Monatsh Chem
DOI 10.1007/s00706-015-1577-6

ORIGINAL PAPER

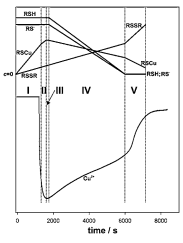
Real-time monitoring of "self-oxidation" of cysteine in presence of Cu^{2+}: novel findings in the oxidation mechanism

Marijo Buzuk[1] · Slobodan Brinić[1] · Nives Vladislavić[1] · Marija Bralić[2] · Maša Buljac[2] · Ivana Škugor Rončević[1]

Received: 29 July 2015 / Accepted: 5 October 2015

Abstract A novel approach for investigation of a mechanism and rate of "self-oxidation" of cysteine in the presence of Cu^{2+} is presented. Continuous monitoring was performed using simple, low-cost, and widely available commercial ion-selective electrode for Cu^{2+}. Presented procedure provides a complete real-time picture of overall oxidation process and has revealed a sequentially organized process, with the domination of certain reactions in each stage. A plausible mechanism, in the light of the previously reported explanation, has been proposed to account for the experimental results together with an adequate scheme of the overall process. The dependence of both the pH (measurements were performed at pH 5, 7, 8) and the concentration of the initially present Cu^{2+} is presented and discussed. Additionally, information into the process was collected by experiments performed in oxygen-free atmosphere and changes in the mechanism of oxidation, at weakly alkaline pH values, were observed. Information presented in this study can be utilized in advanced biochemical monitoring systems, when considering the importance of the position of cysteine and cysteine containing peptides in metabolic processes.

Graphical abstract



Keywords Metal complexes · Amino acids · Bioinorganic chemistry · Sensors

Introduction

The thiol functional group of the amino acid cysteine is highly sensitive to the redox state, mainly due to the reactivity of anionic sulfur to various oxidizing agents that can form multiple types of oxidized species and can perform a countless number of physiological functions. The physiological function of cysteine arises from its tendency to: form cross-links that stabilize protein structure; function as a powerful nucleophile in many enzyme active sites and act in redox signaling—functioning as a regulatory reversible molecular switch [1]. As the thiol group represents most reduced state of sulfur in proteins, conversion (which can be reversible or irreversible) of this group to oxidized species can act as a switch, thus changing the catalytic properties of an enzyme [2]. Also, the thiol side chain can be strongly influenced by the microenvironment of the surrounding protein and at physiological pH it is deprotonated, which is an important attribute for enhancing

✉ Marijo Buzuk
buzuk@ktf-split.hr

[1] Department of General and Inorganic Chemistry, Faculty of Chemistry and Technology, University of Split, Split, Croatia

[2] Department of Environmental Chemistry, Faculty of Chemistry and Technology, University of Split, Split, Croatia

Published online: 03 November 2015

reactivity [2, 3]. As cysteine-dependent proteins appear to be intimately involved in the neurodegenerative processes, a proper redox environment for their activity can provide evidence for their redox control in neurodegenerative diseases [4]. Cysteine has been shown to be the major extracellular antioxidant and major component of various physiologically antioxidant systems and also play an important role in the production of reactive sulfur species (RSS). Beside these species, as a consequence of the imbalance in the metabolism of reactive intermediates, disulfide stress, as a specific type of oxidative stress associated with oxidation of the pair cysteine/cystine, was recently reported [5].

Copper is known to be the cofactor of enzymes involved in respiration processes or in the removal of reactive oxygen species. In addition, copper can be found in some proteins such as hemocyanin, plastocyanin, ceruloplasmin. Since copper ions can easily cycle between cuprous ion and cupric ion, they could take part into non-enzymatic redox processes, thus altering the cell status by acting on intracellular redox potential [6]. In the presence of oxygen, copper ions influence the catalytic oxidation of biomolecules. Thus, they influence the production of reactive oxygen species (ROS) and consequently the level of oxidative stress. The interactions of cysteine and other biological thiols with various transition metals have been given a considerable amount of attention for a long period of time.

The term "self-oxidation of thiols" can be found in some articles from the 1950s [7], although this behavior was first noticed by Mathews and Walker [8]. They reported acceleration in the atmospheric cysteine oxidation when small quantities of metals are present in the solution. Warburg and Sakuma explained that "autoxidation" of cysteine is catalyzed by metals, assuming the formation of an autoxidisable intermediate complex [9]. In addition, some authors found that the oxidation of cysteine is catalyzed by cystine, probably by the formation of an intermediate cysteine–cystine complex [10]. Other authors [11, 12] found that metals can accelerate the oxidation of cysteine, even in anaerobic conditions. Heretofore, oxidations of thiol compounds have been the subject of numerous scientific investigations.

