Nitric Oxide 33 (2013) 74–80

Contents lists available at SciVerse ScienceDirect

Nitric Oxide

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

A model for the nitric oxide producing nitrite reductase activity of hemoglobin as a function of oxygen saturation

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ARTICLE INFO

Article history:
Received 20 April 2013
Revised 18 June 2013
Available online 2 July 2013

Keywords:
Hemoglobin
Nitrite
Nitric oxide
Reductase
Kinetics

ABSTRACT

The production of nitric oxide by the nitrite reductase activity of hemoglobin has been proposed to play a major role in hypoxic vasodilation. The bimolecular reaction rate constant for nitric oxide formation is a complex function of hemoglobin oxygenation stemming from the intrinsic allosteric character of hemoglobin, resulting in an unsymmetrical inverted U shape profile of activity versus oxygen saturation. We present an analysis of the hemoglobin nitrite reductase activity based on the Monod Wyman Changeux (MWC) allosteric model and derive a set of equations that enabled us to express the rate constant of bimolecular reaction of nitrite with hemoglobin as a function of hemoglobin saturation and use this expression to explore the factors controlling the shape of the nitrite reductase activity versus hemoglobin saturation curve. From the value of the maximum reductase activity, we derive equations to calculate microscopic nitrite reductase reaction rate constants for the *R* and *T* quaternary states. We have also developed two methods to parameterize the MWC model based on the Hill equation, with its parameters, h and P_{50}, and the knowledge that these two descriptions of the binding curve coincide in the region of the curve where h is defined. This has allowed the calculation of the hemoglobin nitrite reductase activity rate profiles for the human hemoglobin and for bovine hemoglobin. The properties of these rate profiles are discussed.

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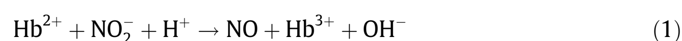
Introduction

Mammals generally respond to hypoxia, an insufficient oxygen supply to the tissues, by dilation of the blood vessels thereby increasing blood flow through the tissues. Such hypoxic vasodilation has been studied for over a century but the mechanism through which lowered oxygen tension leads to vasodilation remains unclear [1]. Many hypotheses have been advanced but none have gained universal acceptance. One mechanism proposed is that nitric oxide (NO), a powerful vasodilator that relaxes vascular endothelia and thus increases blood flow, is produced at an enhanced rate under hypoxia [2]. Production of NO by the nitric oxide synthase (NOS) family of enzymes including endothelial, neural and inducible NOS isozymes, requires oxygen and can become limited by oxygen at low P_{O2} [3]. Increase in NOS activity is thus unable to account for enhanced production of NO under lowered oxygen tension [4], unless there is an alternative mechanism to overcome this.

Hypoxic vasodilation appears to be a function of hemoglobin (Hb) oxygen saturation, rather than a direct response to sensing tissue P_{O2} [5]. Therefore proposals have been advanced that directly involve the hemoglobin molecule in release of NO as oxygen saturation drops. One, by Ellsworth and co workers [6], is that a conformational change in hemoglobin releases ATP, which then indirectly activates eNOS activity. Another, proposed by Stamler and co-workers [7], involves the release of NO from a nitrosothiol group (–SNO) on the βCys-93 residue following the allosteric conversion of hemoglobin as blood P_{O2} falls. An alternative hypothesis suggested by Gladwin and co-workers [8,9], proposes that deoxyhemoglobin can act as a nitrite reductase to directly form NO from nitrite within the erythrocyte.



$$Hb^{2+} + NO_2^- + H^+ \rightarrow NO + Hb^{3+} + OH^- \quad (1)$$

The profile for nitrite reductase activity has been shown to exhibit a maximum at an oxygen tension at which Hb is partially liganded, often depicted as an inverted U shape curve peaking at the vicinity of the P_{50} for Hb [2,5,10–15]. This behavior is unexpected as Eq. (1) would suggest that maximum activity should coincide with maximum deoxyhemoglobin concentration. However, it can be explained by reference to the allosteric properties of Hb that are based on an equilibrium between conformation states, *T* state and *R* state, the latter being stabilized and therefore favored on oxygenation [2,5,10–15]. By proposing that deoxy Hb in the *R* state has greater nitrite reductase activity than Hb inthe *T* state the profile can be rationalized [16]. At 0% saturation, only *T* state Hb which

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http://dx.doi.org/10.1016/j.niox.2013.06.008

Glossary

Hill model

Y the oxygen fractional saturation of hemoglobin

H the Hill coefficient

P_{50} the oxygen partial pressure at which the hemoglobin is 50% oxygen saturated

MWC model

R_i and T_i the molecular number of relaxed (R) and tense (T) state hemoglobin. R and T represent hemoglobin quaternary states. The subscript i represents the number of hemes bound to oxygen

$L = T_0/R_0$ the conformation equilibrium constant for $R_0 \Longleftrightarrow T_0$

K_R and K_T the microscopic (each heme) dissociation equilibrium constants of R and T hemoglobin from oxygen, respectively

$c = K_R/K_T$ the oxygen dissociation constant ratio

$\alpha = P_{O2}/K_R$ the dimensionless oxygen partial pressure, given by the oxygen partial pressure divided by the R state dissociation constant for oxygen

