ABSTRACT

Kinetics and Mechanism of Nitric Oxide release from some Nitrovasodilators

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Nitric oxide (NO) is a very diverse endogenous molecule and is responsible for a number of physiological activities including vasodilation of smooth muscles. It is synthesized in the body from endothelial cells by L-arginine, molecular oxygen, enzymes (the nitric oxide synthases) among other necessary cofactors. Nitric oxide containing compounds (nitrosocompounds) is now a vital alternative source of NO especially in conditions when the body's natural mechanism is unable to deliver.

Nitrosocompounds are generally quite stable by themselves and do not appreciably release NO. However, it was found that in physiological environments and in the presence of known biological reducing agents (L-ascorbic acid, L-cysteine, DL-penicillamine, glutathione, etc.), NO release was significantly improved. Transition metal ions, especially Cu^{2+}, were also found to catalyze the decomposition of nitrosocompounds to afford NO.

In this study we have investigated the kinetics and the mechanism of decomposition of sodium nitroprusside (SNP) and S-nitrosoglutathione (GSNO), including the transnitrosation reactions of GSNO using mainly spectrophotometric (stopped-flow and diode array) and electrochemical methods.

The rates of these reactions are strongly pH dependent and increase with pH. The three forms of ascorbate present in aqueous solution reduce the nitrosocompounds in the order $A^{2-} >> HA^{-} > H_2A$ to afford NO, while the unprotonated thiolate anion (RS^{-}) group of thiols seems to be the reactive component.

Reduction of SNP by L-cysteine proceeds in three stages. The first stage is the equilibrium formation of the SNP-cysteine adduct (RS^{-} group of cysteine coordinated with ^{+}NO group of SNP) followed by electron transfer. CN^{-} was released in the process by the trans labilizing effect of neutral $NO^{\cdot}$ ligand and was detected with a cyanide ion-specific electrode. At low [cysteine], the other stages were not observed, and NO and a cysteine-coordinated Fe(II) complex are the primary products. However, at higher [cysteine] i.e. when cysteine is at about 10 folds greater than SNP, the subsequent stages were pronounced. The second stage also involved formation of another SNP-cysteine adduct followed by electron transfer, this time reducing the coordinated NO to nitroxyl (NO^{-}) ligand. In the third stage, NO^{-} is substituted by the cysteinate anion (CyS^{-}) to produce $[Fe(CN)_4SCy]^{3-}$, possibly another Fe(II) complex and the $NO^{\cdot}$ intermediate.

Nitroxyl (protonated NO^{-}, HNO) is a very unstable intermediate and will then undergo a number of subsequent chemical reactions depending on certain conditions such as the pH, the availability of oxygen , the presence of neutral NO and the concentration of free thiol. Other nitrogen species identified includes nitrite (NO_2^{-})and hydroxylamine (NH_2OH), however nitrous oxide (N_2O), ammonia (NH_3) and N_2 could have also been end products of HNO reactions. However, the latter species were not determined.

The experimental rate and activation parameters are, *First stage*: $K_1 = 2.42 \pm 0.42 \times 10^3$ M^{-1}; $k_1 = 1.57 \pm 0.31 \times 10^{-2}$ s^{-1} at 25 °C, with $\Delta H_1^{\ddagger} = 49 \pm 3$ kJ mol^{-1}; $\Delta S_1^{\ddagger} = -116 \pm 11$ J K^{-1} mol^{-1}. *Second stage*: $K_2 = 3.24 \pm 0.42 \times 10^2$ M^{-1}; $k_2 = 4.95 \pm 0.83 \times 10^{-2}$ s^{-1} at 25 °C, with $\Delta H_2^{\ddagger} = 21 \pm 1$ kJ mol^{-1}; $\Delta S_2^{\ddagger} = -200 \pm 3$ J K^{-1} mol^{-1}. *Third stage*: $k_3 = 0.55 \pm 0.02$ s^{-1} at 25 °C, with $\Delta H_3^{\ddagger} = 49.0 \pm 1.5$ kJ mol^{-1}; $\Delta S_3^{\ddagger} = -85 \pm 5$ J K^{-1} mol^{-1}. Addition of NaCN retards the reaction while Cu^{2+} catalysis was most pronounced in the third stage.

