Metabolic Engineering Communications 1 (2014) 12–18

Contents lists available at ScienceDirect

Metabolic Engineering Communications

journal homepage: www.elsevier.com/locate/mec

Model-driven identification of dosing regimens that maximize the antimicrobial activity of nitric oxide

Jonathan L. Robinson [a], Richard V. Miller [b], Mark P. Brynildsen [a,b,*]

[a] Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ, USA
[b] Department of Molecular Biology, Princeton University, Princeton, NJ, USA

ARTICLE INFO

Article history:
Received 28 June 2014
Received in revised form
21 August 2014
Accepted 22 August 2014
Available online 1 September 2014

Keywords:
Nitric oxide
Antimicrobial
Escherichia coli
Respiration
Nitric oxide dioxygenase
Hmp
Kinetic model

ABSTRACT

The antimicrobial properties of nitric oxide ($NO^{\bullet}$) have motivated the design of $NO^{\bullet}$-releasing materials for the treatment and prevention of infection. The biological activity of $NO^{\bullet}$ is dependent on its delivery rate, suggesting that variable antimicrobial effects can result from identical $NO^{\bullet}$ payloads dosed at different rates. Using a kinetic model of the *Escherichia coli* $NO^{\bullet}$ biochemical network, we investigated the relationship between $NO^{\bullet}$ delivery rate, payload, and cytotoxicity, as indicated by the duration of respiratory inhibition. At low $NO^{\bullet}$ payloads, the model predicted greater toxicity with rapid delivery, while slower delivery was more effective at higher payloads. These predictions were confirmed experimentally, and exhibited quantitative agreement with measured O_2 and $NO^{\bullet}$ concentrations, and durations of respiratory inhibition. These results provide important information on key design parameters in the formulation of $NO^{\bullet}$-based therapeutics, and highlight the utility of a model-based approach for the analysis of dosing regimens.

1. Introduction

$NO^{\bullet}$ is a highly reactive metabolite whose antimicrobial activity has been harnessed by the immune system to neutralize pathogens (Bogdan, 2001; Fang, 2004; Bowman et al., 2011; Robinson et al., 2014). In recent years, numerous materials that enable controlled release of $NO^{\bullet}$ to infection sites, such as nanoparticles (A. Friedman et al., 2011; A.J. Friedman et al., 2011), patches (Sulemankhil et al., 2012), dendrimers (Sun et al., 2012), and topical creams (Ormerod et al., 2011), have been synthesized, characterized, and shown to exhibit efficacy in treating antibiotic-resistant infections (Schairer et al., 2012; Jones et al., 2010). Two important design parameters of such materials include the rate at which $NO^{\bullet}$ is delivered, and the total payload that can be administered. We recently discovered that the utility of a major $NO^{\bullet}$ detoxification system in *Escherichia coli* (Hmp; $NO^{\bullet}$ dioxygenase) (Gardner et al., 1998; Stevanin et al., 2000), and thus the toxicity of $NO^{\bullet}$, depends highly on the $NO^{\bullet}$ delivery rate (Robinson and Brynildsen, 2013). This phenomenon suggests that $NO^{\bullet}$ payloads of the same total quantity but different delivery rates can vary remarkably in their cytotoxicity toward bacteria.

Here, we used quantitative kinetic modeling to explore the relationship between $NO^{\bullet}$ delivery rate, total payload, and antimicrobial activity as measured by the duration of respiratory inhibition by $NO^{\bullet}$. The model predicted that a faster release of $NO^{\bullet}$ is more effective at low payloads, whereas the opposite holds true at high payloads. These predictions were confirmed experimentally by measuring $NO^{\bullet}$ and O_2 consumption in batch cultures of *E. coli* following single or co-treatment with two $NO^{\bullet}$ donors with differing release kinetics. This work provides fundamental knowledge on the dependence of $NO^{\bullet}$ toxicity on delivery rate and total dose, which is a relationship that could prove useful for selection of $NO^{\bullet}$ dosing regimens for antimicrobial therapy.

