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*Review*

# Coordination chemistry of glutathione*

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**The metal ion coordination abilities of reduced and oxidized glutathione are reviewed. Reduced glutathione (GSH) is a very versatile ligand, forming stable complexes with both hard and soft metal ions. Several general binding modes of GSH are described. Soft metal ions coordinate exclusively or primarily through thiol sulfur. Hard ones prefer the amino acid-like moiety of the glutamic acid residue. Several transition metal ions can additionally coordinate to the peptide nitrogen of the γ-Glu-Cys bond. Oxidized glutathione lacks the thiol function. Nevertheless, it proves to be a surprisingly efficient ligand for a range of metal ions, coordinating them primarily through the donors of the glutamic acid residue.**

Reduced glutathione (GSH) is a non-protein tripeptide of the sequence γ-Glu-Cys-Gly. It is omnipresent in biological fluids and serves a variety of fundamental physiological functions. The intracellular concentration of GSH in human cells is often as high as 10 mM. This makes it one of the two most abundant organic substances present in the human body [1, 2]. Within the cell, GSH is most highly concentrated in the nucleus and in mitochondria.

Glutathione is a cofactor for many cellular repair enzymes, forming S-conjugates with xenobiotics, that are subsequently removed from cells by active transport [3]. Another role for GSH resulting from its low redox potential of -0.23 V, is to maintain essential oxidation-prone proteins in their reduced form [4]. These include hemoglobin, thiol redox proteins, structural zinc finger motifs and metallothioneins (MT). GSH can be thus

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**Abbreviations:** GSH, reduced glutathione; GSSG, oxidized glutathione; MT, metallothionein.

treated as a redox buffer of a cell. It is also involved in chromatin condensation and DNA expression regulation [5]. Another function of glutathione is to protect cellular structures from oxidative stress. GSH is a suicide substrate for a variety of reactive oxygen species (ROS), like $O_2^{\bullet -}$, $H_2O_2$, $^{\bullet}OH$, NO etc. It is also the ROS substrate for glutathione peroxidase, a repair enzyme reducing fatty acid peroxides and thereby maintaining the integrity of cellular membranes. GSH is, thus, a major intracellular antimutagen [6, 7].

In the course of the oxidative processes GSH becomes oxidized to its disulfide, oxidized glutathione (GSSG). This disulfide is normally present at low concentrations in the cell, because it is actively removed through the cell membrane and reduced back to GSH by glutathione reductase. Nevertheless, GSSG has several important physiological functions, e.g. as a specific oxidizing agent that helps to form disulfide bridges in proteins and as a regulator of metal content of MT [8–14].

Intra-, inter- and extracellular metal ion transport is a distinct function of GSH which is responsible for delivering a variety of metal ions to apoproteins. This is possible because a GSH molecule possesses all kinds of biological donor atoms and is conformationally flexible. One can distinguish in it as many as eight coordination sites (presented in Fig. 1a): two carboxyls, one thiol, one amino, and two pairs of carbonyl and amide donors within two peptide bonds. A GSSG molecule (Fig. 1b) has all these donor atoms doubled, except for thiols which are replaced by a disulfide moiety.

Four of the GSH donor atoms can protonate and deprotonate in water solution. These are the two carboxylic acids, the amine and the thiol. The protonation processes can be described quantitatively with the use of overall protonation constants (Table 1) as well as microconstants (Fig. 2) [15]. For definitions of particular constants see Annex.

Conformational studies on GSH [16] indicate that, at neutral pH, the particular donor groups of GSH do not interact effectively with each other. The most populated rotamer has the amine and carboxylate of the Glu residue maximally separated from the thiol, and the latter is also *trans* to either of the peptide bonds flanking the Cys residue.

Values of protonation constants (pK) for GSSG [17] are also included in Table 1.

The main objective of this review is to present the interactions of reduced and oxidized glutathione with a range of metal ions. Attention is paid to examples of very stable complexes (that may thus be of biological importance) and to redox activities resulting from metal ion–glutathione bonding (which are relevant to metal toxicology).

## COMPLEXES OF METAL IONS WITH GSH

The eight donor atoms of GSH can be grouped into three classes of binding sites: the glutamic (amino acid-like) set of amine and carboxylate donors, the thiol, and the peptide bonds. (The isolated carboxylate of glycine can be functionally included into the first class, but often participates in coordination with the thiol donor, due to the spatial constraints). The hard metal donors primarily interact with the glutamic moiety, while the soft ones prefer the thiol.

Among the generally weak complexes of group I and II, those of Mg(II) and Ca(II) were studied and were (as expected) found to interact primarily (at lower pH) with carboxylates and secondarily (at higher pH) also with the glutamic amine. Table 2 presents the stability constants for Ca(II) complexes [18].

The stability constants established on the basis of thermodynamic and NMR spectroscopic studies of GSH complexation by soft (heavy) metal ions of the main groups, Pb(II), Sn(IV), Tl(III), and for electronically analogous group 10 metals, Hg(II), Cd(II) and Zn(II), are also presented in Table 2. All these metal ions, except Zn(II), are industrially and environmen-

