Histidine–copper(II) complex (Cu-His₂) is a form of bound copper necessary for cellular copper uptake. Due to the high affinity of histidine to copper(II) ions, the binding of copper(II) by histidine is considered a substantial part of plasma antioxidative defense. Also, cysteine plays a role in the antioxidative system. However, we show here that in the presence of oxygen the histidine–copper(II) complex plus cysteine produces reactive oxygen species (ROS). Cysteine concentration was assayed using a thiol specific silver-mercury electrode. Hydrogen peroxide was assayed amperometrically using platinum electrode. ROS formation was followed by chemiluminescence of luminol-fluoresceine-enhanced system. Addition of cysteine to Cu-His₂ solution at pH 7.4 in the presence of atmospheric oxygen initiates the synthesis of H₂O₂, and generation of ROS, which manifests as a burst of chemiluminescence. The reaction has two stages; in the first stage, cysteine is utilized for the synthesis of an unstable intermediary product which becomes a substrate for ROS formation. Anaerobic conditions inhibit ROS formation. Increased cysteine concentration enhances the lag phase of the oxidative burst without influencing the amount of ROS. The synthesis of ROS (measured by chemiluminescence) is proportional to the concentration of Cu-His₂ employed. ROS production can be repetitively initiated by further additions of cysteine to the reaction medium. The study suggests that Cu-His₂ catalyzes cysteine-dependent reduction of oxygen to superoxide employing an intermediary cysteine-copper(I) complex and enabling Fenton reaction with copper and hydrogen peroxide produced as a secondary product. In effect, Cu-His₂ with cysteine may be a source of ROS in biological media.

Key words: Cysteine copper complex, cysteine oxidation, hydrogen peroxide formation, histidine copper complex

Received: 01 January, 2013; revised: 28 May, 2013; accepted: 02 December, 2013; available on-line: 15 December, 2013

INTRODUCTION

Biological antioxidative defense systems integrate actions of a variety of low-molecular-mass compounds and enzymes that prevent the formation of major biological oxidants. Among the low-molecular-mass antioxidants, compounds with reducing properties (classified as free radical scavengers) play a crucial role. These include common metabolites such as uric acid, bilirubin, biliverdin, carotenoids, flavonoids, cysteine and glutathione. Another group of compounds participating in the antioxidative defense are proteins sequestering transient metal ions from biological fluids. The best known members of this group include ceruloplasmin (de Silva & Aust, 1993) controlling Cu²⁺ ions, and ferritin, lactoferrin and transferrin chelating iron (Halliwell, 1994). Low molecular-mass-antioxidants and metal-binding proteins act concurrently in the body, protecting proteins and other compounds from damage by reactive oxygen species generated by cellular metabolism (Tubaro et al., 1998).

Cysteine, whose concentration in the human plasma is about 250 µM, is a crucial component of the thiol antioxidative system in the extracellular space. It is the main constituent of a specific redox thiol-disulphide buffer which determines properties of numerous proteins and low molecular mass compounds (Biswa et al., 2006). About 3–10% of the total plasma cysteine is present in a free, reduced form (Ueland, 1995; Giustarini et al., 2011). The thiol group of cysteine participates in various scavenging reactions of free radicals, reduction of lipid peroxides, hydrogen peroxide, and in chelating ions of transient metals. Therefore cysteine, either free or as a component of proteins, is necessary for the prevention of Fenton reaction (Hegde et al., 2010).

