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ORIGINAL PAPER

Oxidation of methionine residue in Gly-Met dipeptide induced by $[Au(en)Cl_2]^+$ and influence of the chelated ligand on the rate of this redox process

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Abstract Spectroscopic (^{1}H NMR and UV–vis) and electrochemical (CV) techniques have been applied to study the reaction of Gly-Met dipeptide with $[Au(en)Cl_2]^+$ complex having bidentate coordinated ethylenediamine (en) ligand. The final product in the investigated reaction was Gly-Met sulfoxide, formed by the oxidation of methionine residue with $[Au(en)Cl_2]^+$. The formation of Gly-Met sulfoxide proceeds through two steps including the monodentate coordination of thioether sulfur atom of methionine residue and release of en ligand from Au(III). In order to investigate the influence of the pH and Cl^- concentration on this redox process, the reaction was performed in solution of KCl with the concentration varying from 0.01 to 0.50 M and in the pH range 2.00–5.00. The obtained kinetic data showed that oxidation of methionine residue in Gly-Met was 200 times slower in comparison to the same process with previously investigated $[AuCl_4]^-$ complex. Difference in the oxidation rate of Gly-Met between these two complexes was attributed to the presence of chelated ethylenediamine ligand in $[Au(en)Cl_2]^+$. The finding that the oxidation of sulfur-containing peptides and proteins can be inhibited by structural modifications of gold(III) complexes introducing polydentate nitrogen-containing ligands can contribute in designing new gold(III) complexes with lower toxic side effects.

Keywords Methionine-containing peptide · Gold(III)-induced oxidation · Proton NMR · UV–vis · Cyclic voltammetry

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Introduction

Gold and its complexes have been playing an important role in medicine since ancient times [1]. Some gold(I) complexes, such as auranofin and aurothiomalate, have been used very successfully in the treatment of rheumatoid arthritis [2], while gold(III) complexes have gained considerable attention as potential antitumor agents [3]. During the recent years, a large number of gold(III) complexes, in which the reduction potential of the Au(III) was lowered by the use of polydentate ligands, have been reported to be appreciably stable under physiologically relevant conditions and to manifest relevant cytotoxic activity against different human tumor cell lines [4–16]. The possible involvement of gold(III) complexes in cancer treatment initiated an interest in the area of gold(III) interactions with different biologically important ligands, such as amino acids and peptides [17]. It was shown that the amino acid L-histidine and L-histidine-containing peptides with N3 anchoring site of the imidazole ring are good chelating ligands for Au(III) ion [18–20]. Moreover, the formed gold(III) complexes with L-histidine-containing peptides are very stable under physiologically relevant conditions with promising cytotoxic properties toward different human tumor cell lines [21]. On the other hand, the reactions of Au(III) with sulfur-containing amino acids and peptides primarily proceed through the reduction of Au(III) ion and oxidation of sulfur side chain [22–32]. Thus, Au(III) rapidly oxidizes the thiol-containing amino acid L-cysteine to its disulfide cystine and, afterwards, cleaves the disulfide bond of cystine to give cysteic acid, thereby being reduced to Au(I) and Au(0) [22–26]. Furthermore, Au(III) is capable to oxidize methionine and methionine-containing peptides to the corresponding sulfoxides [27–32]. These interactions are thought to be responsible for the toxic side effects of gold-based drugs.

Recent studies showed that the reactions of $[AuCl_4]^-$ with Gly-Met dipeptide and its *N*-acetyl derivative, Ac-Gly-Met, proceed in two steps [31]. The first step is a very fast coordination of Au(III) ion to the thioether sulfur with formation of an intermediate gold(III)–peptide complex, $[AuCl_3$(R-Gly-Met-*S*)] (R = H and Ac). This intermediate product further reacts with an additional methionine residue to generate corresponding chlorosulfonium cation, which readily undergoes hydrolysis to give the R-Gly-Met sulfoxide as the final product of the redox process. Moreover, the structure of the corresponding sulfoxide as the final product of gold(III)-induced oxidation of methionine-containing peptides was confirmed by X-ray crystallography [32]. In a continuation of our previous investigations [31, 32], in the present study an attempt was made to gain insight into course of the reactions of gold(III) complexes containing polydentate ligands with methionine-containing peptides. For this purpose, ^{1}H NMR spectroscopy, UV–vis spectrophotometry, and cyclic voltammetry (CV) have been used to study the reaction of $[Au(en)Cl_2]^+$ complex (en is bidentate coordinated ethylenediamine) with Gly-Met dipeptide.

