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Minireview

Autoxidation kinetics of aqueous nitric oxide

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Reports on the kinetics of the autoxidation of aqueous nitric oxide are discussed. It is concluded that the correct rate law is $-\mathrm{d[NO]}/\mathrm{d}t = 4k_{\mathrm{aq}}[\mathrm{NO}]^2[\mathrm{O_2}]$ with $k_{\mathrm{aq}} = 2 \times 10^6\ \mathrm{M^{-2} \cdot s^{-1}}$ at 25°C and that a recent report of a rate law zero order in NO is incorrect.

Nitric oxide; Dioxygen; Autoxidation; Kinetics; Genotoxicity; Cytotoxicity; Bioregulation

Nitric oxide (NO) is a species of considerable current interest owing to its wide ranging biochemical activity [1] including physiological roles in blood pressure control [2], neurotransmission [3,4] and cytostatic and cytotoxic activity of stimulated macrophages [5]. An important factor is the fugitive character of NO in oxygenated media, especially given the potential genotoxicity of species generated in NO autoxidation [6,7]. How, for example, can NO participate as a bioregulatory agent in oxygenated media despite its propensity to undergo autoxidation? A proper assessment of nitric oxide autoxidation under biological conditions depends on knowing the reaction kinetics, i.e. rate law and the relevant rate constants in aqueous media. The purpose of this correspondence is to point out that the rate law is now known with some confidence, despite potential confusion resulting from two recent reports that are incorrect. The correct rate law is $-\mathrm{d[NO]}/\mathrm{d}t = 4k_{\mathrm{aq}}[\mathrm{NO}]^2[\mathrm{O_2}]$ with $k_{\mathrm{aq}} = 2 \times 10^6\ \mathrm{M^{-2} \cdot s^{-1}}$ in aqueous solution at 25°C.

The gas phase autoxidation of NO is [8]

$$2\mathrm{NO} + \mathrm{O_2} \rightarrow 2\mathrm{NO_2} \qquad (1)$$

and the corresponding rate law has been shown to be

$$-\frac{\mathrm{d[NO]}}{\mathrm{d}t} = 2k_{\mathrm{g}}[\mathrm{NO}]^2[\mathrm{O_2}] \qquad (2)$$

The stoichiometry cannot be the same in aqueous solution since $\mathrm{NO_2}$ is unstable in this medium. A key question is whether the form of the rate law also differs in aqueous solution.

The first detailed report on the aqueous autoxidation was published in 1975 by Pogrebnaya et al. [9], who found that the stoichiometry is given by

$$4\mathrm{NO} + \mathrm{O_2} + 2\mathrm{H_2O} \rightarrow 4\mathrm{NO_2^-} + 4\mathrm{H^+} \qquad (3)$$

These workers used a continuous-flow method to study the kinetics and monitored the reaction by conductivity measurements. The rate law they obtained is

$$-\frac{\mathrm{d[NO]}}{\mathrm{d}t} = 4k_{\mathrm{aq}}[\mathrm{NO}]^2[\mathrm{O_2}] \qquad (4)$$

with $4k_{\mathrm{aq}} = 9 \times 10^6\ \mathrm{M^{-2} \cdot s^{-1}}$ at 25°C. Thus, although the stoichiometry was found to differ between the gas phase and aqueous solution, the form of the rate law was found to be the same.

The next report on the aqueous kinetics appeared in 1989 [10] as an ancillary to studies of the reaction of NO with deoxyhemerythrin. The stopped-flow method was used to study the kinetics by monitoring absorbance changes at 355 nm due to nitrite formation. It was reported that with $[\mathrm{NO}]_0 = 1 \times 10^{-4}$ M and $[\mathrm{O_2}]_0 = 0.9 \times 10^{-4}$ M the reaction proved to be too fast to observe ($t_{1/2} < 6$ ms at 25°C). Thus, it was deduced that, if the rate law had the same form as reported [9], then $4k_{\mathrm{aq}}$ would be $> 4 \times 10^{10}\ \mathrm{M^{-2} \cdot s^{-1}}$, several orders of magnitude greater than reported. No explanation for the discrepancy was offered.

In another 1989 report, Eguchi et al. [11] apparently encountered no difficulty in using the stopped-flow method to monitor the kinetics of Eq. 3. Their rate law was of the same form as that described by Pogrebnaya et al. [9].