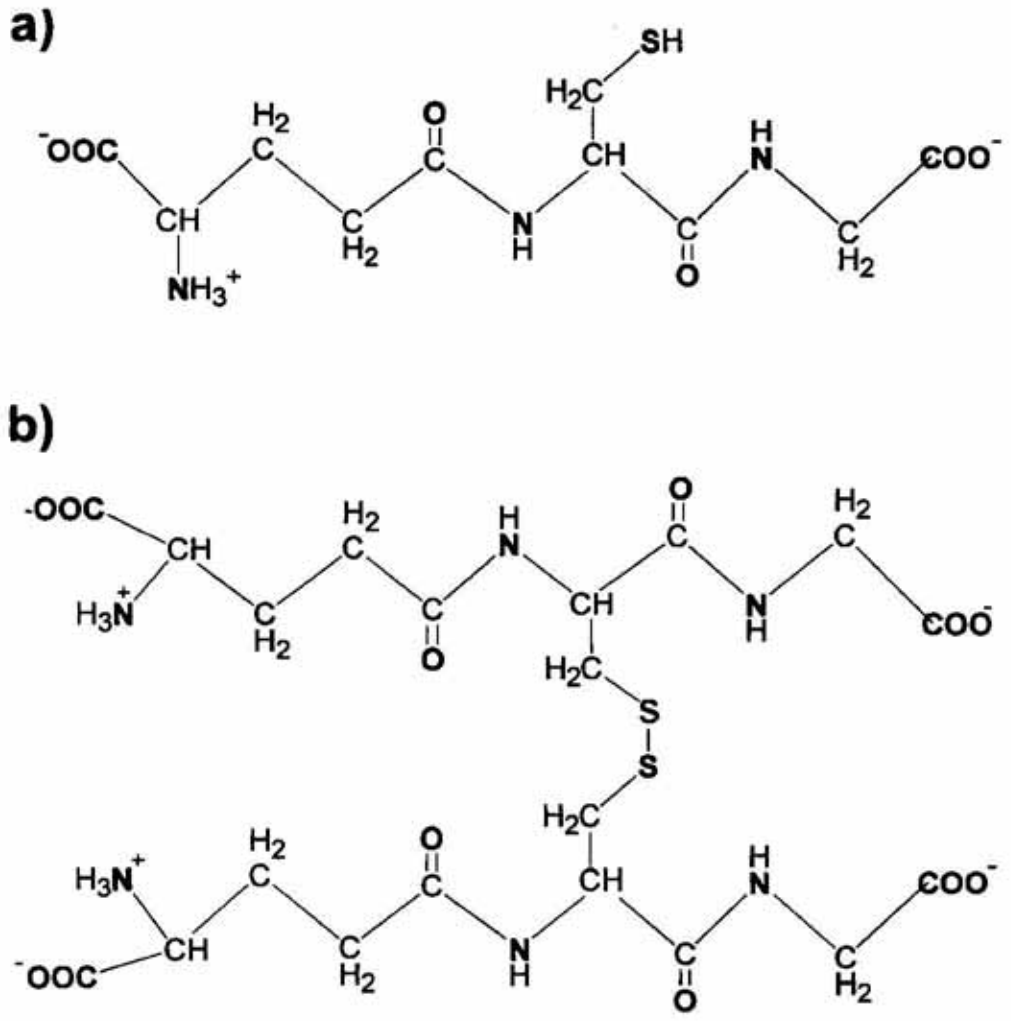



**Figure 1. The structures of reduced glutathione, GSH (a) and oxidized glutathione, GSSG (b).**

tally toxic to humans. Zn(II) is a major physiological metal ion. Taking into account the physiological abundance and roles of GSH, studies on its coordination by the above listed metal ions are very important for elucidating such aspects of their toxicology, as transport, bioaccumulation and impairment of cellular redox systems.

Lead(II) coordinates GSH above pH 4, forming six different complexes at various pH values [19, 20]. The thiol donor is the primary anchor of the metal ion in all of them. In addition, oxygen donors of Gly residue participate in the binding up to pH 9, at which they are substituted by hydroxyl groups.

The aquacomplex of trimethyltin(IV), $[Sn(CH_3)_3(H_2O)_2]^+$, initially exchanges water molecules to bind to the glutamic moiety. The thiol becomes the main binding site at higher pH, yielding a stable complex of MHL stoichiometry (M denotes trimethyltin and HL stands for monoprotonated glutathione molecule). The stability is enhanced by the simultaneous coordination of glycine carboxylate to tin [21]. In contrast, dimethylthallium(III) probably coordinates to the glutamate moiety of GSH in addition to the primary bonding through sulfur [22] (Fig. 3).

The extremely toxic mercury(II) forms the strongest complexes with biological thiol donor ligands, like MT, sulfur proteins and also GSH. Complexes with coordination through sulfur only were found to exist (GS-Hg-SG, $CH_3$-Hg-SG, where G represents the remain-

**Table 1. Micro- and macroscopic equilibrium pronotation constants of GSH and GSSG [15, 36]**

| Glutathione | Pronotation constants | Value |
|---|---|---|
| GSH | $pK_1$ | 2.12 |
| | $pK_2$ | 3.53 |
| | $pK_3$ | 8.66 |
| | $pK_4$ | 9.62 |
| | $pk_1$ | 2.09 |
| | $pk_2$ | 3.12 |
| | $pk_{12}$ | 3.36 |
| | $pk_{21}$ | 2.33 |
| | $pk_{123}$ | 8.93 |
| | $pk_{124}$ | 9.13 |
| | $pk_{1234}$ | 9.28 |
| | $pk_{1243}$ | 9.08 |
| | $pk_5$ | −1.7 |
| GSSG | $pK_1$ | 1.79 |
| | $pK_2$ | 2.39 |
| | $pK_3$ | 3.16 |
| | $pK_4$ | 3.82 |
| | $pK_5$ | 8.44 |
| | $pK_6$ | 9.90 |

der of GSH beyond the thiol group), having a characteristic linear structure [15, 20] (Fig. 4).

Seven stoichiometric forms were found in solutions containing GSH and cadmium(II) ions at various pH values. The thiolate donor is coordinated to Cd(II) at pH as low as 3.5. The glycyl carboxylate coordinates at weakly acidic and neutral pH (below 8.5), while the glutamic moiety coordinates at and above pH 6.5 [19, 23, 24]. The coordinative deprotonation of the γ-Glu–Cys peptide bond was detected in very alkaline solutions [20].

Zinc(II) is generally similar to Cd(II) in its coordination abilities, including a preference for thiol bonding. Zn(II) complexes with GSH can be detected in solution even below pH 4. Below pH 8.5 the coordination sphere includes also the glycyl carboxylate. Tetrahedral {S,O} coordinated complexes MHL and $M(HL)_2$ are also formed. In the neutral and alkaline pH range another kind of complex is also present, having Zn(II) coordinated in the amino acid-like fashion, through the glutamic moiety. The maximum presence of such complexes is centered around pH 9–9.5. At still higher pH the γ-Glu–Cys peptide bond undergoes Zn(II)-assisted deprotonation, yielding $Zn(H_{-1}L)$ and $Zn(H_{-1}L)_2$ (Fig. 5). The hydroxyl-containing mixed complexes are formed only at low metal-to-ligand ratios [8, 9, 18–20, 25, 26, 28] (A. Krężel and W. Bal, 1999, *XV-th Peptides Symposium-"Peptides 99", Waplewo, Poland, Book of Abstracts;* A. Krężel and W. Bal, manuscript in preparation).

The data presented in Table 2 allow to calculate the order of stability of GSH complexes with particular metal ions:

$$CH_3Hg\text{-}SG \sim Hg\text{-}(SG)_2 > Cd\text{-}(SG)_2 > Zn\text{-}(SG)_2 \sim Pb\text{-}(SG)_2 > Me_3Sn\text{-}(SG) > Me_2Tl\text{-}(SG).$$

Platinum(II) coordination to GSH gained wide interest along with the development of platinum anticancer drugs. Studies of the equilibrium involving Pt(II) are very difficult due to the profound inertness of such systems. *Cis*-diamminedichloroplatinum(II) (cisplatin) forms at least three different complexes with GSH at physiological pH [30–34]. The sulfur and the peptide nitrogen of the γ-Glu–Cys bond are involved in coordination in these square-planar complexes (Fig. 6). These studies, pointing to the ease of formation of GSH-containing ternary complexes by Pt(II), helped to understand the processes of cellular resistance to cisplatin involving the glutathione pump system [35].

Palladium(II) also forms square-planar complexes of $ML_2$ stoichiometry with GSH. Similarly to Pt(II), the coordination is effected through the sulfur and the peptide nitrogen of the γ-Glu–Cys bond. A particular feature of Pd(II) coordination is the formation of bridged dimers of the $M_2X_2L_2$ type (X = e.g. $Cl^-$) [36, 37].