However, the available data are too contradictory or scarce to estimate the mechanism of the proposed reaction. These include the dependence of the oxidation rate of thiol compounds on pH [13], the inertness of thiol to oxygen in aqueous alkaline solution and the oxidation of aminothiols in the presence of various variable-valence metals [14–16]. For example, Pecci et al. [17] have proposed simplified reactions when cupric ions have been added in a solution of cysteine at pH 7.4 and found high stability of cuprous bis–cysteine complex—$[(RS^-)Cu^+(^-SR)]^-$, when measurements were performed under a nitrogen atmosphere. They also found that the excess of cysteine has significant effect on the improved stability of the cuprous bis–cysteine complex—$[(RS^-)Cu^+(^-SR)]^-$ in solutions equilibrated with the atmospheric O_2. These experiments were performed by spectrophotometric and electron paramagnetic resonance (EPR) analysis. Rigo et al. [6] investigated the stability of cuprous complexes, in anaerobic conditions, by spectroscopy, NMR and electron spin resonance (ESR), at various Cu^+ (or Cu^{2+}): cysteine ratios. They reported the stability of $Cu^+(^-SR)$ complex (characterized with stoichiometry Cu^{2+}:cysteine = 1:1.2, owing to the formation of polymeric species with bridging thiolate sulfur), up to Cu^{2+}: cysteine ratio of 0.45. When Cu^{2+} exceeds this ratio (even in trace amounts), a fast and complete oxidation of the complex occurs. Nevertheless, it was previously reported [18] that cystine (produced by decomposition of cuprous cysteine complex) could slow down the terminal oxidation of Cu^+ (produced by decomposition of cuprous cysteine complex), which was not reported by the Pecci et al. [17]. Begiyan et al. [19] investigated the kinetic order of the catalytic oxidation of thiol compounds by molecular oxygen in an aqueous solution, in the presence of various metals ion, at various pH values. The authors concluded that, in the neutral medium, oxidation rates depended only on the concentration of variable-valence metal ions. Moreover, the same authors emphasize that, in an alkaline media, with the presence of a large excess of thiol compounds with respect to Cu^{2+}, predominant complex is cuprous bis–cysteine complex—$[(RS^-)Cu^+(^-SR)]^-$. The copper-catalyzed oxidation of cysteine to cystine, in an alkaline solution, was investigated by Cavallini et al. [20] and they reported cysteine–Cu^{2+} complex, with stoichiometry of cysteine to Cu^{2+}—2:1. Hanaki and Kamide [21] proposed a direct and indirect mechanism of cysteine oxidation, based on results of oxidation kinetics of cysteine with H_2O_2 in the presence of Cu^{2+} obtained by the spectrophotometric method. Zwart et al. [22] suggested that, during the catalytic oxidation, at least two different copper complexes are operative. Masoud and El-Hamid [23] describe the synthesis of a pale green Cu^{2+}–cysteine complex in distilled water with a stoichiometry of 1:1.2. Their findings suggest that both carboxylate and thiol groups are involved in the copper binding. However, Dokken et al. [24] suggested that they probably synthesized oxidized Cu^+–cysteine complex or an impure Cu^{2+}–cystine complex similar to that reported by Gale and Winkler [25].

As it is presented above, many contradictory findings concerning the mechanism, condition and role of specific species in the oxidation of thiol compounds can be noticed. In addition, all used techniques were time-consuming, expensive or improper for continuous measurement and

monitoring of these processes. Although many reports concerning potentiometric determination of thiols, based on silver materials [26–29], metal-phthalocyanine (ionophores) [30, 31] or metal-salophenes [32] have been published, reports for potentiometric determination using copper-based materials are unavailable, mainly due to reasons (catalytical oxidation, etc.) mentioned before. However, continuous cysteine oxidation monitoring is not reported.

The method presented in this paper can give a more complete picture and provide new insights into processes involved in cysteine oxidation. For this purpose, a Cu-ion-selective electrode (Cu-ISE) offers significant potential, as concentrations of copper ions are directly dependent on the concentration of various amino acid species. In this work, a simple, cheap, and widely available method for monitoring cysteine oxidation in real time by commercial Orion 94–29 Cu-ISE is presented. Behavior and processes that take place near or on the electrode surface are monitored and explained in the light of previous reports. This work brings a novel method, based on continuous cysteine oxidation monitoring using Cu-ISE, to the investigation of the mechanism, kinetics and rate of the oxidation of thiols, as this topic has attracted attention until the present time [33–39].

Results and discussion

To clarify the stoichiometry of the Cu^{+}:cysteine complex, modified potentiometric titration (using ORION Cu-ISE), by the addition of a certain amount of cysteine into a solution containing Cu^{2+}, at pH 5, was performed. The obtained results are presented in Fig. 1. When mole ratio of Cu^{2+} and added cysteine reaches 1:2, drop in potential was observed, indicating stoichiometry of complex as 1:1, when concerning mechanism proposed by Rigo et al. [6]. However, it must be emphasized that formation of bis-cysteine complex cannot be discarded at a large excess of cysteine (see "Introduction"). Each addition of cysteine, for achieving aimed mole ratio toward Cu^{2+}, was performed in fresh solution of Cu^{2+}, at pH 5. This was done to avoid a possible hysteresis effect, mainly due to fouling of complexes on the electrode surface (at given Cu^{2+} concentration), which can influence sensitivity or provide a "false" response of Cu-ISE. A relatively high concentration of Cu^{2+} was chosen due to better insight in effect.