Ceruloplasmin binds the majority of Cu²⁺ maintaining body fluids free of copper ions, and a fraction of copper ions are also bound to free histidine (Sarkar & Kruck, 1967) and histidine residues of plasma albumin (Deschamps et al., 2003; Moriya et al., 2008). Moreover, some plasma copper ions also associate with cysteine (Sandstead, 1995), threonine, glutamine and asparagine (Casella & Gullotti, 1983; Brumas et al., 1993; Deschamps et al., 2005). As copper(I) is one of the most active substrates for Fenton reaction, ceruloplasmin and other copper chelators are important elements of the antioxidative defense system. Histidine is a component of the active centers of proteins responsible for the binding of transient metals (Cu²⁺, Fe²⁺/³⁺ and others) with high affinity. L-histidine also acts as a hydroxyl radical and singlet oxygen scavenger. Free histidine as well as carnosine and anserine (dipeptides containing histidine) bind copper ions to form high affinity complexes (Wade & Tucker, 1998; Hodak et al., 2009) which participate in amino acid-dependent transmembrane transport of copper (Goode et al., 1989). Free histidine and histidine incorporated into peptides and proteins is an essential component of the antioxidative defense system (Deschamps et al., 2003; Mesu et al., 2006). In the plasma and other body fluids histidine co-

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Abbreviations: Cu-His₂, histidine–copper(II) complex; RLU, Relative Light Units
exists with cysteine and other thiol compounds. However, the properties of the compound histidine–cysteine antioxidative system have not been studied so far. The purpose of the present study was to follow production of reactive oxygen species in a reaction medium combining cysteine and Cu-His₂ in the presence of atmospheric oxygen.

MATERIALS AND METHODS

The study of cysteine reaction with Cu-His, included analysis of varying cysteine and Cu-His concentrations and their ratio on the generation of reactive oxygen species in phosphate buffer pH 7.4.

All reagents were of analytical grade. Working solutions were prepared using glass-distilled water (with resistivity of ≥ 18 MΩ/cm). Stock solution of 1-cysteine (20 mM) (Sigma) was prepared in 55 mM sodium phosphate buffer, pH 7.4 (POCh Gliwice). Dilutions of the stock solution with the same buffer were prepared directly prior to use. Their final pH was adjusted to 7.4 with 0.5 M NaOH. The luminol-fluoresceine enhanced detection system was prepared from 5 mM luminol (Carl Roth) and 5 mM fluoresceine (Fluka). Solution of Cu-His₂ was obtained by mixing buffered solution of CuCl₂ (Sigma) and histidine (Sigma) in a molar ratio of 1:4. The two-fold histidine excess assures a lack of unbound copper ions.

The measurement of chemiluminescence was carried out every 1 or 5 s using a Lumat LB 9507 luminometer. The intensity of chemiluminescence was expressed in Relative Light Units (RLU) integrating the curve of chemiluminescence intensity (Drożdż et al., 1998). Each measurement was performed in triplicate and the reported results are mean of the three values.

Measurement of reactive oxygen species generated in Cu-His₂ solution as a function of cysteine concentration

In a set of experiments samples of 10, 20, 30, 40, 50 µl of 10 mM cysteine solution in 55 mM phosphate buffer pH 7.4 were added to a reaction cell containing 500 µl of the luminol-fluoresceine enhanced detection system and 20 µl of Cu-His₂ (final Cu²⁺ concentration 0.1 mM) to give a final cysteine concentration from 0.01 to 1 mM. Chemiluminescence generated in this reaction was measured every 5 s. Data visualization was performed using Sigma Plot v.11.0 program.

In further experiments the basic experimental system was constituted with a modified concentrations ratio: 500 µl Cu-His₂, final Cu²⁺ concentration 0.1 mM, 20 µl luminol-fluoresceine enhanced detection system and 50 µl of 6 mM cysteine – final conc. 0.5 mM. This system was used as a reference (control) in the next experiments.

ROS formation under restricted access of oxygen was measured as described above but the reagents were first deaerated for 3 min by purging with nitrogen or helium (final O₂ concentration was not measured) and the reaction mixture in the measuring chamber was overlaid with liquid paraffin. All additional reagents added were pipetted under the paraffin layer using a Hamilton micro-syringe. The control included the same reagent system with free oxygen access.

To study the effect of cysteine on the Cu-His₂ absorption spectrum 500 µl of Cu-His₂ solution in 55 mM phosphate buffer pH 7.4 (final Cu²⁺ concentration 0.1 mM) and 50 µl of 6 mM cysteine were added to a 2.5-mI spectrophotometric quartz cuvette. The changes of the Cu-His₂ spectrum were recorded at 300 nm on a HELIOS γ spectrophotometer.