Experimental

Reagents

Distilled water was demineralized and purified to a resistance of greater than 10 MΩ cm^{-1}. The compounds D_2O, DCl, KOD, $H[AuCl_4]\cdot 3H_2O$, and ethylenediamine (en) were purchased from Sigma-Aldrich Chemical Co. The dipeptide glycyl-D,L-methionine (Gly-Met) was obtained from Bachem A.G. Hydrochloric acid, potassium chloride, and absolute ethanol were obtained from Zorka Pharma. All the employed chemicals were of analytical reagent grade.

Synthesis of the $[Au(en)Cl_2]Cl\cdot 2H_2O$ complex

The $[Au(en)Cl_2]Cl\cdot 2H_2O$ complex was synthesized by modification of the previously described methods [33, 34]. To the solution of 0.5 mmol of $H[AuCl_4]\cdot 3H_2O$ (196.9 mg) in absolute ethanol (10.0 mL), an equimolar amount of en ligand (33.8 μL, 99 %, ρ=0.898 g/mL) was added. An orange precipitate, which was formed immediately after addition of en, redissolved after 30 min. The resulting pale yellow solution was stirred in the dark for 2 h at room temperature, and then left standing to slowly evaporate. Pale yellow crystals of the $[Au(en)Cl_2]Cl\cdot 2H_2O$ complex formed after 2 days were removed by filtration and air-dried. The yield was 54 % (107.9 mg). The purity of the complex was checked by elemental microanalysis and NMR (^{1}H and ^{13}C) spectroscopy. Analytical calculations for $[Au(en)Cl_2]Cl\cdot 2H_2O$ = $C_2H_{12}AuCl_3N_2O_2$ (FW 399.45): C, 6.01; H, 3.03; N, 7.01. Found: C, 6.15; H, 2.96; N, 6.87 %. ^{1}H NMR (D_2O, 200 MHz); δ 3.27 (*s*, $enCH_2$). ^{13}C NMR (D_2O, 50 MHz): δ 53.68 ($enCH_2$). All these data were in accordance with those previously reported for the $[Au(en)Cl_2]Cl\cdot 2H_2O$ complex [33, 34].

Measurements

Elemental microanalysis of the gold(III) complex

Elemental microanalysis for carbon, hydrogen, and nitrogen of the synthesized gold(III) complex was performed by the Microanalytical Laboratory, Faculty of Chemistry, University of Belgrade.

^{1}H NMR spectroscopy

All ^{1}H NMR spectra were recorded on a Varian Gemini 2000 spectrometer (200 MHz) using 5-mm NMR tubes. Sodium trimethylsilylpropane-3-sulfonate (TSP, δ 0.00 ppm) was used as an internal reference. The NMR spectra were processed using the Varian VNMR software (version 6.1, revision C). The chemical shifts are reported in parts per million (ppm).

Fresh solutions of the gold(III) complex and Gly-Met dipeptide were prepared separately in D_2O as solvent at an initial concentration of 20 mM and then mixed in a NMR tube in 1:1 molar ratio. All reactions were performed in the pH range 2.00–5.00 and at room temperature (25 °C). In order to investigate the effect of the chloride concentration, the reactions were performed in the presence of potassium chloride, with Cl^- concentration varying from 0.01 to 0.50 M. All rate constants were obtained from the ^{1}H NMR data recorded at appropriate time intervals. The release of bidentate coordinated en ligand from the Au(III) ion was fitted to a first-order process [35] by plotting $\ln(a_0/a_t)$ against t (a_0 is the concentration of the intermediate Au(III)-bound methionine complex and a_t is the concentration of this complex at time t). The concentration of the gold(III) complex was determined by integration of the singlets at 3.27 and 3.38 ppm, corresponding to the CH_2 methylene protons of coordinated and free ethylenediamine, respectively.

In order to compare the rate constant of the reaction between Gly-Met and $[Au(en)Cl_2]^+$ with that previously reported for the reaction between this dipeptide and $[AuCl_4]^-$ [31], herein the $[Au(en)Cl_2]^+$ and Gly-Met were dissolved in 0.01 M DCl at pH 2.00 and 25 °C, and then reacted in 1:1 molar ratio. The value of the rate constant was determined from the ^{1}H NMR measurements, when the data from the early part of the reaction were fitted to a second-order process [35] by plotting $x/a_0(a_0-x)$ against t (where a_0 is the initial concentration of the Gly-Met dipeptide and x is the concentration of the sulfoxide at time t). The concentration of Gly-Met sulfoxide was determined by integration of the singlet for the $-SCH_3$ methyl protons of Gly-Met sulfoxide at 2.71 ppm.

