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Dynamic studies of transnitrosation of thiols of biological importance by the nitrosated 4,4′,4″,4‴-tetrasulfophthalocyaninecobaltate(III) anion in aqueous solution

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ABSTRACT

The kinetics of interaction of Co(III)TSPcNO (TSPc = 4,4′,4″,4‴-tetrasulfophthalocyanine) with various thiols of biological relevance, e.g., reduced glutathione (GSH), captopril (CapSH), *N*-acetyl-*L*-cysteine (NALC), and *L*-cysteine ethyl ester (LCEE) have been investigated spectrophotometrically. The observed rate constants for transnitrosation are all first-order with respect to the respective thiols. The second-order rate constants which were determined at physiological temperature, 37 °C are 258 ± 8, 159 ± 3, 66.7 ± 1.3 and 37.4 ± 0.6 $M^{-1}\,s^{-1}$, respectively. The second-order rate constants decreased according to the sequence LCEE > CapSH > GSH > NALC. The activation parameters ($\Delta H^{\neq}$ and $\Delta S^{\neq}$) were derived from the Eyring's equation. The experimental activation parameters were then correlated by an isokinetic plot, for the reduction of $[Co(III)TSPc(NO^{-})]^{4-}$ by the thiols, making use of the expression: $\Delta H^{\ddagger} = \Delta G_0^{\ddagger} + \beta_0 \Delta S^{\ddagger}$ where $\Delta G_0^{\ddagger}$ is the intrinsic free energy of activation, and β_o the isokinetic temperature. The plot which showed very good linearity ($R^2 = 0.997$), gave values of $\Delta G_o^{\ddagger}$ ($61 \pm 1\,J\,K^{-1}\,mol^{-1}$) from the intercept, and β_o (260 ± 11 K) from the slope. It is concluded that a common mechanism is adhered to in the reduction of Co(III)TSPcNO, irrespective of the type of thiol being used, to give the corresponding *S*-nitrosothiol, which is further confirmed by high performance liquid chromatography with mass spectrometric detector.

1. Introduction

Reactions leading to transnitrosation, i.e., transfer of NO in biological systems have generated considerable interest in recent years because of the important role of S-nitrosothiols in bioregulatory processes [1]. Biological thiols are reactive and ubiquitous, existing as products of sulfur metabolism. They possess similar chemical properties as alcohols (R-OH) in terms of pK, redox potentials, and the ability to form free-radicals [2]. These properties within the –SH group account for the uniqueness of thiol chemistry [2].

Thiol groups in general are required for the activity of many biologically important proteins, and are important in functioning as reducing agents and cellular antioxidants [3]. Whilst reduced glutathione is the principal thiol and redox buffer in mammalian cells and serum albumin [3], *L*-cysteine [4] exists predominantly as the extracellular low-molecular weight thiol.

Thiols are known to scavenge reactive oxygen species [5], and in particular have been shown to have anti-platelet effects at millimolar concentration levels [6]. Of more critical importance, is the ability of thiols to form S-nitrosothiols (RSNOs). The easiest and most convenient way to synthesize RSNOs is to nitrosate thiols via sodium nitrite under acidic conditions Eq. (1) [7].

$$RSH + HNO_2 \overset{H^+}{\rightleftharpoons} RSNO + H_2O \quad (1)$$

The generation of RSNOs also comes about via the S-transnitrosation of thiols [8].

$$RSNO + R'SH \rightleftharpoons R'SNO + RSH \quad (2)$$

The products of such reactions Eq. (2) are known to be detected at submolar levels in plasma and broncho-alveolar lavage fluid, presumably formed from the S-nitrosation of thiols, and cysteine residues of proteins [8]. The occurrence of transnitrosation has been confirmed by characterizing the disulfides formed as end products, as shown in Eq. (3) [7,9].

$$RSNO + R'SH \longrightarrow RSSR + RSS'R + R'SSR' \quad (3)$$

Principal targets of NO in bioregulatory systems are not only heme and non-heme iron centers, but also other transition metal centers such as Co (III). The proposition is made, that in cellular systems, metal–NO adducts play important roles in the nitrosating of various nucleophiles [10].