**Table 2. Stability constants for GSH complexes with soft metal ions**

| $M^{n+}$ | $\log \beta_{MHL}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $MH_2L$ | MHL | ML | $MH_{-1}L$ | $MH_2L_2$ | $MHL_2$ | $ML_2$ | $MH_{-1}L_2$ |
| $(Me)_2Tl^+$ (III) [a] | | 11.19 | 2.39 | | | | | |
| $(Me)_3Sn(H_2O)_2^+$ (IV) [b] | | 14.17 | | | | | | |
| $Ca^{2+}$ [c] | 20.68 | 12.89 | 3.84 | -6.46 | | | | |
| $Cd^{2+}$ [d] | | 17.02 | 10.18 | 0.29 | 33.03 | 25.09 | 15.35 | 3.17 |
| $Zn^{2+}$ [d] | | 14.76 | 8.57 | -0.07 | 30.62 | 23.27 | 13.59 | 3.63 |
| $Pb^{2+}$ [d] | | 17.14 | 10.57 | | 32.10 | 24.66 | 15.00 | 4.50 |
| $MeHg^+$ (II) [a] | 28.68 | 25.24 | 15.99 | | | | | |

[a][22]; [b][21]; [c][18]; [d][19].

Nickel(II) is another metal ion capable of forming square-planar, diamagnetic complexes with GSH, with coordination through sulfur and nitrogen donors. In this case, however, octahedral, paramagnetic complexes with nitrogen/oxygen coordination are also present in equilibrium [38–43] (A. Krężel and W. Bal, manuscript in preparation). It is generally assumed that the low pH (5–6.5) complex has a stoichiometry of NiHL and amino acid-like coordination through the glutamic moiety. At the physiological pH range monomeric or dimeric square-planar complexes are also formed, depending on concentration and metal-to-ligand ratio. In alkaline conditions (pH 10–12) other diamagnetic

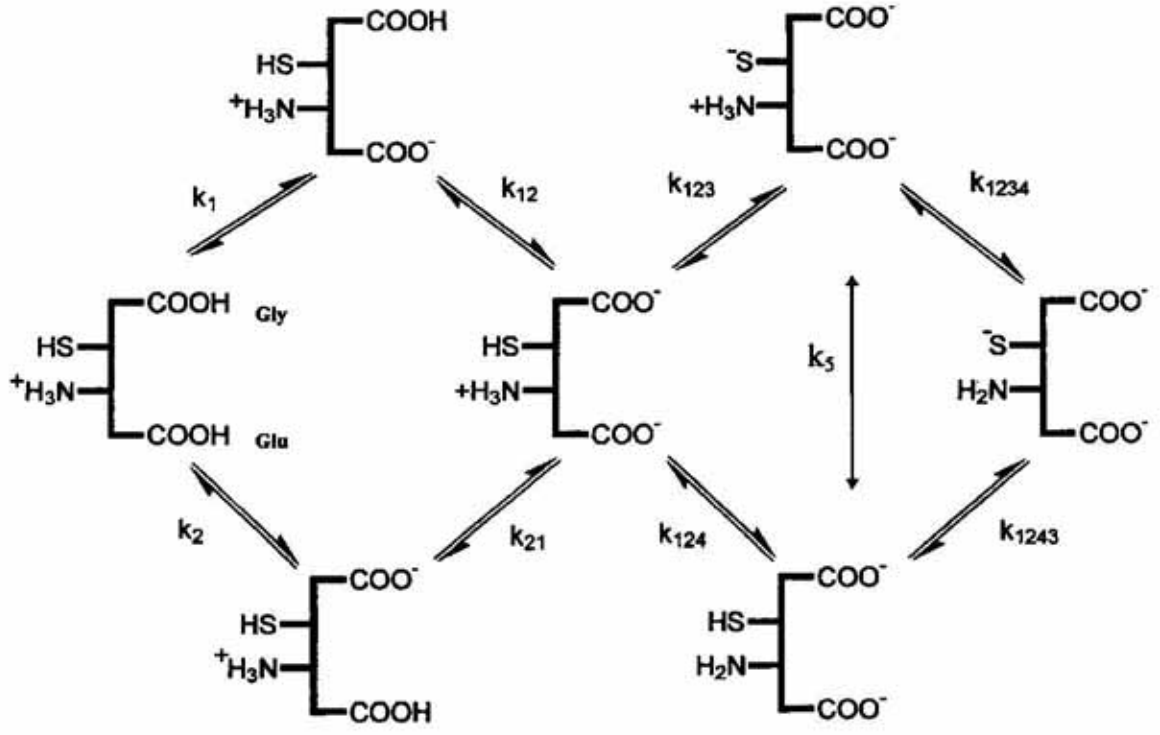



**Figure 2. Scheme of molecular protonation of GSH [15, 17].**

Symbols on the arrows denote particular microconstants. See Annex for definition.

complexes prevail, having Ni(II) coordinated through amide nitrogens.

Nickel(II) complexes (the square-planar ones

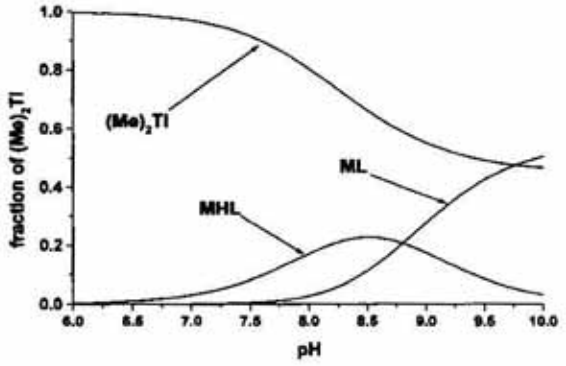



**Figure 3. Species distribution for $Tl(Me)_2$–GSH complexes, calculated for 5 mM:0.1 mM (GSH: $M^{n+}$) molecular ratio, based on the published stability constants [22].**

in particular) exhibit oxidation-promoting properties. In the presence of ambient oxygen, Ni(II) markedly accelerates GSH oxidation to GSSG, while in the presence of $H_2O_2$ Ni(II)–GSH complexes catalyze the formation of oxygen free radicals [45–48].

Copper(II) was found to form tetragonal amino acid-like CuHL and $Cu(HL)_2$ complexes

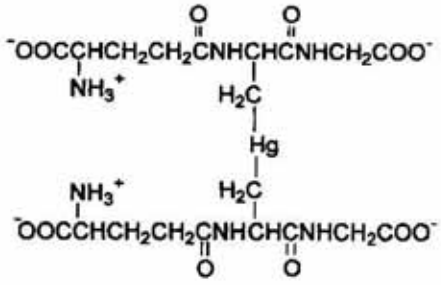


**Figure 4. The Hg(II)–GSH complex, exhibiting a characteristic linear sulfur coordination [15].**

at pH below 6.5. At higher pH Cu(II) is efficiently reduced by GSH, Cu(I) and GSSG being the reaction products. When an excess of GSH is present, coordination of Cu(I) to GSH occurs, with thiol sulfur as the only donor [37, 49, 50]. NMR and EXAFS studies revealed an interesting trigonal structure for this complex, with Cu(I) coordinated to three sulfur donors [51]. In the solid state, as well as in solution, clusters with multiple sulfur bridges are formed, mimicking somewhat the situation observed for copper metallothioneins.