Voltammetric measurements by cyclic voltammetry

Cyclic voltammetric measurements were performed with an Autolab potentiostat (PGSTAT 302N). The working electrode for the cyclic voltammetric measurements was glassy carbon (GC) with 3 mm inner and 9 mm outer diameter of the PTFE sleeve. Prior to use, the GC electrode was wet-polished on an Alpha A polishing cloth (Mark V Lab) with small particles of alumina (0.05 μm diameter). The electrode was washed twice with doubly distilled water and then with the background electrolyte solution. The washed electrode was placed into a voltammetric cell with supporting electrolyte solution. The reference electrode was a saturated calomel electrode type 401 (Radiometer, Copenhagen) and the counter electrode was a platinum wire.

The supporting electrolyte used to perform the cyclic voltammetric experiments was 0.50 M KCl at pH 2.00. The concentration of the gold(III) complex in the final solution was $1.00 \cdot 10^{-3}$ M. The conditions were as following: E_{begin}=−0.5 V, E_{end}=1.5 V, and E_{step}=0.003 V at a scan rate of 0.070 V s^{-1}. All experiments were repeated at least three times. The data were collected and analyzed using the Origin 8 program.

UV–vis spectrophotometry

The UV–vis spectra were recorded on a PerkinElmer Lambda 35 double-beam spectrophotometer equipped with thermostated 1.00-cm quartz Suprasil cells. Stock solutions of the gold(III) complex and Gly-Met were prepared directly before use in 0.01–0.50 M KCl at 2.00≤pH≤5.00 and 25 °C. The UV–vis spectra were recorded in the first 1 h after mixing of $[Au(en)Cl_2]^+$ and Gly-Met in 1:1, 1:2, and 1:3 molar ratios, respectively, as well as after 3, 24, and 48 h over the wavelength range 200–500 nm. The concentration of the gold(III) complex in the final solutions was in the range $1.67 \cdot 10^{-4}$–$5.00 \cdot 10^{-4}$ M. Data were collected and analyzed using Origin 8 and Microsoft Office Excel 2007 programs.

pH measurements

All pH measurements were performed at room temperature (25 °C). The pH meter (Iskra MA 5704) was calibrated with a Fischer certified buffer solution of pH 4.00. All pH* values measured in D_2O solutions were recalculated into pH valid for H_2O by using the previously reported equation: pH=0.929pH*+0.42 [36].

Results and discussion

In general, gold(III) complexes are relatively unstable and easily reducible to gold(I) species and metallic gold, due to their high reduction potential and fast rate of hydrolysis [2]. Stability of the Au(III) ion can be achieved by the appropriate choice of the ligands, in most cases containing nitrogen donor atoms [4–16]. Herein, a gold(III) complex containing polydentate ligand, namely $[Au(en)Cl_2]^+$, has been synthesized and utilized in order to verify whether the introduction of a chelate ligand enhances the stability of gold(III) complexes in their reactions with methionine-containing peptides. The solution behavior of $[Au(en)Cl_2]^+$ complex and course of its reaction with Gly-Met dipeptide have been investigated in the presence of KCl with the concentration varying from 0.01 to 0.50 M at 2.00≤pH≤5.00 and 25 °C by ^{1}H NMR, UV–vis, and CV techniques. This investigation has not been performed at pH>5.00, due to the fact that $[Au(en)Cl_2]^+$ complex, under these conditions, forms a brown precipitate insoluble in water at room temperature [33].

The reaction of $[Au(en)Cl_2]^+$ with Gly-Met

In the reaction of $[Au(en)Cl_2]^+$ complex with an equimolar amount of Gly-Met dipeptide, under the abovementioned experimental conditions, Gly-Met sulfoxide was observed as the final product (Fig. 1 and Table 1). This product resulted from the oxidation of methionine residue in the dipeptide induced by Au(III) and its formation can be successfully followed by ^{1}H NMR spectroscopy (Fig. 1). The singlet at 2.71 ppm was assigned to the –*S*CH_3 methyl protons of Gly-Met sulfoxide, which is in agreement with that previously reported for $[H^+$Gly-Met sulfoxide$][AuCl_4]$ characterized by ^{1}H NMR spectroscopy and X-ray crystallography [31, 32].

