SUPPORTING INFORMATION

Role of Arginine Guanidinium Moiety in Nitric Oxide Synthase Mechanism of Oxygen Activation

Claire Giroud*, Magali Moreau*, Tony A. Mattioli[†], Véronique Balland[Φ], Jean-Luc Boucher*, Yun Xu*, Dennis J. Stuehr, and Jérôme Santolini[†]

[†] iBiTec-S; LSOD, C. E. A. Saclay; 91191 Gif-sur-Yvette Cedex; France

*UMR 8601 CNRS, University Paris Descartes, 45 rue des Saints Peres, 75270 Paris, France

Lerner Research Foundation, Cleveland Clinic, Cleveland, OH, USA

[Φ]Laboratoire d'Electrochimie Moléculaire, University Paris Diderot, UMR 7591, 15 rue J.-A. de Baïf, 75205 Paris cedex 13, France

Corresponding author: Jérôme Santolini. iBiTec-S; LSOD, C. E. A. Saclay; 91191 Gif-sur-Yvette Cedex; France. Fax: 33 - 1 - 69088717. Email: jerome.santolini@cea.fr

Figure S1: Effect of L-Arg analogues binding on Fe^{III} iNOSoxy resonance Raman spectra. Panels A and B display the low- and high-frequency regions of RR spectra, respectively, of $Fe^{III}iNOS_{oxy}$ in the presence of representative compounds. In panel A, curves were fitted to Gaussian functions. Experiments were achieved in the presence of H_4B, except for the (-/-) experiment that was achieved in the absence of both analogue and H_4B. Excitation wavelength was 363.8 nm and full protocol is described under Experimental Section.

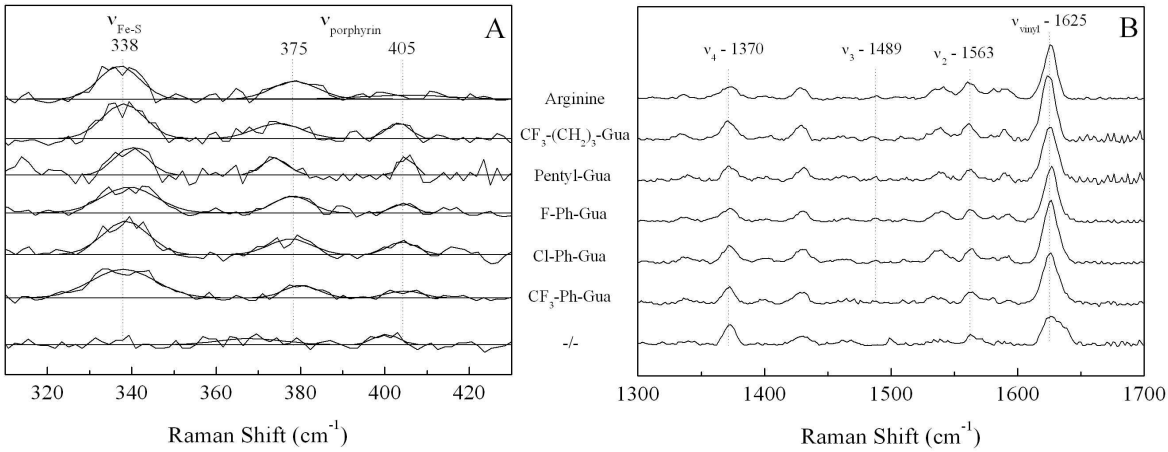

Figure S2: Effect of L-Arg analogues binding on iNOSoxy $Fe^{II}CO$ resonance Raman spectra. Panels A and B display the low- and high-frequency regions of resonance Raman spectra, respectively. Excitation wavelength was 441.6 nm. Experiments were achieved in the presence of H_4B, except for the (-/-) experiment that was achieved in the absence of both L-Arg analogues and H_4B as described under Experimental Section. Stars denote peaks associated with photo-dissociation.

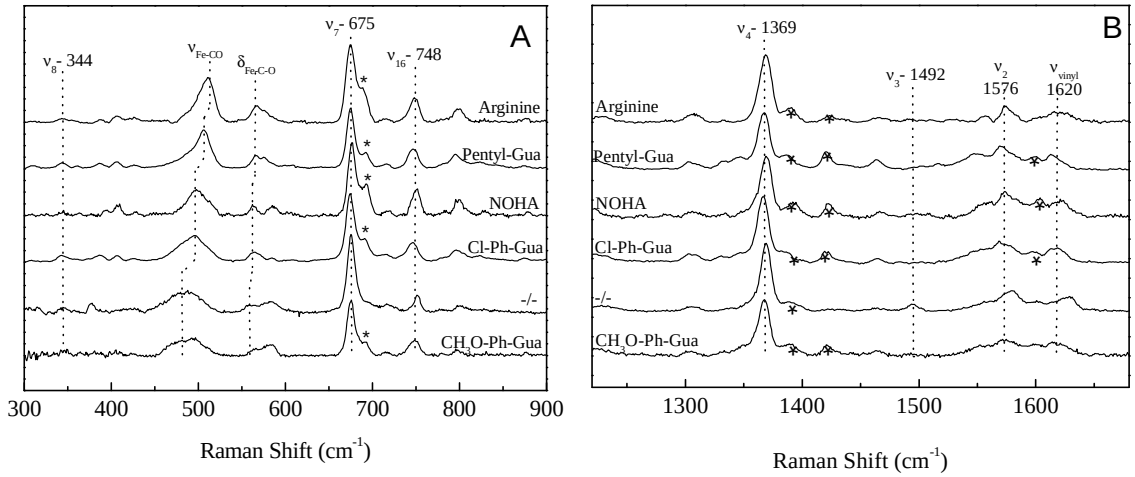

Table S1: pK_a values of L-Arg and various guanidines measured in water at 25°C. Detailed protocol is described under Experimental Section.

