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Review

Nitric-oxide synthase: A cytochrome P450 family foster child

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Abstract

Nitric-oxide synthase (NOS), the enzyme responsible for mammalian NO generation, is no cytochrome P450, but there are striking similarities between both enzymes. First and foremost, both are heme-thiolate proteins, employing the same prosthetic group to perform similar chemistry. Moreover, they share the same redox partner, a diflavoprotein reductase, which in the case of NOS is incorporated with the oxygenase in one polypeptide chain. There are, however, also conspicuous differences, such as the presence in NOS of the additional cofactor tetrahydrobiopterin, which is applied as an auxiliary electron donor to prevent decay of the oxyferrous complex to ferric heme and superoxide. In this review similarities and differences between NOS and cytochrome P450 are analyzed in an attempt to explain why NOS requires BH4 and why NO synthesis is not catalyzed by a member of the cytochrome P450 family.

Keywords: Nitric-oxide synthase; Tetrahydrobiopterin; Uncoupling

Two decades have passed since the realization of the importance of nitric oxide in human biology. After it was recognized that this diatomic gas performs crucial functions in a wide array of physiological processes, including signal transduction and the immune response [1–4], the hunt for its biological source was on, and it was soon discovered that NO was synthesized from the amino acid L-arginine by an enzyme that, predictably, was baptized nitric-oxide synthase (NOS). This enzyme turned out to be a real gem for structural chemists, enzymologists, and pharmacologists alike. In its active center, NOS contains a heme of the same type as found in cytochrome P450 (P450), and it is this property that justifies its inclusion in this volume. Here we will focus on similarities and differences with other members of the P450 family of proteins. For other aspects of nitric oxide synthases interested readers can choose from a wide range of reviews [2,5–12].

1. General properties

NOS catalyzes NO synthesis in two distinct cycles with *N*-hydroxy-L-arginine (NHA) as an intermediate product that is processed to citrulline and NO without being released from the enzyme [2,5–9]. Both cycles consume one molecule of O_2 and both require the input of exogenous electrons – two in the first and one in the second cycle – that are furnished by NADPH. NOS is a modular enzyme that consists of an N-terminal oxygenase and a C-terminal reductase domain. Catalysis takes place at a cysteinyl sulfur-coordinated b-type heme in the oxygenase domain. The heme iron binds O_2 as a sixth ligand in the distal pocket, which also serves as the site for substrate binding. In close proximity, (*6R*)-*5*,6,7,8-tetrahydro-L-biopterin (BH4) is bound as an additional cofactor. The required electrons are provided by the reductase domain, which shuttles electrons from NADPH to the heme via two flavin cofactors, one FAD moiety that accepts electrons two at a time from NADPH, and one FMN moiety that transfers them one at a time to the heme. The two domains are separated by a short amino acid sequence that must bind calmodulin to enable interdomain electron transfer. NOS is only active as a homodimer, because electron transfer can only occur from the reductase domain of one subunit to the oxygenase domain of the second subunit [7,9]. The stability of the dimer is enhanced by a zinc ion that is coordinated to four cysteinyl sulfurs in the dimer interface [9]. A schematic illustration of NOS structure and cofactor content is presented in Fig. 1.

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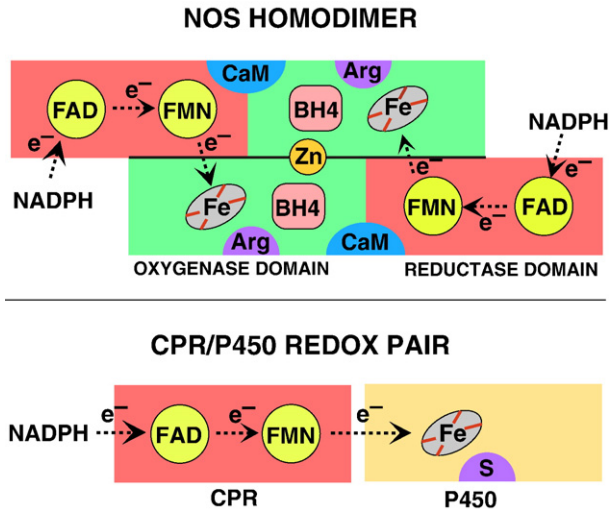

Fig. 1. Schematic presentation of the NOS homodimer including all cofactors and the electron transfer pathway. For comparison, the CytP450/P450 reductase redox pair is shown in a similar fashion. Note the presence of calmodulin, BH4, and Zn^{2+} as additional cofactors in NOS. The color-coding of the figure illustrates the lack of structural homology, apart from the heme, between the NOS oxygenase domain and P450.

