11504 *Inorg. Chem.* **2009**, *48*, 11504–11506
DOI: 10.1021/ic9018376

Inorganic Chemistry
Communication

The Side-On Copper(I) Nitrosyl Geometry in Copper Nitrite Reductase Is Due to Steric Interactions with Isoleucine-257

Anna C. Merkle and Nicolai Lehnert*

Department of Chemistry, University of Michigan, 930 North University Avenue, Ann Arbor, Michigan 48109-1055

Received September 17, 2009

Density functional theory calculations were used to investigate the binding mode of copper(I) nitrosyl (Cu(I)−NO) in copper nitrite reductase (CuNIR). The end-on Cu(I)−NO geometry (**2**) was found to be the global energy minimum, while the side-on binding mode (**1**) corresponds to a local minimum. Isoleucine-257 severely interacts sterically with the Cu(I)−NO unit when bound end-on but not in the side-on case. In addition, the side-on geometry is also stabilized by a hydrogen bond between aspartic acid-98 and NO, estimated to be ∼3 kcal/mol. The steric constraint of the CuNIR active site is mainly responsible for the observed side-on coordination of NO in the CuNIR crystal structure. We speculate that a small conformational change of the active site that slightly changes the position of isoleucine-257 would allow NO to bind end-on. This explains the observed end-on binding of NO to copper(I) when CuNIR is in solution.

The reduction of nitrite (NO_2^-) to nitric oxide (NO) is catalyzed by two classes of nitrite reductases (NIRs) utilizing either iron or copper. Copper nitrite reductase (CuNIR) is a trimer with two copper sites per subunit, a type 1 copper site for electron transfer, and a type 2 copper center where NO_2^- is reduced to NO.[1] In the resting state, the type 2 copper is bound to three histidines and a water molecule to form a tetrahedral copper(II) complex. Aspartic acid (Asp) forms a hydrogen bond to the water molecule and is believed to facilitate the reduction of NO_2^- to NO.[1] Under conditions of elevated NO concentrations, CuNIR is also believed to function as a NO reductase. Here, a copper(I) nitrosyl (Cu(I)−NO) complex is likely catalytically active. The Cu(I)−NO binding mode in CuNIR was initially inferred from model complexes using tris(pyrazol)borate type ligands, where CuNO end-on angles of 160−175° were observed.[2] It was therefore a great surprise when side-on bound NO (Cu−N−O angles: 67.4° and 70.7°) was discovered in CuNIR crystal structures.[3] The side-on binding mode was believed to be promoted by a hydrogen bond of NO with Asp-98 along with interactions with Ile-257 and His-255 (Figure 1).[3a] This finding has facilitated much debate on the binding mode of NO in CuNIR in solution.[2b,4−8] Density functional theory (DFT) calculations indicate that Cu(I)−NO is preferentially end-on bound by 3−10 kcal/mol.[2b,6−8] This poses the important question of how the protein active site promotes NO side-on binding as found in the crystal structure or whether this is instead an artifact, in particular because the structure changes to end-on in solution.[5] In previous DFT work, this important question has not been addressed.[4,7,8]

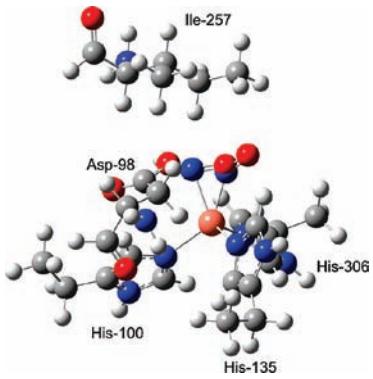

Figure 1. CuNIR active site with side-on bound NO (**1**), overlaid with the end-on bound structure (**2**). In these cases, only NO was optimized (see text).

*To whom correspondence should be addressed. E-mail: lehnertn@umich.edu.

