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The reaction mechanism of nitrosothiols with copper(I)

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Abstract Copper and other transition metal ions and their complexes are catalysts for the decomposition of nitrosothiols. In this way they catalyze the biological functions of nitrosothiols. The kinetics and mechanism of the reaction of two nitrosothiols, S-nitrosothiolactic acid and S-nitrosogluthathione (GSNO), with copper(I) are reported. The kinetics of the reaction of Cu(MeCN)$_n^+$ (n=0–3) with the nitrosothiols were studied. The results indicate that Cu$^+$$_{aq}$ is the active species in the GSNO system, with $k$(Cu$^+$$_{aq}$+GSNO)=$(9.4 \pm 2.0) \times 10^7$ dm$^3$ mol$^{-1}$ s$^{-1}$. The results also indicate that the Cu(MeCN)$_n^+$ (n=0–3) complexes react with S-nitrosothiolactic acid. Transient species are formed in these processes. The results suggest that these species contain copper(I) and thiol. The results shed light on the catalytic role of copper complexes in the decomposition of S-nitrosothiols.

Key words Nitric oxide · Nitrosothiols · Copper(I) · Catalysis

Abbreviations GSH: glutathione · GS$: glutathione anion · GSNO: S-nitrosogluthathione · NO$: nitric oxide RSH: thiols · RSNO: nitrosothiols · TSH: thiolactic acid · TS$: thiolactate anion · TSNO: S-nitrosothiolactic acid

Introduction

Nitric oxide (-NO) was shown recently to be a key reactant in a variety of physiological processes, e.g. in the regulation of blood flow, in the central nervous system, and in some biological defense mechanisms [1–6]. NO is formed from arginine by the enzyme nitric oxide synthase and is stored as nitrosothiols (RSNO):

$$RSNO \xrightleftharpoons{e^{-}.H^+} NO + RSH \quad (1)$$

Examples of nitrosothiols with important biological roles are: S-nitrosoalbumin, which is present in micromolar concentrations in the plasma [7], and S-nitrosogluthathione, which is present in the alveolar lining fluid at micromolar concentrations [7]. Both serve as “buffers” and reservoirs of NO. It is known that copper, and other transition metal, complexes are catalysts for the decomposition of nitrosothiols [1, 4, 8, 9, 10, 11, 12, 13, 14]. Thus they catalyze the biological functions of NO. A mechanism for the reaction between nitrosothiols and copper has been proposed in the literature [11]: the rate-determining step in this process is the reaction between RSNO and copper(I).

It seemed therefore of interest to study the kinetics of this step directly by mixing two RSNO compounds: S-nitrosothiolactic acid (TSNO) and S-nitrosogluthathione (GSNO) with Cu(MeCN)$_n^+$ (n=0–3) complexes. These nitrosothiols were chosen as it was suggested that their reactions with Cu(I) are relatively slow [10, 11, 13]. The results obtained clearly demonstrate that both compounds are reduced by Cu$^+$$_{aq}$ in a fast reaction.
Materials and methods

Materials

All solutions were prepared from A.R. grade chemicals and from distilled water which was further purified by passing through a Millipore MilliQ setup (final resistivity >10 MΩ cm⁻¹).

Cu(I) solutions were prepared via comproportionation of Cu_{aq}^{2+} with Cu_{0}; acetonitrile was used as a ligand. The experiments were done with a range of Cu(MeCN)$_n^{+}$ concentrations, (0.1–1.0) × 10⁻³ mol dm⁻³, that were in excess compared to the RSNO concentrations.

The RSNO compounds were synthesized by electrophilic nitrosation of the corresponding thiols (RSH) according to the procedure described in the literature [15, 16]; HNO$_2$ was the carrier of NO$^+$.

RSH$^+$+HNO$_2$ $\rightarrow$ RSNO$^+$+H$_2$O \hspace{1cm} (2)

TSNO was prepared [15] by adding NaNO$_2$ (20 mmol) in 20 mL of water to thiolactic acid (TSH) (10 mmol) dissolved in methanol containing 1 mol dm⁻³ HCl with 2 mL of conc. H$_2$SO$_4$ during 20 min with stirring at 25 °C. After 15 min more this solution was purified of salts and other reagents by extraction of the TSNO into dichloromethane. TSNO was thus obtained as a solution in dichloromethane. Aqueous solutions were obtained by dissolving aliquots of this solution in water. The concentration of dichloromethane in these solutions was always < 0.015 mol dm⁻³. The resulting solutions have an absorption band with a maximum at 335 nm, in accord with literature reports concerning compounds with a SNO group. For calculating the concentration of the solutions it was assumed that the extinction coefficient of TSNO at 335 nm is 1000 dm³ mol⁻¹ cm⁻¹, as most materials with SNO groups have an extinction coefficient in this region [10, 16].

GSNO was obtained as a solid in accord with the procedure described in the literature [16]. Thus to a stirred ice-cold solution of glutathione (GSH) (1.53 g) in water (8 mL) containing 2 mol dm⁻³ HCl was added, in one portion, sodium nitrite (0.345 g). After 40 min at 5 °C the red solution was treated with acetone (10 mL) and stirred for further 10 min. The resulting fine pale-red precipitate was filtered off and then washed with ice-cold water, acetone, and ether to afford GSNO. Its maximum absorbance was at 335 nm with an extinction coefficient of (920/C127 10) dm³ mol⁻¹ cm⁻¹, in very good agreement with the value reported in the literature [16].

The concentrations of the nitrosothiols do not influence the kinetic measurements.

Kinetics

All solutions contained 0.1 mol dm⁻³ phosphate buffer in the pH range 2.5–4.0 (no pH dependence of the results was observed) and were saturated with helium.

The kinetic measurements for the decomposition of TSNO were carried out by monitoring the disappearance of the absorbance at 335 nm; for GSNO the formation of an intermediate at 290 nm was followed. Two instruments were used: (1) a Hewlett Packard 8452A diode array spectrophotometer for the slower processes and (2) a stopped-flow SX 18 MV apparatus (Applied Photophysics). All experiments were carried out at room temperature, 22 ± 2 °C.

Results

The compounds GSNO and TSNO are relatively stable in the absence of reducing agents; therefore the addition of chelators to the solutions in order to scavenge transition metal ions present in the blank solutions was not required. When copper(II) is added to the solutions, relatively slow decomposition processes are observed in accord with those reported in the literature (Fig. 1). Thus the half-life time of GSNO in the presence of 2.5 × 10⁻³ mol dm⁻³ CuSO$_4$ is ≥ 1 h. When GSNO is mixed with copper(I) acetonitrile complexes, the fast formation of an intermediate is observed. The spectrum of this intermediate is shown in Fig. 2. The yield of the intermediate, as measured by its optical density, is proportional to [GSNO] as long as [Cu(I)] ≥ [GSNO].

Owing to the formation of this intermediate it is difficult to observe the kinetics of disappearance of GSNO at 335 nm, as the difference in the molar absorption coefficients is too small. Instead, the kinetics of the formation of the intermediate were followed at 290 nm (Fig. 3). The rate of this process is proportional to [Cu(MeCN)$_n$] (n=0–3) (Fig. 4). From the