Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance

Enzymes of the L-Arginine to Nitric Oxide Pathway[1,2]

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ABSTRACT L-Arginine is the biological precursor of nitric oxide (NO), which serves as an important signal and effector molecule in animals. This review summarizes some structure-function aspects of the mammalian nitric oxide synthases, which are enzymes that catalyze the oxidation of L-arginine to NO and L-citrulline. These include aspects related to: *1*) the chemical transformations of L-arginine during enzyme catalysis, *2*) binding of L-arginine or its structural analogs to the nitric oxide synthases, and *3*) how L-arginine levels may affect product formation by the nitric oxide synthases and how this can be modulated by structural analogs of L-arginine. J. Nutr. 134: 2748S–2751S, 2004.

KEY WORDS: • *L-arginine* • *nitric oxide* • *flavoprotein* • *reactive oxygen species* • *hemeprotein*

The widespread interest in nitric oxide (NO) biology can be traced back to nutrition and toxicology research conducted in the early 1980s that established that mammals are indeed capable of NO biosynthesis (1,2). L-Arginine (Arg)[4] was subsequently found to be the precursor of NO and related N-oxides (nitrite, nitrate) that are produced by mammals (3–5). This represents a new metabolic pathway for Arg and is of current interest because of the tremendous biological and medical importance of NO.

Some general properties of the NO synthases

A family of enzymes called the NO synthases (NOSs, EC 1.14.13.39) catalyze the oxidation of Arg to NO and L-citrulline, with NADPH and O_2 serving as cosubstrates (6). The NOSs first hydroxylate a terminal guanidino nitrogen of Arg to generate *N*-hydroxy-L-arginine (NOHA) as an enzyme-bound intermediate. NOHA is then oxidized further by the enzyme to generate NO plus L-citrulline (**Fig. 1**). Three related NOSs are expressed in mammals, along with several splice variants (7). NOS-like enzymes are also coded for in the genomes of most life forms, including bacteria (8,9). The mammalian NOSs have a similar structure and composition. They are all homodimeric, heme-containing flavoproteins. The NOS flavins transfer NADPH-derived electrons to the heme (**Fig. 2**). This enables the heme to bind and activate dioxygen in both steps of NO synthesis. 6-(*R*)-Tetrahydrobiopterin (H4B) is also a required cofactor, and it is tightly bound in NOS next to the heme (10,11). H4B has structural and redox roles in the NOS (11,12). The K_s values for Arg and NOHA binding to NOSs range from 1 to 20 μmol/L (6). The electron transfer reactions of NOS are regulated by a calcium-binding protein (calmodulin), which can bind to some NOSs in a reversible manner (6). This enables cells to couple their NO synthesis to changes in intracellular calcium ion concentration. Calmodulin binds tightly to other NOSs at all cellular calcium ion concentrations; thus, their NO synthesis is regulated primarily through control of gene expression (13).

Arg binding site in NOS

Crystal structures of NOS heme domains that contain bound Arg or NOHA show in detail how either of these substrates bind in the enzyme active site (14–16,10). There is a funnel-shaped entrance to the enzyme active site that allows substrate and dioxygen access to the heme. In general, NOS binds Arg or NOHA in a similar extended conformation, with the guanidino (or hydroxyguanidino) moiety located above the heme iron and the amino acid portion held away from the heme (**Fig. 3**). The location of the guanidino group above the heme iron is consistent with the heme iron being the site where dioxygen binds and becomes activated in NOS, such that the resulting heme iron-oxy species can react directly with the guanidino moiety. Arg and NOHA are held in place by hydrogen bonds that form between conserved residues of NOS and both the amino acid and guanidino ends of the substrates. Several of the binding residues are located in a substrate-binding helix of NOS (16). The substrate hydrogen-bonding network extends into the H4B binding site, consistent with H4B and Arg binding to NOS in a cooperative manner (12) and with the finding that point mutations in the H4B binding site of NOS often lower the binding affinity toward Arg (17). The α amino groups of Arg and NOHA also

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[4] Abbreviations used: Arg, L-arginine; H4B, 6-(*R*)-tetrahydrobiopterin; NO, nitric oxide; NOHA, *N*-hydroxy-L-arginine; NOS, nitric oxide synthase (EC 1.14.13.39).