Time–potential curves presenting the dependence of Cu^{2+} versus time were obtained using Cu-ISE as working electrode. Knowing the "approximate" stoichiometry of Cu^{+}:cysteine complexes, it is possible to get insight into the processes that take place in the solution. In Fig. 2, the influence of cysteine addition, at pH 5, is presented. Interestingly, at longer monitoring time, steady state was not achieved, but additional changes in curve shape were observed. These changes can be useful in the interpretation and understanding of the overall process, and also as a sequential reaction concerning cysteine "fate". According to the information obtained from Fig. 1, and reported data, the following reactions are used for the explanation of observed processes:

$$RSH \rightleftarrows RS^{-} + H^{+} \quad (1)$$

$$2RS^{-} + 2Cu^{2+} \rightleftarrows 2RS* + 2Cu^{+} \quad (2)$$

$$RS^{*} + RS^{*} \rightleftarrows RSSR \quad (3)$$

$$2RS^{-} + 2Cu^{+} \rightleftarrows 2RSCu \quad (4)$$

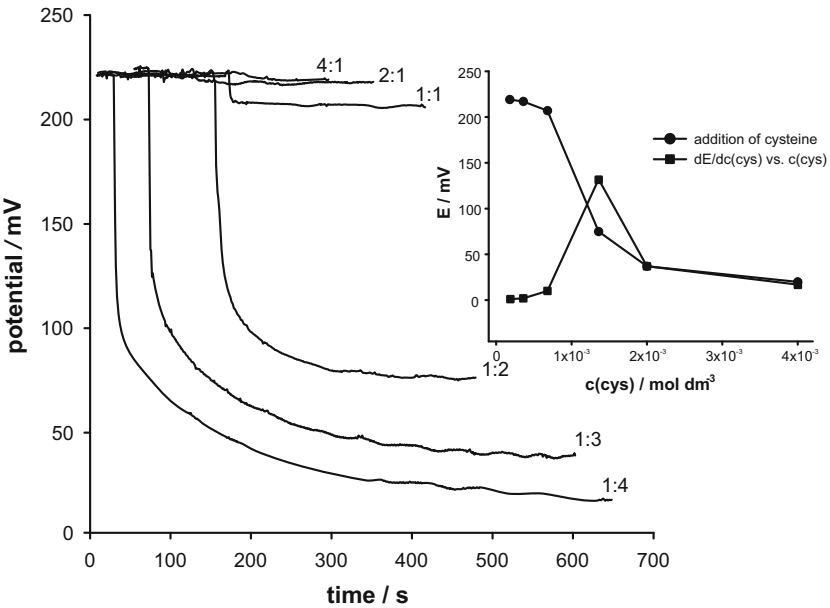


Fig. 1 Determination of stoichiometry of Cu–cysteine complex. *Curves* represent different mole ratio of Cu^{2+}:cysteine. Initial concentration of Cu^{2+} was 6.8×10^{-4} mol dm^{-3}, at pH 5. *Small figure* presents dependence of final potentials vs. *c*(cysteine) and its first derivation

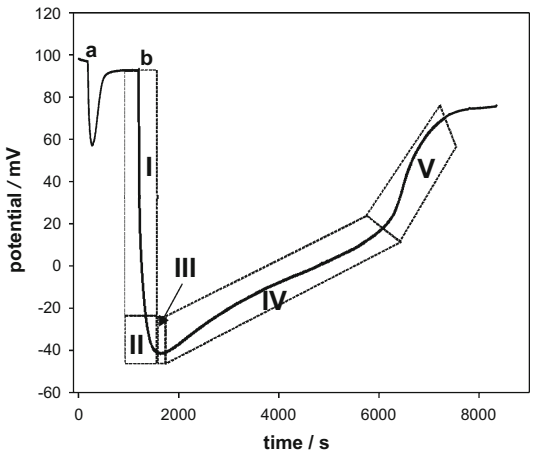


Fig. 2 Time-dependent response of Cu-ISE with addition of cysteine. *Curve* is divided into five stages, with corresponding reactions in Scheme 1. Initial concentration of Cu^{2+} was 2.0×10^{-7} mol dm^{-3}, pH 5. Addition of cysteine: at point (*a*) to reach 2.0×10^{-6} mol dm^{-3} cysteine and at point (*b*) to reach 2.0×10^{-5} mol dm^{-3} cysteine

$$RSCu + RS^- \rightleftarrows [(RS^-)Cu^+(^-RS)]^- \quad (5)$$

$$2[(RS^-)Cu^+(^-RS)]^- + 2O_2 \rightleftarrows 2RSCu + RSSR + 2O_2^{*-} \quad (6)$$

$$2RSCu + 2Cu^{2+} \rightleftarrows RSSR + 4Cu^+ \quad (7)$$

$$4Cu^+ + O_2 + 4H^+ \rightleftarrows 4Cu^{2+} + 2H_2O \quad (8)$$

$$RSCu + O_2 + 2H^+ \rightleftarrows 1/2RSSR + H_2O_2 + Cu^{2+} \quad (9)$$

where RSH stands for cysteine; RS^- stands for cysteine in thiolate form; RS* stands for thiyl radical of cysteine; RSSR stands for cystine.

Also, measurements were performed at a high ratio of cysteine:the initial Cu^{2+} (tenfold and hundredfold higher) which is needed for providing sufficient buffer capacity of system for getting insight into processes.

