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CO exchange of the oxyferrous complexes of endothelial nitric-oxide synthase oxygenase domain in the presence of 4-amino-tetrahydrobiopterin

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Abstract

Tetrahydrobiopterin (BH4) is an essential cofactor of nitric-oxide synthase (NOS) that serves as a 1-electron donor to the oxyferrous–heme complex. 4-Amino-tetrahydrobiopterin (4-amino-BH4) inhibits NO synthesis, although it has similar redox properties. We recently reported that 4-amino-BH4 is capable of electron transfer to $Fe(II)\cdot O_2$ in cryogenic single-turnover [J. Biol. Chem. 278 (2003) 48602]. We also suggested that BH4 serves as a proton donor to the $Fe(II)\cdot O_2^-$ complex, and that 4-amino-BH4 cannot perform this second essential function. To corroborate these claims and to further characterize the intermediates observed after oxygenation of NOS in the presence of 4-amino-BH4, we added CO immediately after O_2 addition to the reduced oxygenase domain of endothelial NOS at −30 °C. This resulted in complete formation of a P450-type $Fe(II)\cdot CO$ complex with either Arg or N^G-hydroxy-L-arginine as the substrate. In the presence of 4-amino-BH2, which is redox-inactive, the same procedure yielded ferric heme with either substrate, without formation of any $Fe(II)\cdot CO$ complex. We conclude: (i) O_2 binding to ferrous heme in the presence of 4-amino-BH2 is essentially irreversible; (ii) 4-amino-BH4 can reduce the oxyferrous complex; (iii) O_2^-, rather than H_2O_2 is the immediate product of uncoupled catalysis in the presence of 4-amino-BH4.

Keywords: NO-synthase; Tetrahydrobiopterin; Ferrous-oxy heme; CO exchange

1. Introduction

In mammals, including humans, the biosynthesis of nitric oxide (NO) is catalyzed by nitric oxide synthase (NOS; EC 1.14.13.39; for recent reviews, see [1–3]) via a two-step mechanism. In the first step L-arginine (Arg) is converted to N^G-hydroxy-L-arginine (NHA). In the second step NHA is further oxidized to NO and citrulline. Both reactions consume 1 equiv. of O_2, as well as NADPH-derived electrons (2 and 1 equiv. in the first and second reactions, respectively). NOS has a bidomain structure, with catalysis taking place at a P450-type heme in the oxygenase domain, whereas reducing equivalents are transferred from NADPH to the heme via two flavins (FAD and FMN) in the reductase domain.

Although the NOS oxygenase domain resembles cytochrome P450 in many ways, it differs by requiring tetrahydrobiopterin (BH4) as a cofactor (for recent reviews on the role of BH4 in NOS, see [4,5]). BH4 serves as a requisite electron donor to the ferrous–oxygen complex that is formed as an early intermediate in the reaction cycles with Arg and NHA [6–12]. In line with the crucial role of BH4 as an electron donor in NOS catalysis, various tetrahydropteridines other than BH4 support NO synthesis, whereas redox-inactive

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7,8-dihydropteridines are BH4-competitive inhibitors [4, and references therein]. An exception to this rule is the inhibitor 4-amino-tetrahydrobiopterin (4-amino-BH4; [13–15]), which is a tetrahydropteridine with electrochemical properties not unlike those of BH4 [16]. We recently demonstrated the formation of a 4-amino-BH3 radical in single-turnover studies with the oxygenase domain of endothelial NOS ($eNOS_{oxy}$) in the presence of Arg and of a ferrous-NO complex in the presence of NHA [17]. From these observations we concluded that 4-amino-BH4 can indeed serve as an electron donor to the heme in both reaction cycles. Furthermore, we proposed a role for BH4 as an obligate proton donor and suggested that inhibition by 4-amino-BH4 is due to its inability to carry out this function [17]. There have been previous hints at an additional function of BH4 as a proton donor [18,19]. However, definitive evidence for the involvement of BH4 in protonation is still lacking. Significantly, in a recent study using the inducible NOS oxygenase domain no 4-amino-BH3 radical was observed in single-turnover, which would imply that 4-amino-BH4 is redox-inactive in that isoform [11].