The investigation of the interaction of thiols with CoTSPcNO was undertaken in order to establish a mechanism of transfer of NO from the nitrosated product of CoTSPc.

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2. Experimental

Co(II)TSPc was prepared and purified according to the method established by Weber and Busch [11]. Solutions of the nitrosated complex were prepared *in situ* similarly to the preparation of NO solutions. NO gas was passed for approximately 3 h through 50 mL of a 5 mM solution of CoTSPc containing H_2EDTA^{2-} (100 μM). NO was generated by adding a saturated solution of $NaNO_2$ to 200 mL of a 0.2 M solution of HCl. All solutions used were deaerated by passing $N_2(g)$ through the closed system for approximately 45 min. The NO produced was passed through a 5 M solution of KOH to ensure the removal of higher oxides of nitrogen [12,13].

The NO stretching frequency obtained from infrared analysis of an aqueous solution was 1639 cm^{-1} which compares well with literature [12] (lit: 1500 to 1900 cm^{-1}), indicating the presence of the NO^- moiety and hence a cobalt(III) center [2,14].

Solutions of thiols (captopril, *N*-acetyl-*L*-cysteine, *N*-acetyl-*D*,*L*-penicillamine and *L*-cysteine ethyl ester) were freshly prepared before each spectrophotometric run, by dissolving the solid in H_2EDTA^{2-} solutions, and making up to the mark on the volumetric flask with deionized water. There were instances where stock solutions were prepared, from which required amounts were pipetted into volumetric flasks containing H_2EDTA^{2-} solutions. Solutions were used as soon as possible to minimize aerial oxidation.

2.1. Reaction stoichiometry

In a spectrophotometric titration, the reductants (thiols) and complex (Co(III)TSPcNO) were reacted at different ratios, with the complex concentration being held constant. This study was carried out in deaerated water at fixed temperature, and monitored at 669 nm. The absorbances recorded at the end of the particular reactions were plotted against the [reductant]/[complex] ratios to determine the number of moles of reductant reacting with each mole of nitroso-compound, as indicated by the breakpoint in the graphs plotted.

2.2. Kinetic measurements

The reactions were monitored spectrophotometrically at 669 nm, where the largest absorbance change occurred. This was carried out on a Hewlett Packard 8453 Diode Array spectrophotometer fitted with a Hi-Tech Scientific SFA-20 Rapid Kinetics Accessory (RKA) unit. All experimental solutions involving kinetic and thermodynamic measurements were thermostatted (±0.1 °C), using a RM6 Lauda Brinkmann thermostatted water bath. Solution pairs (same pH) of thiol and Co(III)TSPcNO were placed in the respective syringes on the RKA unit. The concentration of solutions was twice the desired final concentration and contained Na_2H_2EDTA (50 or 100 μM) to sequester traces of the contaminating metal ions (example Cu^{2+}) that may catalyse the reaction.

All kinetic experiments were carried out under pseudo-first-order conditions, with the thiol in at least 10-fold excess. Deionized water was used to make up all solutions. Values of pseudo-first order rate constants (k_{obs}) were obtained from the absorbance–time traces, which were fitted to Eq. (4) where A_o, A_∞ and A_t, are the absorbance values at initial, final and varying times, respectively.

$$A_t = A_\infty + (A_o - A_\infty)exp(-k_{obs}.t) \quad (4)$$

2.3. Product determination

In NO release reactions, the nitrite anion is known to be a by-product, and is determined via the standard diazotization reaction procedure [15]. Standard solutions (5–20 μM nitrite) were used to prepare a calibration curve according to literature. 0.5 mL of sulphanilamide solution (0.5 g sulphanilamide dissolved in 100 mL of 20% v/v hydrochloric acid) was added to 25.0 mL of a neutral nitrite solution. 0.5 mL of N-(l-naphthyl)-ethylenediamine dihydrochloride solution (0.3 g of the solid reagent dissolved in 100 mL of 1% v/v hydrochloric acid) was added to each flask. Absorbance readings were recorded at 541 nm on a HP 8453 Diode Array spectrophotometer after 10 min had elapsed. A blank solution was used to zero the instrument.