Measurement of cysteine concentration using electrochemical method

Cysteine concentration was determined using a silver mercury electrode and a reference Ag/AgCl electrode in saturated KCl solution by a procedure described earlier (Drożdż et al., 2007). The analyses were carried out in a work cell (2.5 ml) filled up with 55 mM phosphate buffer pH 7.4 and a magnetic micro-stirring bar inside. After several minutes of incubation with working buffer to obtain electrochemical equilibrium between the buffer and the electrode, an electro-voltaic cell was produced by inserting a liquid junction connecting the work cell with the reference-electrode cell. The baseline electric potential (in mV) was measured for 3 to 30 min, using an Elmetron CP-401 millivoltmeter. Once the baseline potential was measured, cysteine solution of varying concentrations was added to make a reference cysteine standard curve. The final cysteine concentration was in the range of 0.01 to 20 mM. The electrochemical potential was stable within ± 2.0 mV.

Time-dependent cysteine concentration changes were also assessed when various cysteine amounts were added to the reaction medium containing Cu-His₂ in 55 mM phosphate buffer. These measurements were carried out following addition of 50 µl, 100 µl, 200 µl, 400 µl, 600 µl, 800 µl or 1000 µl aliquots of 10 mM cysteine solution into the work-cell containing 1200 µl of Cu-His₂ at 0.01 mM Cu²⁺. The electrode potential was measured every 1 s until termination of the reaction. Measurement results were automatically re-calculated into cysteine concentration (mM) and expressed as a function of cysteine concentration versus reaction time.

Measurement of hydrogen peroxide formation by amperometry

H₂O₂ concentration was measured by amperometry employing a platinum electrode activated with o-phenylenediamine (o-PD) as described by Liu and Zweiler (2001). The electrode was activated by immersion in an o-phenylenediamine dihydrochloride buffer along with an auxiliary second platinum electrode and a silver/chloride reference electrode. The reference starting potential of the activated platinum electrode versus the silver/chloride reference electrode was +900 mV. The activated platinum electrode was washed with distilled water and stored in 55 mM phosphate buffer pH 7.4. The auxiliary platinum electrode was stored in distilled water.

The measurement of H₂O₂ was carried out in a work-cell containing 9 ml of phosphate buffer pH 7.4. The electrode starting potential versus the reference Ag/AgCl electrode was 650 mV at room temperature. All measurements were performed in triplicate. The reference relationship between the H₂O₂ concentration and current was: \( H₂O₂ (\mu M) = 2.817 \times 10^{-3} \times \text{current (mA)} - 29.527 \). In order to measure \( H₂O₂ \) synthesis in the presence of cysteine, first 500 µl of 10 mM cysteine solution in 55mM phosphate buffer pH 7.4 was added to 9 ml of 55 mM phosphate buffer, incubated for 40 s (delay), then 400 µl of Cu-His₂ solution was added. If not stated otherwise, the final copper concentration employed was 0.1 mM. In serial measurements, consecutive aliquots of cysteine were added in 1-min intervals.
RESULTS

ROS synthesis in the cysteine — Cu-His$_2$ reaction system

Cysteine and Cu-His$_2$ in phosphate buffer pH 7.4 react and generate ROS, whose appearance is manifested as a chemiluminescence burst owing to the luminol-fluoresceine detection system. The reaction is specific for Cu-His$_2$ and does not occur without histidine as a complexing agent for copper ions. However, the addition of cysteine does not directly initiate the ROS synthesis since the burst of the chemiluminescence appears after some delay, the length of which depends on the final cysteine concentration (Fig. 1). Increasing the cysteine concentration from 200 to 1000 µM prolonged the delay of the light burst from 10 to 120 s (Fig. 2). The duration of the chemiluminescence increased proportionally to the cysteine concentration. At the highest cysteine concentration employed (1 mM) the light emission began at 120 s, achieved its maximum at 165 s, and terminated at about 200 s. The cysteine concentration also determined the intensity of the light emitted and the amount of ROS formed (calculated as the area under the RLU curve). However, with increasing cysteine concentration the amount of ROS formed per micromole of cysteine actually decreased (Table 1).

