayment, I. Threedimensional structure of a TN5-transposase-related proteins determined at 2.9-A resolution. *J. Biol. Chem.*, **1999**, *274*, 11904-11913.

[70] Gorman, M.A.; Morera, S.; Rothwell, D.G.; de La Fortelle, E.; Mol, C.D.; Tainer, J.A.; Hickson, I.D.; Freemont, P.S. The crystal structure of the human DNA repair endonuclease HAP1 suggests the recognition of extra-helical deoxyribose at DNA abasic sites. *EMBO J.*, **1997**, *16*, 6548-6558.

[71] Diez, J.; Diederichs, K.; Greller, G.; Horlacher, R.; Boos, W.; Welte, W. The crystal structure of a liganded trehalose/maltose-binding protein from the hyperthermophilic Archaeon Thermococcus litoralis at 1.85 A. *J. Mol. Biol.*, **2001**, *305*, 905-915.

[72] Schubot, F.D.; Kataeva, I.A.; Blum, D.L.; Shah, A.K.; Ljungdahl, L.G.; Rose, J.P.; Wang, B.C. Structural basis for the substrate specificity of the feruloyl esterase domain of the cellulosomal xylanase Z from Clostridium thermocellum. *Biochem. Biophys. Res. Commun.*, **2001**, *40*, 12524-12532.

[73] Gu, Y.; Reshetnikova, L.; Li, Y.; Wu, Y.; Yan, H.; Singh, S.; Ji, X. Crystal structure of shikimate kinase from Mycobacterium tuberculosis reveals the dynamic role of the LID domain in catalysis. *J. Mol. Biol.*, **2002**, *319*, 779-789.

[74] Zeth, K.; Ravelli, R.B.; Paal, K.; Cusack, S.; Bukau, B.; Dougan, D.A. Structural analysis of the adaptor protein ClpS in complex with the N-terminal domain of ClpA. *Nat. Struct. Biol.*, **2002**, *9*, 906-911.

[75] Garrett, T.P.J.; McKern, N.M.; Lou, M.; Elleman, T.C.; Adams, T.E.; Lovrecz, G.O.; Zhu, H.-J.; Walker, F.; Frenkel, M.J.; Hoyne, P.A.; Jorissen, R.N.; Nice, E.C.; Burgess, A.W.; Ward, C.W. Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha. *Cell*, **2002**, *110*, 763-773.

[76] Fernandez-Tornero, C.; Lopez, R.; Garcia, E.; Gimenez-Gallego, G.; Romero, A. A novel solenoid fold in the cell wall anchoring domain of the pneumococcal virulence factor LytA. *Nat. Struct. Biol.*, **2001**, *8*, 1020-1024.

[77] Lukacik, P.; Roversi, P.; White, J.; Esser, D.; Smith, G.P.; Billington, J.; Williams, P.A.; Rudd, P.M.; Wormald, M.R.; Harvey, D.J.; Crispin, M.D.M.; Radcliffe, C.M.; Dwek, R.A.; Evans, D.J.; Morgan, B.P.; Smith, R.A.G.; Lea, S.M. Complement regulation at the molecular level: the structure of decay-accelerating factor. *Proc. Natl. Acad. Sci.*, **2004**, *101*, 1279-1284.

[78] Vogt, J.; Schulz, G.E. The structure of the outer membrane protein OmpX from Escherichia coli reveals possible mechanisms of virulence. *Structure*, **1999**, *7*, 1301-1309.

[79] Lee, S.; Sowa, M.E.; Watanabe, Y.; Sigler, P.B.; Chiu, W.; Yoshida, M.; Tsai, F.T.F. The structure of ClpB: a molecular chaperone that rescues proteins from an aggregated state. *Cell*, **2003**, *115*, 229-240.

[80] Varughese, K.I.; Ruggeri, Z.M.; Celikel, R. Platinum-induced space-group transformation in crystals of the platelet glycoprotein Ib alpha N-terminal domain. *Acta Crystallogr. Sect. D*, **2004**, *60*, 405-411.

[81] Patel, S.D.; Rajala, M.W.; Rossetti, L.; Scherer, P.E.; Shapiro, L. Disulfide-dependent multimeric assembly of resistin family hormones. *Science*, **2004**, *304*, 1154-1158.

[82] Baikalov, I.; Schroder, I.; Kaczor-Grzeskowiak, M.; Grzeskowiak, K.; Gunsalus, R.P.; Dickerson, R.E. Structure of the Escherichia coli response regulator NarL. *Biochemistry*, **1996**, *35*, 11053-11061.

