Blood Cells, Molecules, and Diseases 50 (2013) 8–19

Contents lists available at SciVerse ScienceDirect

Blood Cells, Molecules, and Diseases

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

The oxidative denitrosylation mechanism and nitric oxide release from human fetal and adult hemoglobin, an experimentally based model simulation study

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ARTICLE INFO

Article history:
Submitted 2 August 2012
Revised 12 August 2012
Available online 13 September 2012

(Communicated by J.F. Hoffman, Ph.D., 14 August 2012)

Keywords:
Nitrite
Fetal hemoglobin
Sickle cell disease
Nitric oxide
Vasodilation

ABSTRACT

Generation of unbound nitric oxide (NO) via the oxidative denitrosylation (ODN) mechanism is proposed to involve the simultaneous reaction of nitrite with oxy and deoxy hemoglobin ($Hb(O_2)$) (k_1) and Hb (k_{13})) to yield respectively, $\bullet NO_2$ and $Hb^{+2}(NO)$. These two reaction pathways are coupled when $\bullet NO_2$ reacts with $Hb^{+2}(NO)$ to yield $Hb^{+3}(NO)$ (k_{22}), a species that releases NO rapidly. Here, I have constructed an experimentally based molecular model of the ODN mechanism (k_1–k_{31}), focusing on the high nitrite reductase activity of R-state hemoglobin. This model was used to test the hypothesis that human fetal hemoglobin (HbF) can generate unbound NO faster and to a greater extent than HbA, consequent to a 25-fold larger value of k_1, which was determined in an earlier study. The results show that despite the use of identical values for k_{22}, there was a 44-fold larger apparent rate of reaction of $\bullet NO_2$ with HbF(NO) compared to HbA(NO), for reactions simulated at 410 μM nitrite and 100 μM hemoglobin (heme basis), 50% oxygen saturation at pH 7.4 and 37 °C. This faster reaction was associated with the generation of about 11 μM peak unbound NO. In contrast, HbA failed to generate unbound NO rapidly under the same conditions. However, raising the concentration of nitrite into the millimolar range did generate unbound NO in the HbA simulation, in agreement with the experimental literature, and that result was associated with acceleration in the rate of reaction of $\bullet NO_2$ with HbA(NO). Unbound NO could be generated at 410 μM nitrite in the HbA simulation by lowering the pH. This too was associated with an acceleration in the rate of reaction of $\bullet NO_2$ with HbA(NO). Furthermore, generation of unbound NO could be assigned to the pH-dependent increase in k_1, independent of the associated increase in k_{13}. Finally, selective exchange of the HbA value of k_1 for the HbF value, keeping all other constants and conditions unchanged, generated kinetic patterns for the various species of the "k_1-modified" HbA simulation, which were virtually indistinguishable from those seen in the HbF simulation. Taken together, these findings show that rapid and extensive generation of unbound NO within the ODN mechanism is controlled by the value of k_1. The faster and more extensive generation of unbound NO by HbF at micromolar nitrite concentration suggests a possible second function for HbF in sickle cell disease, namely enhanced vasodilation. The failure of 410 μM nitrite to generate unbound NO in the HbA simulation at pH 7.4, contrasts with evidence in the literature showing that exposure of intact red cells to 100 to 200 μM nitrite in PBS, promoted NO release into the gas phase. I point out that this difference in outcome may be due to the higher activity of HbA when bound to the cytoplasmic domain of the red cell membrane anion transport protein SLC4A1 (band 3) and to the demonstrated capacity of band 3 to transport nitrite.

Introduction

Hypoxia-induced vasodilation is a physiological response that allows oxygen supply to meet tissue oxygen demand. Nitric oxide (NO) is proposed to function as one of the signaling molecules involved in that process. NO is generated from L-arginine in an oxygen-dependent fashion by endothelial NO synthase [1]. However, NO synthase activity is reduced when the supply of oxygen is depleted to levels present under conditions of chronic hypoxia [2], suggesting the need for additional oxygen-sensitive mechanisms for producing NO in order to maintain vascular tone, and to regulate blood flow in a high oxygen demand environment.