Two general kinds of GSH complexes were found for cobalt(II) – the octahedral amino-acid-like ones, similar to those found e.g. for Ni(II), and tetrahedral ones, involving Cys and Gly donors. Monomer-dimer equilibrium was found for Co(II), with mixed-geometry $Co_2HL_3$ and $Co_2L_3$ species (Fig. 7) [37, 52].

Iron(III) undergoes reduction to iron(II) in the presence of GSH, similarly as does Cu(II). Fe(II) coordinates GSH with the use of carboxylate donors at pH 3–7. The complexes are weak, however, and hydrolysis occurs at

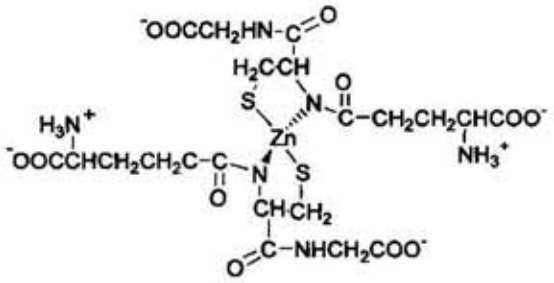


**Figure 5. The high-pH Zn(II)–GSH complex, with amide nitrogen coordination to Zn(II), based on $^{13}C$ NMR [20].**

pH 8, yielding a $Fe(OH)_2$ precipitate. On the other hand, a transient interaction between iron and sulfur occurs during Fe(III) reduction or Fe(II) air oxidation (Fig. 8). Sulfur participates in electron transfer and sulfur-center radical coordinates to the iron ion [53, 54].

Chromium(VI) in the form of chromate ion is efficiently reduced by GSH to Cr(III), as expected. However, the first one-electron step of this complicated reaction is very specific, and a metastable Cr(V)–GSH complex can be identified. This complex, of tetragonal pyramid geometry (Fig. 9) is a very destructive intracellular oxidative toxin in the case of chromate intoxication. The redox reaction further proceeds *via* short-lived Cr(IV) intermediates, and oxygen and sulfur-centered radicals are produced in its course [55, 58].

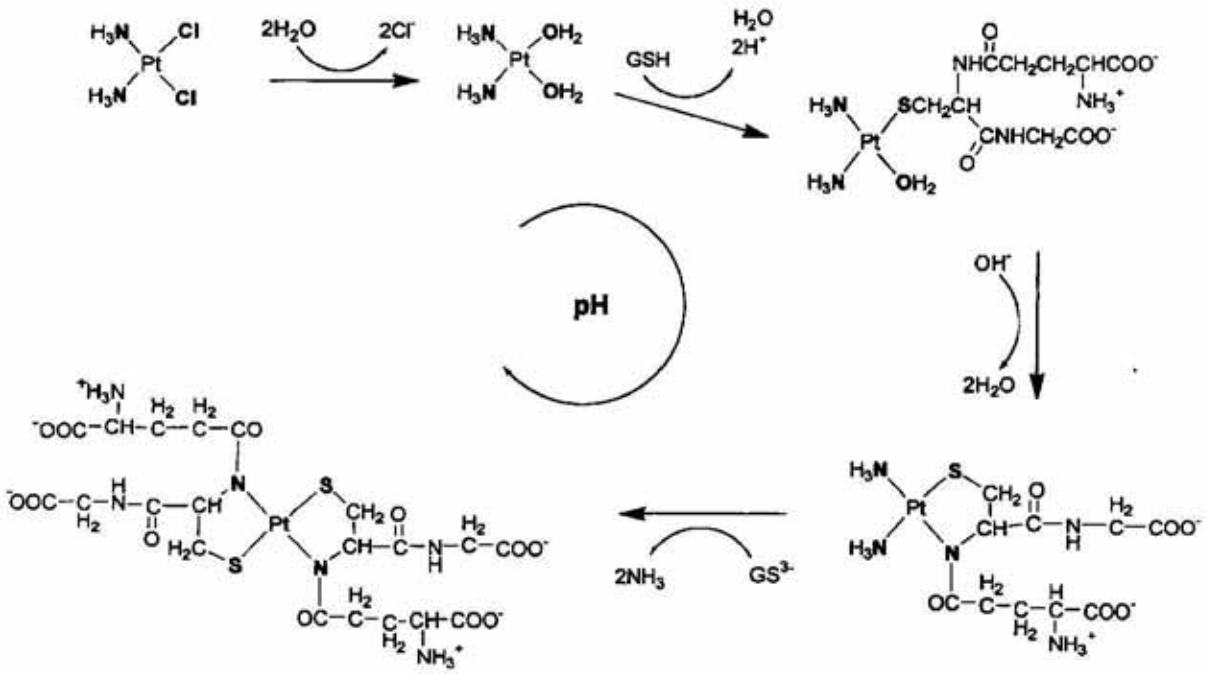



**Figure 6. The binding modes of cisplatin to GSH [30–34].**

## COMPLEXES OF METAL IONS WITH GSSG

Oxidized glutathione has no thiol function, thus it does not contain the main coordination site for many of the metal ions described above. Instead, it can offer two glutamic {N,O} chelate systems brought close to each other by the disulfide bridge. This arrangement can offer an additional stabilizing factor in the form of a large, 19-membered ring. Nevertheless, GSSG is usually disregarded as a ligand, compared to GSH, and the literature on its complexes is much smaller.

Zinc coordinates GSSG through the glutamic donors, yielding "open" and "closed" types of complexes (in the "closed" complex the metal ion is coordinated to both glutamic moieties of one GSSG molecule, Fig. 10; in the "open" complexes GSSG behaves like a simple amino acid). MHL, ML, $MHL_2$ and $ML_2$ complexes were detected [17] (A. Krężel and W. Bal, manuscript in preparation). Quite surprisingly, the stabilities of these complexes are comparable to those formed by GSH. This indicates that the 19-membered macrochelate can compensate for the lack of the thiolate donor.

Similar GSSG complexes were found for Ni(II) and Co(II). Consistently with the absence of the thiol donor, no low-spin complexes were found for Ni(II) [17, 59]. No tetragonal complexes were seen for Co(II) either. On the other hand, the high stability of GSSG complexes with these metal ions is confirmed by the absence of hydrolysis even at very high pH values [17, 59]. With Co(II), GSSG behaves as an oxidant, slowly yielding Co(III) and GSH. This reaction is reversible and the resulting solutions contain a mixture of all four components. Both GSH and GSSG complexes with Co(II) can form dioxygen bridged dimers, a phenomenon often encountered in cobalt chemistry [60].