The outer-sphere reduction of nitroprusside by L-ascorbic acid also involved three stages with NO release in the last stage. *First stage*: $K_{IT} = 74.0 \pm 1.3$ M^{-2}; $k_1 = 1.67 \pm 0.11 \times 10^9$ dm^3 mol^{-1} s^{-1} at 25 °C, with $\Delta H_1^{\ddagger} = 31.6 \pm 3.4$ kJ mol^{-1}; $\Delta S_1^{\ddagger} = 38 \pm 2$ J K^{-1} mol^{-1}. *Second stage*: $k_2 = 3.97 \pm 0.04 \times 10^5$ dm^3 mol^{-1} s^{-1} at 25 °C, with $\Delta H_2^{\ddagger} = 94.0 \pm 2.0$ kJ mol^{-1}; $\Delta S_2^{\ddagger} = 182 \pm 6$ J K^{-1} mol^{-1}. *Third stage*: $k_3 = 2.08 \pm 0.04 \times 10^5$ dm^3 mol^{-1} s^{-1} at 25 °C, with $\Delta H_3^{\ddagger} = 92.3 \pm 2.7$ kJ mol^{-1}; $\Delta S_3^{\ddagger} = 171 \pm 9$ J K^{-1} mol^{-1}.

The release of CN^- was also evident in this process. NO release showed significant pH dependency with an increase up to ~ pH 7 before decreasing again. The catalytic effect of some group 1 cations on the anion-anion electron transfer between ascorbate and nitroprusside show increasing catalysis down the group, where $Cs^+ > K^+ > Na^+ > Li^+$. This was ascribed to the decrease in the enthalpies of hydrations as we descend the group since deaquation is necessary to facilitate electron-transfer between the reacting anions. The ion-triplet formation constants, K_{IT}, also increase down the group for this same reason. Self exchange calculations, $k_{11} = 1.31 \times 10^4$ dm^3 mol^{-1} s^{-1} for the $[Fe(CN)_5NO]^{2-/3-}$ redox couple and $k_{11} = 1.97 \times 10^3$ dm^3 mol^{-1} s^{-1} for the

$[Fe(CN)_4NO]^{2-/3-}$ system is comparable to previous studies with other cyanometallates and is clearly showing an outer-sphere electron transfer mechanism.

The rate of the reaction between GSNO and L-ascorbic acid is very slow at low pH's but increases drastically above ~ pH 6. The second-order rate constant, k_a, via H_2A was found to be negligible. At 25 °C, k_b (HA^-) = 5.23 ± 1.47 × 10^{-3} dm^3 mol^{-1} s^{-1}; k_c (A^{2-}) = 1.22 ± 0.04 × 10^3 dm^3 mol^{-1} s^{-1}, with activation parameters $\Delta H_b^{\ddagger}$ = 54.4 ± 4.3 kJ mol^{-1}; $\Delta S_b^{\ddagger}$ = -105 ± 16 J K^{-1} mol^{-1}, $\Delta H_c^{\ddagger}$ = 80.5 ± 7.5 kJ mol^{-1}; $\Delta S_c^{\ddagger}$ = 83.3 ± 7.4 J K^{-1} mol^{-1}. The experimental rate and activation parameters clearly suggest that this reaction follows an outer-sphere electron transfer mechanism. GSNO is relatively stable at trace Cu^{2+} concentrations, but catalytic decomposition becomes significant above *ca.* 10 μmol dm^{-3} Cu^{2+}. However, EDTA was added so as to nullify any effect of trace quantities present in buffers or deionized water. In comparison, ascorbic acid showed greater potency than Cu^{2+} ions at inducing decomposition of GSNO to afford NO.

The reaction of GSNO with amino thiols is quite interesting. At low thiol concentrations NO is the sole product, however at higher concentrations the derivatives of NO^-, which includes, N_2O, NH_2OH, NO_2^- and NH_3 are produced. We were able to detect the formation of NO_2^- and NH_2OH as end products, but were not able to directly detect N_2O and NH_3 due to certain limitations. Both NO and NO^- are responsible for many biological activities. NO/ NO^- release from GSNO induced by L-cysteine is relatively slow and occurs via transnitrosation. At 25 °C, K_e = 78 ± 11 M^{-1}; k_2 = 1.34 ± 0.09 dm^3 mol^{-1} s^{-1}, with $\Delta H^{\ddagger}$ = 41.5 ± 3.2 kJ mol^{-1}; $\Delta S^{\ddagger}$ = -103 ± 10 J K^{-1} mol^{-1}. K_e being the

transnitrosation (between GSNO and RSH) equilibrium constant, and k_2 the second order rate constant for the decomposition of the newly formed S-nitrosothiol. NO/NO^- release from GSNO induced by DL-penicillamine is slower than L-cysteine; $K_e = 342 \pm 12\ M^{-1}$ and $k_2 = 0.71 \pm 0.03\ dm^3\ mol^{-1}\ s^{-1}$ at 25 °C; $\Delta H^{\ddagger} = 25.2 \pm 1.0\ kJ\ mol^{-1}$; $\Delta S^{\ddagger} = -160 \pm 4\ J\ K^{-1}\ mol^{-1}$. The activation parameters here demonstrate the associative nature of the transnitrosation mechanism.