In search of an independent method to answer the central question if 4-amino-BH4 can serve as an electron donor to the oxyferrous complex in NOS, we performed CO exchange experiments immediately after O_2 addition to reduced $eNOS_{oxy}$ in the presence of Arg or NHA at cryogenic temperatures. We found that a stable ferrous–CO complex was formed in the presence of 4-amino-BH4, but not in the presence of its redox-inactive counterpart 4-amino-BH2. From these studies we draw the following conclusions: (i) formation of the red-shifted $Fe(II)\cdot O_2$ complexes in the presence of 4-amino-BH4 and 4-amino-BH2 is essentially irreversible, suggesting that the complexes may have considerable $Fe(III)\cdot O_2^-$ character; (ii) 4-amino-BH4 can reduce the oxyferrous complex in the presence of either Arg or NHA; (iii) the immediate product of uncoupling in the presence of 4-amino-BH4 is O_2^- rather than H_2O_2.

2. Materials and methods

2.1. Materials

The oxygenase domain of bovine $eNOS_{oxy}$ was expressed in and purified from *Escherichia coli* [9]. All chemicals were from Sigma except for the pteridines (Schircks Laboratories, Jona, Switzerland), Ar, O_2, and CO (Aga, Toulouse, France).

2.2. Sample preparation and low-temperature optical spectroscopy

Low-temperature UV/visible absorption spectra of the reaction between reduced $eNOS_{oxy}$ and O_2, CO, or Ar were measured in a Cary 3E (Varian, Palo Alto, CA) spectrophotometer, adapted to low-temperature studies, according to previously published procedures [6,8,20]. NOS samples of 3–5 μM in a buffer consisting of 50 mM potassium phosphate (pH 7.4), 1 mM 3-[3-(cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 1 mM 2-mercaptoethanol, 0.5 mM ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA), and 50% ethylene glycol, and in the presence or absence of substrates (250 μM Arg or 500 μM NHA) and pteridines (50 μM) as indicated, were deoxygenated under argon atmosphere and reduced by sodium dithionite (1 mM final concentration) at room temperature. After complete reduction the temperature was decreased to −30 °C. Oxygenation was performed with a pre-cooled O_2-containing syringe (30 mL). Immediately after measurement of the UV/vis spectrum, CO was added with a second pre-cooled syringe (typically within 1 min after O_2 addition), and spectra were measured immediately, and after regular intervals (up to 80 min). In some instances the temperature of the samples was transiently increased afterwards to allow the reaction to go to completion.

2.3. Characteristics of the experimental set-up

It may be worthwhile to discuss in advance some of the features of the experimental procedures applied in this study. Although the use of a relative large excess of sodium dithionite offers a facile method to reduce the heme, the possibility of artifacts must be considered. As is evident from the totally different observations with 4-amino-BH4 and 4-amino-BH2 (vide infra), one such potential artifact, the reduction of 4-amino-BH2 by dithionite to 4-amino-BH4, clearly does not occur, in agreement with the very low reduction potential (well below −1.0 V [16]) of the 4-amino-BH2/4-amino-BH4 couple. Furthermore, as shown previously [6], the reduction of the heme by dithionite is extremely slow at −30 °C, which guarantees that any dithionite that might survive bubbling the sample with oxygen (with a greater than 20,000-fold molar excess of O_2), will be unable to react with the enzyme. Consequently, the applied method offers a reliable means to study the ability of CO exchange of the oxyferrous complex, provided the latter complex is stable on the time-scale of the experiment (tens of seconds). This last provision implies that the method is not applicable to study CO exchange for the oxy-complexes observed with BH4, which are too short-lived [6,8].

3. Results

In an attempt to determine the electronic state of the 428/431-nm species obtained under cryogenic conditions

with 4-amino-BH4 and Arg, CO was added immediately after its formation. As shown in Fig. 1, in the presence of O_2 the Soret band at 412 nm of dithionite-reduced $eNOS_{oxy}$ was replaced by a band at 428 nm with significantly lower amplitude, in line with previous observations [8,17]. Addition of CO resulted in the slow ($t_{1/2} \sim 27$ min) appearance of a spectrum typical of thiolate-ligated ferrous–CO heme ($\lambda_{max} = 443/444$ nm).