Copper(II) is the only metal ion for which a direct interaction with the disulfide bridge was detected. At low pH (as low as 2.5) Cu(II) binds to the glutamic moiety, forming a

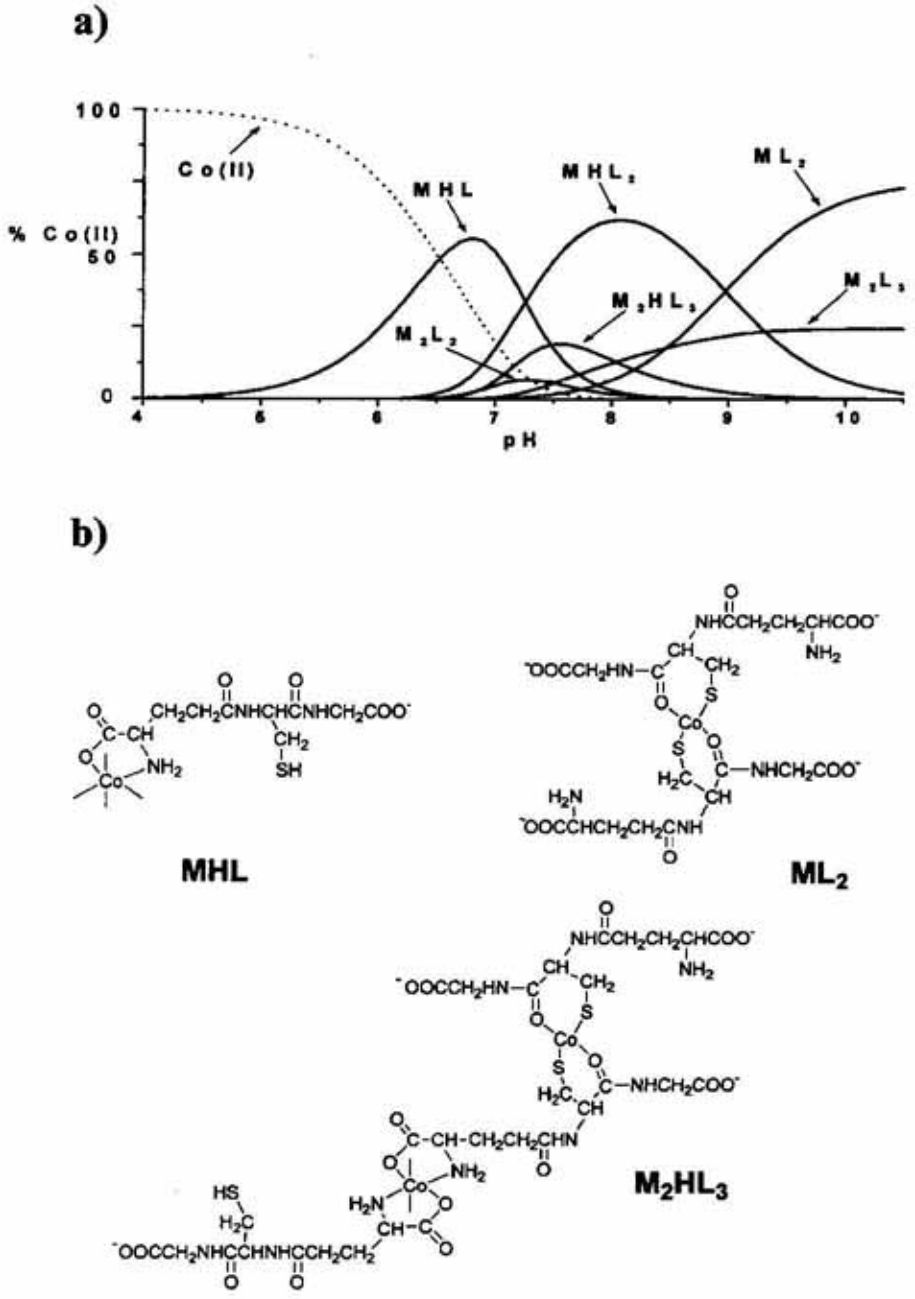



**Figure 7. Species distribution for Co(II) complexes with GSH (a), and structures of complexes present at physiological pH [52].**

"closed" $MH_3L$ complex [17, 59]. The sulfur coordination occurs at neutral and alkaline pH. In these conditions the coordination geometry is completely different from that at low pH. Cu(II) coordinates GSSG through one amino nitrogen, the carboxylate group of Gly, two amide nitrogens and one of the bridging sulfur atoms (Fig. 11) [61, 62]. Due to the presence of two identical "GS" parts in the GSSG molecule a simultaneous coordination of two Cu(II) ions to one GSSG molecule is possible, and that leads to the formation of polynuclear forms.

## SUMMARY

The data presented in this short review allow to conclude that glutathione, both in the reduced and the oxidized form, is a strong and

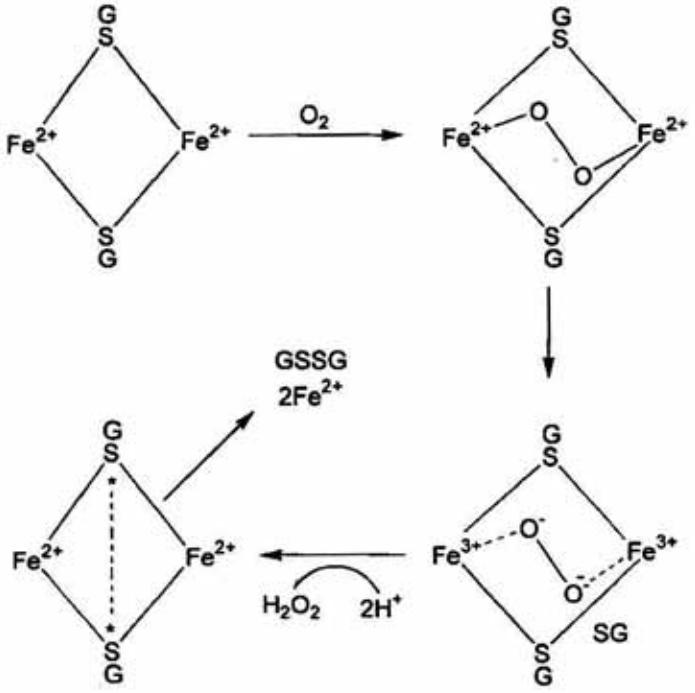



**Figure 8. Catalytic effect of Fe(II) on GSH oxidation in the presence of $O_2$ [53, 54].**

versatile complexing agent. GSH exhibits two general coordination modes. It binds soft metal ions with the thiol donor and auxilliary oxygen donors of the glycine residue. The coordination of the glutamic donors to such metals occurs at higher pH, upon Glu amine deprotonation, and clearly is of secondary importance. Harder metal ions, including those of the first transition row, have the binding order reversed – with initial coordination to the glutamic moiety which is followed by thiol coordination. The latter is often accompanied by the coordinative deprotonation of the Glu–Cys amide nitrogen, at high pH, especially in the case of such metal ions as Ni(II), Co(II), Zn(II) and Cd(II). With GSSG all the binding occurs through the glutamic moiety. GSSG can be thus regarded from the coordination point of view as a doubled amino-acid analog. The most efficient binding can be seen for the first transition row elements, like Cu(II), Ni(II) and Zn(II).