The formation of Gly-Met sulfoxide in the investigated reaction proceeds through two consequent steps. The first step of the redox process is the coordination of the sulfur atom of methionine residue of Gly-Met dipeptide to the Au(III) ion. This was noticed in the ^{1}H NMR spectrum by the appearance of a new singlet due to the –*S*CH_3 methyl protons of Au(III)-bound methionine species, $[Au(en)Cl(Gly\text{-}Met\text{-}S)]^{2+}$. The chemical shift of this singlet is in the region between chemical shifts of –*S*CH_3 protons of the free dipeptide (2.11 ppm) and these protons of Gly-Met sulfoxide (2.71 ppm). This singlet was shifted downfield during time depending on the concentration of the oxidized product. As a consequence of the formation of the intermediate Au(III)-bound methionine species, a release of chelated ethylenediamine ligand from Au(III) was observed. The detachment of en ligand from the Au(III) can be easily detected in the ^{1}H NMR spectrum by the simultaneous decline of the resonance at 3.27 ppm due to the methylene protons of bidentate coordinated en ligand, and the growth of the resonance at 3.38 ppm corresponding to these protons for free en (Table 1). Upon addition of ethylenediamine to the reaction mixture, the resonance at 3.38 was enhanced. The release of en ligand from Au(III) ion can be attributed to the *trans* influence of the coordinated thioether sulfur atom. In order to confirm that release of en ligand occurred only in the presence of Gly-Met dipeptide, the

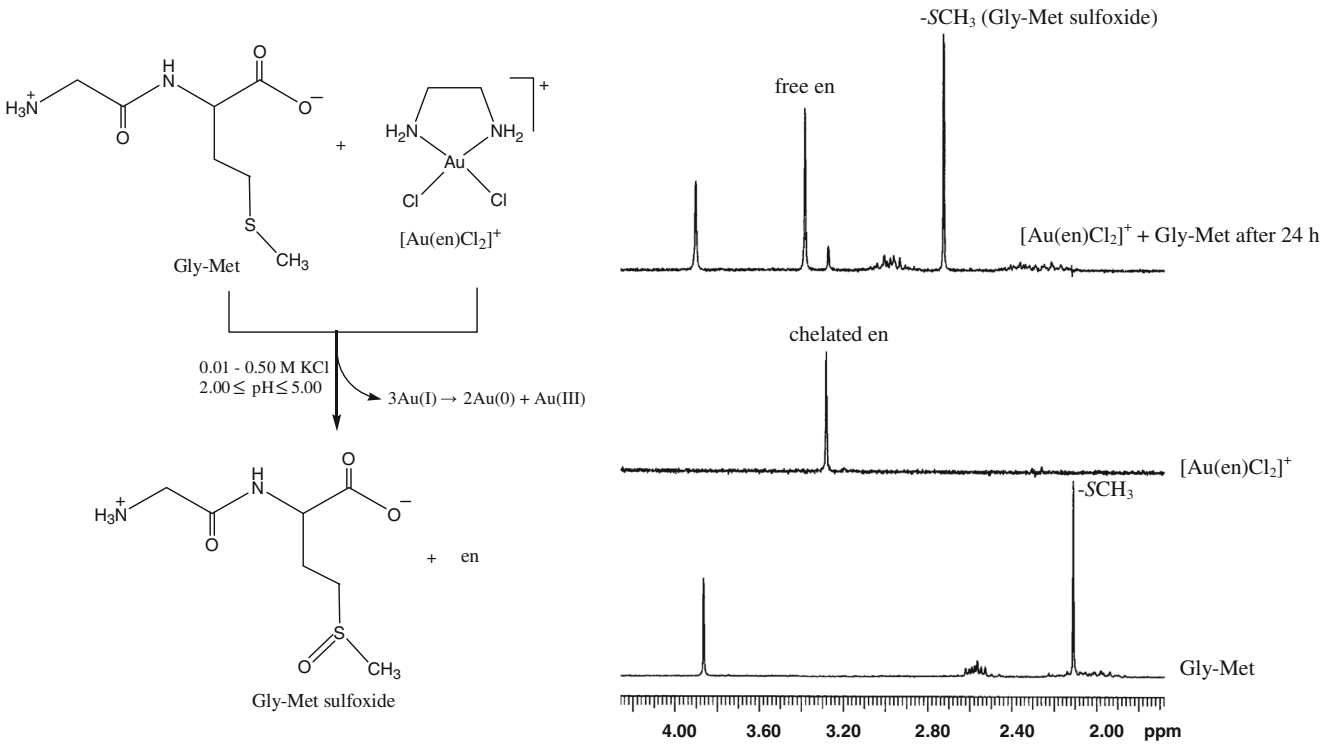

Fig. 1 Schematic presentation of the oxidation of methionine residue in Gly-Met dipeptide in the presence of $[Au(en)Cl_2]^+$ complex and parts of the proton NMR spectra for the reactants and products formed after 24 h. The dipeptide and gold(III) complex were mixed in 1:1 molar ratio in 0.50 M KCl at pH 2.00 and 25 °C