2. Materials and methods

2.1. Kinetic modeling

Model simulations and analyses were performed in MATLAB (The MathWorks, Inc.). To simulate [$NO^{\bullet}$] and [O_2] dynamics, we employed a model of $NO^{\bullet}$ stress that we previously developed (Robinson and Brynildsen, 2013), and added a respiratory module that allowed simulation of O_2 consumption from the batch culture system.

* Correspondence to: 205 Hoyt Laboratory, 25 William Street, Princeton, NJ 08544, USA. Tel.: +1 609 258 1995; fax: +1 609 258 0211.
E-mail address: mbrynild@princeton.edu (M.P. Brynildsen).

http://dx.doi.org/10.1016/j.meteno.2014.08.001

2.1.1. Incorporation of a respiratory module

Aerobic respiration was incorporated through the addition of 4 reactions (NADH dehydrogenases NDH-1 and NDH-2, and O_2 consumption by cytochromes *bo* and *bd*-I), 14 kinetic parameters, and 4 species (Table S1). The third *E. coli* cytochrome oxidase, *bd*-II, was not included, as its activation is generally limited to conditions of phosphate starvation, entry into stationary phase, or anaerobiosis (Atlung and Brondsted, 1994; Dassa et al., 1991). NADH dehydrogenase I (NDH-I) contains 9 iron-sulfur ([Fe–S]) clusters (two [2Fe–2S] and seven [4Fe–4S]) (Euro et al., 2008), and therefore could potentially be inhibited by $NO^{\bullet}$, which is known to react rapidly with [Fe–S] clusters (Gardner et al., 1997; Foster and Cowan, 1999; Crack et al., 2011). This effect was addressed by incorporating a term into the NDH-1 rate equation which scales the reaction rate by the ratio of undamaged to total [Fe–S] clusters. Of the respiratory kinetic parameters, 4 were found to be variable within the literature, and only approximate concentrations of NADH dehydrogenases I and II, ubiquinone-8, ubiquinol-8 and cytochromes *bo* and *bd* were available. We therefore optimized this set of 10 uncertain parameters with experimentally-measured $[O_2]$ data from aerobic, exponential-phase *E. coli* cultivated in our system in the absence of nitrosative stress (Table S2 and Fig. S1).

2.1.2. Simulation of NONOate co-administration

To simulate the simultaneous delivery of two NONOates with different $NO^{\bullet}$-release kinetics, an additional NONOate species and dissociation reaction were incorporated into the model.

2.2. Experimental measurements

2.2.1. Chemicals

The growth media used in all experiments was MOPS minimal media (Teknova) with 10 mM glucose. DPTA NONOate, (Z)-1-[*N*-(3-aminopropyl)-*N*-(3-ammoniopropyl)amino]diazen-1-ium-1,2-diolate, and PAPA NONOate, (Z)-1-[*N*-(3-aminopropyl)-*N*-(n-propyl)amino]diazen-1-ium-1,2-diolate (Cayman Chemical), were dissolved in ice-cold 10 mM NaOH prior to use. Potassium cyanide (KCN; Sigma Life Science) was dissolved in sterile, deionized H_2O at a concentration of 1 M.

2.2.2. Bacterial strains

All experiments in this study were performed with *E. coli* K-12 MG1655.

2.2.3. Bioreactor

A disposable batch bioreactor, identical to that described previously (Robinson and Brynildsen, 2013), was used to facilitate measurements with the $NO^{\bullet}$ and O_2 sensors. Briefly, the bioreactor consisted of a sterile, 50 mL polypropylene conical tube (Falcon®) suspended in a magnetically-stirred water bath maintained at 37 °C. The 50 mL conical tube was open to the air, contained 10 mL of culture, and was stirred with a 0.5 in. magnetic stir bar.

2.2.4. Detection of $NO^{\bullet}$ and O_2

The concentrations of $NO^{\bullet}$ and O_2 were measured continuously ($\geq$1 sample per second) in the bioreactor culture using a $NO^{\bullet}$ sensor (2 mm ISO-NOP, World Precision Instruments, Inc.) and an O_2 sensor (3 mm OXROB10 Oxygen Probe with FireStingO2 meter, PyroScience GmbH), respectively. The sensors were suspended approximately 3 mm below the culture liquid surface, and clamped in place throughout the duration of the assay.

