

ABSTRACT

Transnitrosation and Redox Reactions Involving Some Potent Nitrovasodilators.

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S-Nitrosothiols (RSNOs) have emerged as important biological carriers of nitric oxide (NO), and consequently provide an alternative source of NO, especially in conditions where the natural biological source of NO is not sufficiently delivering enough (NO) to effect its maximum physiological activities which include vasodilation of smooth muscles, inhibition of platelet aggregation and macrophage activity.

This study deals with the detailed investigation of the kinetics and mechanism of the decomposition of two S-nitrosothiols, S-nitroso-N-acetyl-D,L-penicillamine (SNAP) and S-nitrosocaptopril (SNOCap) in the presence of known biological reducing agents (L-ascorbic acid and thiols, such as cysteine and captopril).

It is known that in the absence of these biological reductants, as well as transition metal ions, especially Cu^{2+} , these S-nitrosothiols are relatively very stable. The kinetic and mechanistic studies were carried out with the aid of spectrophotometric (Stopped-Flow and Diode-Array)

and electrochemical methods. These studies were undertaken so as to arrive at a more general understanding of the mechanisms of NO release that may be present when S-nitrosothiols are present in physiological environments.

Reduction of SNAP by L-cysteine and captopril occurs via two stages. The first stage in both cases is the fast transnitrosation reaction, while the second stage is the slow decomposition of the newly formed CySNO and SNOCap, respectively in the presence of excess thiol. Both stages were clearly distinguishable and ^{were} easily monitored separately. Transnitrosation occurred via nucleophilic attack on the nitroso-N atom by the thiolate anion (RS^-) group of the excess thiol present; and is a pH and [thiol] dependent reaction. The transnitrosation reaction is a reversible reaction with the tendency for the equilibrium to break down to give an irreversible process at high thiol concentration. The reactivity of CyS^- and $CapS^-$ with SNAP are somewhat similar, with second-order rate constants at 37 °C: k_f (forward reaction) = $7.69 \pm 0.23 \times 10^2 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, k_r (reverse reaction) = $171 \pm 63 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, and $K_{eq} = 4.50 \pm 1.66$. Activation parameters are: $\Delta H_f^\ddagger = 54 \pm 1 \text{ kJ mol}^{-1}$, $\Delta S_f^\ddagger = -17 \pm 1 \text{ J K}^{-1} \text{ mol}^{-1}$; $\Delta H_r^\ddagger = 68 \pm 7 \text{ kJ mol}^{-1}$, $\Delta S_r^\ddagger = 16 \pm 2 \text{ J K}^{-1} \text{ mol}^{-1}$; for the reaction between SNAP and cysteine. The reaction between SNAP and captopril resulted in the following data at 37 °C: k_f

$= 7.85 \pm 0.14 \times 10^2 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ (and compares well with k_f for the SNAP/cysteine reaction); there was no viable result for k_r . $\Delta H_f^\ddagger = 49 \pm 2 \text{ kJ mol}^{-1}$ and $\Delta S_f^\ddagger = -32 \pm 2 \text{ J K}^{-1} \text{ mol}^{-1}$. The activation parameters in both cases demonstrate the associative nature of the transnitrosation mechanism.

The second-order rate constants for the second stage of the reaction between SNAP and cysteine could not be evaluated due to the complexity of the reactions involved, however second-order rate constant at 37°C , for the second-stage of the reaction between SNAP and captopril is $74.4 \pm 7.4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ and was calculated from the product of the equilibrium constant (K) for the intermediate formation (SNOCap---CapS) and the first-order rate constant (k_f) for the subsequent decay of the intermediate. $\Delta H_1^\ddagger = 37 \pm 1 \text{ kJ mol}^{-1}$ and $\Delta S_1^\ddagger = -181 \pm 44 \text{ J K}^{-1} \text{ mol}^{-1}$.

Both reaction systems resulted in the formation of NO, NO_2^- , NH_2OH , N_2O and possibly NH_3 . NO_2^- and NH_2OH were confirmed by colourimetric methods, while N_2O was detected by GC-mass spectrometry as the head-space gas of the reactions under anaerobic conditions. NH_3 could not be detected due to certain limitations. NO was measured using an ISO-NOP electrode attached to an ISO-NO Mark II metre.