behavior of $[Au(en)Cl_2]^+$ complex in aqueous solution of KCl (0.01–0.50 M) in the pH range 2.00–5.00 has been investigated. Under these experimental conditions, no release of ethylenediamine ligand from Au(III) complex was observed during 48 h.

Furthermore, the formation of the intermediate Au(III)-methionine species was confirmed by CV measurements. As a result of the coordination of thioether sulfur atom to Au(III), the characteristic cathodic peak I at 0.15 V, corresponding to the reduction of $[Au(en)Cl_2]^+$ complex ($[Au(en)Cl_2]^+ + 3e^- \rightarrow Au(0) + en + 2Cl^-$ [33]) was shifted toward more negative potential of 0.05 V (peak II, Fig. 2a and Table 2). The anodic peak II′ at 0.80 V, detected in the cyclic voltammogram, corresponds to the oxidation of Au(0) to $[AuCl_4]^-$ [31, 33, 37], while the second cathodic peak III at 0.40 V is assigned to the reduction of the already formed $[AuCl_4]^-$ complex to Au(0) in accordance with the previously reported data [31, 33, 37] (Table 2). The presence of metallic gold on the electrode surface also confirmed the reduction of Au(III) complex to Au(0). In accordance to the previous results [37–39], it was not possible to detect $[AuCl_2]^-$ complex, due to its disproportionation to $[AuCl_4]^-$ and Au(0) in aqueous solution:

$$3[AuCl_2]^- \leftrightarrow 2Au(0) + [AuCl_4]^- + 2Cl^- \quad (1)$$

The disproportionation of $[AuCl_2]^-$ complex was found to be slow in the early stage, but then rapidly increased to the values that remained approximately constant with further reaction progress ($\log K = 7.4$ at 25 °C) [39, 40]. When the reaction was followed during time, the current responses for the cathodic peaks II and III increased (Fig. 2b), which is in accordance with the progress of the reduction of Au(III) species to Au(0).

In addition, the reaction of $[Au(en)Cl_2]^+$ with Gly-Met has been monitored by recording the UV–vis spectra at appropriate

Table 1 ^{1}H NMR characterization of $[Au(en)Cl_2]^+$, Gly-Met and products of their reaction in 1:1 molar ratio

Reactants/products	^{1}H NMR chemical shifts (δ, ppm)[a]
$[Au(en)Cl_2]^+$	3.27 (*s*, enCH$_2$)
Gly-Met	2.11 (*s*, –*S*CH$_3$), 2.18 (*m*, MetβCH$_2$)
	2.58 (*m*, MetγCH$_2$), 3.87 (*s*, GlyCH$_2$)
	4.32 (*t*, MetαCH$_2$)
free en	3.38 (*s*, enCH$_2$)
Gly-Met sulfoxide	2.71 (*s*, –*S*CH$_3$), 2.40 (*m*, MetβCH$_2$)
	3.00 (*m*, MetγCH$_2$), 3.87 (*s*, GlyCH$_2$)
	4.32 (*t*, MetαCH$_2$)

All spectra were recorded at pH 2.00 in 0.50 M KCl in D_2O with TSP as the internal standard

[a] No influence of the pH (2.00–5.00) and KCl concentration (0.01–0.50 M) was observed on the chemical shifts (*s* = singlet; *t* = triplet; *m* = multiplet)

