Biosynthesis of Nitric Oxide—Quantum Chemical Modelling of N^{ω}-Hydroxy-L-arginine Formation

Ewa Wasielewska, Małgorzata Witko, Grażyna Stochel and Zofia Stasicka*

Abstract: The electronic structure (charge distribution, bond indices, character of the frontier orbitals) and geometry (bond distances and angles) of L-arginine and *N*-methyl-L-arginine were determined by means of the INDO procedure. The method was also adopted to model the conversion of L-arginine into *N*-hydroxy-L-arginine in biological systems. This revealed that the approach of diatomic O species does not result in reaction, whereas the approach of either an O atom or an O^{2-} ion leads to insertion of oxygen and formation of hydroxy-L-arginine. The insertion of oxygen between the nitrogen and hydrogen atoms leads to more stable products than insertion into the C–H bond. The same results were obtained for *N*-methyl-L-arginine, and are consistent with the hypothesis that the inhibitive effect of *N*-substitution in L-arginine is of no importance for the first step in the biosynthesis of NO (hydroxylation process). The mechanistic considerations based on the charge distribution and frontier orbital characteristics led to the conclusion that the most probable mechanism of L-arginine hydroxylation consists in electrophilic attack of $[FeO]^{3+}$ at the N^{ω}–H bond, initiated by the reduction of L-arginine^{+}, followed by insertion of oxygen and product oxidation.

Keywords
amino acids · biosynthesis · hydroxylations · nitric oxides · semiempirical calculations

Introduction

One of the most exciting findings in biological chemistry during last few years was the discovery that nitric oxide, a potentially toxic molecule, is responsible for an astonishing range of physiological processes in humans.[1–4] For example, it plays a role in vascular system regulation, nervous system mediation and immunological activity.

The biological sources of nitric oxide are endogenous as well as exogenous. The conversion of L-arginine (L-Arg) to NO and L-citrulline (Cit), catalyzed by a family of homodimeric dioxygenases called NO synthetases (NOS, EC 1.14.13.39),[4, 9–11] is a widely accepted hypothesis for the endogenous synthesis of nitric oxide.[1–18] Compounds related to arginine (e.g. *N*-methyl-L-arginine, *N*-nitro-L-arginine, *N*-cyclopropyl-L-arginine) are effective inhibitors of NO synthetases. Four cofactors (heme, FMN, FAD and H_4biopterin), two cosubstrates, an oxidant (O_2) and a reducer (NADPH) participate in the biosynthesis of NO from L-arginine. Isotopic studies have shown that nitric oxide is derived from one of the two equivalent guanidine nitrogen atoms of L-arginine[5, 6] and that dioxygen is the source of oxygen atoms incorporated into NO and L-citrulline.[7] It has also been shown that the first intermediate in the reaction is *N*-hydroxy-L-arginine (NOH-Arg).[8] Furthermore, when NOH-Arg is N-labelled on the NH–OH nitrogen, NO is exclusively derived from this nitrogen.[8, 9] NOS-catalyzed conversion of L-arginine to L-citrulline and NO is thus a 5-electron oxidation of one of the guanidine nitrogen atoms of L-arginine. Production of 1 mole of NO consumes 2 moles of O_2 (8-electron oxidation) and 1.5 mole NADPH (3-electron reduction), which constitutes a 5-electron oxidation system [Eq. (1)].

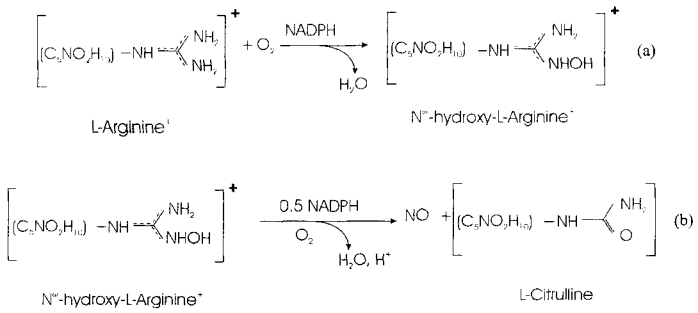
$$3H + 2O_2 + >C=\overset{-3}{N}H \longrightarrow H_2O + \overset{+2}{N}O + >C=O \qquad (1)$$