The amount of ROS generated depends not only on total amount of cysteine but also on dosing mode of the constant amount of thiol. When 50 µl of 6 mM cysteine was divided into two, five or ten portions and consecutively added to the Cu-His$_2$ solution after the end of the previous chemiluminescence burst, the amount of ROS formed (estimated as a sum of all oxidative bursts) concentration from 200 to 1000 µM prolonged the delay of the light burst from 10 to 120 s (Fig. 2). The duration of the chemiluminescence increased proportionally to the cysteine concentration. At the highest cysteine concentration employed (1 mM) the light emission began at 120 s, achieved its maximum at 165 s, and terminated at about 200 s. The cysteine concentration also determined the intensity of the light emitted and the amount of ROS formed (calculated as the area under the RLU curve). However, with increasing cysteine concentration the amount of ROS formed per micromole of cysteine actually decreased (Table 1).

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increased progressively with the number of aliquots (Fig. 3, Table 2). The experiment described above suggests that Cu-His₂ acts as a catalyst mediating oxidation of cysteine following each new addition of the substrate. Measured by chemiluminescence generation of ROS takes place at the end of each cycle after depletion of cysteine and is performed by active, reduced form of the copper complex and cumulated hydrogen peroxide. The proposed catalytic function of Cu-His₂ in cysteine-dependent ROS formation is in agreement with the finding that the intensity of chemiluminescence was directly proportional to the Cu²⁺ concentration.

The ROS formation always began after some delay from the time of cysteine addition. Potentiometric analysis of cysteine concentration changes in the reaction medium containing Cu-His₂ indicated that the cysteine concentration decreases during the first reaction stage and falls to zero before the ROS synthesis begins (Fig. 4). It is known that copper ions catalyze oxidation of cysteine (Kachur et al., 1999; Munday et al., 2004), while the highly nucleophilic thiolate anion is a potential substrate for cysteine oxidation. Once the synthesis of H₂O₂ started again. Each addition of cysteine initiated H₂O₂ formation. We propose that oxygen is the other substrate necessary for cysteine-mediated ROS formation in the presence of Cu-His₂.

### H₂O₂ synthesis in the presence of cysteine and Cu-His₂

In our oxidation model a decrease in cysteine concentration is accompanied by production of H₂O₂ as measured by an amperometric system (Fig. 4, Fig. 6). Electrochemical follow up of synthesized H₂O₂ showed that the synthesis terminated at a certain level which depended on the concentration of cysteine added to the medium. Once the synthesis of H₂O₂ terminated (about 5 min after addition of cysteine), addition of a new portion of cysteine resulted in new synthesis of H₂O₂ that continued for another 2 min. In effect, the total H₂O₂ concentration in the sample increased (not shown). However, addition of cysteine before the termination of the initial synthesis of H₂O₂ halted its synthesis for a time necessary for the free cysteine to disappear from the reaction medium. Then, the synthesis of H₂O₂ started again. Each finding that duration of the first “silent” stage of the reaction depended positively on the cysteine concentration in the solution. As long as copper was bound to cysteine, it prevented oxygen reduction, but when the cysteine was oxidized, copper(I) was released to the medium and a rapid synthesis of ROS occurred through Fenton reaction. Copper(II) produced in this reaction was bound again into the complex with histidine.

### Involvement of oxygen in ROS synthesis

Deaeration of the reaction solutions with nitrogen or helium prior to the addition of cysteine and limitation of atmospheric oxygen access by a liquid paraffin layer caused a remarkable decrease in ROS formation (Fig. 5). This observation strongly indicates that an intermediary cysteine copper complex specifically reduced oxygen. Formation of H₂O₂ in the reaction medium suggested that oxygen was reduced to the ‘O₂− radical, which eventually dismutated to H₂O₂. Our experiments showed that each addition of cysteine initiated H₂O₂ formation. We propose that oxygen is the other substrate necessary for cysteine-mediated ROS formation in the presence of Cu-His₂.