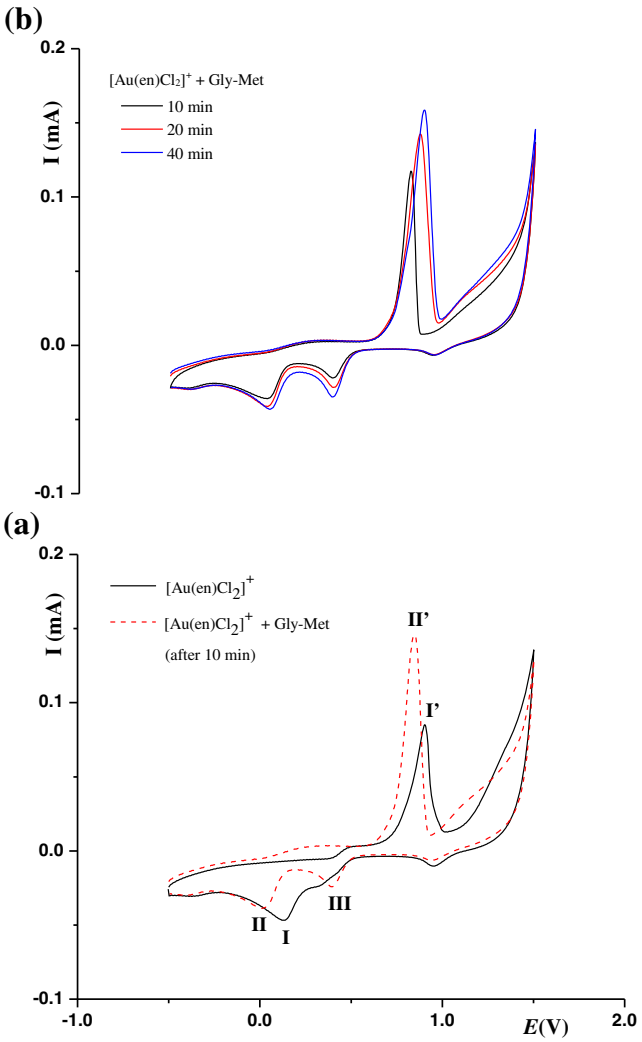

Fig. 2 Cyclic voltammograms of **a** $[Au(en)Cl_2]^+$ and its reaction with an equimolar amount of Gly-Met and **b** during progress of the reaction, recorded at a GC electrode in 0.50 M KCl at pH 2.00 and 25 °C

time intervals. When an equimolar amount of the dipeptide was added to a solution of the $[Au(en)Cl_2]^+$ complex, the absorbance of maximum at λ=305 nm decreased during time, indicating a decrease of the concentration of Au(III) species due to its reduction. As an illustration of this finding, the UV–vis spectra of the investigated reaction in 0.50 M KCl at pH 2.00 as a function of time are presented in Fig. 3. The reaction between $[Au(en)Cl_2]^+$ and Gly-Met has been studied in an excess of the dipeptide. The gold(III) complex and Gly-Met were mixed in 1:2 and 1:3 molar ratios, respectively, and the UV–vis spectra were recorded at appropriate time intervals. As it was shown in the inserted chart (Fig. 3), the absorbance of maximum at λ=305 rapidly decreased with increase of the dipeptide concentration, which is in accordance with the previously reported results for the reaction between this dipeptide and $[AuCl_4]^-$ complex [31]. However, the influence of an excess of $[Au(en)Cl_2]^+$ complex on the peptide oxidation could not be observed due to the fact that an initially formed $[AuCl_2]^-$ undergoes disproportionation to $[AuCl_4]^-$ and Au(0) (see Eq. 1).

The influence of the pH and chloride concentration on the rate of release of ethylenediamine from $[Au(en)Cl_2]^+$ in the reaction with Gly-Met

The release of ethylenediamine from $[Au(en)Cl_2]^+$ in the reaction with Gly-Met is an important step in the formation of Gly-Met sulfoxide. The rate of this process has been investigated in the presence of different chloride concentration (0.01–0.50 M KCl) in the pH range 2.00–5.00 (Table 3). The first-order rate constants were calculated from the integral values of the methylene protons for the free and coordinated ligand (Fig. 1). As it can be seen in Table 3, the observed rate constants markedly decrease with increasing the pH. Thus, the release of en ligand in 0.50 M KCl at pH 2.00 ($k=(36.23\pm1.07)\cdot10^{-6}$ s^{-1}) is approximately 15 times faster in comparison to the same process at pH 5.00 ($k=(2.35\pm0.07)\cdot10^{-6}$ s^{-1}). This difference can be attributed to the fact that higher H^+ concentration favors the release of en ligand by protonation of its amino groups [41]. Furthermore, it is obvious that the increase of Cl^- concentration from 0.01 M ($k=(1.29\pm0.18)\cdot10^{-6}$ s^{-1}) to 0.50 M ($k=(36.23\pm1.07)\cdot10^{-6}$ s^{-1}) accelerates the detachment of en ligand from Au(III) ion (Table 3). As a consequence of this detachment, the percentage of the Gly-Met sulfoxide increased with decreasing the pH (Fig. 4a) and increasing Cl^- concentration (Fig. 4b). The amount of sulfoxide obtained after 2 h of the reaction in 0.50 M KCl at pH 2.00 (52 %) was approximately 3.7 times larger than