Stage I

The curve presented in Fig. 2 can be divided into five stages, according to the dominating reactions in each stage. Stage I is characterized by formation of complexes. Thus, a fast decrease of Cu^{2+} concentration, according to reactions (2, 4), and potentially (5), is the dominant process. During this stage, the decomposition of complex, according to reaction (7) is negligible, as rates of complexes formation considerably overwhelm its decomposition. It must be emphasized that the addition of 2×10^{-7} cysteine did not produce a significant potentiometric response, which was expected, considering the reactions (2, 4), and (5). The small decrease in Cu^{2+}, due to its complexation, can be compensated by Cu^{2+} originated from the dissolution of Cu-ISE.

Stage II

At this stage, a slower rate of processes explained in stage I, associated with a significantly low Cu^{2+} concentration, can be observed. As the concentration of cysteine is tenfold greater than Cu^{2+}, most of Cu^{2+} in the solution is controlled by the stability of the complex in the excess of cysteine. Thus, the concentration of "free" Cu^{2+} is significantly lower than the initial one. Besides "free" Cu^{2+} (originated from initially added Cu^{2+}), the rate of reaction starts to be controlled by Cu^{2+} originating from the electrode dissolution. The concentrations of RSH and RS^- in this stage can be considered constant, or the concentrations changes are negligible. The concentration of the complex remained constant since its decomposition (by molecular oxygen) can be compensated by its formation, due to the dissolution of Cu-ISE, as an "infinite source" of Cu^{2+}.

Stage III

The narrow stage III is characterized with constant potential, indicating the constant concentration of Cu^{2+} in the solution. The only source of Cu^{2+} at this stage is Cu-ISE. All dissolute Cu^{2+} from electrode were immediately complexed by RS^-. Thus, the concentration of Cu^{2+} remained constant and controlled by the stability of the complex [through reaction (4)]. Moreover, the reactions (6) and (7) started to take place. Consequently, as a final product of these reactions, Cu^{2+} must be obtained [see reaction (8)]. However, an increase of its concentration was not detected by Cu-ISE. Bearing in mind the excess of cysteine (in form of RSH/RS^-), all Cu^+ produced by the reaction (7) will be complexed (buffered) by cysteine. Thus, the dominant reactions at this stage are those involving cysteine (RS^- and RSH). Hence, as a final product of its oxidation, cystine is produced.

Stage IV

As it has been mentioned above, simultaneously, during the reaction of the formation of complexes, the reaction of its decomposition (7) also takes place. The increase in the potential (Cu^{2+} concentration) at this stage can be attributed to reaction (8), preceded by reaction (7), since at this stage the concentration of cysteine is not sufficient to bind the Cu^+ released in the reaction (7). This phenomenon was proven by the experiment carried out in a nitrogen atmosphere (see Fig. 6) under which reactions (6) and (8) are hampered. Thus, the potential is determined only by the

equilibrium (which remained unchanged) concentration of Cu^{2+} from reactions (4) and (5).

Stage V

At the beginning part of this stage, all cysteine are in the form of complexes (RSCu or $RS^-Cu^+RS^-$) and cystine (RSSR) and "free" cysteine (as RS^- or RSH) is not available (buffer capacity is loosed). This implies that the decomposition of complexes is the dominant reaction. Furthermore, Cu^{2+} produced by reaction (8) has a catalytic role in the decomposition of RSCu [see Eq. (7)] and the mechanism at this stage can be described as an autocatalytic decomposition of complexes (Cu^{2+} as a final product serves as reactant in decomposition of complexes). Thus, rapid decomposition and release of Cu^{2+} could be expected. This was indeed proven by an increase in Cu^{2+} concentration.

After stage V, reactions (7) and (8) ceased and only Cu^{2+} and RSSR were present in the solution. The slight increase in the potential can be attributed to the autotitration of Cu-ISE.

The influence of pH on the behavior and rates of reaction is presented in Fig. 3.

Similar behavior at higher pH values can be noticed, but it was followed by increased reaction rates in all stages. This increase at higher pH values was also reported by Bagiyan et al. [19], together with the changes in the reaction mechanism. In addition, they explained these behaviors with the characteristic features of complex formation of thiol compounds with metal. However, the characteristic "peak", after the addition of cysteine, can be observed at pH 7 and 8. Although the concentrations of "free" Cu^{2+} in the solution at pH 7 and 8 are lower than at pH 5, the "total" concentration of Cu^{2+} (as "free" Cu^{2+} and in form of hydroxo complexes) in the solution is higher due to the improved contribution of the electrode dissolution at higher pH values. This fact can be useful for the explanation of the "peak" appearance. With the addition of cysteine, all "free" Cu^{2+} is instantaneously bound into the complex. However, additional Cu^{2+} can be released from Cu^{2+}–hydroxo complexes, due to the shift of the Cu^{2+}–hydroxo complexes $\left(Cu^{2+} + nOH^- \rightleftarrows Cu(OH)_n^{n-2}\right)$ equilibrium toward its dissociations, which was noticed as a rapid increase in potential. After this point, the reactions that take place are similar as explained in Fig. 2.