With NHA, qualitatively similar observations were made. In the presence of NHA and 4-amino-BH4, O_2 addition to reduced $eNOS_{oxy}$ caused a considerably smaller red-shift (from 412 to 423 nm), in confirmation of prior observations [8,17]. After addition of CO followed by 80 min incubation, complete transition to a P450-type ferrous-CO complex ($\lambda_{max} = 443$ nm) had occurred. However, in this case partial formation of a blue-shifted spectrum ($\lambda_{max} = 417$ nm) preceded formation of the Fe–CO complex (Fig. 2). The kinetics were comparable to those observed with Arg ($t_{1/2} \sim 8$ min for the formation of the blue-shifted intermediate, $t_{1/2} \sim 17$ min for the formation of the final ferrous–CO complex).

When 4-amino-BH4 was replaced by its oxidized counterpart 4-amino-7,8-dihydro-L-biopterin (4-amino-BH2) no ferrous–CO complexes were detected with Arg or NHA. With Arg, the oxygenated intermediate ($\lambda_{max} = 433$ nm) was converted slowly to high-spin ferric heme ($\lambda_{max} = 390$ nm, Fig. 3). With NHA, transformation of the oxygenated intermediate ($\lambda_{max} = 425/6$ nm) in the presence of CO yielded a compound with absorbance maximum at 417 nm, that was only readily converted to high-spin ferric heme after a transient increase to room temperature. Essentially identical results were obtained in the presence of 4-amino-BH2 when argon was added instead of CO (not shown).

The oxy-complexes in the presence of Arg exhibited similar lifetimes as observed with 4-amino-BH4 ($t_{1/2}$ of ~33 and ~32 min with CO and argon, respectively). The rates of conversion of the oxy-complex in the presence of NHA and 4-amino-BH2 to the species absorbing at 417 nm were faster ($t_{1/2}$ of ~3.8 and ~3.1 min with CO and argon, respectively). The fact that the formation of this species, the nature of which is unclear, was not affected by the presence of CO, indicates that no CO exchange took place with the oxy-complex in the presence of NHA and 4-amino-BH2.

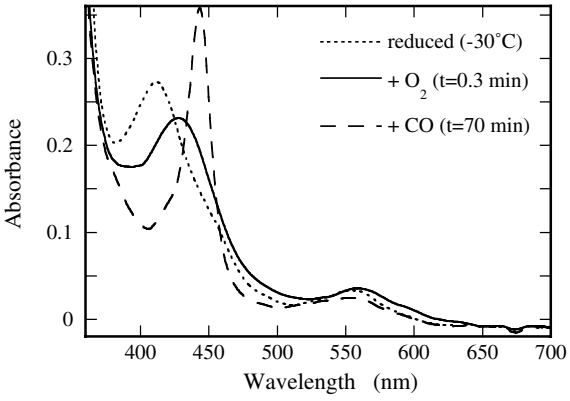


Fig. 1. Reaction of $eNOS_{oxy}$ with O_2 and CO in the presence of Arg and 4-amino-BH4 at −30 °C. Shown are the initial spectrum of the reduced enzyme (. . . . . .) and the spectra immediately (0.3 min) after O_2 addition (——), and 70 min after CO addition (– – –). See Section 2 for experimental details.