### Table 1. ROS generation at different concentrations of cysteine.

Amount of ROS generated was estimated as amount of chemiluminescence (area under curve) at constant Cu-His₂ concentration (final Cu²⁺ 0.1 mM).

| Cysteine concentration (mM) | Area under curve (RLU) ± S.D. | RLU/ mM of cysteine ± S.D. |
|-----------------------------|-----------------------------|---------------------------|
| 0.2                         | 5453 ± 299                  | 2726 ± 1497               |
| 0.4                         | 12186 ± 1051                | 30465 ± 2628              |
| 0.5                         | 16110 ± 641                 | 26850 ± 1069              |
| 0.7                         | 18113 ± 324                 | 22641 ± 405               |
| 0.9                         | 16979 ± 130                 | 16979 ± 130               |

**Figure 5. Influence of molecular oxygen on formation of ROS in the Cu-His₂-cysteine system.**

ROS formation by cysteine and Cu-His₂ depends on oxygen access (unlimited [O] or restricted [●]) to the reaction medium. Experimental conditions were as described in Fig. 1, but oxygen was removed from the reagents (Cu-His₂ and luminal and fluoresceine) by deaeration with helium and in sample 2 the reaction medium was separated from the atmosphere by a 3-mm layer of liquid paraffin. Cysteine was injected under the paraffin layer (final concentration 0.5 mM). Chemiluminescence was measured at 5-s intervals.

**Figure 6. Generation of H₂O₂ in reaction of cysteine with Cu-His₂ at unlimited access of oxygen.**

Cysteine was added to 9 ml of 55 mM phosphate buffer pH 7.4 to obtain final concentration equal to 0.5 mM. After 40 s Cu-His₂ was added to obtain Cu(II) concentration of 0.1 mM [●]. H₂O₂ concentration was measured by amperometric method at 1-s intervals.
new addition of small amounts of cysteine caused accumulation of $H_2O_2$ in the reaction medium (Table 4). However, in the presence of cysteine also some reduction of $H_2O_2$ occurred, which was observed as a progressing decrease of the $H_2O_2$ electrode signal.

DISCUSSION

L-histidine binds copper, cobalt, zinc, cadmium and other bivalent transitory metal ions into stable tridentate complexes (Hofstetter et al., 2011). Blood plasma practically does not contain unbound copper since it is associated with histidine residues in the binding center of ceruloplasmin or those of albumin. Cu-His2 that constitutes about 5% of the total blood plasma copper content coordinates some of the exchangeable pool of copper in the blood (Deschamps et al., 2005). However, as shown in this study, even histidine-bound copper(II) in a high affinity complex can participate in generation of free radicals in the presence of cysteine. This process includes cysteine-dependent reduction of oxygen leading to the synthesis of superoxide and hydrogen peroxide. At a physiological pH protonated thiols express low reactivity toward oxygen and hydrogen peroxide, but introduction of Cu-His2 leads to the activation of the cysteine reducing ability, similarly as it was documented for Cu$^{2+/+}$, Fe$^{3+/2+}$ ions in PBS solution and other transient metals (Lynch & Frei, 1997; Ullah et al., 2011). One of the possible mechanisms of the cysteine-dependent ROS formation in the presence of Cu-His2 may include reduction of copper(II) to copper(I) (Fig. 7). However, prior to this process, formation of an unstable intermediary cysteine-copper compound, either free or bound to histidine, may represent a primary product of reaction of cysteine with Cu-His2.