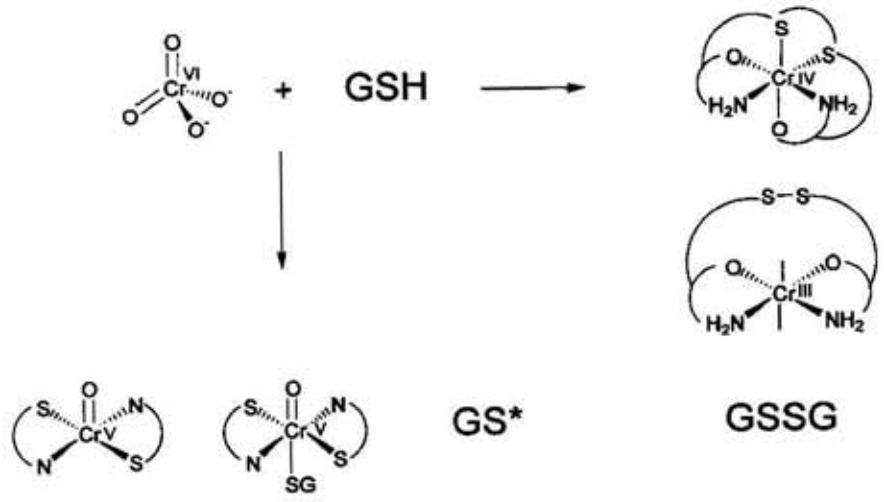



**Figure 9. The reduction of Cr(VI) by GSH, yielding a biologically stable Cr(V) complex with GSH [55–58].**

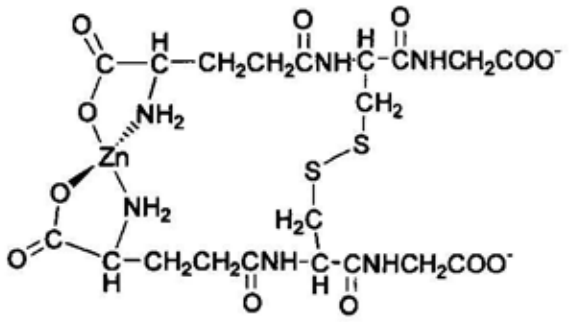


**Figure 10. The proposed solution structure of the ZnL complex with GSSG [17, 59].**

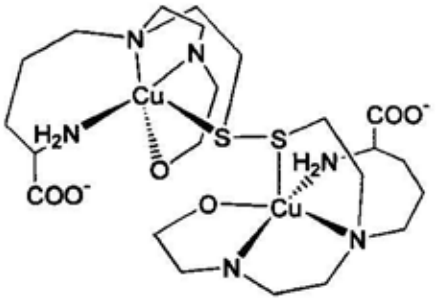


**Figure 11. The structure of Cu(II) complex with GSSG [61, 62].**

From the biological point of view, the probably most important issue has not been approached by coordination chemistry yet. The evidence (including chemical studies presented above) is accumulating for glutathione being a physiological metal ion transporter. The metal uptake and release processes are fundamental for such a role. These processes would almost certainly involve the formation of ternary complexes with metal storage molecules on one end and with target molecules (e.g. metalloenzymes) on the other. Other ternary complexes, e.g. with cellular amino acids, may form during transport. The modelling of such phenomena is the major task which should be undertaken by the coordination chemistry of glutathione.

## REFERENCES


1. Meister, A. & Anderson, M.E. (1983) Glutathione. *Annu. Rev. Biochem.* **52**, 711–760.
2. Rabenstein, D.L. (1989) Metal complexes of glutathione and their biological significance; in *Glutathione*, Chapter V, pp. 147–186, New York.
3. Ballatori, N. (1994) Glutathione mercaptides as transport forms of metals. *Adv. Pharm.* **27**, 271–296.
4. Bartosz, G. (1995) *The second face of oxygen.* Warszawa, PWN (in Polish).
5. Bellomo, G., Vairetti, M., Stivala, L., Mirabelli, F., Richelmi, P. & Orrenius, S. (1992) Demonstration of nuclear compartmentalization of glutathione in hepatocytes. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4412–4416.
6. Munday, R. (1994) Bioactivation of thiols by one-electron oxidation. *Adv. Pharm.* **27**, 237–270.
7. Kasprzak, S.K. (1991) The role of oxidative damage in metal carcinogenicity. *Chem. Res. Toxicol.* **4**, 604–616.
8. Jacob, C., Maret, W. & Vallee, B.L. (1999) Selenium redox biochemistry of zinc-sulfur coordination sites in proteins and enzymes. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 1910–1914.
9. Maret, W. (1994) Oxidative metal release from metallothionein *via* zinc-thiol/disulfide interchange. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 237–241.
10. Savas, M.M., Shaw III, C.F. & Petering, D.H. (1993) The oxidation of rabbit liver metallothionein-II by 5,5′-dithiobis(2-nitrobenzoic acid) and glutathione disulfide. *J. Inorg. Biochem.* **52**, 235–249.
11. Maret, W., Jacob, C., Vallee, B.L. & Fischer, E.H. (1999) Inhibitory sites in enzymes: Zinc removal and reactivation by thionein. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 1936–1940.
12. Maret, W. & Vallee, B.L. (1998) Thiolate ligands in metallothionein confer redox activity on zinc clusters. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3478–3482.

13. Jiang, L.J., Maret, W. & Vallee, B.L. (1998) The glutathione redox couple modulates zinc transfer from metallothionein to zinc-depleted sorbitol dehydrogenase. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3483–3488.

14. Jacob, C., Maret, W. & Vallee, B.L. (1998) Control of zinc transfer between thionein, metallothionein, and zinc proteins. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3489–3494.

15. Rabenstein, D.L. (1973) Nuclear magnetic resonance studies of the acid–base chemistry of amino acids and peptides. I. Microscopic ionization constants of glutathione and methylmercury-complexed glutathione. *J. Am. Chem. Soc.* **95**, 2797–2803.

16. Fujiwara, S., Formicka-Kozłowska, G. & Kozłowski, H. (1977) Conformational study of glutathione by NMR. *Bull. Chem. Soc. Jap.* **50**, 3131–3135.

17. Varnagy, K. & Sovago, I. (1988) Transition metal complexes of amino acids and derivatives containing disulphide bridges. *Inorg. Chim. Acta* **151**, 117–123.