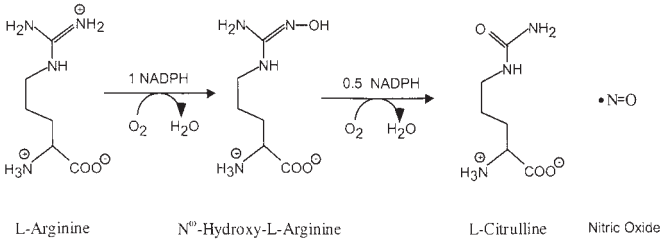

FIGURE 1 The two reactions of NO synthesis as catalyzed by NOS. The NADPH and oxygen requirements of each reaction are shown.

hydrogen bond with a heme propionate group, but the functional significance of this interaction is unclear. Point mutagenesis studies demonstrate the relative importance of some of the NOS substrate binding residues for Arg binding (18–20). In particular, there is a conserved glutamic acid whose side-chain carboxylate forms hydrogen bonds with two of the guanidino nitrogens of Arg or NOHA. Removing this interaction by point mutagenesis completely disables Arg binding to NOS. Mutational substitution at other residues involved in Arg or NOHA binding typically has a less drastic effect.

Effects of Arg binding on NO synthase

Arg binding causes a number of observable changes in NOSs. It stabilizes the NOS ferric heme iron in a high-spin electronic state, which is typically manifested by the shifting of the heme Soret absorbance band to a lower wavelength (21). Arg binding increases NOS affinity for H4B, consistent with their binding in a cooperative manner (12). Arg binding increases the reduction potential of the NOS heme iron (22). This is important for some NOSs because it makes their heme reduction by the flavins thermodynamically favorable (**Fig. 4**). Arg binding also stabilizes the dimeric structure of some NOSs (23,24), and stabilizes heme-NO complexes that form during NOS catalysis (25,26).

Substrate analogs of Arg

Various Arg derivatives can serve as substrates for NOS, including non–amino acid analogs (**Fig. 5**) (27,28). In general, NOSs display a lower binding affinity toward these analogs, show isoenzyme selectivity, and have slower V_{max} values regarding NO synthesis. The *N*-hydroxyguanidine analogs typically bind more tightly than the guanidine analogs and support greater NO synthesis. However, NO synthesis from the guanidine analogs is likely compromised due to dissociation of the *N*-hydroxyguanidine intermediate from NOS during catalysis. These data indicate that NOS interactions with the

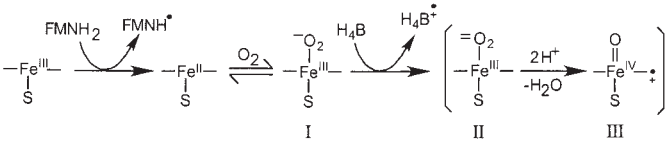

FIGURE 2 Mechanism of oxygen activation by the NOS heme. The ferric heme first receives an electron from the FMN hydroquinone (FMNH2) that is located in the NOS flavoprotein domain. This enables dioxygen to bind, forming the ferric-superoxy species (*I*). This species then receives an electron from tetrahydrobiopterin (H4B) to generate heme peroxo (*II*) and perferryl (*III*) species that are thought to react with Arg or NOHA.