The amount of generated intermediate necessary for the reduction of oxygen molecule to $\dot{O}_2$ depends on the amount of cysteine added. The superoxide radical produced in the presence of cysteine is immediately dismutated and production of $H_2O_2$ may be demonstrated.
by amperometry. When cysteine is completely depleted from the reaction medium the H$_2$O$_2$ formed can undergo Fenton reaction with complexed copper (I) to give the hydroxyl radical, which in our luminol-fluoresceine detection system generated a burst of chemiluminescence. After oxidation of copper (I) to copper(II) reconstitution of Cu-His occurs and the reaction cycle may start again if a new portion of cysteine is supplied. Our results are in accord with the suggestion by Theophanides and Anastasopoulou (2002) that copper ions attached to albumin or to free amino acids in the presence of biological reductants can interact with O$_2^-$ or H$_2$O$_2$ leading to the formation of hydroxyl radicals. 

Oxidation of cysteine in the presence of histidine-bound copper may occur through a mechanism known from previous studies on oxidation of amino acids in the presence of transition metals forming complexes with a thiol (Kachur et al., 1999). Reduced cysteine (RSH) binds metal ions. The cysteine thiol group may competitively bind to histidine-copper complex to form a thiolate complex. The nucleophilic properties of the cysteine -SH residue increase when it is transformed to a thiolate anion (RS$^-$) (Ueland, 1995) and a thiolate-copper complex may oxidize an oxygen molecule and yield Cu$^{2+}$. A similar mechanism was proposed by Pecci et al. (1997) who demonstrated that Cu$^{2+}$ ions form with cysteine a cuprous bis-cysteine complex which reduces oxygen, cytochrome and nitroblue tetrazolium. This process can also lead to reduction of copper(II) to Cu(I). The copper(I) bis-cysteine complex is relatively stable under anaerobic conditions, but introduction of oxygen leads to complex formation of Cu(I)-oxygen adducts which facilitate two-electron transfer to give cysteine and H$_2$O$_2$. The copper remains reduced until all cysteine is oxidized. Khosravani and Borechard (1998) studied metal-catalyzed oxidation of cysteine and histidine in the presence of H$_2$O$_2$ and proposed that combination of copper(II) and a strong reductor leads to the generation of Cu(I) which becomes a substrate for Fenton type reaction:

$$\text{Cu}^{2+} + \text{RH} \rightleftharpoons \text{Cu}^{+} + \text{R} - + \text{H}^+$$

$$\text{Cu}^{2+} + \text{A}^{-} \rightleftharpoons \text{Cu}^{+} + \text{A}$$

The species formed in the reactions presented above can then react with O$_2$ to form ROS, including O$_2^-$, OH and H$_2$O$_2$.

Another factor promoting reduction of copper(II) to copper(I) in the presence of cysteine may be the influence of histidine on the properties of the complexed copper. Histidine decreases the redox potential of the Cu(I)/Cu(II) couple, thus facilitating reduction of copper(II) and its entering into Fenton reaction (Gaubert et al., 2000). It means that in the presence of Cu-His$_2$ oxygen may undergo one-electron reduction to form O$_2^-$: Therefore Cu-His$_2$ seems to have contrasting properties, acting both as a chelator removing Cu$^{2+}$ from biological fluids, and as a copper(I) source that facilitates Fenton reaction and hydroxyl radical formation. Such an effect is observed in Zn,Cu superoxide dismutase (EC 1.15.1.1) which contains copper(I) bound to four imidazole residues of histidine. In ceruloplasmin, copper(II) is bound to histidine 426. However, reduction of the complexed copper may occur and in effect ceruloplasmin also has oxidative properties, which are utilized in the oxidation of Fe(II) to Fe(III) (Shukla et al., 2006). The influence of histidine on the susceptibility of the complexed copper to reduction increases when the histidine is located at the N-terminal position of a polypeptide chain (Ueda et al., 2000). Finally, free Cu-His$_2$ at physiological concentrations (about 25 µM) acts as a superoxide scavenger, while at higher concentrations (250 µM) it catalyzes dismutation of O$_2^-$ to H$_2$O$_2$ and finally hydroxyl radical formation (Ueda et al., 1994).