A generally accepted working hypothesis assumes that the reactive form of arginine in aqueous solution at pH ≈ 7 is a monovalent cation with an ionized amino acid group and that the NO synthesis proceeds in two steps (see Scheme 1).[2, 5–17]

The first step, an overall two-electron oxidation, is a hydroxylation resulting in the formation of *N*-hydroxy-L-arginine as an enzyme-bound intermediate. The second step, an overall 3-electron oxidation, involves electron removal, oxygen insertion and carbon–nitrogen bond scission to form L-citrulline and an NO free radical. Mechanistic speculations on the biosynthesis of nitric oxide have been based mainly on information of the primary structure of NOS.[2, 3, 5–18] Each NOS subunit is composed of reductase and oxygenase domains.[2, 12, 17] The reductase domain contains binding sites for NADPH, FAD and FMN. The oxygenase domain is presumed to contain binding

[*] Prof. Dr. Z. Stasicka, Prof. Dr. G. Stochel, Dr. E. Wasielewska
Department of Inorganic Chemistry, Jagiellonian University
Ingardena 3, 30-060 Kraków (Poland)
Fax: Int. code +(12)335-392
e-mail: stasicka@trurl.ch.uj.edu.pl
Prof. Dr. M. Witko
Institute of Catalysis and Surface Chemistry
Polish Academy of Sciences, Kraków (Poland)

  0947-6539/97/0304-0609 $ 17.50+.50/0



Scheme 1. Proposed mechanism of NO biosynthesis.

sites for heme, H_4biopterin and arginine. The amino acid sequence of the reductase domain is similar to the sequences present in the mammalian protein cytochrome P-450 reductase.[2, 17] The similarity of the NOS reductase domain to cytochrome P-450 reductase suggests that the function of the flavins is to store and transfer electrons from NADPH to a catalytic site in the oxygenase domain. As in cytochrome P-450, the NOS heme in its resting state contains a pentacoordinated ferric atom, which is bound to the protein through a thiolate anion of cysteine. Also by analogy to the cytochrome P-450 enzyme, the iron centre, after its reduction to the ferrous form, is likely to bind and activate the dioxygen molecule.[2, 12–17, 19, 20]

For the mechanism of NO biosynthesis proposed to date,[2, 5–17] it has been suggested that it is heme which generates distinct oxidants to form and metabolize the intermediate NOH–Arg$^+$, namely, first an electrophilic species $[FeO]^{3+}$, which hydroxylates nitrogen of L-Arg$^+$ to form NOH–Arg$^+$ (Scheme 1a), and then a nucleophilic oxygenating species $[FeOO]^+$, which attacks the electron-deficient carbon of the guanidine group of NOHArg$^+$ to form ultimately NO and Cit (Scheme 1b).[14]

In order to obtain a better insight into the molecular nature of the first step of nitric oxide biosynthesis, that is, formation of *N*-hydroxy-L-arginine from L-arginine and oxygen, we used quantum chemical modelling. This approach was also applied to the question of whether this step is responsible for the different behaviour of L-arginine and of its derivatives, which are known NOS inhibitors. The electronic properties of the systems studied were derived from calculations performed with a semiempirical INDO-type method.

Method and Model

The electronic and molecular structures were obtained by the ZINDO method. The details of the method are described elsewhere (see, for example, refs. [21–24] and references therein). We give only a quick overview here.

The ZINDO method is characterized by the inclusion of all the one-centre exchange terms necessary for rotational invariance and accurate spectroscopic predictions. The method uses a basis set of Slater-type orbitals (STO), which are then envisioned as being symmetrically orthogonalized to one another. A basis set of single Slater-type orbitals (STO) is characterized by the choice of exponential constants. For hydrogen a value of 1.2 is taken, whereas for elements of the second and third rows the exponents derived from Slater's rules are used. One-centre core integrals are calculated from ionization processes. One-centre resonance integrals are set to zero, whereas the two-centre one-electron integrals are calculated. The nuclear attraction integrals are proportional to two-centre Coulomb integrals between the appropriate atoms. The two-electron two-centred nonvanishing integrals are evaluated over STO's as are the one-centre integrals.