[83] Kumaraswami, M.; Howe, M.M.; Park, H.W. Crystal structure of the Mor protein of bacteriophage Mu, a member of the Mor/C family of transcription activators. *J. Biol. Chem.*, **2004**, *279*, 16581-16590.

[84] Papagrigoriou, E.; Gingras, A.R.; Barsukov, I.L.; Bate, N.; Fillingham, I.J.; Patel, B.; Frank, R.; Ziegler, W.H.; Roberts, G.C.; Critchley, D.R.; Emsley, J. Activation of a vinculin-binding site in the talin rod involves rearrangement of a five-helix bundle. *EMBO J.*, **2004**, *23*, 2942-2951.

[85] Fujimoto, Z.; Kaneko, S.; Momma, M.; Kobayashi, H.; Mizuno, H. Crystal structure of rice alpha-galactosidase complexed with D-galactose. *J. Biol. Chem.*, **2003**, *278*, 20313-20318.

[86] Pedersen, L.; Henriksen, A. Acyl-CoA oxidase 1 from Arabidopsis thaliana. Structure of a key enzyme in plant lipid metabolism. *J. Mol. Biol.*, **2005**, *345*, 487-500.

[87] Locher, M.; Lehnert, B.; Krauss, K.; Heesemann, J.; Groll, M.; Wilharm, G. Crystal structure of the Yersinia enterocolitica type III secretion chaperone SycT. *J. Biol. Chem.*, **2005**, *280*, 31149-31155.

[88] Tarbouriech, N.; Ruggiero, F.; Deturenne-Tessier, M.; Ooka, T.; Burmeister, W.P. Structure of the Epstein-Barr virus oncogene BARF1. *J. Mol. Biol.*, **2006**, *359*, 667-668.

[89] Goto, T.; Abe, Y.; Kakuta, Y.; Takeshita, K.; Imoto, T.; Ueda, T. Crystal structure of Tapes japonica Lysozyme with substrate analogue: structural basis of the catalytic mechanism and manifestation of its chitinase activity accompanied by quaternary structural change. *J. Biol. Chem.*, **2007**, *282*, 27459-27467.

[90] Casini, A.; Mastrobuoni, G.; Temperini, C.; Gabbiani, C.; Francese, S.; Moneti, G.; Supuran, C.T.; Scozzafava, A.; Messori, L. ESI mass spectrometry and X-ray diffraction studies of adducts between anticancer platinum drugs and hen egg white lysozyme. *Chem. Commun.*, **2007**, 156-158.

[91] Yang, X.; Zhou, J.; Sun, L.; Wei, Z.; Gao, J.; Gong, W.; Xu, R.M.; Rao, Z.; Liu, Y. Structural basis for the function of DCN-1 in protein Neddylation. *J. Biol. Chem.*, **2007**, *282*, 24490-24494.

[92] Bachhawat, P.; Stock, A.M. Crystal structures of the receiver domain of the response regulator PhoP from Escherichia coli in the absence and presence of the phosphoryl analog beryllofluoride. *J. Bacteriol.*, **2007**, *189*, 5987-5995.

[93] Martinez, M.A.; Zaballa, M.E.; Schaeffer, F.; Bellinzoni, M.; Albanesi, D.; Schujman, G.E.; Vila, A.J.; Alzari, P.M.; De Mendoza, D. A novel role of malonyl-ACP in lipid homeostasis. *Biochemistry*, **2010**, *49*, 3161-3167.

[94] Angkawidjaja, C.; You, D.J.; Matsumura, H.; Kuwahara, K.; Koga, Y.; Takano, K.; Kanaya, S. Crystal structure of a family I.3 lipase from Pseudomonas sp. MIS38 in a closed conformation. *FEBS Lett.*, **2007**, *581*, 5060-5064.

[95] Lo, Y.C.; Ko, T.P.; Su, W.C.; Su, T.L.; Wang, A.H.J. Terpyridine-platinum(II) complexes are effective inhibitors of mammalian topoisomerases and human thioredoxin reductase 1. *J. Inorg. Biochem.*, **2009**, *103*, 1082-1092.

[96] Boal, A.K.; Rosenzweig, A.C. Crystal structures of cisplatin bound to a human copper chaperone. *J. Am. Chem. Soc.*, **2009**, *131*, 14196-14197.

Received: October 04, 2010 Revised: December 10, 2010 Accepted: January 11, 2011