Nitrite reductase activity

D the fraction of hemoglobin in deoxy states

k_N the macroscopic bimolecular hemoglobin nitrite reductase reaction rate coefficient

k_R and k_T the microscopic bimolecular (per heme) nitrite reductase reaction rate constants for R and T quaternary state, respectively

$N = k_R/k_T$ the microscopic bimolecular (per heme) nitrite reductase rate constant ratio

Derived parameters

Y_h the hemoglobin oxygen saturation at which the Hill coefficient is defined

P_{O2h} the oxygen partial pressure at which the Hill coefficient is defined

α_h the dimensionless oxygen partial pressure at which the Hill coefficient is defined

$\alpha_{50} = P_{50}/K_R$ the dimensionless oxygen partial pressure at which the hemoglobin is 50% oxygen saturated

$k_{N\max}$ the maximum macroscopic bimolecular hemoglobin nitrite reductase reaction rate constant

$P_{O2\max}$ the oxygen partial pressure at which the nitrite reductase activity of hemoglobin is maximal

$Y_{\max}$ the hemoglobin oxygen saturation at which the nitrite reductase activity of hemoglobin is maximal

$\alpha_{\max}$ the dimensionless oxygen partial pressure at which the nitrite reductase activity of hemoglobin is maximal

$\alpha_{\max 0}$ the maximum $\alpha_{\max}$ value for hemoglobin

$\alpha_{\max R}$ the dimensionless oxygen partial pressure at which the nitrite reductase activity of R state hemoglobin is maximal

has the lower nitrite reductivity, is present and as saturation increases two opposing effects become evident, (1) the activity is lowered because the total concentration of deoxy Hb is lowered and (2) the activity increases as deoxy R state becomes populated. It is the changing balance between these effects that give the asymmetrical bell shaped curve that rises to a maximum at oxygen saturation above 0% and thereafter falls to zero at 100% saturation at which no deoxy Hb is present.

Although it is sometimes stated, and even graphed [5], that nitrite reductase activity peaks at the hemoglobin P_{50}, and declines symmetrically from this peak, this is a pedagogical cartoon and by no means a requirement of such a model [10]. Indeed the relationship cannot be symmetrical, given the requirements for zero activity at 100% oxygen saturation (no deoxyhemoglobin species in either the R state or T state tetramers) and a non zero activity at 0% saturation (four deoxyhemoglobin species per T state tetramer).

To explore this phenomenon quantitatively models based on the Monod Wyman Changeux (MWC) model of allostery have been advanced that are able to simulate the profile and require as an input the values of the parameters of the MWC allosteric model, namely L, c and K_R, where R_i and T_i: the molecular number of relaxed (R) and tense (T) state hemoglobin. R and T represent hemoglobin quaternary states; the subscript i represents the number of hemes bound to oxygen; $L = T_0/R_0$: the conformation equilibrium constant for $R_0 \Longleftrightarrow T_0$; K_R and K_T: the microscopic (each heme) dissociation constants of R and T hemoglobin from oxygen, respectively; $c = K_R/K_T$: the oxygen dissociation constant ratio. The values for these quantities have in general not been determined on the same Hb (or blood) sample that was used to determine the nitrite reductase activity and have thus been chosen, somewhat arbitrarily, to fall within the range reported for HbA so that they generate profiles that mimic those found experimentally. In this paper we extend these existing models with the objective of providing a set of equations and procedures that has predictive power and which rests upon obtained consistent estimates of L, c and K_R from the easily obtained mid-section of oxygen binding curves. To determine these parameters one usually requires very carefully and precisely determined oxygen saturation (Y) curves from $Y = 0.01–0.99$ and as such are not always available. Therefore, we have developed methods that can provide good, self consistent, estimates of the allosteric parameters L, c and K_R from the relatively easily acquired knowledge solely of P_{50}, the oxygen tension (conventionally given in mmHg) that half saturates the hemoglobin and h (the Hill coefficient) a measure of cooperativity determined from the maximum slope of the Hill plot ($\log(Y/D)$ versus $\log(P_{O2})$), where D is the fraction of hemoglobin in deoxy states. These quantities are more readily obtained from the central part of the oxygen binding curve and are experimentally assessable to most laboratories working with Hb or whole blood. In order to simulate the nitrite reductase activity as a function of hemoglobin saturation, we also need reliable measurements of k_R and k_T, the micro rate constant for nitrite reduction by a deoxy heme species when the hemoglobin tetramer is in either the R state or T state respectively. We use the terms k_R and k_T to maintain consistency with the literature, but it is important to note that these are distinct from K_R and K_T, the intrinsic dissociation constants of oxygen from the R and T states of hemoglobin (see later).

The literature contains a range of values for k_R and k_T (see Table 1) [3,10–15] determined using different experimental protocols [17–19]. The differences in these reported values may be due to differences in the experimental conditions and/or the models used to analyze the data. We therefore also sought an improved method to calculate the k_R and k_T values.

Given these parameters for a given Hb or whole blood sample we report a set of procedures and analytical equations derived from the model that may be used to determine allosteric constants and, with the nitrite reductase rate constants of the R (k_R) and T (k_T)

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