Another phenomenon can be observed at the end of the overall process. It is obvious that all Cu^{2+} initially present has not been released by proposed reactions at the end of process. The explanation of this behavior can be found in the report presented by Kolthoff et al. [18]. They proposed a novel equilibrium of cystine (RSSR)/Cu^+ (where Cu^+ reduces RSSR) which can slow down the terminal oxidation of Cu^+. Also, various Cu^{2+}–cystine complexes can be formed with constants that can be found in the technical report by Berthon [40]. However, this discrepancy is more pronounced at higher pH values; therefore, suggesting the dependence of stoichiometry of cystine/Cu^+ or Cu^{2+}–cystine complex on pH. The other possible explanation is that, at pH 5, the reaction (10) affects the Cu^{2+} concentration.

$$H_2O_2 + 2Cu^+ + 2H^+ \rightleftarrows 2H_2O + 2Cu^{2+} \quad (10)$$

The standard electromotive force of the reaction (10) is 1.617 V and in solutions with a pH greater than 7 this reaction is significantly suppressed (reaction is shifted toward left). Thus, the contribution of this reaction, as a source of Cu^{2+}, is decreased. H_2O_2 is the product of the spontaneous dismutation of O_2^{*-} [from reaction (6)], as reported by Bielski [41].

In Fig. 4, the influence of different initial Cu^{2+} concentrations at various pH is presented. A faster reaction rate with an increase in the Cu^{2+} concentration was observed at all measured pH. Interestingly, at pH 5, wider stages II and III were observed. This is a consequence of a

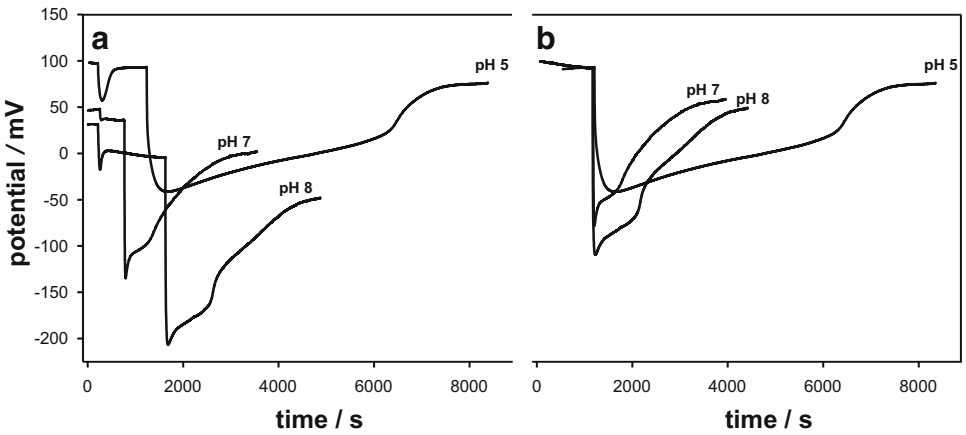


Fig. 3 **a** Time-dependent responses of Cu-ISE at different pH with addition of cysteine. Initial concentration of Cu^{2+} was 2.0×10^{-7} mol dm^{-3}. Cysteine was added for reaching concentrations of the 2.0×10^{-6} and 2.0×10^{-5} mol dm^{-3}. **b** Comparison of curves presented in **a** for *c*(cysteine) 2.0×10^{-5} mol dm^{-3}

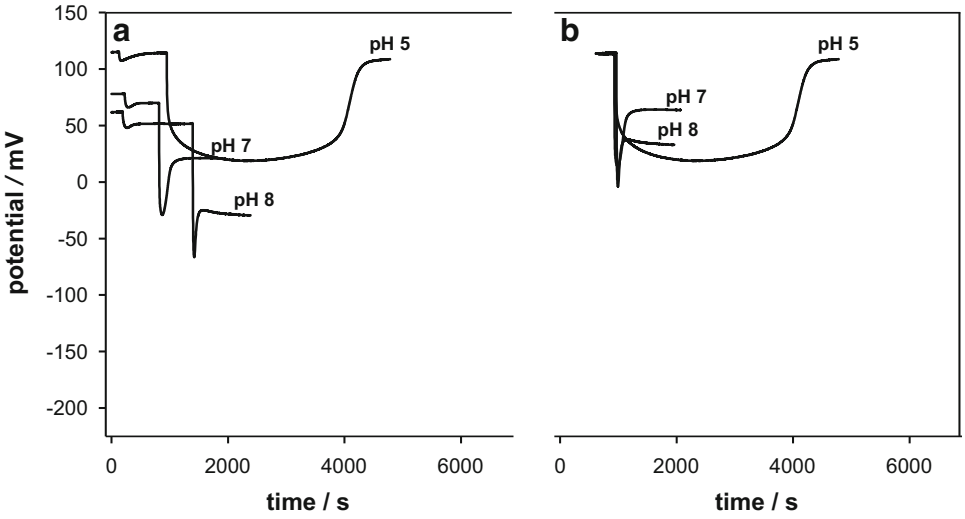


Fig. 4 **a** Time-dependent responses of Cu-ISE, at different pH, with addition of cysteine. Initial concentration of Cu^{2+} was 2.0×10^{-6} mol dm^{-3}. Cysteine was added for reaching concentrations of the 2.0×10^{-6} and 2.0×10^{-5} mol dm^{-3}. **b** Comparison of curves presented in **a** for *c*(cysteine) 2.0×10^{-5} mol dm^{-3}