Table 2 Positions and redox reactions associated with observed cathodic and anodic peaks in cyclic voltammograms of $[Au(en)Cl_2]^+$ complex and its reaction with an equimolar amount of Gly-Met recorded at a GC electrode in 0.50 M KCl at pH 2.00 and 25 °C

Gold(III) complex/reaction	Process	Peak	*E* (V)	Redox reaction	Reference
$[Au(en)Cl_2]^+$	Reduction	I	0.15	$[Au(en)Cl_2]^+ + 3e^- \rightarrow Au(0)+en+2Cl^-$	[33]
	Oxidation	I′	0.90	$Au(0)+4Cl^- \rightarrow [AuCl_4]^- +3e^-$	[31, 33, 37]
$[Au(en)Cl_2]^+$ + Gly-Met	Reduction	II	0.05	$[Au(en)Cl(Gly\text{-}Met\text{-}S)]^{2+} + 3e^- \rightarrow Au(0)+Gly\text{-}Met$ sulfoxide$+en+Cl^-$	[This work]
	Reduction	III	0.40	$[AuCl_4]^- +3e^- \rightarrow Au(0)+4Cl^-$	[31, 33, 37]
	Oxidation	II′	0.80	$Au(0)+4Cl^- \rightarrow [AuCl_4]^- +3e^-$	[31, 33, 37]

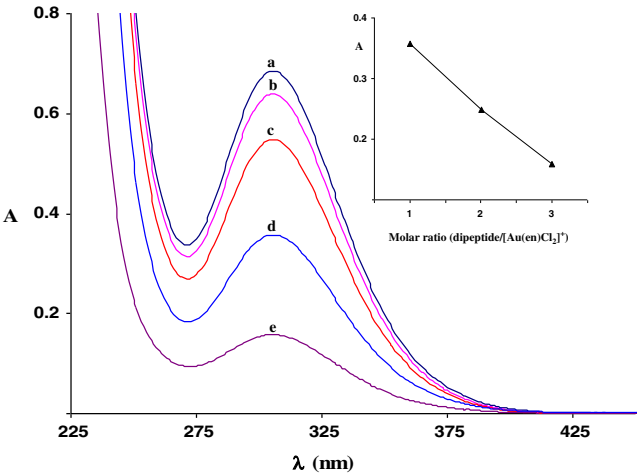

Fig. 3 The UV–vis spectra for the reaction of $[Au(en)Cl_2]^+$ with an equimolar amount of Gly-Met in 0.50 M KCl at pH 2.00 and 25 °C as a function of time: **a** 1 min, **b** 20 min, **c** 1 h, **d** 3 h, **e** 24 h. The inserted chart shows dependence of the absorbance in relation to the increasing concentration of Gly-Met

at pH 5.00 (14 %) under the same Cl^- concentration. On the other hand, the increase of Cl^- concentration from 0.01 to 0.50 M KCl results in the increase of the sulfoxide concentration from 15 to 52 %, respectively (at pH 2.00 after 2 h of progress of the reaction). Finally, it can be stated that the increase of the concentration of H^+ and Cl^- ions accelerates the investigated redox process.

Comparative study of Gly-Met oxidation in the presence of $[Au(en)Cl_2]^+$ and $[AuCl_4]^-$

In order to investigate the influence of chelated ethylenediamine ligand on the redox potential of $[Au(en)Cl_2]^+$, we compared the rate constant for the oxidation of methionine residue in Gly-Met in the presence of $[Au(en)Cl_2]^+$ complex with that previously reported for $[AuCl_4]^-$ under the same experimental conditions [31]. The $[Au(en)Cl_2]^+$ and Gly-Met were dissolved in 0.01 M DCl at pH 2.00 and 25 °C, and then mixed in 1:1 molar ratio. It was found that the oxidation of methionine residue with

Table 3 The influence of the pH and chloride concentration on the first-order rate constants ($k\,10^{-6}$ s^{-1}) for the release of en ligand from $[Au(en)Cl_2]^+$ in the redox reaction with an equimolar amount of Gly-Met at 25 °C

pH[a]	$k\,10^{-6}$ s^{-1}	[Cl−] (mol/dm^3)[b]	$k\,10^{-6}$ s^{-1}
2.00	36.23±1.07	0.01	1.29±0.18
3.00	12.84±0.51	0.05	4.88±0.10
4.00	7.80±0.33	0.10	9.58±0.52
5.00	2.35±0.07	0.50	36.23±1.07