Compound	field parameter σ_I	pK_a measured	pK_a estimated	Ref
L-Arg	0.03	12.48		(52)
Pentyl-Gua **3**	0.01	-	12,6	(a)
CH_2F-$(CH_2)_3$-Gua **2**	0.05	-	12,1	(a)
CF_3-$(CH_2)_3$-Gua **1**	0.07	-	11,8	(a)
Cyclopropyl-Gua **4**	0.07	-	11,8	(a)
Ph-Gua	0.12	10.8		(a)
Ph-Gua		10.77		(49)
CH_3OPh-Gua **5**	0.13	11.0		(a)
FPh-Gua **6**	0.17	10.8		(a)
ClPh-Gua **7**	0.18	10.3		(a)
CF_3Ph-Gua **8**	0.19	10.0		(a)
NO_2Ph-Gua **9**	0.26	9.3		(a)
NO_2Ph-Gua **9**		9.13		(62)

(a) : this work

Table S2: Heme midpoint potentials of iNOSoxy in the presence of various guanidines.
All experiments were achieved in the presence of H_4B as described under Experimental Section.

Compound	λ_{Soret} (nm)	$E^{0'}$ (mV) *vs* NHE	Ref
L-Arg	397	- 270 ± 5	(a)
L-Arg	396	- 263	(63)
CF_3-$(CH_2)_3$-Gua **1**	395	- 265 ± 5	(a)
CH_2F-$(CH_2)_3$-Gua **2**	397	- 267 ± 5	(a)
Cyclopropyl-Gua **4**	398	- 269 ± 5	(a)
CH_3OPh-Gua **5**	399	- 269 ± 5	(a)
FPh-Gua **6**	398	- 262 ± 5	(a)
SEITU	396	< - 305	(a)
SEITU	398	- 322	(63)
L-NAME	400	- 500 < E°' < - 305	(a)
L-NAME	400	< - 460	(63)

(a) : this work

Table S3: Porphyrin and Fe-S vibration modes of ferric iNOSoxy in the presence of various L-Arg analogues. NOS experiments were achieved in the presence of H_4B, except for the (-/-) experiment which was achieved in the absence of both analogues and H_4B. Data were obtained with a laser excitation at 363.8 nm as described under Experimental Section. Frequencies are reported in cm^{-1}.

Protein	L-Arg analogues	ν_{Fe-S}	γ_{12}	ν_7	ν_{16}	ν_4	ν_3	ν_2	ν_{vinyl}	Ref
iNOSoxy	L-Arg	337	498	676	754					(65)
	L-Arg	337	495	675	753	1372	1489	1562	1625	(a)
	CF_3-$(CH_2)_3$-Gua **1**	337	498	674	753	1371	1486	1562	1624	(a)
	Pentyl-Gua **3**	339	499	675	753	1372	1487	1562	1625	(a)
	F-Ph-Gua **6**	339	498	675	754	1372	1488	1562	1627	(a)
	Cl-Ph-Gua **7**	339	498	675	751	1371	1486	1560	1625	(a)
	CF_3-Ph-Gua **8**	338	495	675	752	1372	1487	1563	1626	(a)
	-/-	nd	496	676	751	1373	-	1562	1626	(a)
eNOS	L-Arg	338		678		1370	1489	1563	1625	(64)
bsNOS	L-Arg	342	498	676	754					(65)
P450	Camphor	351		677	756	1368	1488	1570	1623	(67)

(a) : this work.

Table S4: Porphyrin vibration modes for iNOSoxy Fe^{II}-CO complexes in the presence of L-Arg analogues. Detailed protocol is described under the Experimental Section. All experiments were achieved in the presence of H_4B, except for the (-/-) experiment that is achieved in the absence of both L-Arg analogues and H_4B. Data were obtained with a laser excitation at 441.6 nm. Frequencies are given in cm^{-1}.

Protein	Compound	ν_8	ν_7	ν_{16}	ν_4	ν_3	ν_2	ν_{10}	ν_{vinyl}	Ref
iNOSoxy	L-Arg	344	674	748	1369	1492	1576	1601	1620	(a)
	3	340	678	750	1367	1463	1569	1600	nd	(40)
	1	340	678	750	1367	1463	1569	1600	nd	(40)
	4	nd	675	748	1367	1492	1573	1597	1617	(a)
	NOHA	344	676	750	1369	1491	1575	1603	1622	(a)
	6	340	678	750	1367	1463	1569	1600	nd	(40)
	7	340	678	750	1367	1463	1569	1600	nd	(40)
	8	343	676	750	1368	1492	1575	1600	1622	(a)
	-/-	345	675	751	1369	1495	1579	1601	1628	(a)
	5	346	675	748	1367	nd	1575	1598	1617	(a)
	9	345	676	749	1368	nd	1576	1599	1619	(a)
nNOS	L-Arg	345	676	752	1369	1493	1572	1602	1618	(58)
	NOHA	345	676	751	1369	1493	1572	1602	1618	(58)
	-/-	343	676	751	1369	1493	1572	1602	1618	(58)
saNOS	L-Arg	344	676	751	1370	1495	1573	1603	1622	(55)
	-/-	344	676	751	1370	1489	1573	1600	1626	(55)
P450	Camphor	352	676	749	1371	1497	1588	nd	nd	(67)
	-	348	677	754	1372	1498	1583	nd	1626	(67)

(a) : this work.