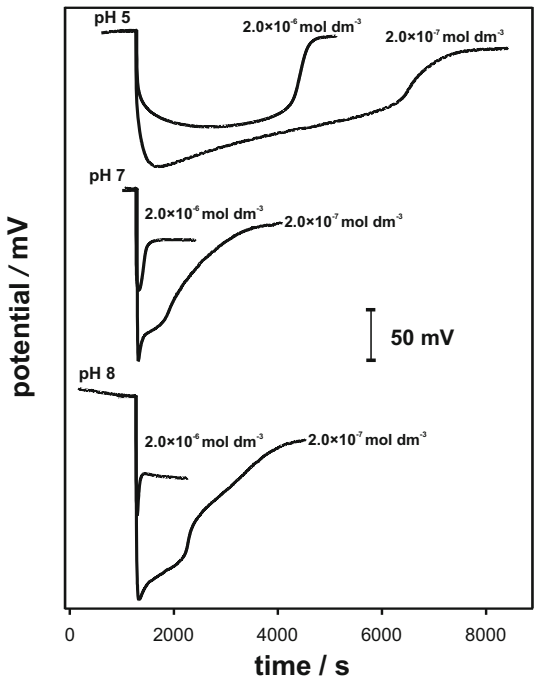


Fig. 5 Comparison of potentiometric responses at different pH and different initial Cu^{2+} concentration

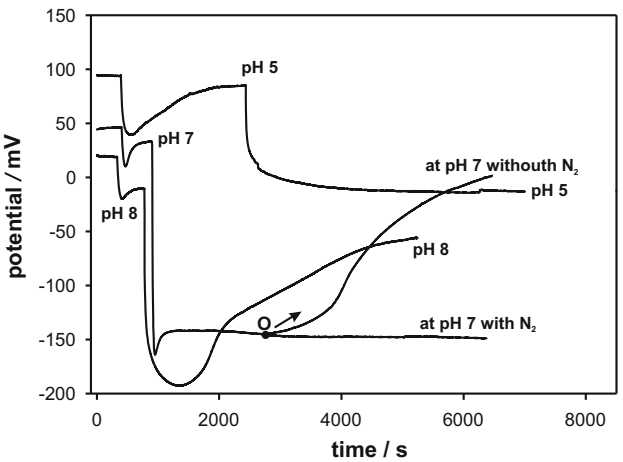
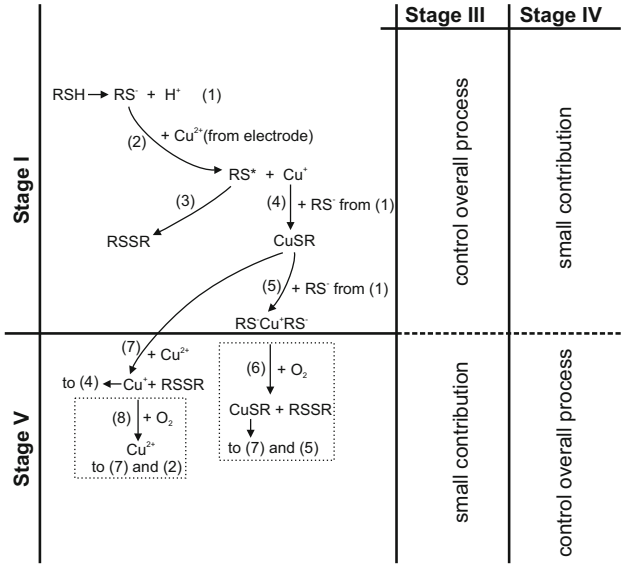


Fig. 6 Time-dependent responses of Cu-ISE at different pH with addition of cysteine. Measurements were performed in N_2 atmosphere. Initial concentration of Cu^{2+} was 2.0×10^{-7} mol dm^{-3}. Cysteine was added for reaching concentrations of the 2.0×10^{-6} and 2.0×10^{-5} mol dm^{-3}. At point (*O*), at pH 7, purging with N_2 was ceased

higher "free" Cu^{2+} concentration in the solution which implies longer time for its consumption by the reaction (7). After significant decreases in cysteine concentration, a fast (faster than at lower initial Cu^{2+}) increase in potential can be observed since the reaction rate is governed by the "free" Cu^{2+} concentration. Also, at this Cu^{2+} concentration and pH 7 and 8, the discrepancy of the initial and final potential was more pronounced than in the case of 2×10^{-7} Cu^{2+}, as at higher concentration of Cu^{2+}, according to the reaction (9), the equilibrium has shifted toward left (Fig. 5).