2.2.5. $NO^{\bullet}$ stress assay

Bacterial growth and $NO^{\bullet}$ treatment methods and conditions were identical to those reported previously (Robinson and Brynildsen, 2013), with two modifications to facilitate O_2 measurements: (1) cells were treated with $NO^{\bullet}$ immediately after resuspension in the bioreactor rather than allowing a ~45 min adjustment period before treatment, to ensure greater consistency among initial $[O_2]$; (2) the cells were delivered to a final OD_{600} of 0.1 rather than 0.05, to amplify $[O_2]$ changes caused by cellular processes, and thus improve their detection. For the KCN-treated O_2 consumption assay (Fig. S2), 1 mM KCN was delivered to the cells during resuspension, and to the 10 mL of fresh media in the bioreactor prior to inoculation.

2.2.6. Measurement of extracellular parameters

Kinetic parameters associated with extracellular processes specific to the experimental system were determined from $[O_2]$ and $[NO^{\bullet}]$ measurements in cell-free growth media (Table S3), as described previously (Robinson and Brynildsen, 2013). Briefly, the volumetric mass transfer coefficient of O_2 (k_La_{O2}) was calculated from the $[O_2]$ curve following media degassing with N_2. The $NO^{\bullet}$ volumetric mass transfer coefficient ($k_La_{NO\cdot}$), autoxidation rate constant ($k_{NO\cdot-O_2}$), and dissociation rate of DPTA ($k_{NONOate,DPTA}$), were optimized to fit the $[NO^{\bullet}]$ curve and final (12 h) NO_2^- concentration resulting from the addition of 500 μM DPTA to cell-free media (Fig. S3). The dissociation rate of PAPA ($k_{NONOate,PAPA}$) was also determined, using an $[NO^{\bullet}]$ curve measured following the treatment of cell-free media with 500 μM PAPA (Fig. S3).

2.3. Parametric analysis and optimization

2.3.1. Optimization of uncertain cellular parameters

Uncertain parameters were defined as those whose value varied widely in the literature, or were unavailable. Given the difference in $NO^{\bullet}$ treatment protocol from our previous study (Robinson and Brynildsen, 2013) (dosing immediately after resuspension into the bioreactor, rather than allowing a pre-growth period), as well as the inclusion of the new respiratory module and an additional dataset ($[O_2]$ curve), 37 uncertain cellular parameters were optimized to simultaneously capture the $[NO^{\bullet}]$ and $[O_2]$ dynamics of an aerobic, exponential-phase *E. coli* culture treated with 500 μM DPTA (Table S4). The optimization procedure was identical to the previous approach except that $[O_2]$ data was included and optimized simultaneously with the $[NO^{\bullet}]$ curve. The set of parameter values yielding the minimum sum of the squared residuals (SSR) between experimental and simulated $[NO^{\bullet}]$ and $[O_2]$ curves were selected as the optimal set.

2.3.2. Parametric analysis

Parametric analyses were conducted to determine which parameters were informed from the optimization of the respiratory module with untreated O_2 consumption data (Table S2), and optimization of the cellular parameters with the 500 μM DPTA $[NO^{\bullet}]$ and $[O_2]$ curves (Table S4). Parameters were individually varied within their literature or physiological ranges, and the resulting effect on SSR between simulated and measured concentrations was calculated. Those exhibiting at least a 5% change in SSR were considered to be informed as a result of the optimization.

3. Results

Given the importance of $NO^{\bullet}$ delivery kinetics on the distribution of $NO^{\bullet}$ in *E. coli* (Robinson and Brynildsen, 2013), we used a quantitative kinetic model to explore the relationship between $NO^{\bullet}$ delivery rate and antimicrobial efficacy, and examined how this relationship is affected by total $NO^{\bullet}$ payload. One of the key