Mammalian NOS comes in three isoforms [1,2,4–11]. Neuronal and endothelial NOS (nNOS and eNOS) are constitutively expressed, and their activity is under strict regulatory control in keeping with their role in signal transduction—for more detailed information on the many aspects of NOS regulation numerous reviews are available [4,9,10,13–16]. Both constitutive enzymes are sensitive to the calcium ion concentration, because calmodulin binds to these isoforms only in the presence of Ca^{2+} [4,9,10,14,16]. Both are also affected by phosphorylation of specific serine and threonine residues [4,9,10,14,16]. NOS activity is modulated by tyrosine phosphorylation as well [16,17]. Furthermore, the localization and, consequently, the activity of eNOS are controlled by myristoylation and palmitoylation events in a small extension at the N-terminus [4,9,14,15]. The much longer extension of nNOS contains a PDZ binding motif that is crucial for subcellular targeting and protein–protein interactions of this isoform [9,15]. Indeed, nNOS and eNOS are regulated by stimulatory and inhibitory interactions with a range of proteins and it is becoming increasingly clear that both enzymes are functioning within the settings of isoform-specific signalosomes [4,9,10,14–16]. In contrast to the constitutive isoforms, the inducible isoform (iNOS) is only expressed in response to cytokines. The affinity of iNOS for calmodulin is so much higher than that of eNOS and nNOS that it binds calmodulin in the virtual absence of free Ca^{2+} [9,10,15]. As a result, iNOS lacks the tight control characteristic of the constitutive isoforms and is able to churn out large quantities of NO for an extended period, in line with its function in the immune response. Although inducible and constitutive NOS are regulated at the transcriptional and post-translational level, respectively, this division is not absolute, as moderate expressional control of eNOS and nNOS [13,16], and post-translational control of iNOS are documented [9,15].

A recent addition to the list of post-translational modifications is the *S*-nitrosation of the cysteine residues involved in binding of the zinc ion [18–20]. In agreement with the role of Zn^{2+} in dimer stability, *S*-nitrosation was accompanied by monomerization and loss of activity. In this way *S*-nitrosation may represent an autoregulatory feedback mechanism, if the nitrosating species originates from NOS catalysis.

It has been reported that a NO-synthase, distinct from the three well-characterized NOS isoforms, exists in mitochondria [9,21]. This putative isoform, abbreviated as mtNOS, is potentially important in view of mounting evidence that the mitochondrial respiratory chain represents one of the main targets of NO. However, almost a decade after the first reports, the identity and even the existence of mtNOS are still a matter of controversy [21–23]. According to a recent proposal [24] the mitochondrial enzyme might not be related to any of the mammalian NOS isoforms but to an NO synthesizing enzyme that was newly discovered in the green plant *Arabidopsis thaliana* [25]. Although this would solve the conundrum why no clear affiliation of mtNOS with the other isoforms could thus far be established, the jury will still be out on mtNOS until a functional enzyme is purified and characterized, particularly as the *Arabidopsis* enzyme has not been fully characterized itself, and its activity appears to be low.

2. NOS and the cytochrome P450 family of proteins: similarities and differences

Strictly speaking, the label cytochrome P450 stands for a huge and widely distributed group of closely related enzymes. In a broader sense, it has come to encompass other proteins containing a thiolate-ligated heme that are not structurally related to P450 and that do not even have to display the typical 450 nm UV/vis absorption maximum in the presence of CO that gave P450 its name [26]. Only in this wider sense NOS belongs to the P450 family, since there is no sequence homology between NOS and P450 nor are there any similarities in the overall protein structure (Fig. 2). NOS does resemble P450 in as far as it catalyzes the mono-oxygenation of its substrate, but the resemblance is rather superficial. NOS does not exhibit the substrate promiscuity typical of many P450s, as it is quite specific for Arg and NHA, with only a limited number of other compounds, specifically some *N*-alkyl- and *N*-aryl-*N*′-hydroxyguanidines, serving as good alternative substrates [27]. Conversely, although *N*-hydroxylation is part of the vast repertoire of P450-catalyzed reactions, it is hardly typical [28,29], and Arg hydroxylation is not known to be catalyzed by any P450. The second reaction cycle with its odd electron stoichiometry is even harder to fit within the framework of classical P450 chemistry, and although P450 was found to transform NHA to NO and citrulline, it does so mainly by uncoupled generation of O_2^- that subsequently reacts with NHA in solution [30].

Perhaps most striking among the similarities between P450 and NOS is the fact that both the NOS oxygenase domain (NOS_{oxy}) and mammalian (microsomal) P450 utilize the same electron donor as a redox partner [31]. Whereas NOS_{oxy} and