Experiments described above indicate that cysteine is not directly involved in the synthesis of H$_2$O$_2$. On the contrary, cysteine inhibits H$_2$O$_2$ synthesis, probably by decomposing the superoxide produced by oxygen reduction. This process, however, occurs in parallel to the reaction of cysteine with Cu-His$_2$, yielding an intermediary product causing one-electron reduction of molecular oxygen. Such properties are shown by certain cysteine-copper(I) complexes, conceivably formed as the reaction by-products. Once cysteine is depleted from the reaction medium, O$_2^-$ produced in the reaction of the intermediary product with oxygen can accumulate and disproportionate to H$_2$O$_2$, which is detected in the reaction medium.

Association of copper ions with albumin, carbohydrates or enzymes can lead to in-site ROS formation \textit{in vivo}. Site-specific metal-catalyzed oxidation that affects some specific amino acid residues located at the metal binging sites has been observed for histidine, arginine, methionine, lysine, proline and cysteine (Trigwell et al., 2001). Cysteine, which forms complexes with Cu(II) or Fe(III), is the most susceptible to metal-catalyzed oxidation. In effect, generation of ROS occurs within complexes of metal and cysteine, leading to oxidation of the specific local amino acid residues (Stadman, 1990). Results obtained in our studies contribute to better understanding of the mechanisms of the copper-mediated damage produced under aerobic conditions.

Acknowledgements

This study was supported by Jagiellonian University Medical College institutional funds.

Conflict of interests: none

REFERENCES

Biwott S, Chida AS, Rahman I (2006) Redox modifications of protein-thiols: emerging roles in cell signaling. Biochim Pharmacol 71: 551–564.

Brumus V, Alley N, Berthon GA (1993) New investigation of copper(I)-serine, copper(II)-histidine-serine, copper(II)-asparagine, and copper(II)-histidine-asparagine equilibria under physiological conditions, and implications for simulation models relative to blood plasma. J Inorg Biochem 52: 287–296.

Casella L, Gallotti M (1983) Coordination modes of histidine 4. Coordination structures in the copper(I)-l-histidine(12) system. J Inorg Biochem 18: 19–31.

De Silva DM, Aust SD (1993) Ferritin and ceruloplasmin in oxidative damage: review and recent findings. Can J Physiol Pharmacol 71: 715–720.

Deschamps P, Kulkarni PP, Gautam-Basak M, Sarkar B (2005) The saga of copper(II)-l-histidine. Coord Chem Rev 249: 895–909.

Deschamps P, Kulkarni PP, Sarkar B (2003) The crystal structure of a novel copper(II) complex with asymmetric ligand derived from l-histidine. Inorg Chem 42: 7366–7368.

Drozdz R, Parmentier C, Hachad H, Leroy P, Siest G, Wellman M (1998) Gamma glutamyltransferase dependent generation of reactive oxygen species from a glutathione/transferrin system. Free Radic Biol Med 25: 786–792.

Drozdz R, Ząbek-Adamska A, Naskalski JW (2007) Potentiometric determination of cysteine with thiol sensitive silver-mercury electrode. Acta Biochim Polon 54: 202–212.

Gaubert S, Bouchaut M, Brumas V, Berthon G (2000) Copper-ligand interactions and the physiological role of reactive processes. Part 3. Influence of histidine, salicylic acid and anibranilic acid on copper-driven Fenton chemistry \textit{in vitro}. Free Radic Res 32: 451–461.

Giustarini D, Dalle-Donne I, Milzani A, Rossi R (2011) Low molecular mass thiols, disulfides and protein mixed disulfides in rat tissues: \textit{Influence of histidine, salicylic acid and anibranilic acid on copper-driven Fenton chemistry \textit{in vitro}}. Free Radic Res 32: 451–461.

Gustarini D, Dalle-Donne I, Milzani A, Rossi R (2011) Low molecular mass thiols, disulfides and protein mixed disulfides in rat tissues: \textit{Influence of sample manipulation, oxidative stress and ageing. Med Agering Der 132: 141–148.