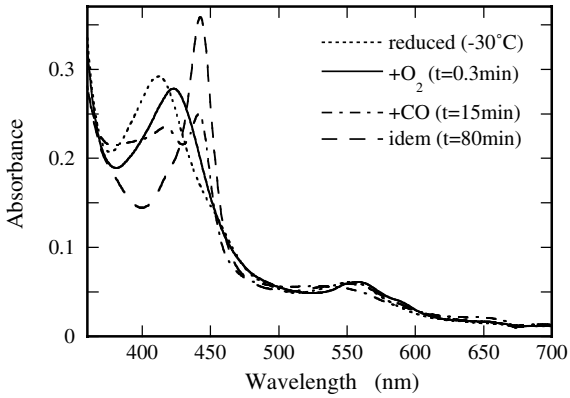


Fig. 2. Reaction of $eNOS_{oxy}$ with O_2 and CO in the presence of NHA and 4-amino-BH4 at −30 °C. Shown are the initial spectrum of the reduced enzyme (. . . . . .) and the spectra immediately (0.3 min) after O_2 addition (——), and 15 (– – –) and 80 (– – –) min after CO addition. See Section 2 for experimental details.

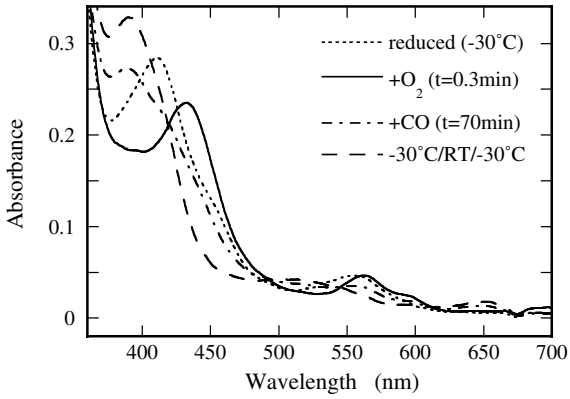


Fig. 3. Reaction of $eNOS_{oxy}$ with O_2 and CO in the presence of Arg and 4-amino-BH2 at −30 °C. Shown are the initial spectrum of the reduced enzyme (. . . . . .) and the spectra immediately (0.3 min) after O_2 addition (——), 70 min after CO addition (– – –), and after a transient increase to room temperature (– – –). See Section 2 for experimental details.

4. Discussion

Although 4-amino-BH4 is the best-studied BH4-competitive inhibitor of NOS [13–15], its mode of action is not completely understood. Whereas other pterin-based inhibitors of NOS differ from BH4 in being unable to reduce the oxyferrous complex, which is the main

role of BH4, the electronic properties of 4-amino-BH4 and of BH4 are similar [16], suggesting that 4-amino-BH4 should be able to serve as an electron donor to the heme. In order to test that assumption we studied the ability of $eNOS_{oxy}$ to form a ferrous-CO complex after initiation of the reaction between the reduced enzyme and O_2. As a control we performed similar experiments with 4-amino-BH2, which represents the two-electron oxidized redox-inactive state of the pteridine. The rationale behind the experiment was that any state isoelectronic with the ferrous enzyme, such as $Fe(II)\cdot O_2$ or $Fe(III)\cdot O_2^-$, should be able to yield a $Fe(II)\cdot CO$ complex after dissociation of O_2, whereas intermediates further along the reaction cycle should not be able to do so. Consequently, one might expect a CO complex in the presence of 4-amino-BH2, the redox-incompetent counterpart of 4-amino-BH4, but not in the presence of 4-amino-BH4. Much to our surprise we observed the exact opposite: CO complex formation with 4-amino-BH4 but not with 4-amino-BH2. There are several important conclusions we can draw from this.

4.1. Identification of the intermediates observed with 4-amino-BH4 as oxyferrous complexes

Since 4-amino-BH2 cannot function as an electron donor, the 433 and 425/6 nm complexes obtained with this pteridine in the presence of Arg and NHA, respectively, must be oxyferrous complexes. The spectra obtained immediately after O_2 addition in the presence of Arg and 4-amino-BH4 (Fig. 1) or 4-amino-BH2 (Fig. 3) were very similar, which suggests that the species observed with 4-amino-BH4 were oxyferrous complexes too. On the basis of the close resemblance of the intermediate spectra observed with NHA and 4-amino-BH4 (Fig. 2) and 4-amino-BH2 (Fig. 4), the same conclusion can be drawn for the reaction with NHA as well. Moreover, the remote possibility that a 1-electron reduced oxy complex ($Fe(II)\cdot O_2^-$) with absorption spectrum virtually identical to that of the oxyferrous complex ($Fe(II)\cdot O_2$) is accumulating in the presence of 4-amino-BH4 can be ruled out on the basis of previous observations that the heme is EPR-silent in this state [17]. The kinetics indicate that the decay of the oxyferrous complex was rate-limiting under all conditions, with lifetimes in good agreement with previous observations in the absence of CO [8].