The reaction between SNOCap and cysteine occurred in a similar fashion as that between SNAP and cysteine, with the presence of two clearly-defined stages, easily monitored in the UV region of the spectrum. The first stage is transnitrosation via the CyS^- species and occurred with the formation of a somewhat partially stable intermediate prior to CySNO formation, giving an equilibrium constant of $K = 178 \pm 11 \text{ dm}^3 \text{ mol}^{-1}$ and $k_f = 2.40 \pm 0.07 \text{ s}^{-1}$ at 37°C with calculated $k_2 = 4.27 \pm 0.29 \times 10^2 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$; and $\Delta H_f^\ddagger = 28 \pm 2 \text{ kJ mol}^{-1}$ and $\Delta S_f^\ddagger = -147 \pm 17 \text{ J K}^{-1} \text{ mol}^{-1}$, establishing an associative mechanism. The second stage is again very complicated and yielded no evaluation for possible rate constants. The transnitrosation rates for the reactions of SNAP with cysteine and captopril are faster than those for the reaction between SNOCap and cysteine. This directly relates to the greater stability of SNOCap over SNAP, and hence its greater resistance to reduction by the said mentioned thiols.

The first stage reaction (Transnitrosation) between GSNO and cysteine was only investigated since the second stage had already been studied, and gave a second-order rate constant of k_f (forward reaction) = $2.70 \pm 0.17 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ at 37.4°C , and activation enthalpy and entropy : $\Delta H_f^\ddagger = 35 \pm 6 \text{ kJ mol}^{-1}$ and $\Delta S_f^\ddagger = -68 \pm 11 \text{ J K}^{-1} \text{ mol}^{-1}$, respectively. This reaction is, as emphasized previously, driven by the

thiolate anion (Cys^-), and is a pH and [cysteine] dependent reaction. Based on the second-order rate constant this reaction appears to be the fastest of the transnitrosation reactions investigated in this project.

The reactions of SNAP and SNOCap with L-ascorbic acid constitute the redox reactions, and occur under conventional spectrophotometric time-scale with the release of NO by slow reduction of both RSNOs by the action of ascorbic acid. NO was the principal N-containing product and was measured in yields of approximately 70 %. The three forms of ascorbate present in aqueous solution, reduce both SNAP and SNOCap in the order $\text{A}^{2-} \gg \text{HA}^- > \text{H}_2\text{A}$, with the reactivity of SNOCap being much slower to that of SNAP under similar conditions (i.e. similar pHs, temperature, and [ascorbate]). The reactions followed the same mechanism and were [ascorbate] and pH dependent. The mechanism involves nucleophilic attack by HA^- and/or A^{2-} at the nitroso nitrogen to give NO, RS^- and A^- as products of the reaction. The second-order rate constant, k_a via H_2A was found to be negligible. At 37 °C, SNAP: $k_b(\text{HA}^-) = 9.81 \pm 1.39 \times 10^{-3} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, $k_c(\text{A}^{2-}) = 662 \pm 38 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$; with activation parameters $\Delta H_b^\ddagger = 93 \pm 7 \text{ kJ mol}^{-1}$, $\Delta S_b^\ddagger = 15 \pm 2 \text{ J K}^{-1} \text{ mol}^{-1}$; $\Delta H_c^\ddagger = 51 \pm 5 \text{ kJ mol}^{-1}$ and $\Delta S_c^\ddagger = -28 \pm 3 \text{ J K}^{-1} \text{ mol}^{-1}$. SNOCap: $k_b(\text{HA}^-) = 2.57 \pm 1.29 \times 10^{-2} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, $k_c(\text{A}^{2-}) = 49.7 \pm 1.3 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$; with activation

parameters $\Delta H_b^\ddagger = 63 \pm 11 \text{ kJ mol}^{-1}$, $S_b^\ddagger = -71 \pm 20 \text{ J K}^{-1} \text{ mol}^{-1}$; $\Delta H_c^\ddagger = 103 \pm 7 \text{ kJ mol}^{-1}$ and $\Delta S_c^\ddagger = 118 \pm 8 \text{ J K}^{-1} \text{ mol}^{-1}$.

The effect of trace amounts of copper ions was eliminated by the addition of EDTA, however the investigation on the effect of $\text{Cu}^{2+}/\text{Cu}^+$ on the decomposition of SNOCap showed that there was not much effect.

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