Chapter 57
Analytical Expression for the NO Concentration Profile Following NONOate Decomposition in the Presence of Oxygen

Zimei Rong and Zhihui Ye

Abstract We have derived an analytical expression for the time dependent NO concentration from NONOate donors in the presence of oxygen for the process of NO release from NO donors following autoxidation. This analytical solution incorporates the kinetics of the releases with the autoxidation and is used to fit the simulated NO concentration profile to the experimental data. This allows one to determine the NO release rate constant, k_1, the NO release stoichiometric coefficient, v_{NO}, and the NO autoxidation reaction rate constant, k_2. This analytical solution also allows us to predict the real NO concentration released from NO donors under aerobic conditions, while v_{NO} is reportedly two under aerobic conditions, it falls to lower values in the presence of oxygen.

Keywords NONOate • Decomposition • Nitric oxide • Autoxidation • Mathematical mode

1 Introduction

The brain responds to hypoxia with an increase in cerebral blood flow (CBF). Many vasodilatory mechanisms have been proposed for this hypoxic vasodilation but none has gained universal acceptance. Nitric oxide (NO) was suggested to play a significant role in this response [1]. Both the formation and removal of this gas are pO_2 dependent and, therefore, CBF is a complex function of oxygen concentration. To test this hypothesis quantitatively, we incorporated the NO vasodilatory mechanism

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into the BRAINSIGNALS model [2], which was designed to assist in the interpretation of multimodal noninvasive clinical measurements. Previously, we incorporated NO generation by deoxyhemoglobin [3] and NO metabolism by oxidised cytochrome *c* oxidase (CCO) [4] and oxyhemoglobin [5] into the BRAINSIGNALS model to reproduce the experimental human CBF versus pO_2 curves digitised from literature [6]. However, we were still unable to reproduce the exact CBF versus pO_2 curve and, in particular, the threshold phenomenon: CBF remaining unchanged until pO_2 decreased to a threshold value triggering a rise in CBF.

We considered looking for either an increase in NO production with decreasing pO_2 or a decrease in NO metabolism with decreasing pO_2 from a steeper NO versus O_2 curve, to reproduce the threshold in a CBF versus pO_2 curve. NO autoxidation is a third order reaction, i.e., first order with respect to oxygen and second order with respect to NO. For our simulation, we need the reaction rate constant, which varies a lot according to different researchers [7]. We reviewed the literature on NO autoxidation, which was studied with two systems, either a mixture of NO and oxygen, or NO released from donors followed by an aerobic removal. For the former, injecting the concentrated NO solution as a bolus may lead to heterogeneous NO oxidation at the tip of the syringe [8]. In the latter case, NO donors were used to provide continuous NO sources for characterization of NO autoxidation [9, 10] and NO formation and autoxidation has been modelled by numerical methods [9, 10].

In this work, we obtain an analytical expression that describes NO release from NO donors and NO oxidation, and use this to simulate NO concentration profiles and hence simultaneously determine three parameters: the NO release rate constant (k_1), the NO release stoichiometric coefficient (v_{NO}), and the NO autoxidation rate constant (k_2).

2 Mathematical Model

In a biochemical system, an NO donor such as ProliNO (disodium 1-(hydroxy-NNO-azoxy)-L-proline) releases NO which is then oxidised by O_2. We will first consider the release of NO by ProliNO.

$$\text{ProliNO} \rightarrow v_{NO}\text{NO} \tag{57.1}$$

where v_{NO} is the stoichiometric coefficient of NO release, i.e., v_{NO} moles of NO molecules are released from one mole of ProliNO. ProliNO decomposition follows first order kinetics with a decay rate constant k_1 in s^{-1} as:

$$\frac{d\left[\text{ProliNO}\right]}{dt} = -k_1\left[\text{ProliNO}\right] \tag{57.2}$$

The solution to Eq. (57.2) can be expressed as:

$$[\text{ProliNO}] = [\text{ProliNO}]_0 \exp(-k_1 t) \tag{57.3}$$

The rate of NO formation can be obtained as:

$$\frac{d[\text{NO}]}{dt} = v_{\text{NO}} k_1 [\text{ProliNO}] = v_{\text{NO}} k_1 [\text{ProliNO}]_0 \exp(-k_1 t) \tag{57.4}$$

We use x to represent NO concentration in M, and P ($v_{\text{NO}}[\text{ProliNO}]_0$) to represent the maximum NO concentration in the absence of decay, i.e., the initial ProliNO concentration ($t=0$) multiplied by v_{NO}. Equation (57.4) can be simplified as

$$\frac{dx}{dt} = k_1 P \exp(-k_1 t) \tag{57.5}$$

Now we consider NO autoxidation as:

$$4\text{NO} + \text{O}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{NO}_2^- + 4\text{H}^+ \tag{57.6}$$