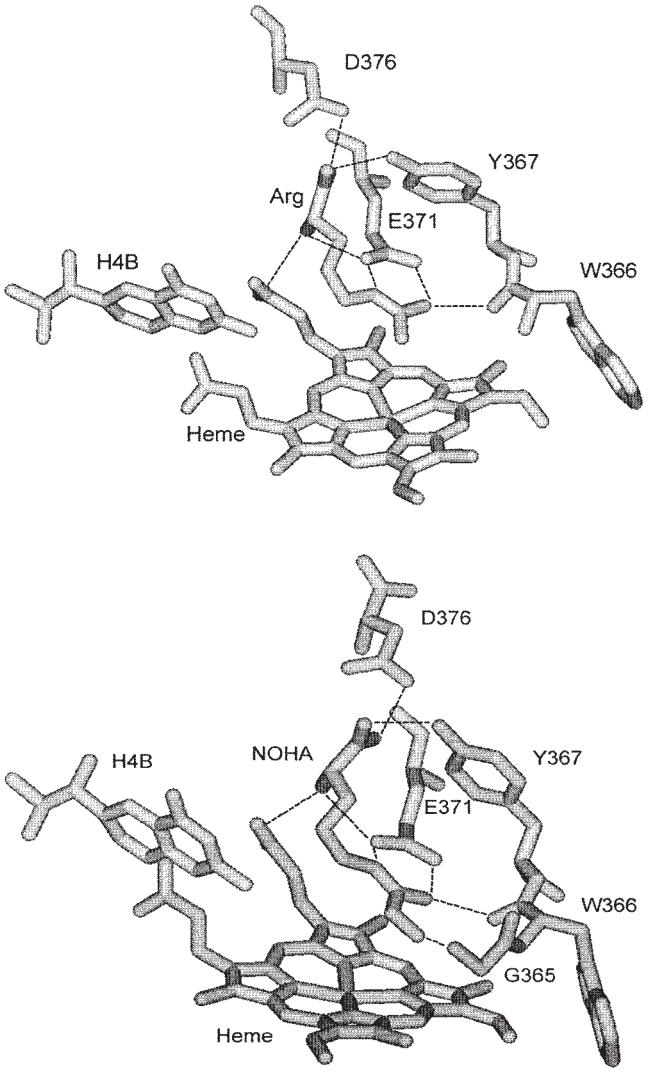

FIGURE 3 Arg and NOHA binding in inducible NOS. The crystal structure data indicate how Arg (*top*) or NOHA (bottom) binds within the active site of NOS in relation to the enzyme heme and H4B groups. Protein residues that make hydrogen bond contacts with Arg or NOHA are indicated, with the hydrogen bonds shown as thin lines. Data are from Crane et al. (10) and Crane et al. (14).

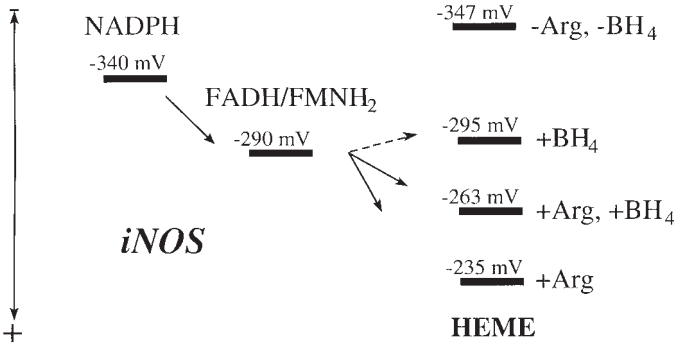

FIGURE 4 Thermodynamic profile for heme reduction in inducible NOS. Estimated reduction potentials for NADPH and the NOS flavoprotein (FADH/FMNH2) are indicated. The right-hand portion of the figure shows how bound Arg or H4B increases the reduction potential of the ferric heme iron. Electron transfer toward a more positive potential is favored; arrows indicate the direction of electron transfer. Data are from Presta et al. (22).

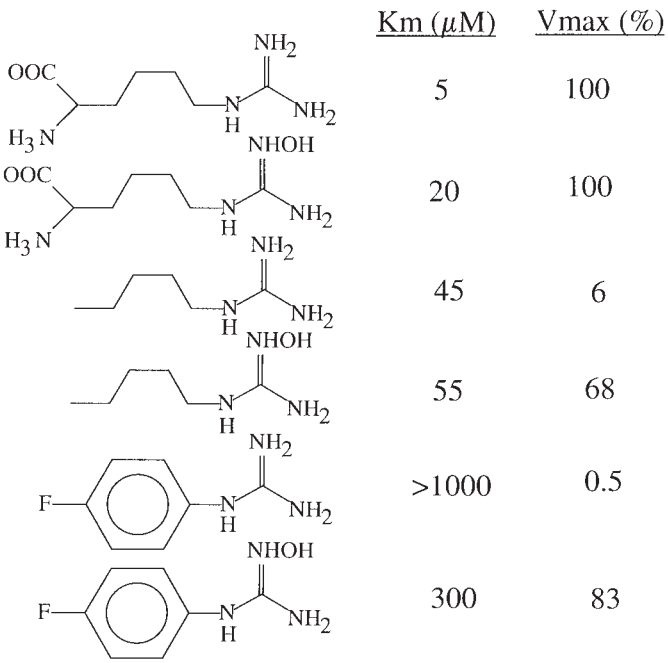

FIGURE 5 Arg analogs that are substrates for NOS. Structures are shown with the corresponding K_m and relative V_{max} values for NO synthesis. Charges are not shown. Data are from Dijols (28).

amino acid portion of Arg are not essential for enzyme catalysis.