The ZINDO program has two different semiempirical procedures: a method for calculating spectroscopic properties and a method for calculating geometries. In the present calculations, the latter method was used. The set of parameters defined in this method allow us to discuss the relative energies of ground-state molecules as well as to perform the geometry optimization. We looked for ground-state structures using Newton–Raphson and Hessian methods. Vacuum calculations were performed. No influence of a reaction field was taken into account. The ZINDO method was compared with ab initio HF and DFT methods for large systems containing main groups and transition elements.[25, 26] It was found that it provides the same qualitative results as the ab initio treatments. Since the INDO method has also been proved to be suitable to interpret the behaviour of biological systems,[27–29] we restrict ourselves in this paper to the semiempirical approach.

The electronic structures were analyzed using Mulliken population analysis (atomic charges, atomic bond indices). The favourable reaction with oxygen species was chosen by discussing the total energies of the oxygen-containing species in their optimal geometries.

In order to model conversion of L-arginine into *N*-hydroxy-L-arginine we used the isolated arginine and *N*-methyl-L-arginine in both cationic and anionic forms. To study the oxygen insertion we examined the approach of oxygen to selected bonds in these molecules.

Results

Isolated molecules: The optimization INDO procedure performed for isolated L-arginine (L-Arg$^+$) and *N*-methyl-L-arginine (L-NMA$^+$) cations led to the structural parameters presented in Figure 1.

The results show that the lower energy forms of the isolated cations are those with a neutral amino-acid group $C_4N_3H_{11}$-CH(COOH)(NH_2), and not the ionized forms

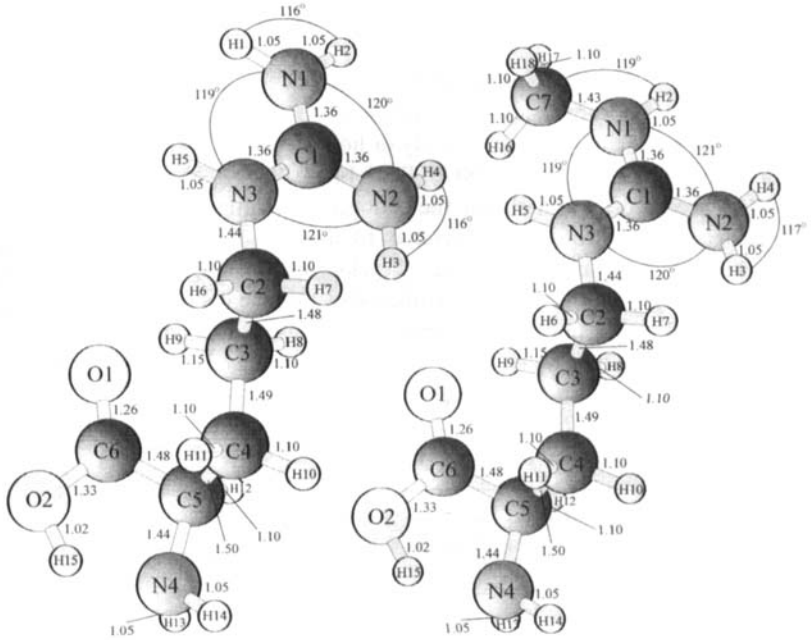


Figure 1. Geometrical structure of L-arginine (left) and *N*-methyl-L-arginine (right) obtained by the INDO method (bond lengths in Å).

© VCH Verlagsgesellschaft mbH, D-69451 Weinheim, 1997 0947-6539/97/0304-0610 $ 17.50+.50/0 Chem. Eur. J. 1997, 3, No. 4

$C_4N_3H_{11}$-CH(COO)$^-$(NH_3^+), which are expected to exist in aqueous solution at pH ≈ 7. However, the difference in energy between the forms is only 0.27 eV and there are no real differences in the electronic structure of their guanidine groups, which are of importance in the biosynthesis of nitric oxide. Moreover, the bond and angle parameters calculated for the lower energy form are in excellent agreement with those obtained by X-ray protein structure refinement[128] (Table 1).