The rate equation of NO autoxidation can be expressed as:

$$\frac{dx}{dt} = -k_3 [\text{O}_2] x^2 = -4k_4 [\text{O}_2] x^2 = -k_2 x^2 \tag{57.7}$$

where k_3 (NO consumption) and k_4 (oxygen consumption) are the NO autoxidation rate constants with units $\text{M}^{-2}\ \text{s}^{-1}$ and k_2 is the NO autoxidation rate constant, k_3 that incorporates the oxygen concentration in unit $\text{M}^{-1}\ \text{s}^{-1}$. Historically, k_2, k_3 and k_4 have been used by different researchers to characterize NO autoxidation.

The overall reaction is a cascade chemical reaction. We need to solve a combined equation with an initial condition $x(t=0)=0$ as:

$$\frac{dx}{dt} = Pk_1 \exp(-k_1 t) - k_2 x^2 \tag{57.8}$$

For simplicity we introduce an intermediate, dimensionless, parameter b as:

$$b = 2\sqrt{Pk_2 / k_1} \tag{57.9}$$

and an intermediate, dimensionless, variable y as:

$$y = b \exp\left(-\frac{k_1 t}{2}\right) \tag{57.10}$$

The solution is given as:

$$x = \frac{2P}{b}\exp\left(-\frac{k_1 t}{2}\right)\frac{I_1(b)K_1(y) - I_1(y)K_1(b)}{I_1(b)K_0(y) + I_0(y)K_1(b)} \tag{57.11}$$

where $I_0(I_1)$ is a modified Bessel function of the first kind with the order of zero (one). $K_0(K_1)$ is a modified Bessel function of the second kind with the order of zero (one) [15]. $b(y)$ is the value at which to calculate the function. These two special engineering functions are built in Microsoft Excel with syntaxes of BESSELI(z, n) and BESSELK(z, n), where z is the variable and n is the order of the Bessel functions.

We can determine the rate constant k_1, b and P by fitting the simulated NO concentration x to experimental data i.e., minimising the standard deviation.

$$\sigma = \sum_{i=1}^{n}\left(x_{\mathrm{sim}i} - x_{\mathrm{exp}i}\right)^2 \tag{57.12}$$

where $x_{\mathrm{exp}} = x_{\mathrm{obs}} - x_{\mathrm{base}}$, since a base line correction is usually needed.

For a best fit, the initial parameters for k_1, b and P are needed. P is the initial ProliNO concentration. There are several ways to obtain the initial parameters k_1 and $b(k_2)$. Firstly, for an existing question, the published data can be used as the initial parameters. Secondly, we can estimate the initial parameters by assuming only NO release (k_1), at the beginning part of the curve, and only NO oxidation (k_2), at the later part of the curve. Finally, we can differentiate Eq. (57.11) to obtain the maximum point (t_{m}, x_{m}).

$$x_{\mathrm{m}} = \frac{2P}{b}\exp\left(-\frac{k_1 t_{\mathrm{m}}}{2}\right) \tag{57.13}$$

$$I_1(b)\left(K_1(y_{\mathrm{m}}) - K_0(y_{\mathrm{m}})\right) = K_1(b)\left(I_1(y_{\mathrm{m}}) + I_0(y_{\mathrm{m}})\right) \tag{57.14}$$

Substituting Eq. (57.13) into Eq. (57.14), we obtain Eq. (57.15)

$$I_1(b)\left(K_1\left(b^2 x_{\mathrm{m}}/2P\right) - K_0\left(b^2 x_{\mathrm{m}}/2P\right)\right) = K_1(b)\left(I_1\left(b^2 x_{\mathrm{m}}/2P\right) + I_0\left(b^2 x_{\mathrm{m}}/2P\right)\right) \tag{57.15}$$

From Eq. (57.15) we can calculate the initial value b, which is substituted into Eq. (57.13) to calculate the initial value k_1.

3 Results and Discussion

The autoxidation of NO was considered too slow to be biologically relevant because the reaction rate depends on the NO concentration squared and the physiologically low NO concentration [8]. The rate of NO autoxidation could be increased by a

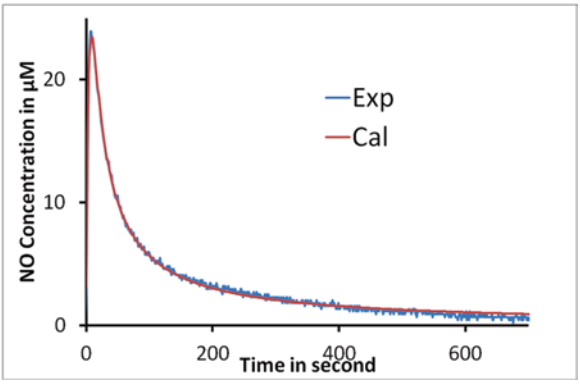

Fig. 57.1 The simulated and observed NO concentration profile after 40 μM proliNO adding to a 100 mM potassium phosphate buffer with pH 7.4 at 30 °C with oxygen concentration of 0.212 mM

higher reaction rate constant in membrane than in water or by higher local NO and O_2 concentrations in hydrophobic media.