In the last several years, attention has focused on the hypothesis that the allosteric transition consequent to hemoglobin deoxygenation may play a significant role in linking oxygen demand to the transport, or generation of NO by hemoglobin (see [3] for a review). The specific allosteric model is that proposed by Monod, Wyman and Changeux [4], where two alternate conformational states of the hemoglobin tetramer (the R-state and the T-state) have different ligand affinities and reactivities (R-state: high affinity, fast "on", slow "off" kinetics, and T-state: low affinity, slow "on", fast "off" kinetics for ligands whose binding to hemoglobin is not limited by diffusion).

Abbreviations: NO, nitric oxide; $\cdot NO_2$, nitrogen dioxide radical; N_2O_3, nitrous anhydride (dinitrogen trioxide); $ONOO^-$, peroxynitrite; NO_3^-, nitrate; ONOOH, peroxynitrous acid; N_2O, nitrous oxide; H_2O_2, hydrogen peroxide; R-SH, organic thiol group; R-SNO, S-nitrosyl derivative; IHP, inositol hexaphosphate; CDB3, cytoplasmic domain of band 3; PMB, p-hydroxymercuribenzoate; ODN, oxidative denitrosylation.

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http://dx.doi.org/10.1016/j.bcmd.2012.08.006

Two hypotheses have been advanced to explain how hemoglobin allosteric effects may account for NO-mediated hypoxia-induced vasodilation. The first hypothesis [5] suggested that hemoglobin may function as an allosterically linked NO carrier, using the differential reactivity at β93 –SH [6,7] to promote the formation of SNO-Hb in the R-state, and its release upon deoxygenation and conversion to the T-state. Although this mechanism is attractive in many respects, recent evidence using a mouse model has indicated that SNO-hemoglobin formation at β93 –SH is not essential for red blood cell-dependent hypoxic vasodilation [8]. This conclusion was arrived at on the basis of studies where human HbA was expressed in the mouse either as the wild-type protein with cysteine at β-93, or with alanine at the β-93 position [8].

An alternate hypothesis [9,10] for NO generation and release by hemoglobin suggested that an allosterically regulated hemoglobin-based nitrite reductase activity was involved, wherein deoxy hemoglobin reduces nitrite to NO and in turn is converted to ferric (met) hemoglobin. Vasodilation of rat aortic rings, formation of NO gas, and formation of Hb(NO) all result from the nitrite reductase activity of deoxy Hb [9]. Nitrite and red cell dependent vasodilation was shown to have maximal activity around the hemoglobin P_{50}, which is about 30 mm Hg for humans, with the deoxy R-state having a significantly greater reductase activity than the deoxy T-state [3].

One major obstacle in theories linking hemoglobin deoxygenation to NO release from the red cell is that NO binds very rapidly to deoxy hemoglobin [6], and is released slowly [11,12]. High affinity binding of NO to deoxy hemoglobin is inconsistent with release of NO from deoxygenating red cells, especially within the short transit times needed to cross the capillary bed where vasodilation is critical.

A recent study has provided a new insight into the paradox of NO entrapment by deoxy hemoglobin. Grubina and co-workers [13] studied the reaction of hemoglobin over a range of nitrite concentrations between 1 mM and 10 mM depending on the experiment, using hemoglobin that was partially saturated with oxygen. Under these conditions nitrite could react with both oxy and deoxy hemoglobin simultaneously. They suggested that products of the reaction of nitrite with $Hb(O_2)$ (in particular the nitrogen dioxide radical $\bullet NO_2$) could oxidize the heme iron of ferrous Hb(NO) to yield ferric Hb(NO), which releases NO rapidly [13]. The overall process is known as the oxidative denitrosylation (ODN) mechanism.

I proposed that the ODN mechanism may play an important role in the clinically beneficial effect of high fetal hemoglobin levels in sickle cell disease [14]. A critical feature of that proposal centered on the established difference between the reaction of nitrite with $HbF(O_2)$ compared $HbA(O_2)$. Several studies have demonstrated that the kinetics of nitrite [15–17] and other ligand reactions [18–22] are faster for human HbF when compared to human HbA. The time course for the reaction $HbF(O_2)$ with nitrite has been shown to be similar to the reaction of $HbA(O_2)$, with the exception that the apparent half time for $HbF(O_2)$ was about 2.5-fold shorter [15–17]. In one study, the bimolecular kinetics for the reaction of nitrite with ovine deoxy HbF were measured and it was found that nitrite reacted about 2.4-fold faster compared to ovine deoxy HbA [23].