For additional insight into these processes, same experiments were performed in anaerobic environment, by bubbling N_2 gas, to avoid participation of molecular O_2 in the oxidation (as it is explained in "Experimental"). Results presented in Fig. 6 reveal that the overall process, under N_2, was hampered in stage III. Thus, according to Scheme 1, the dominant reactions would be those where O_2 is not involved (or has a small contribution), and those

Scheme 1



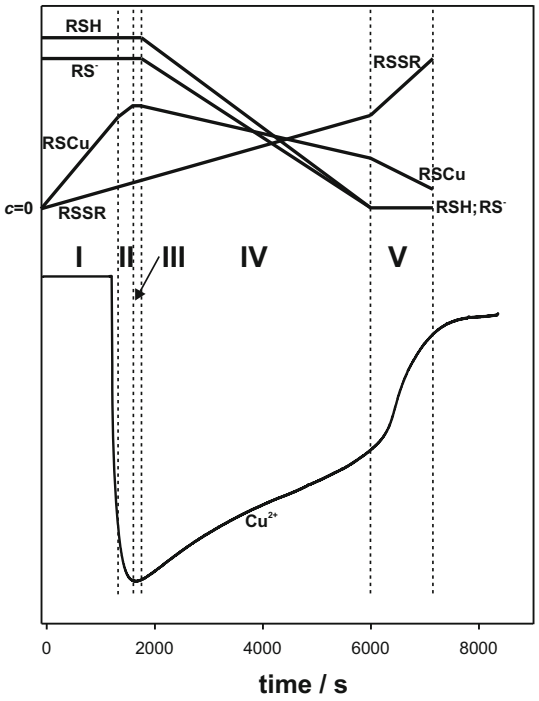


Fig. 7 Main species concentrations trends over time

that take place in stages I and III. At a lower cysteine concentration (2×10^{-6} M), the oxidation of cysteine does not cease, but it can be significantly slower. This is the case for pH 5 and 7. After the purging stopped, an increase in the potential was noticed (at point O; pH 7). Surprisingly, this behavior was not noticed at pH 8, indicating different mechanism of oxidation. This can be explained with alkaline hydrolysis of formed cystine [42], generating a cysteine (RS^-) and a cysteine sulfenic acid (RSOH). This hydrolysis influences the shift of equilibrium in reaction (7). Hence, more Cu^+ is produced which reacts with cysteine (produced by hydrolysis of cystine) making a cycle process. At the end of this process, registered potential arises from "free" Cu^+. Its concentration is determined by several equilibriums, including reactions (7, 4) and the reaction of cystine hydrolysis.

Finally, trends in concentrations of main species, involved in this process, are presented in Fig. 7.

It must be emphasized that electrode responses (calibration of Cu-ISE; see "Experimental") toward Cu^{2+}, at all pH, after addition of cysteine were unchanged (same as before cysteine addition), which implies no formation of complexes on the surface or other surface modifications of Cu-ISE.

Conclusion

In this work, a novel and simplified method of real-time monitoring of the process of "self-oxidation" of cysteine, in the presence of Cu^{2+}, is presented. Using this approach, additional information, helpful for the clarification of the overall process was acquired. It is proposed that the overall oxidation process consists of several steps (stages) with different dominant reaction in each of them. A crucial role of reactive oxygen in the oxidation process, at pH 5 and 7, was proven, as well as changes in the mechanism of oxidation at higher pH, with a reduced influence of oxygen and an improved role of alkaline hydrolysis of cystine. Also, due to the equilibrium of final oxidation products (cystine) and formed Cu^+, the terminal oxidation of Cu^+ can be slowed down or even hampered. This phenomenon is more pronounced with an increase in pH. The experiments performed in the solution bubbled with N_2 revealed that the overall process can be ceased in stage III, characterized with a small contribution of oxygen in the overall process.

The information presented in this study can be helpful in future investigations concerning oxidation processes of sulfydryl compounds in the living cell, for monitoring and quantifying cysteine modifications as a key for understanding their regulatory and pathophysiologic function or to provide evidence for the activity and redox control of cysteine-dependent enzymatic systems in neurodegenerative diseases.

Experimental

All chemical were of analytical reagent grade. All solutions were prepared with redistilled water. Acetate buffer (pH 5; 0.1 mol dm^{-3}) was prepared by mixing previously

prepared solutions of potassium acetate and acetic acid, all purchased from Kemika (Croatia). Potassium dihydrogen phosphate and potassium hydrogen phosphate (all purchased from Sigma-Aldrich, Inc.) were used for the preparation of phosphate buffers (0.1 mol dm^{-3}; pH 7 and 8). Stock solution of the copper nitrate (1×10^{-3} mol dm^{-3} Cu^{2+}) was prepared by the dissolution of $Cu(NO_3)_2 \times 5H_2O$ (Sigma-Aldrich, Inc.) in potassium nitrate (Kemika, Croatia) solution (0.1 mol dm^{-3}). Solution of cysteine (Merck) was prepared daily by the dissolution of appropriate amount of the cysteine in redistilled water, previously deaerated with N_2.

Apparatus

Potentiometric measurements were carried out with a potentiostat (Autolab PGSTAT 302 N) connected to a PC and driven by GPES 4.9 Software (Eco Chemie). Measurements were performed with double junction electrode (Mettler Toledo InLab 301 electrode) as reference and ORION 94–29 ISE (Cu^{2+} selective) as working electrode. All experiments were carried out at 25 °C with or without purging with N_2 (99.9999 %). Time interval between two recorded potentiometric signals was 1 s.