Bond lengths (Table 1 and Figure 1) and atomic bond indices (Table 2) consistently point to stronger bonding within the guanidine group (between C1 and the N1, N2 and N3 atoms) than between C2 and N3 or C5 and N4. Of particular interest is the somewhat stronger bond between C1 and N3 (bond index 1.33) than between C1 and N1 or N2 (bond index 1.27, Table 2).

According to the net-charge calculations (Table 2), relatively high positive charges are found on two carbon atoms of the functional groups (C1 and C6) and nearly equal negative charges on all the nitrogen atoms except N3, which is ≈25% less negative than other N atoms.

The frontier orbitals (Table 3) are dominated by the functional group contributions: the HOMO of the cationic forms is mainly localized on the oxygen atoms of the carboxylic group (54%), whereas the contribution of the guanidine molecular orbitals is less than 1%. In contrast, the LUMO orbital consists of more than 90% of the guanidine group orbitals.

The atomic orbital (AO) contribution to either frontier orbital is completely reversed in the anionic form of L-arginine (L-Arg$^-$): here the HOMO orbital is mainly localized on the guanidine group, whereas the LUMO is localized on the carboxylic group (Table 3).

Table 1. Structural parameters of the guanidine group of L-arginine$^+$. Comparison of the calculated bond lengths [Å] and bond angles [°] with those obtained by X-ray protein structure refinement [30] (numbering of atoms as in Figure 1).

	INDO	X-ray		INDO	X-ray
C1–N1	1.36	1.33	N1-C1-N3	119	120
C1–N2	1.36	1.33	N2-C1-N3	121	120
C1–N3	1.36	1.33	N1-C1-N2	120	120
C2–N3	1.44	1.46	C1-N3-C2	122	124
C2–C3	1.48	1.52	N3-C2-C3	112	112

Table 3. Characteristics of the frontier orbitals in L-arginine and *N*-methyl-L-arginine calculated by the INDO method (numbering of atoms as in Figure 1).

	L-Arg$^+$	L-NMA$^+$	L-Arg$^-$
E(HOMO) (au)	−0.36	−0.36	−0.15
AO contrib. to HOMO (%)			
C1			39
N1			10
N2			10
N3			12
O1	44	44	
O2	10	10	
C3	14	12	
C5	7	5	
C6	4	4	
E(LUMO) (au)	+0.08	+0.08	+0.44
AO contrib. to LUMO (%)			
C1	57	58	
N1	12	11	
N2	12	11	
N3	12	13	
O1			26
O2			6
C3			1
C5			1
C6			45

Substitution of the hydrogen atom at N1 (or N2) by a methyl group has no real influence on bond lengths and angles, atomic bond indices, net charges or characteristics of the frontier orbitals (Table 2, Table 3 and Figure 1). Only the charge on the nitrogen atom bonded to the methyl group, becomes less negative, as expected.

Reaction with oxygen species: To model conversion of L-arginine into *N*-hydroxy-L-arginine, the insertion of oxygen from a species approaching a particular site on the arginine cation (L-Arg$^+$) was first analyzed. Since the form of oxygen involved in the reaction (Scheme 1a) is not well defined, we investigated the effect of all possible forms, starting with molecular O_2 and ending with anionic O^{2-}. The monoatomic species, O and O^{2-}, were found to lead to oxygen insertion, whereas dioxygen species, such as O_2, O_2^- or O_2^{2-}, appeared to be ineffective in the process (the reactions with these species resulted in the formation of peroxo-type adducts).

Table 2. Net charges of atoms and atomic bond indexes in L-arginine$^+$ (L-Arg$^+$) and *N*-methyl-L-arginine$^+$ (L-NMA$^+$) obtained by INDO method (numbering of atoms as in Figure 1).

