Nitric Oxide Kinetics in Biological Systems

by

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Abstract

The kinetics of nitric oxide (NO) reactions in aqueous solutions are important in determining the role of NO as both a biological messenger and a cytotoxic agent, since NO reacts with molecular oxygen, superoxide, and various other species such as iron containing proteins. The aqueous reaction of NO with O_2 was studied utilizing real-time measurements of NO and the principal product, nitrite (NO_2^-). Kinetic information obtained from these studies was used to develop a model to quantify several extracellular species formed in the presence of macrophages, which generate both NO and superoxide.

Novel methods for measureing gaseous and aqueous NO concentrations were developed for studying NO kinetics. These methods utilized the NO permeability of a polydimethylsiloxane membrane, in which one side of a flat sheet membrane was exposed to the NO concentration source, while the other side was exposed to a high vacuum created by one of two detectors, a mass spectrometer or a NO-specific chemiluminescence detector. The mass transfer of NO from the bulk solution to the detector, via the membrane, was quantified. The results showed a fast response time and a low NO flux, which made real-time NO measurements possible with only a negligible loss of NO from the solution. In addition to NO measurements, a membrane system was also utilized for delivering NO at low dose rates (mimicking rates at which NO is synthesized by macrophages) to study the effects of NO toxicity and mutagenicity on various cells. Delivery of NO was achieved by exposing the lumen of the polydimethylsiloxane tubing to a known gaseous NO concentration, while the tubing was immersed in aqueous solution.

The reaction of NO with O_2 was confirmed to be second order in NO and first order in O_2 in both the gas and aqueous phase. Rate constants ($\boldsymbol{k}_1$), expressed as $-R_{NO} = 2\boldsymbol{k}_1[NO]^2[O_2]$ were determined as 6100 ± 40 M^{-2} s^{-1} and 6200 ± 30 M^{-2} s^{-1} for the gas phase and $2.1 \pm 0.4 \times 10^6$ M^{-2} s^{-1} and $2.4 \pm 0.3 \times 10^6$ M^{-2} s^{-1} for the aqueous phase at 23° C and 37°C respectively. Pseudo-steady state approximations for certain reactive intermediates in the aqueous phase, principally N_2O_3 and NO_2, was shown to be valid.

As reported in the literature, nitrous anhydride (N_2O_3) is a likely intermediate in the aqueous reaction of NO with O_2 at acidic pH. It is known that N_2O_3 can nitrosate secondary amines, such as morpholine, to form carcinogenic nitrosamines. It is well established that nitrosamine formation is enhanced by chloride and thiocyanate at acidic pH, but there has been little information on the effects of these anions, and other anions such as phosphate and perchlorate, on nitrosation at physiological pH. Accordingly the

effects of various anions on the rate of N-nitrosation of morpholine, a model amine, were studied at near-neutral pH. The results with morpholine showed that N_2O_3 is the likely reactive intermediate in aqueous NO/O_2 reaction at physiological pH. Chloride and phosphate were shown to scavenge N_2O_3 via a proposed hydrolysis reaction. Thus, these anions diminished, rather than enhanced, nitrosation of morpholine at physiological pH by reducing the availability of N_2O_3 for nitrosation.

Macrophages in culture can be made to synthesize both NO and superoxide anion, thereby mimicking certain aspects of their response to infections in the body. The kinetic model based on data in the cell-free systems was tested further by measuring NO, NO_2^-, O_3^-, and nitrosomorpholine concentrations in the extracellular fluid of a macrophage suspension culture. The model was successful in explaining the concentrations of the various nitrogen oxide species. The model shows that relative release rates of superoxide and NO strongly affect the ratio of NO_3^- to NO_2^- formation. The reaction of NO with peroxynitrite ($ONOO^-$), with a rate constant ($-R_{NO} = \boldsymbol{k}_{12}[NO]\,[ONOO^-]$ of $4.0 \pm 2.1 \times 10^5\ M^{-1}\ s^{-1}$, was consistent with observations at pH 7.4 and 37 °C. Rate of morpholine nitrosation in the presence of macrophages were overpredicted by the kinetic model, by roughly a factor of ten. A probable cause of this discrepancy is the presence of one or more chemical species found in the presence of activated macrophages which reduce the N_2O_3 available for nitrosation. Further work will be needed to identify the species responsible.

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