Measurements

Base solution contained 90 cm^3 solution of KNO_3 [$c(KNO_3) = 0.1$ mol dm^{-3}] + 10 cm^3 of appropriate buffer solution. Measurements were performed in the following manner:

1. Calibration of working electrode: working electrode was calibrated) toward Cu^{2+}, by sequential addition of stock Cu^{2+} solution into base solution, to achieve the following concentrations: 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} mol dm^{-3}.
2. Procedure of measurements: fresh base solution was spiked with the appropriate amount of Cu^{2+} (measurements were performed at two Cu^{2+} concentrations: 2.0×10^{-7} and 2.0×10^{-6} mol dm^{-3}). These solutions were sequentially spiked with cysteine for achieving cysteine concentrations of the 2.0×10^{-6} and 2.0×10^{-5} mol dm^{-3}. Additions of cysteine aliquot were performed sequentially, after achieving stabile potential.
3. Calibration of working electrode: After the measurements described in paragraph 2, the working electrode was rinsed in distilled water and tested on Cu^{2+} in same manner as described in paragraph 1.

To investigate repeatability, three consecutive measurements were performed.

All described procedures were carried out in two ways: in solutions with equilibrium with atmospheric oxygen and in deaerated solutions. The deaeration was done with purified nitrogen (pyrogallol, distilled water, base solution) and a nitrogen blanket was kept above the solution during the measurement.

References

1. Mauro LC, Kate SC (2013) In: Jacob U, Reichmann D (eds) Oxidative stress and redox regulation. Springer, The Netherlands, p 1
2. Klomsiri C, Karplus PA, Poole LB (2011) Antioxid Redox Signal 14:1065
3. Paulsen CE, Carroll KS (2013) Chem Rev 113:4633
4. Guttmann RP, Powell TJ (2012) Int J Cell Biol. doi:10.1155/2012/703164
5. Moreno ML, Escobar J, Izquierdo-Álvarez A, Gil A, Pérez S, Pereda J, Zapico I, Vento M, Sabater L, Marina A, Martínez-Ruiz A, Sastre J (2014) Free Radical Biol Med 70:265
6. Rigo A, Corazza A, di Paolo ML, Rossetto M, Ugolini R, Scarpa M (2004) J Inorg Biochem 98:1495
7. Shoberl A (1955) Proc Int Text Res Conf 155C:157
8. Mathews AP, Walker S (1909) J Biol Chem 6:299
9. Warburg O, Sakuma S (1923) Pftuiger's Arch 200:203
10. Dixonn M, Tunnicliffe HE (1923) Proc R Soc Lond B 94:266
11. Toda S (1926) Biochem Z 172:34
12. Harrison DC (1927) Biochem J 21:335
13. Benesch RE, Benesch R (1955) J Am Chem Soc 77:5877
14. Wallace TJ, Schriesheim A (1965) Tetrahedron 21:2271
15. Hanaki A (1995) Bull Chem Soc Jpn 68:831
16. Kachur AV, Koch CJ, Biaglow JE (1999) Free Radic Res 31:23
17. Pecci L, Montefoschi G, Musci G, Cavallini D (1997) Amino Acids 13:355
18. Kolthoff JM, Stricks W (1951) J Am Chem Soc 73:1728
19. Bagiyan GA, Koroleva IK, Soroka NV, Ufimtsev AV (2003) Russ Chem Bull 52:1135
20. Cavallini D, Marco CD, Duprè S, Rotilio G (1969) Arc Biochem Biophys 130:354
21. Hanaki A, Kamide H (1973) Chem Phar Bull 21:1421
22. Zwart J, van Wolput JHMC, van der Cammen JCJM (1981) J Mol Catal 11:69
23. Masoud MS, El-Hamid OHA (1989) Transit Met Chem 14:233
24. Dokken KM, Parson JG, McClure J, Gardea-Torresdey JL (2009) Inorg Chim Acta 362:395
25. Gale RJ, Winkler CA (1977) Inorg Chim Acta 21:151
26. Drożdż R, Naskalski R, Ząbek-Adamska A (2007) Acta Biochim Pol 54:205
27. Bralić M, Radić NJ (1994) Croat Chem Acta 67:543
28. Komljenović J, Radić NJ (1998) Fresenius J Anal Chem 360:675
29. Rajbhandari A, Yadav AP, Manandhar K, Pradhananga RR (2009) Sci World 7:19
30. Shrakhoian S (2001) Anal Chem 73:5972
31. Khaloo SS, Amini MK, Tangestaninejad S, Shahrokhian S, Kia R (2004) J Iran Chem Soc 1:128
32. Amini AK, Khorasani JH, Khaloo SS, Tangestaninejad S (2003) Anal Biochem 320:32
33. Zeida A, Babbush R, Lebrero MCG, Trujillo M, Radi R, Estrin DA (2012) Chem Res Toxicol 25:741
34. Dewan A, Bora U, Kakati DK (2012) Heteroat Chem 23:231
35. Corma A, Rodenas T, Sabater MJ (2012) Chem Sci 3:398
36. Golub E, Freeman R, Willner I (2013) Anal Chem 85:12126

37. Nipane SV, Mali MG, Gokavi GS (2014) Ind Eng Chem Res 53:3924
38. Mishra R, Rupendranath B, Mukhopadhyay S (2012) J Phys Org Chem 25:1193
39. Prudent M, Girault HH (2009) Metallomics 1:157
40. Berthod G (1995) Pure Appl Chem 67:1117
41. Bielski BHJ, Allen AO (1977) J Phys Chem 81:1048
42. Hogg PJ (2003) Trends Biochem Sci 28:210