The experimental samples were treated similarly and the calibration curve used to calculate the concentration of nitrite present in solution.

Under acidic conditions, the nitrite ions cause diazotization of sulphanilamide (4-aminobenzenesulphonamide) to occur, and the end product is coupled with N-(1-naphthyl)-ehtylenediamine dihydrochloride) [16,17]. The intensity of the reddish coloured solution is directly proportional to the concentration of the nitrite anion, and is quantified spectrophotometrically.

2.4. Identification of S-nitrosocaptopril: LC/DAD/MS measurements

The confirmation of the formation of S-nitrosocaptopril was done by LC/DAD/MS, using an Agilent 1100 Series LC coupled to an Agilent 1100 Series MS Detector. DAD: 332 ± 10, ref. 360 ± 100. MSD: SIM mode, − ve ESI; 245; frag. 70 V; gain 40 V. MSD: SCAN mode, 200–300 range, frag. 80 V, gain 60 V. Pump: 0.5 mL/min, 95% water: 5% methanol, run time = 10 min. Column: 25 °C, Zorbax XDB-C18, 15 cm, 4.6 mm i.d., 5 mm particle size. Injection volume: 25 mL.

3. Results and discussion

3.1. Nature of the reaction

On mixing the colourless solution of the thiols, GSH, CapSH, NALC and LCEE, with the blue–green solution of Co(III)TSPcNO, a pale blue solution of the product formed within minutes. This is accompanied by a decrease in absorbance at 669 nm, and a corresponding increase at 300 nm. The repetitive scan representing the reaction of CapSH with Co(III)TSPcNO (Fig. 1) shows three isosbestic points at 288, 339 nm and 640 nm. Repetitive scans for the other thiols are shown in the supplementary data (Figs. S1, S2 and S3).

As displayed in Fig. 1 (a–b), the decrease in absorbance at 669 nm is accompanied by a blue shift from 669 to 661 nm. An equilibrium mixture of the monomer/dimer system of Co(II)TSPc is known to absorb in the region of 659 nm [12]. This strongly suggests the loss of NO from the complex with concerted reduction of the metal center to Co(II).

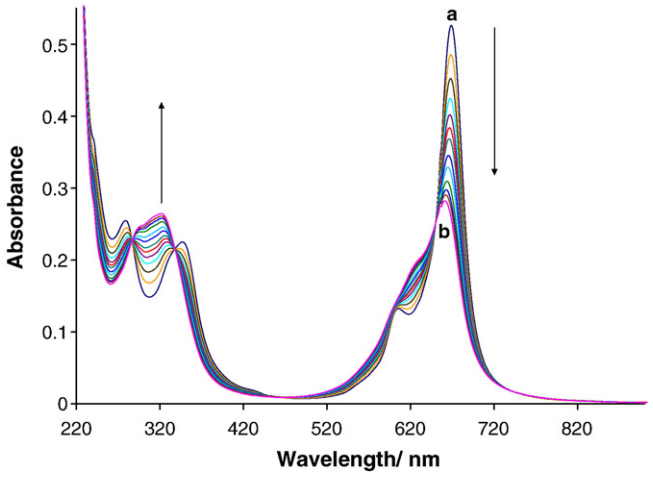

Fig. 1. Repetitive scan for the interaction of captopril with Co(III)TSPcNO in water: (a) spectrum immediately after mixing (λ_{max} = 669 nm); (b) 25 min after mixing (λ_{max} = 661 nm). [CapSH] = 50 μM, [Co(III)TSPcNO] = 5 μM, θ = 20.6 °C and [H_2EDTA^{2-}] = 50 μM.