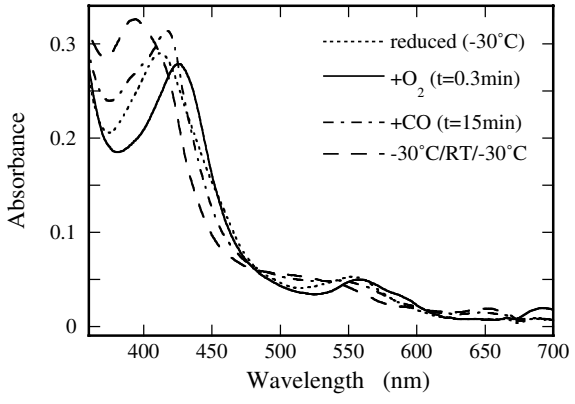


Fig. 4. Reaction of $eNOS_{oxy}$ with O_2 and CO in the presence of NHA and 4-amino-BH2 at −30 °C. Shown are the initial spectrum of the reduced enzyme (.....) and the spectra immediately (0.3 min) after O_2 addition (——), 15 min after CO addition (– – –), and after a transient increase to room temperature (–·–·–). See Section 2 for experimental details.

4.2. Tentative identification of the reduced dioxygen $eNOS_{oxy}$ complexes as $Fe(III)\cdot O_2^-$

The inability of the oxyferrous complexes obtained in the presence of 4-amino-BH2 to yield a CO complex implies that O_2 cannot dissociate from these compounds, and that the complexes decay exclusively to Fe(III) and O_2^-. The quasi-irreversibility of O_2 binding is also supported by the observation that no Fe(II) was recovered when O_2 was replaced by argon immediately after oxygenation. These observations implicate the oxyferrous complexes as the compounds responsible for uncoupling and strongly support their assignment as $Fe(III)\cdot O_2^-$. Predominant $Fe(III)\cdot O_2^-$ character of the oxyferrous complex might also help explain the unusually red-shifted Soret band. In stark contrast to the apparent irreversibility of O_2 binding in this paper, values ranging from 18 to 38 s^{-1} were derived for the dissociation rate constants for O_2 from full-length eNOS [21]. The discrepancy does not derive from the different experimental conditions (aqueous stopped-flow studies at 10 °C vs. cryogenic studies at −30 °C in 50% ethylene glycol), but may be due to the identity of the pterin. [1] In recent stopped-flow/rapid-scan studies we found O_2 dissociation to be essentially irreversible with 4-amino-BH4, whereas dissociation rate constants in the presence of BH4 were similar to those reported by Abu-Soud et al. (unpublished observations).

4.3. Electron donation by 4-amino-BH4 to the oxyferrous complex in both reaction cycles

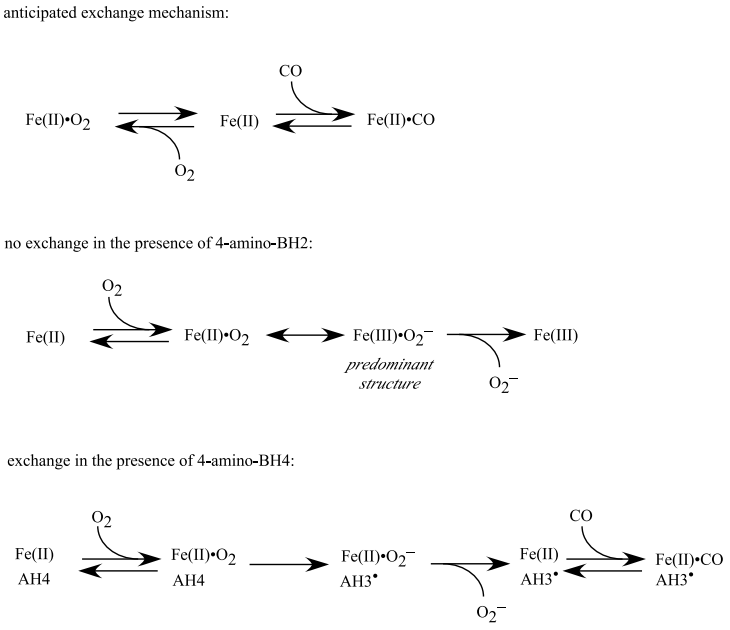
Although similar oxyferrous compounds were formed with both pteridines, a $Fe(II)\cdot CO$ complex formed in the presence of 4-amino-BH4 but not of 4-amino-BH2.