In 1992 a kinetics investigation of NO autoxidation using an electrochemical method to detect NO was described [12]. Initial NO concentrations were 10 and 20 μM with $\mathrm{O_2}$ in 5- to 10-fold excess in phosphate

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buffer at pH 7.4. A rate law zero order with regard to NO, i.e. rate = $k[O_2]$, was reported [12] with no reference to discrepancies with prior studies [9–11]. One can use the rate law and constants from our studies cited below to estimate a half-life for the reaction at initial NO and O_2 concentrations used in the electrochemical study. This estimate gives a half-life of 130 s, approximately that which was observed [12], so the detection technique apparently was not grossly malfunctioning. However, the reported data include an internal inconsistency. In Fig. 2 of that report are shown two kinetic traces of the NO signal as a function of time which are decidedly nonlinear; a rate law zero-order with respect to [NO] should give linear traces.

Very recently NO autoxidation has been investigated independently in our respective laboratories [13,14]. In one study, Wink et al. [13] used the stopped-flow method to monitor absorbance changes at 216 nm due to nitrite formation in pH 7.4 aqueous phosphate buffer with 100 μM NO and 1.0 mM O_2 and with 0.17–1.7 mM NO and 40 μM O_2. The third order rate law obtained was the same as that reported by Pogrebnaya et al. [9] and by Eguchi et al. [11]. Moreover, the rate constant ($4k_{aq} = 6 \pm 1.5 \times 10^6\ M^{-2} \cdot s^{-1}$ at 22°C) was close to that reported by Pogrebnaya et al. Related experiments using trapping agents to study the kinetics of oxidation and nitrosation by key reactive intermediates in the NO/O_2 reaction gave the same rate law and rate constants and furthermore extended the range of applicability to much lower concentrations 3–10 μM NO.

In the other study, by Awad and Stanbury [14], the stopped-flow method also was used, although somewhat differently. In one set of experiments the absorbance at 355 nm due to nitrite was used to monitor the kinetics, while in another set the conductivity of the solution was used. When the conductivity method was used, the solutions were not buffered, and the initial concentrations were 40 or 100 μM NO and 0.6 mM O_2. When the absorbance method was used, the solutions were either acidic (0.06 M HCl) or alkaline (0.05 M NaOH) and the initial concentrations were 47–300 μM NO and 0.6 mM O_2, or 0.8 mM NO and 17 μM O_2. Under all these conditions, the time-dependent data were consistent with Eq. 4, i.e. the same rate law as reported by Wink et al. [13], Eguchi et al. [11], and Pogrebnaya et al. [9]. Moreover, the rate constant obtained ($4k_{aq} = 8.4 \times 10^6\ M^{-2} \cdot s^{-1}$ at 25°C) was close to the values reported by Wink et al. and by Pogrebnaya et al.

Although it is unclear why there is disagreement in the studies represented by refs. 10 and 12, the weight of the evidence clearly supports a rate law for NO autoxidation in aqueous solution which is second order with respect to [NO] (i.e. Eq. 4) with a third order rate constant $4k_{aq} \cong 8 \times 10^6\ M^{-2} \cdot s^{-1}$. Further insights into key chemical properties of NO autoxidation include the demonstrations by Awad and Stanbury [14] that the reaction rate in aqueous solution is pH-independent over the range 1–13 and by Wink et al. [13] that NO oxidation and concomitant nitrosation of amines occur at the same rates and that the substrates used had no influence on the lifetime of NO. Lastly, an earlier study of NO autoxidation in tetrachloromethane showed third order kinetics with a rate constant close to that seen in aqueous solution [15], despite the product being nitrogen dioxide rather than nitrite ion. Thus, the dynamics of reaction between NO and O_2 are remarkably indifferent to the medium although medium dependent pathways partitioning the reactive intermediates do determine the eventual products.

Characterizing the kinetics of NO autoxidation enhances ones fundamental understanding of how nitric oxide can serve in a variety of physiological processes. At the low concentrations found for bioregulatory processes such as blood pressure regulation and neurotransmission, NO should be sufficiently long-lived (100–500 s) to allow for reaction with heme proteins such as guanylate cyclase. In contrast, high levels of NO are produced under certain conditions, e.g. by stimulated macrophages. This situation would lead to rapid reaction with O_2 and concomitant oxidation and nitrosation chemistry which could contribute to cytotoxic and genotoxic activities [6,7]. Thus, delineating the correct rate law for NO autoxidation in aqueous media provides important insight regarding how this reactive molecule can play important bioregulatory roles yet can have cytotoxic or even mutagenic properties under other conditions.

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