Nonsubstrate analogs of Arg

A great number of Arg derivatives are not substrates for NOS and act instead as competitive inhibitors or in some cases as mechanism-based inactivators (29). Many of these compounds no longer contain the guanidino moiety, which is essential for NO synthesis. In some cases, the NOS binding affinity toward these analogs is greater than that toward Arg itself. **Figure 6** depicts the structures of some representative analogs. NOS crystal structures that contain some of the bound inhibitors are available (30).

Consequences of suboptimal Arg concentration

At nonsaturating Arg concentrations, a calmodulin-bound NOS will continue to bind and activate dioxygen at its heme

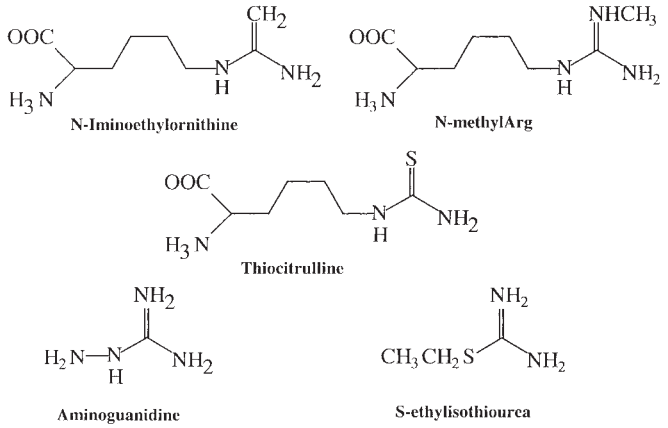

FIGURE 6 Structures of Arg analogs that are NOS inhibitors. Charges are not shown.

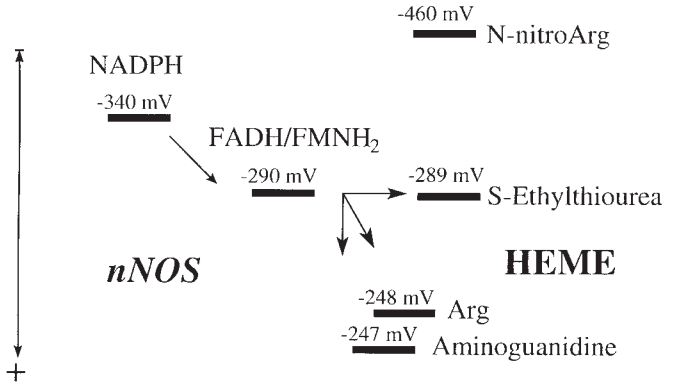

FIGURE 7 Effect of some NOS inhibitors on the thermodynamics of neuronal NOS heme reduction. Estimated reduction potentials for NADPH and the NOS flavoprotein (FADH/FMNH2) are indicated. The right-hand portion of the figure compares how bound Arg and Arg analog inhibitors poise the reduction potential of the ferric heme iron. Electron transfer toward a more positive potential is favored; arrows indicate the direction of electron transfer. Data are from Presta et al. (22).

and subsequently will release reactive oxygen species such as superoxide and H_2O_2 (31,32). The frequency and biological effect of this process are under scrutiny. An active NOS operating at a subsaturating Arg concentration can generate NO and superoxide at the same time. These products can conceivably react to generate the cellular toxin peroxynitrite (33,34).

Certain Arg analogs can inhibit the production of reactive oxygen species by NOS, whereas others either allow it or enhance it (35,36). Some of the analogs that inhibit reactive oxygen species production by NOS act by lowering the heme midpoint reduction potential, rendering the transfer of electrons from the NOS flavins thermodynamically unfavorable (**Fig. 7**) (22). This represents one strategy to control production of reactive oxygen species by NOS under conditions of low Arg concentration.

Regulating Arg availability for the NOSs

In some cells, the expression of cationic amino acid transporters is increased coincident with NOS expression (37,38). Arginase may also be expressed and typically is the chief competitor for Arg in cells and tissues (39–41). In certain environments, arginase expression can deplete Arg levels to the point where NO synthesis by NOS is compromised (42,43). Citrulline can be converted to Arg in many tissues, and this may provide a significant source of Arg in some cases (44). Endogenous methylarginines are also present in tissues at concentrations that may inhibit Arg binding to NOS (45–47). Arg demethylase enzymes may serve to regenerate small amounts of Arg (48). Factors regulating Arg availability are discussed in detail in some of the other papers of this series.

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