18. Touche, M.L.D. & Williams, D.R. (1976) Thermodynamic considerations in co-ordination. Part XXV. Formation of ternary complexes containing two dissimilar metal ions and the implication for metal-metal stimulation phenomena *in vivo*. *J. Chem. Soc. Dalton Trans.* 1355–1359.

19. Corrie, A.M., Walker, M.D. & Williams, D.R. (1976) Thermodynamic considerations in coordination. Part XXII. Sequestering ligands for improving the treatment of plumbism and cadmiumism. *J. Chem. Soc. Dalton Trans.* 1012–1015.

20. Fuhr, J., Rabenstein, D.L. (1973) Nuclear magnetic resonance studies of the solution chemistry of metal complexes. IX. The binding of cadmium, zinc, lead, and mercury by glutathione. *J. Am. Chem. Soc.* **95**, 6944–6950.

21. Hynes, M.J. & O'Dowd, M. (1987) Interactions of the trimethyltin (IV) cation with carboxylic acids, amino acids, and related ligands. *J. Chem. Soc. Dalton Trans.* 563–566.

22. Bugarin, M.G. & Filella, M. (1999) The formation constants of dimethylthallium (III)-glutathione complexes in aqueous solution. *J. Inorg. Biochem.* **73**, 17–29.

23. Li, Z.S., Lu, Y.P., Zhen, R.G., Szczypka, M., Thiele, D.J. & Rea, P.A. (1997) A new pathway for vacuolar cadmium sequestration in *Saccharomyces cerevisiae*: YCF1-catalyzed transport of bis(glutathionato)cadmium. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 42–47.

24. Kadima, W. & Rabenstein, D.L. (1990) Nuclear magnetic resonance studies of the solution chemistry of metal complexes. 26. Mixed ligand complexes of cadmium, nitrilotriacetic acid, glutathione, and related ligands. *J. Inorg. Biochem.* **38**, 277–288.

25. Diaz-Cruz, M.S., Mendieta, J., Monjonell, A., Tauler, R. & Esteban, M. (1998) Study of the zinc-binding properties of glutathione by differential pulse polarography and multivariate curve resolution. *J. Inorg. Biochem.* **70**, 91.

26. Dominey, L.A. & Kustin, K. (1983) Kinetics and mechanism of Zn(II) complexation with reduced glutathione. *J. Inorg. Biochem.* **18**, 153–160.

27. Krężel, A. & Bal, W., reference in the text.

28. Gockel, P., Gelinsky, M., Vogler, R. & Vahrenkamp, H. (1998) Solution behaviour and zinc complexation of tripeptides with cysteine and/or histidine at both termini. *Inorg. Chim. Acta* **272**, 115–124.

29. Krężel, A. & Bal, W. (1999) reference omitted.

30. Odenheimer, B. & Wolf, W. (1982) Reactions of cisplatin with sulfur-containing amino acids and peptides I. Cysteine and glutathione. *Inorg. Chim. Acta* **66**, L41–L43.

31. Appleton, T.G., Connor, J.W., Hall, J.R. & Prenzler, P.D. (1989) NMR study of the reactions of *cis*-diamminediaquaplatinum(II) cat-

ion with glutathione and amino acids containing a thiol group. *Inorg. Chem.* **28**, 2030–2037.

**32.** Lempers, E.L.M. & Reedijk, J. (1990) Reversibility of binding of cisplatin-methionine by diethyldithiocarbamate or thiourea: A study with model adducts. *Inorg. Chem.* **29**, 217–222.

**33.** Berners-Price, S.J. & Kuchel, P.W. (1990) Reaction of *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ with reduced glutathione studied by $^{1}H$, $^{13}C$, $^{195}Pt$ and $^{15}N$-{$^{1}H$} DEPT NMR. *J. Inorg. Biochem.* **38**, 305–326.

**34.** Berners-Price, S.J. & Kuchel, P.W. (1990) Reaction of *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ with reduced glutathione inside human red blood cells, studies by $^{1}H$ and $^{15}N$-{$^{1}H$} DEPT NMR. *J. Inorg. Biochem.* **38**, 327–345.

**35.** Corden, B. (1987) Reaction of platinum(II) antitumor agents with sulfhydryl compounds and the implications for nephrotoxicity. *Inorg. Chim. Acta* **137**, 125–130.

**36.** Sovago, I. & Martin, R.B. (1981) Transition metal ion induced deprotonation of amide hydrogenes in sulfhydryl containing compounds. *J. Inorg. Nucl. Chem.* **43**, 425–429.

**37.** Chow, S.T., McAuliffe, C.A. & Sayle, B.J. (1975) Metal complexes of amino acids and derivatives-IX. Reactions of the tripeptide, glutathione, with divalent cobalt, nickel, copper and palladium salts. *J. Inorg. Nucl. Chem.* **37**, 451–454.

**38.** Kozłowski, H., Decock-Le Reverend, B., Ficheux, D., Loucheux, C. & Sovago, I. (1987) Nickel(II) complexes with sulfhydryl containing peptides. Potentiometric and spectroscopic studies. *J. Inorg. Biochem.* **29**, 187–197.

**39.** Letter, J.E., Jr. & Jordan, R.B. (1975) Complexing of Nickel(II) by cysteine, tyrosine and related ligands and evidence for zwitterion reactivity. *J. Am. Chem. Soc.* **97**, 2381–2390.

**40.** Formicka-Kozłowska, G., May, P.M. & Williams, D.R. (1980) Potentiometric studies on nickel(II)-glutathionate interactions. *Inorg. Chim. Acta* **46**, L51–L53.

**41.** Jeżowska-Trzebiatowska, B., Jaruga-Baranowska, M., Ostern, M. & Kozłowski, H. (1981) Polarographic studies on Ni(II)-glutathione system in aqueous solutions. *Polish J. Chem.* **55**, 2477–2483.

**42.** Jeżowska-Trzebiatowska, B., Formicka-Kozłowska, G. & Kozłowski, H. (1976) Metal-glutathione interaction in water solution. NMR and electron spectroscopy study of Ni(II)-glutathione complexes in aqueous solution. *Chem. Phys. Lett.* **42**, 242–245.

**43.** Ostern, M.I. & Jaruga-Baranowska, M. (1983) Complex structure and catalytic hydrogen ion reduction in Ni(II)-glutathione system. *Electrochim. Acta* **28**, 1173–1175.

**44.** Krężel, A. & Bal, W. (1999) reference omitted.

**45.** Li, W., Zhao, Y. & Chou, I.N. (1996) $Mg^{2+}$ antagonism on $Ni^{2+}$-induced changes in microtubule assembly and cellular thiol homeostasis. *Toxicol. Appl. Pharmacol.* **136**, 101–111.

**46.** Shi, X., Dalal, N.S. & Kasprzak, K.S. (1993) Generation of free radicals in reactions of Ni(II)-thiol complexes with molecular oxygen and model lipid hydroperoxides. *J. Inorg. Biochem.* **50**, 211–225.