	Net charge					Atomic bond index				
Atom	L-Arg$^+$	L-NMA$^+$	Atom	L-Arg$^+$	L-NMA$^+$	Bond	L-Arg$^+$	L-NMA$^+$	Bond	L-Arg$^+$	L-NMA$^+$
C1	0.54	0.50	H5	0.25	0.25	C1–N1	1.27	1.29	C2–H6	0.96	0.95
C2	−0.01	−0.03	H6	0.07	0.07	C1–N2	1.27	1.26	C2–H7	0.96	0.96
C3	−0.25	−0.25	H7	0.11	0.11	C1–N3	1.33	1.32	C3–H8	0.96	0.96
C4	−0.12	−0.12	H8	0.22	0.22	C2–N3	0.95	0.95	C3–H9	0.85	0.85
C5	−0.02	−0.02	H9	0.07	0.07	C2–C3	1.03	1.03	C4–H10	0.96	0.96
C6	0.47	0.47	H10	0.09	0.09	C3–C4	1.01	1.02	C4–H11	0.96	0.96
N1	−0.44	−0.30	H11	0.08	0.08	C4–C5	0.97	0.97	C5–H12	0.94	0.94
N2	−0.44	−0.44	H12	0.09	0.09	C5–C6	0.99	0.99	N4–H13	0.97	0.97
N3	−0.32	−0.32	H13	0.19	0.19	C5–N4	1.00	1.00	N4–H14	0.96	0.96
N4	−0.43	−0.43	H14	0.19	0.19	C6–O1	1.70	1.70	O2–H15	0.86	0.86
O1	−0.40	−0.40	H15	0.30	0.30	C6–O2	1.15	1.14	N1–C7	–	1.00
O2	−0.37	−0.37	C7	–	−0.17	N1–H1	0.94	0.94	C7–H16	–	0.97
H1	0.28	0.28	H16	–	0.14	N1–H2	0.94	–	C7–H17	–	0.97
H2	0.28	0.24	H17	–	0.12	N2–H3	0.94	0.93	C7–H18	–	0.97
H3	0.28	0.28	H18	–	0.12	N2–H4	0.94	0.94			
H4	0.28	0.28				N3–H5	0.93	0.92			

Although the reaction pathways and their energies were not investigated, the energy of the hydroxy isomers produced by directing the O-species to different N–H and C–H bonds was used to find the most probable site of insertion of oxygen. The procedure led to the conclusion that insertion is possible in all the cases; however, more stable products (by 1.35 eV) are formed when N–H rather than C–H bonds are attacked.

The modelling of the insertion of oxygen, illustrated by a few snapshots of the geometry optimization in Figure 2 (top), reveals that the approach of oxygen towards the N atom results in the formation of N–O and O–H bonds via intermediate triangular forms in which the N–H bond becomes increasingly weaker whilst the N–O and O–H bonds become increasingly stronger. The results also showed that the energy of the product is not affected by the direction of approach of the oxygen, that is, it is the same for the insertion of oxygen between N2 and H3 or N2 and H4. Insertion of O^{2-} followed by the removal of two electrons, or insertion of an O atom into the anionic form of L-Arg$^-$ leads to the same energy effect as insertion of the O atom.

Modelling of the oxygen insertion process for *N*-methyl-arginine, produced results close to those of L-arginine (Figure 2, bottom). No real difference in energy between the guanidine nitrogen atoms was found here either. On the other hand, insertion of oxygen into the C–H bond of the methyl group appeared to be energetically less favourable than insertion into the N–H bond (by 1.42 eV).