Although all of the early studies on the reaction of nitrite with $Hb(O_2)$ have compared half times to define differences between $HbF(O_2)$ and $HbA(O_2)$, I have pointed out that such comparisons are qualitative at best due to the complexity of the reaction (i.e. lag periods) [14]. To establish this point quantitatively, I simulated the time course for the reaction using the experimentally based model of Keszler and co-workers [24] for the reaction of nitrite with human $HbA(O_2)$. I varied the value for the rate constant of the initial, rate-limiting step (k_1) until I could reproduce the 2.5-fold difference in half times between $HbF(O_2)$ and $HbA(O_2)$ reported in the literature. This was possible, but only after raising the value of k_1 for $HbF(O_2)$ by 25-fold [14]. A similar analysis of nitrite with oxy Hb Bart's (a γ-chain tetramer) required that k_1 be increased by 63-fold in order to account for a 4.4-fold shorter half-time compared to $HbA(O_2)$ [14].

On the basis of the initial study [14], I suggested that unbound NO should be generated faster and to a greater extent by HbF when compared to HbA, during the simultaneous reactions of nitrite with $Hb(O_2)$ and deoxy Hb, within context of the ODN mechanism [13], and that this may contribute to improved vascular tone in individuals homozygous for sickle hemoglobin. Here, I have simulated that scenario by constructing an extended version of the ODN mechanism using experimental evidence from an extensive survey of the literature, populated it with rate constants also generated from the literature, and then tested the effect of varying the rate constants on the time courses of selected species of the model.

Materials and methods

Model simulation studies were performed using "Chemical Kinetics, Version 1.01", provided on-line by the IBM Almaden Research Center in California: http://www.almaden.ibm.com/st/computational_science/ck.

The general settings were as follows: number of particles was set at 10^6, state of simulation was recorded at intervals of 100 events, and the random number speed was set at 12,947. The limit for stopping the calculation was 4×10^9 events, and the stop time varied. "Equilibrium detect" was enabled for a test cycle length of 100 events and a selection frequency of 90%. Plots of "species concentration" versus time were created, and the raw data imported in tabular form into the Sigma Plot program (SPSS Science, Chicago, IL) for analysis and presentation. Simulations were repeated at least twice to assure reproducibility.

Theoretical considerations and calculations

Theoretical considerations and calculations used to establish the experimental basis for the oxidative denitrosylation model used in this study are given in the Supplementary Data file associated with this paper.

Results

The simulation reactions of $HbA(O_2)$ and $HbF(O_2)$ with nitrite (Table 1)

$HbA(O_2)$

The panels on the left hand side of Fig. 1 show the concentration versus time behavior of selected species during the reaction of $HbA(O_2)$ with nitrite. These results represent only one reaction pathway of the ODN mechanism (i.e. the mechanisms and constants given in Table 1). While virtually all experimental work in the literature had focused on the presence of lag periods in the reaction of nitrite with $Hb(O_2)$ [15–17,24], it became apparent from this simulation that there were also significant lag periods in the appearance of met Hb and nitrate. The measured lag times for $HbA(O_2)$, met HbA and nitrate were virtually identical (average ~57 s) (see the legend to Fig. 1). The same was true of the half time values (average ≈ 72 s). The rate of the reaction at around the halfway point in the reaction (defined as the absolute value of the slope around the halfway point in the overall reaction) showed identical velocities for $HbA(O_2)$ and met HbA (average ≈ 1.64 μM s^{-1}). However, the rate for nitrate formation (~2.47 μM s^{-1}) was about 1.5-fold faster (see the legend to Fig. 1). There was no significant production of unbound NO during the reaction of fully saturated $HbA(O_2)$ with nitrite, despite the presence of a step for production of unbound NO in Table 1 (step 3). Apparently, NO is consumed sufficiently rapidly in step 4 of Table 1 to prevent accumulation.

$HbF(O_2)$

The panels on the right hand side of Fig. 1 show the concentration versus time behavior of various selected species during the reaction of $HbF(O_2)$ with nitrite (using the mechanisms and constants given in Table 1). The distinctive feature of these time courses was the presence of an ~3-fold shorter lag time for $HbF(O_2)$, met HbF and nitrate (average ≈ 18 s) compared to the same species of HbA (average ≈ 57 s).