Equation (57.11) can be used to simulate the NO concentration profile for a process combining NO generation and autoxidation. From a best fit of the simulated NO concentration profile to experimental data as shown in Fig. 57.1 [11] with Excel determined at oxygen concentration of 212 μM, the following values were obtained: $k_1 = 0.265\ s^{-1}$, $b = 0.857$, and $P = 31.345$ μM. From the obtained P value, we can calculate $v_{NO} = P/[\text{ProliNO}]_0 = 0.78$. From the value of b, we get $k_2 = 1.55 \times 10^3\ M^{-1}\ s^{-1}$, and $k_3 = 7.31 \times 10^6\ M^{-2}\ s^{-1}$.

Pogrebnaya was the first researcher to propose stoichiometrical NO autoxidation in aqueous solution and reported the kinetic reaction parameter $k_3 = 9 \times 10^6\ M^{-2}\ s^{-1}$ [12]. A wide range of kinetic reaction constants $k_3 = 6 \times 10^6\ M^{-2}\ s^{-1}$ [13], $k_3 = 8 \times 10^6\ M^{-2}\ s^{-1}$ [10], $k_3 = 9.2 \times 10^6\ M^{-2}\ s^{-1}$ [9], $k_3 = 12 \times 10^6\ M^{-2}\ s^{-1}$ [14], and $k_3 = 17 \times 10^6\ M^{-2}\ s^{-1}$ [8] were reported to be independent of pH [14]. Our NO autoxidation reaction rate constant is consistent with the reported values [8–14].

The rate of NO release from NO donors depends more upon experimental conditions such as pH. Therefore the characterization of NO release becomes more significant compared with the autoxidation which is independent of pH. DEANO releases NO at rate constants of $k_1 = 1.4 \times 10^{-3}\ s^{-1}$ [9] and $k_1 = 1.3 \times 10^{-3}\ s^{-1}$ [10], and were much slower than ProliNO with a rate constant of $k_1 = 0.265\ s^{-1}$. ProliNO release NO and NO autoxidation were studied [8, 11] but NO release rate constants were not reported.

In principle, 2 mol of NO can be released from 1 mol of the donor molecule. In reality, the stoichiometric coefficient is usually less than 2 and depends on the experimental conditions. v_{NO} of DEANO was determined to be 1.0 [9] but varies from 1.5 [10] to 2.0 [9]. Under anaerobic conditions, v_{NO} of ProliNO was measured to be 1.94 [11], which is very different from v_{NO} of 0.78 under aerobic conditions. From the analytical solution, it can be found that the NO concentration depends on v_{NO} in a complex way. Therefore v_{NO} can be determined from a fit of the simulated curve to the experimental data.

NO donors decompose at physiological pH and continuously release NO to allow studying of the effect of NO on biological systems even in long-term experiments. Therefore, the prediction of NO concentration evolution in such systems becomes very important. Schmidt et al. [9] developed a numerical model for NO decomposition and autoxidation in aqueous solution and also presented a graphical plot of NO concentration evolution, which was used to estimate the actual NO concentration. The analytical solution provides an improved way to forecast NO concentration produced from NO donors under aerobic conditions. Thus, it may contribute to elucidation of hypoxic vasodilation: the CBF increases to maintain the constant cerebral metabolic rate of O_2 when $[O_2]$ decreases [15].

4 Conclusions

We have derived an analytical solution for NO donor decomposition in aerobic solutions. This solution was used to characterize the decomposition rate constant of NO donors and the stoichiometric coefficient of NO release, and the reaction rate constant of NO autoxidation. NO donors can be used as continuous NO sources in biochemical systems and the analytical solution can be used to predict the actual NO concentration in such systems. In general, characterization of the kinetics of NO autoxidation enhances one's fundamental understanding of how nitric oxide can serve in many physiological processes. Furthermore, this mathematical model provides a simple way to study the variation of v_{NO}, clearly reported in the literature, with such parameters as $[O_2]$. This variation is important to characterize if one is to use NONOates to generate NO in biological system with different $[O_2]$.

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