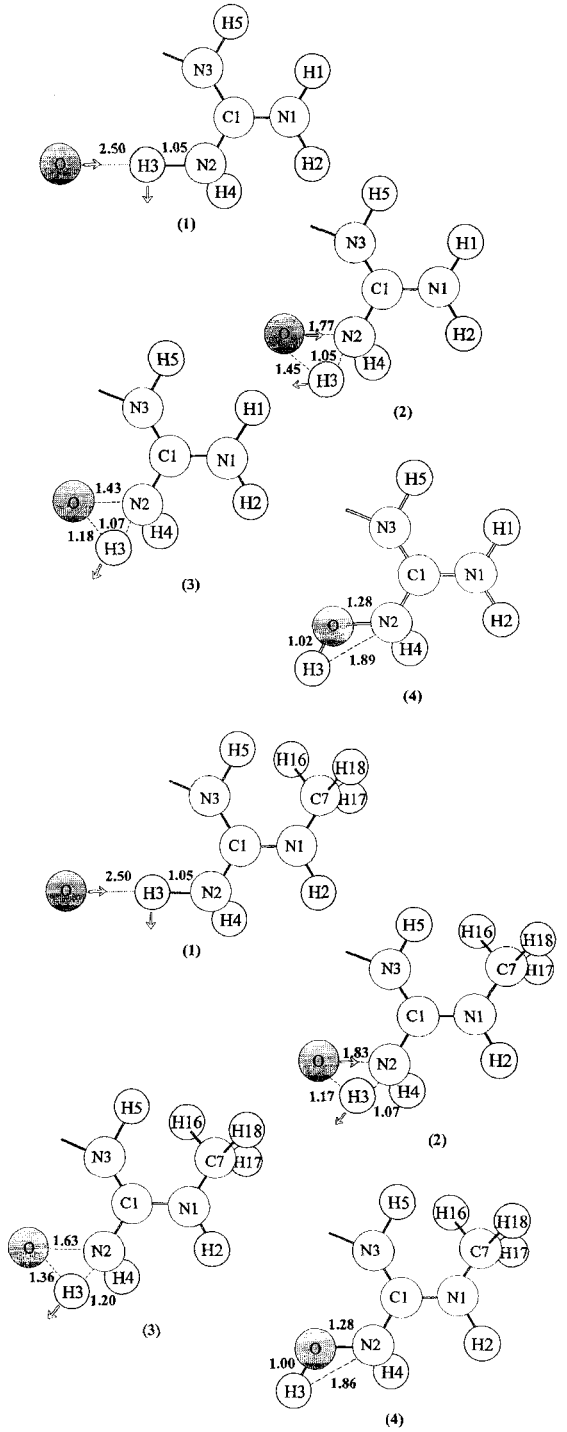


Figure 2. Insertion of atomic oxygen into the N–H bond—snapshots of the changes in the geometry (1–4) in L-arginine (top) and *N*-methyl-L-arginine (bottom) obtained by the INDO method (bond lengths in Å).

Discussion

INDO modelling supports the proposed mechanism of conversion of L-arginine into hydroxyarginine proceeding through oxygen insertion. The results of this study show that the N–H bond, rather than the C–H bond, is preferred as the site of the insertion of oxygen. N–H bond cleavage is preceded by an increase in the coordination number of N from 3 to 4. The mechanism is similar to that of hydrocarbon hydroxylation recently reported to proceed via pentacoordinated carbon species, catalyzed among others by the $[FeO]^{3+}$ active centre of cytochrome P-450.[31, 32]

When considering the possible mechanism of insertion of oxygen originating from the $[FeO]^{3+}$ of the NO synthetase, the electrophilic character of the reactive centre must be taken into account.[14] A mechanism involving electrophilic attack of $[FeO]^{3+}$ at L-arginine is supported by the fact that N1 and N2 have high negative charges (Table 2) and by the fact that monoatomic and not diatomic oxygen species effectively lead to insertion. However, in this type of mechanistic pathway the highest occupied orbitals must be involved, and the INDO calculations showed that not only the HOMO, but also other occupied orbitals of similar energy are localized mainly at the carboxylic group (Table 3). The LUMO orbitals are almost completely localized at the guanidine centre. In view of the above, and excluding the hypothesis of hydroxylation by nucleophilic attack, two possibilities have to be considered: 1) The isolated arginine molecule is not an adequate model of the real system, or 2) electrophilic $[Fe-O]^{3+}$ attack is preceded by the reduction of L-arginine.

To check the first of these hypotheses, the effect of bond formation on the characteristics and energy of the frontier orbitals was investigated. No particular effect was observed as a result of protonation or dimerization involving the amino-acid group. In all cases the contribution of the guanidine group to the HOMO orbital of the product was negligible. Therefore, it was concluded that either the real environment interacts with arginine much more strongly than assumed here, or bonding by the amino-acid group has no real effect on the frontier orbital

© VCH Verlagsgesellschaft mbH, D-69451 Weinheim, 1997 0947-6539/97/0304-0612 $ 17.50+.50/0 Chem. Eur. J. 1997, 3, No. 4

scheme, that is, the model based on the isolated molecule is adequate to interpret the mechanism of NO biosynthesis.