[1] In similar experiments to be described elsewhere, complete oxidation without CO exchange was observed in the presence of NHA and BH2, whereas the reaction in the presence of NHA without pterin yielded a mixture of oxidized heme and the ferrous–CO complex. The likelihood of the oxyferrous complex dissociating to Fe(II) and O_2, as reflected in its ability to form a ferrous–CO complex, appears to correlate with the absorption spectrum, with 'blue' complexes ($\lambda_{max} = \sim$ 416–419 nm) but not 'red' ones ($\lambda_{max} = \sim$ 426–432 nm) allowing O_2 dissociation (unpublished observations).

This can be explained if 4-amino-BH4 can function as an electron donor as is illustrated in Scheme 1. As with 4-amino-BH2, formation of the oxygen complex is virtually irreversible, precluding O_2/CO exchange. However, in this case CO binding may nevertheless occur after reduction of the $Fe(II)\cdot O_2/Fe(III)\cdot O_2^-$ complex by the pterin cofactor, since the doubly reduced oxy complex ($Fe(II)\cdot O_2^-$) will yield ferrous heme after dissociation of O_2^-. The same arguments are valid for the reactions in the presence of NHA as well. These conclusions are in accordance with the electrochemical properties of 4-amino-BH4 [16] and are supported by the fact that a 4-amino-BH3 radical and a $Fe(II)\cdot NO$ complex can be observed by EPR in single-turnover studies in the presence of Arg and NHA, respectively [17].

4.4. Origin of the 417 nm intermediate in the presence of 4-amino-BH4 and NHA

One of the more enigmatic observations in the present study is the transient appearance of an absorption band at 417 nm in the CO exchange reaction in the presence of NHA and 4-amino-BH4 (Fig. 2). Definitive identification of this band is not possible from the present data. The spectrum resembles that of low-spin ferric heme, but such an assignment is problematic in view of the fact that its disappearance is accompanied by complete formation of the $Fe(II)\cdot CO$ complex. We recently observed the formation of a $Fe(II)\cdot NO$ complex in low-temperature EPR studies of the reaction of $eNOS_{oxy}$ with O_2 in the presence of NHA and 4-amino-BH4, and comparison of the EPR and optical spectra demonstrated that this complex most likely had a protonated cysteine sulfur for a proximal ligand [17]. The intermediate 417 nm Soret band observed here would be consistent with that assignment [22,23]. Alternatively, the spectrum might originate from the $Fe(II)\cdot CO$ complex that would be formed by CO exchange of such a complex. Protonation of the proximal thiolate has been suggested to explain the spectral properties of the CO complex that is the hallmark of the inactive form of cytochrome P450, shortly known as P420 [23,24]. In support of an assignment of the intermediate as a P-420-like $Fe(II)\cdot CO$ complex (i.e., with neutral cysteine as a weak proximal ligand), the observed spectrum is remarkably similar to that of the P420 $Fe(II)\cdot CO$ complexes of cytochrome $P450_{CAM}$ and the oxygenase domain of neuronal NOS, including the presence of a weak absorbance band near 650 nm [24,25]. Either of the assignments proposed here (a ferrous–NO or –CO complex with neutral thiol as the proximal ligand) would support our previous findings [17] and demonstrate that the presumed protonation of the proximal sulfur ligand is reversible, since a P450-type CO complex is eventually formed. Reversal of the P450-to-P420 conversion of NOS in the presence of substrate and BH4 has been reported before [25]. However, a better