**47.** Shi, X., Mao, Y., Ahmed, N. & Jiang, H. (1995) HPLC investigation on Ni(II)-mediated DNA damage in the presence of *t*-butyl hydroperoxide and glutathione. *J. Inorg. Biochem.* **57**, 91–102.

**48.** Ross, S.A. & Burrows, C.J. (1998) Nickel complexes of cysteine- and cystine-containing peptides: Spontaneous formation of disulfide-bridged dimers at neutral pH. *Inorg. Chem.* **37**, 5358–5363.

**49.** Jeżowska-Trzebiatowska, B., Formicka-Kozłowska, G. & Kozłowski, H. (1977) NMR and EPR study of the Cu(II)-glutathione interac-

tion in water solution. *J. Inorg. Nucl. Chem.* **39**, 1265–1268.

**50.** Sivertsen, T. (1980) Copper-induced GSH depletion and methaemoglobin formation *in vitro* in erythrocytes of some domestic animals and man. A comparative study. *Acta Pharmacol. Toxicol.* **46**, 121–126.

**51.** Corazza, A., Harvey, I. & Sadler, P.J. (1996) $^{1}H$, $^{13}C$-NMR and X-ray absorption studies of copper(I) glutathione complexes. *Eur. J. Biochem.* **236**, 697–705.

**52.** Harman, B. & Sovago, I. (1983) Metal complexes of sulphur-containing ligands. V. Interactions of cobalt(II) ion with L-cysteine and its derivatives. *Inorg. Chim. Acta* **80**, 75–83.

**53.** Hamed, M.Y. & Silver, J. (1983) Studies on the reactions of ferric iron with glutathione and some related thiols. Part II. Complexes formation in the pH range three to seven. *Inorg. Chim. Acta* **80**, 115–122.

**54.** Hamed, M.Y., Silver, J. & Wilson, M.T. (1983) Studies on the reactions of ferric iron with glutathione and some related thiols. Part III. A study of the iron catalyzed oxidation of glutathione by molecular oxygen. *Inorg. Chim. Acta* **80**, 237–244.

**55.** Kitagawa, S., Seki, H., Kamentani, F. & Sakurai, H. (1988) EPR study on the interaction of hexavalent chromium with glutathione or cysteine: Production of pentavalent chromium and its stability. *Inorg. Chim. Acta* **152**, 251–255.

**56.** Bose, R.N., Moghaddas, S. & Gelerinter, E. (1992) Long-lived chromium(IV) and chromium(V) metabolites in the chromium(VI)-glutathione reaction: NMR, ESR, HPLC and kinetic characterization. *Inorg. Chem.* **31**, 1987–1994.

**57.** Zhitkovich, A., Voitkun, V. & Costa, M. (1995) Glutathione and free amino acids form stable complexes with DNA following exposure of intact mammalian cells to chromate. *Carcinogenesis* **16**, 907–913.

**58.** Cupo, D.Y. & Watterhahn, K.E. (1985) Modification of chromium(VI)-induced DNA damage by glutathione and cytochromes P-450 in chicken embryo hepatocytes. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6755–6759.

**59.** Formicka-Kozłowska, G., Kozłowski, H. & Jeżowska-Trzebiatowska, B. (1979) Metal-glutathione interaction in aqueous solution. Nickel(II), cobalt(II), and copper(II) complexes with oxidized glutathione. *Acta Biochim. Polon.* **26**, 239–248.

**60.** Gillard, R.D. & Phipps, D.A. (1997) Optically active co-ordination compounds. Part XXI. The oxygenation of cobalt(II)-tripeptide complexes. *J. Am. Chem. Soc.* **119**, 1074–1082.

**61.** Kroneck, P. (1975) Models for the electron paramagnetic resonance nondetectable copper in "blue oxidases". A binuclear copper(II) complex with oxidized glutathione. *J. Am. Chem. Soc.* **97**, 3839–3841.

**62.** Miyoshi, K., Sugiura, Y., Ishizu, K., Iitaka, Y. & Nakamura, H. (1980) Crystal structure and spectroscopic properties of violet glutathione-copper(II) complex with axial sulfur coordination and two copper sites *via* a disulfide bridge. *J. Am. Chem. Soc.* **102**, 6130–6136.

## ANNEX

**1.** Protonation reaction of a ligand **L** can be quantitativly described by the equilibrium constant, $K$, as well as by the stability constant, $\beta$:

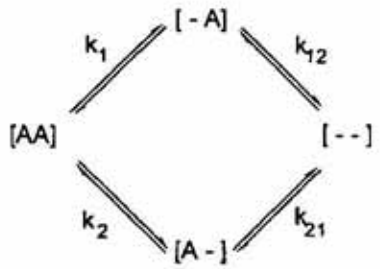
$$L^{n-} + H^{+} \xleftrightarrow{K_1} HL^{(n-1)-}$$

$$\beta_1 = K_1 = \frac{[HL^{(n-1)-}]}{[L^{n-}][H^{+}]}$$

In a general case of molecule binding **i** hydrogen ions:

$$\beta_i = \frac{[H_iL]}{[L][H^+]^i}$$

**2.** The microscopic ionization scheme of a diprotic acid can be shown schematically as:




where **A** is a group with proton. On the left is 1, on the right 2 acidic group(s). The ionization reaction to which a given equilibrium constant refers is indicated by the subscript. The last number in the subscript denotes the group involved in the ionization step under consideration while the preceding number denotes the group from which the proton has already ionized. The microscopic protonation constants can be defined as:

$$k_1 = \frac{[H^+][-A]}{[AA]} \qquad k_2 = \frac{[H^+][-A]}{[AA]}$$

$$k_{12} = \frac{[H^+][--]}{[-A]} \qquad k_{21} = \frac{[H^+][--]}{[-A]}$$

Relations between micro- and macroconstants are defined as:

$$K_1 = k_1 + k_2$$

$$K_2 = \frac{k_{12}k_{21}}{k_{12}+k_{21}}$$

**3.** Similar constants can be defined for complexes:

$$M^{n+} + H_2L \xleftrightarrow{K_1} MH_2L^{n+}$$

$$\beta_1 = \frac{[MH_2L^{n+}]}{[M^{n+}][H^+]^2[L]}$$

In general, for a complex containing **a** metal ions, **b** hydrogen ions and **c** ligand molecules:

$$\beta_{abc} = \frac{[M_aH_bL_c]}{[M]^a[H]^b[L]^c}$$

Note that for reactions with hydrogen ion displacement from amide groups by a metal ion **a** can assume negative values. This is because amide protons do not dissociate freely, and therefore cannot be introduced into the ligand formula.

Reaction of ligand with metal ion can be written as a proton competition reaction:

$$M^{n+} + H_iL = [MH_mL]^{(n-i+m)} + (i-m)\,H^+$$

Equilibrium constant for such reactions is denoted $^*K$. Values for $^*K$ constants can be easily derived from stability constants:

$$\log {}^*K = \log\{\beta(MH_mL)\} - \log\{\beta(H_iL)\}.$$