We now turn to the second hypothesis. It is highly unlikely that electrophilic attack on the carboxylic group of the L-Arg^+ cation would result in the insertion of oxygen into the guanidine group. Moreover, for such an electrophilic attack, an agent would be required to mediate the interactions between the two positive centres. A more likely hypothesis is that the hydroxylation accompanied by overall nitrogen oxidation (Scheme 1a) is initiated by the reduction of L-arginine. In the reduction product L-Arg^-, the HOMO orbitals are localized at the guanidine group (Table 3) and are thus set up for electrophilic attack by the $[FeO]^{3+}$ centre. This hypothesis is supported by the fact that reducing and electron-transferring cofactors are present and cooperate with NO synthetase. The conversion of arginine to *N*-hydroxyarginine (Figure 3) should then start with reduction of

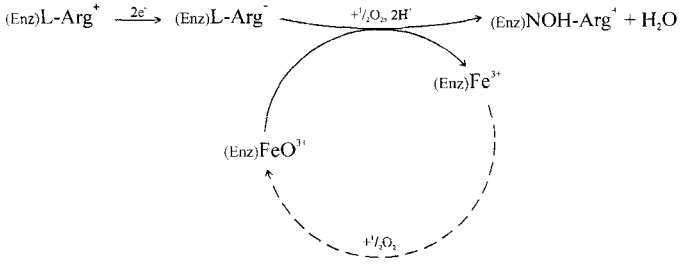


Figure 3. Scheme of the conversion of L-arginine (L-Arg^+) to *N*-hydroxy-L-arginine (NOH-Arg^+) in the biological system investigated.

(Enz)Arg^+ followed by electrophilic attack by the (Enz)$[FeO]^{3+}$ group of cytochrome resulting in insertion of oxygen and production of *N*-hydroxyarginine. The actual electronic structure of the attacking oxygen atom bound to the Fe centre has not been precisely determined; it should, however, be within the two limiting cases calculated here, namely, that of the neutral oxygen atom and the O^{2-} anion, which were both shown to undergo insertion. The (Enz)Fe^{3+} species formed may be a source of the (Enz)FeO^{3+} electrophilic centre[11, 19, 20] or may take part in the second stage of the nitric oxide biosynthesis (Scheme 1b).[2, 12, 14] These stages are not the subject of this paper and are assumed to proceed according to the mechanisms suggested earlier.[14]

Conclusions

The mechanism of L-arginine hydroxylation derived from INDO calculations (Figure 3) substantiates many experimental findings, such as, the electrophilic attack by the heme $[Fe-O]^{3+}$ group, the insertion of oxygen into the guanidine N–H bonds, the activity of the dimeric form of the NO synthetase[18] and the role of reducing agents and electron transmitters in endogeneous nitric oxide production.

The fact that similar results were obtained for *N*-methylarginine indicates that the *N*-substitution at L-arginine is of no importance for the first step of the NO biosynthesis.

Acknowledgement: Financial support from KBN (grant no. 0976/P-3/93/04) and from COST/PECO (grant no. D3/0005/93) is very gratefully acknowledged.

Received: October 19, 1996 [F498]