[a] 0.50 M KCl

[b] pH 2.00

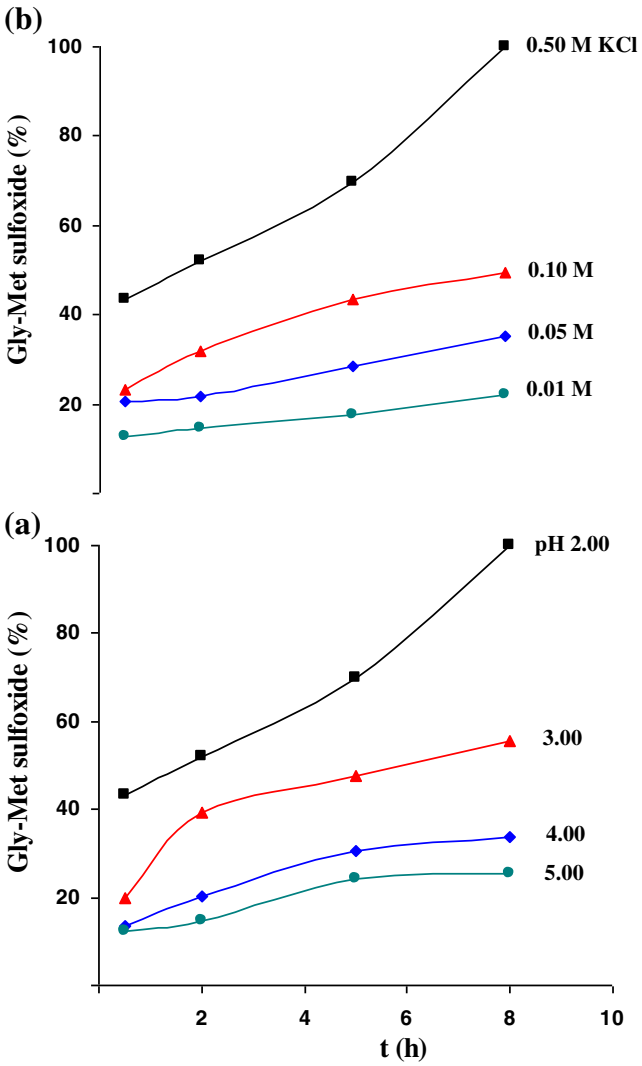

Fig. 4 Time dependence of the amount of Gly-Met sulfoxide (%) formed in the reaction between $[Au(en)Cl_2]^+$ and Gly-Met (1:1 molar ratio) on **a** pH (2.00–5.00) and **b** concentration of KCl (0.01–0.50 M)

$[Au(en)Cl_2]^+$ ($k_2=(1.74\pm0.05)\cdot10^{-3}$ M^{-1} s^{-1}) was about 200 times slower that with $[AuCl_4]^-$ ($k_2=(363.00\pm74.00)\cdot10^{-3}$ M^{-1} s^{-1}) [31]. Large difference in the oxidation rates between these two complexes undoubtedly confirms that the bidentate coordinated en ligand stabilizes Au(III) ion, which results in slower peptide oxidation in the presence of $[Au(en)Cl_2]^+$ in comparison with $[AuCl_4]^-$ complex.

Conclusions

From the present investigation of the reaction of $[Au(en)Cl_2]^+$ complex with Gly-Met the following conclusions can be drawn. The final product in this reaction is Gly-Met sulfoxide resulted from the oxidation of methionine side chain induced by Au(III) ion. The formation of this product proceeds in two consequent steps involving the coordination of methionine

sulfur atom and detachment of ethylenediamine ligand from the Au(III) complex. The investigation shows that the $[AuCl_2]^-$ complex, formed in the reaction with an equimolar amounts of reactants, has a strong tendency to disproportionate, forming $[AuCl_4]^-$ and metallic gold, Au(0). The oxidation of methionine residue in Gly-Met is strongly dependent on the pH and chloride concentration. In comparison with $[AuCl_4]^-$, the oxidation of Gly-Met dipeptide in the presence of $[Au(en)Cl_2]^+$ complex is 200 times slower. This finding leads to the conclusion that the oxidation of thioether sulfur atom in methionine-containing peptides can be inhibited by chelation of Au(III) ion with polydentate ligands containing nitrogen donor atoms. Considering this, our latest results could have an impact on designing new gold(III) complexes as potential antitumor agents with lower toxic side effects.

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