Scheme 1. Proposed reaction mechanism for CO/O_2 exchange in the presence of 4-amino-BH4. We anticipated CO binding to Fe(II) after dissociation of O_2 in the control experiments in the presence of 4-amino-BH2 (upper scheme). The absence of such a reaction suggests that O_2 dissociation does not occur, perhaps because of electron redistribution from the heme to the O_2 ligand after oxygenation, resulting in dissociation of O_2^- rather than O_2 (middle scheme). Complete formation of $Fe(II)\cdot CO$ in the presence of 4-amino-BH4 may be explained by the ability of this pteridine to serve as an electron donor to the oxy-complex, which would leave the heme in a ferrous state after O_2^- dissociation (lower scheme). The scheme illustrates the proposed reactions in the presence of Arg. In the presence of NHA a $Fe(II)\cdot NO$ complex is perhaps intermediately formed (see main text).

characterization of the intermediate is necessary to verify these speculations.

4.5. The product of uncoupling in the presence of 4-amino-BH4

It has been suggested that, whereas the uncoupled reaction yields O_2^- in the absence of BH4, uncoupling in the presence of pteridines produces H_2O_2 [10,26]. Under the conditions of the present study, such a reaction should have resulted in the concomitant formation of H_2O_2 and ferric heme in the presence of 4-amino-BH4. The complete formation of the Fe(II)·CO complex in the presence of 4-amino-BH4 with either substrate demonstrates that, at least with this pteridine and at cryogenic temperatures, O_2^- is the sole product of the uncoupled reaction. Presently, it is unclear if the unprecedented ability of the Fe(II)·O_2^- complex to dissociate to ferrous heme and superoxide, suggested in a previous study [17] and indirectly confirmed here, is peculiar to NOS in the presence of 4-amino-BH4. Additional studies are under way in our laboratories to corroborate this phenomenon and to establish whether it is unique to 4-amino-BH4 or represents a more general property of NOS catalysis.

In summary, the results reported here confirm that 4-amino-BH4 can reduce the oxyferrous complex of $eNOS_{oxy}$. Indirectly, they also support our previous proposal that BH4 serves as a combined electron and proton donor, and that the inhibitory properties of 4-amino-BH4 derive from its inability to substitute BH4 as a proton donor.

5. Abbreviations

NOS	nitric-oxide synthase
$eNOS_{oxy}$	the oxygenase domain of recombinant bovine endothelial NOS
BH4	tetrahydrobiopterin [(6*R*)-5,6,7,8-tetrahydro-6-(L-*erythro*-1′,2′-dihydroxypropyl)pterin]
4-amino-BH4	4-amino-tetrahydrobiopterin [(6*R*)-2,4-diamino-5,6,7,8-tetrahydro-6-(L-*erythro*-1′,2′-dihydroxypropyl)pteridine]
4-amino-BH2	4-amino-dihydrobiopterin [(6*R*)-2,4-diamino-7,8-dihydro-6-(L-*erythro*-1′,2′-dihydroxypropyl)pteridine]
NHA	N^G-hydroxy-L-arginine
CHAPS	3-[3-(cholamidopropyl)dimethylammonio]-1-propanesulfonate
EDTA	ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid
UV/vis	ultraviolet/visible
EPR	electron paramagnetic resonance