[1] J. S. Stamler, D. J. Singel, J. Loscalzo, *Science* **1992**, *258*, 1898.
[2] P. L. Feldman, O. W. Griffith, D. J. Stuehr, *Chem. Eng. News* **1993**, *71*, 26.
[3] A. R. Butler, D. Lyn, H. Williams, *Chem. Soc. Rev.* **1993**, *22*, 233.
[4] Y. Henry, C. Ducrocq, J. C. Drapier, D. Servent, C. Pellat, A. Guissani, *Eur. Biophys. J.* **1991**, *20*, 1.
[5] R. Iyengar, D. J. Stuehr, M. A. Marletta, *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 6369.
[6] M. J. Palmer, D. S. Ashton, S. Moncada, *Nature* **1988**, *333*, 664.
[7] N. S. Kwon, C. F. Nathan, C. Gilker, O. W. Griffith, D. E. Matthews, D. J. Stuehr, *J. Biol. Chem.* **1990**, *265*, 13442.
[8] D. J. Stuehr, N. S. Kwon, C. F. Nathan, O. W. Griffith, P. L. Feldman, J. Wiseman, *J. Biol. Chem.* **1991**, *266*, 6259.
[9] R. A. Pufahl, P. G. Nanjappan, R. W. Woodard, M. A. Marletta, *Biochemistry* **1992**, *31*, 6822.
[10] B. Mayer, M. John, B. Heinzel, E. R. Werner, H. Wachter, G. Schultz, E. Bohme, *FEBS Lett.* **1991**, *288*, 187.
[11] T. D. Porter, M. J. Coon, *J. Biol. Chem.* **1991**, *266*, 13469.
[12] M. A. Marletta, *J. Biol. Chem.* **1993**, *268*, 12231.
[13] H. G. Korth, R. Sustmann, C. Thater, A. R. Butler, K. U. Ingold, *J. Biol. Chem.* **1994**, *269*, 17776.
[14] P. L. Feldman, D. J. Stuehr, O. W. Griffith, J. M. Fukuto, in *Biochemical, Pharmacological and Clinical Aspects of Nitric Oxide* (Eds.: B. A. Weissman, N. Allon, S. Shapira) Plenum Press, New York **1995**, pp. 13–20.
[15] B. Mayer, in *Biochemical, Pharmacological and Clinical Aspects of Nitric Oxide* (Eds.: B. A. Weissman, N. Allon, S. Shapira) Plenum Press, New York **1995**, pp. 37–48.
[16] O. W. Griffith, in *Biochemical, Pharmacological and Clinical Aspects of Nitric Oxide* (Eds.: B. A. Weissman, N. Allon, S. Shapira) Plenum Press, New York **1995**, pp. 21–36.
[17] D. J. Stuehr, O. W. Griffith, *Adv. Enzymol. Relat. Areas Mol. Biol.* **1992**, *65*, 287.
[18] K. D. Kröncke, K. Fehsel, V. Kolb-Bachofen, *Biol. Chem. Hoppe-Seyler* **1995**, *376*, 327.
[19] F. P. Guengerich, *J. Biol. Chem.* **1991**, *266*, 10019.
[20] M. Akhar, J. N. Wright, A. Z. Shyadehi, P. Robichaud, *Pure. Appl. Chem.* **1994**, *66*, 2387.
[21] J. Ridley, M. C. Zerner, *Theoret. Chim. Acta* **1973**, *32*, 111.
[22] A. D. Bacson, M. C. Zerner, *Theoret. Chim. Acta* **1979**, *53*, 21.
[23] M. C. Zerner, G. H. Loew, R. F. Kirchner, U. T. Mueller, *J. Am. Chem. Soc.* **1980**, *102*, 589.
[24] W. D. Edwards, M. C. Zerner, *Theoret. Chim. Acta* **1987**, *72*, 347.
[25] M. Witko, K. Herman, R. Tokarz, *J. Electr. Spectr. and Related Phenomena* **1994**, *69*, 89.
[26] M. Witko, *Cat. Today*, **1996**, *32*, 89.
[27] W. D. Edwards, B. Weiner, M. C. Zerner, *J. Am. Chem. Soc.* **1986**, *108*, 2196.
[28] W. D. Edwards, B. Weiner, M. C. Zerner, *J. Phys. Chem.* **1988**, *92*, 6188.
[29] K. K. Stavrev, M. C. Zerner, *Chem. Eur. J.* **1996**, *2*, 83.
[30] R. A. Engh, R. Huber, *Acta Crystallogr.* **1991**, *A47*, 392.
[31] A. F. Shestakov, A. E. Shilov, *J. Mol. Cat. A: Chem.* **1996**, *105*, 1.
[32] B. Wilkinson, M. Zhau, N. D. Priestley, H. T. Nguyen, H. Morimoto, P. G. Williams, S. I. Chan, H. G. Floss, *J. Am. Chem. Soc* **1996**, *118*, 921.