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References

[1] S. Pfeiffer, B. Mayer, B. Hemmens, Angew. Chem. Int. Ed. 38 (1999) 1714–1731.
[2] D.J. Stuehr, Biochim. Biophys. Acta 1411 (1999) 217–230.
[3] W.K. Alderton, C.E. Cooper, R.G. Knowles, Biochem. J. 357 (2001) 593–615.
[4] A.C.F. Gorren, B. Mayer, Curr. Drug Metab. 3 (2002) 133–157.
[5] C.-C. Wei, B.R. Crane, D.J. Stuehr, Chem. Rev. 103 (2003) 2365–2383.
[6] N. Bec, A.C.F. Gorren, C. Voelker, B. Mayer, R. Lange, J. Biol. Chem. 273 (1998) 13502–13508.
[7] A.R. Hurshman, C. Krebs, D.E. Edmondson, B.H. Huynh, M.A. Marletta, Biochemistry 38 (1999) 15689–15696.
[8] A.C.F. Gorren, N. Bec, A. Schrammel, E.R. Werner, R. Lange, B. Mayer, Biochemistry 39 (2000) 11763–11770.
[9] P.P. Schmidt, R. Lange, A.C.F. Gorren, E.R. Werner, B. Mayer, K.K. Andersson, J. Biol. Inorg. Chem. 6 (2001) 151–158.
[10] C.-C. Wei, Z.-Q. Wang, Q. Wang, A.L. Meade, C. Hemann, R. Hille, D.J. Stuehr, J Biol. Chem. 276 (2001) 315–319.
[11] A.R. Hurshman, C. Krebs, D.E. Edmondson, M.A. Marletta, Biochemistry 42 (2003) 13287–13303.
[12] C.-C. Wei, Z.Q. Wang, C. Hemann, R. Hille, D.J. Stuehr, J. Biol. Chem. 278 (2003) 46668–46673.
[13] E.R. Werner, E. Pitters, K. Schmidt, H. Wachter, G. Werner-Felmayer, B. Mayer, Biochem. J. 320 (1996) 193–196.
[14] B. Mayer, C. Wu, A.C.F. Gorren, S. Pfeiffer, K. Schmidt, P. Clark, D.J. Stuehr, E.R. Werner, Biochemistry 36 (1997) 8422–8427.
[15] S. Pfeiffer, A.C.F. Gorren, E. Pitters, K. Schmidt, E.R. Werner, B. Mayer, Biochem. J. 328 (1997) 349–352.
[16] A.C.F. Gorren, A.J. Kungl, K. Schmidt, E.R. Werner, B. Mayer, Nitric Oxide 5 (2001) 176–186.
[17] M. Sørlie, A.C.F. Gorren, S. Marchal, T. Shimizu, R. Lange, K.K. Andersson, B. Mayer, J. Biol. Chem. 278 (2003) 48602–48610.
[18] B.R. Crane, A.S. Arvai, S. Gosh, E.D. Getzoff, D.J. Stuehr, J.A. Tainer, Biochemistry 39 (2000) 4608–4621.
[19] R. Davydov, A. Ledbetter-Rogers, P. Martásek, M. Larukhin, M. Sono, J.H. Dawson, B.S.S. Masters, B.M. Hoffman, Biochemistry 41 (2002) 10375–10381.
[20] A.C.F. Gorren, N. Bec, R. Lange, B. Mayer, Methods Enzymol. 353 (2002) 114–121.
[21] H.M. Abu-Soud, K. Ichimori, A. Presta, D.J. Stuehr, J. Biol. Chem. 275 (2000) 17349–17357.
[22] H.L. Voegtle, M. Sono, S. Adak, A.E. Pond, T. Tomita, R. Perera, D.B. Goodin, M. Ikeda-Saito, D.J. Stuehr, J.H. Dawson, Biochemistry 42 (2003) 2475–2484.
[23] R. Perera, M. Sono, J.A. Sigman, T.D. Pfister, Y. Lu, J.H. Dawson, Proc. Natl. Acad. Sci. USA 100 (2003) 3641–3646.
[24] S.A. Martinis, S.R. Blanke, L.P. Hager, S.G. Sligar, G. Hui Bon Hoa, J.J. Rux, J.H. Dawson, Biochemistry 35 (1996) 14530–14536.
[25] L. Huang, H.M. Abu-Soud, R. Hille, D.J. Stuehr, Biochemistry 38 (1999) 1912–1920.
[26] J. Vásquez-Vivar, N. Hogg, P. Martásek, H. Karoui, K.A. Pritchard Jr., B. Kalyanaraman, J. Biol. Chem. 274 (1999) 26736–26742.