(1) Wasser, I. M.; de Vries, S.; Moënne-Loccoz, P.; Schröder, I.; Karlin, K. D. *Chem. Rev.* **2002**, *102*, 1201.
(2) (a) Ruggiero, C. E.; Carrier, S. M.; Antholine, W. E.; Whittaker, J. W.; Cramer, C. J.; Tolman, W. B. *J. Am. Chem. Soc.* **1993**, *115*, 11285. (b) Fujisawa, K.; Tateda, A.; Miyashita, Y.; Okamoto, K.; Paulat, F.; Praneeth, V. K. K.; Merkle, A.; Lehnert, N. *J. Am. Chem. Soc.* **2008**, *130*, 1205.
(3) (a) Tocheva, E. I.; Rosell, F. I.; Mauk, A. G.; Murphy, M. E. P. *Science* **2004**, *304*, 867. (b) Antonyuk, S. V.; Strange, R. W.; Sawers, G.; Eady, R. R.; Hasnain, S. S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12041.
(4) Wasbotten, I. H.; Ghosh, A. *J. Am. Chem. Soc.* **2005**, *127*, 15384.
(5) Usov, O. M.; Sun, Y.; Grigoryants, V. M.; Shapleigh, J. P.; Scholes, C. P. *J. Am. Chem. Soc.* **2006**, *128*, 13102.
(6) (a) Ghosh, S.; Dey, A.; Usov, O. M.; Sun, Y.; Grigoryants, V. M.; Scholes, C. P.; Solomon, E. I. *J. Am. Chem. Soc.* **2007**, *129*, 10310. (b) Ghosh, S.; Dey, A.; Sun, Y.; Scholes, C. P.; Solomon, E. I. *J. Am. Chem. Soc.* **2009**, *131*, 277.
(7) Silaghi-Dumitrescu, R. *J. Inorg. Biochem.* **2006**, *100*, 396.
(8) (a) Sundararajan, M.; Surendran, R.; Hilliers, I. H. *Chem. Phys. Lett.* **2006**, *418*, 96. (b) Periyasamy, G.; Sundararajan, M.; Hilliers, I. H.; Burton, N. A.; McDouall, J. J. W. *Phys. Chem. Chem. Phys.* **2007**, *9*, 2498.

Published on Web 11/25/2009

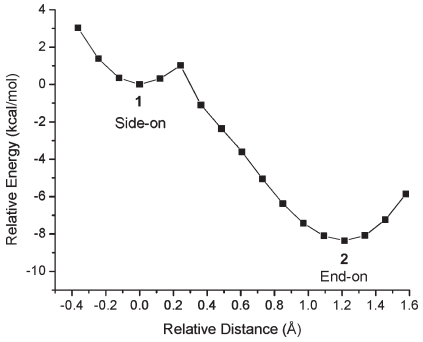

Figure 2. PES scan connecting **1** and **2**. Here, the N atom of NO was moved along a linear path connecting structures **1** and **2**.

Table 1. Geometric Parameters of the Optimized Structures

structure[a]	energy [kcal/mol]	Cu−N−O [deg]	hydrogen bond [Å]
1(X-ray)	+25.2	67.4	1.77
1	0	76.5	2.13
2	−8.4	133.9	2.79
3	−10.8	137.3	
4	−16.1	131.6	2.42

[a] For a detailed explanation, see the Supporting Information.

Here, we present a DFT (BP86/TZVP) study on the side-on and end-on binding modes of NO in CuNIR to investigate this point in detail. Our model of the CuNIR active site is based on the NO-bound crystal structure,[3a] which includes amino acid residues His-100, His-135, His-306, Asp-98, and Ile-257 (see Figure 1). Details of the model are available in the Supporting Information. Starting from the exact crystal structure, model **1(X-ray)**, we optimized the NO ligand to determine whether the DFT calculations would reproduce the side-on binding mode observed experimentally. To our surprise, the optimization resulted in a side-on bound NO (Cu−N−O angle: 76.5°). The resulting structure, **1**, corresponds to a local minimum on the potential energy surface (PES). This is confirmed by a PES scan (Figure 2), which shows an energy barrier of +1.0 kcal/mol to change the geometry from side-on to end-on. This is in agreement with DFT results for a model complex, where a similar barrier was calculated.[8b] *This result shows that the side-on Cu(I)−NO structure, in fact, exists as a local minimum.* As we continue along the PES scan path, the nitrosyl snaps to the end-on bound structure **2**, producing *a global minimum*, 8.4 kcal/mol lower than that of **1** (see Table 1). This number is again in agreement with previous DFT work (see above).[2b,6,8] We then performed further DFT calculations on **2**, which identified additional end-on structures that are generally 6−8 kcal/mol lower in energy than **1** (see the Supporting Information). The differences in energies and structures are due to the movement of NO around the active site, localizing in distinct pockets around Ile-257, as indicated in Figure 3. *This clearly shows that end-on-bound NO is sterically restricted by Ile-257, which therefore is a key player in determining the CuNO geometry*. Usov et al. speculated, on the basis of ENDOR experiments, that NO experiences a noncovalent perturbation by the Ile bulky side chain in the end-on bound structure in solution.[5] The closeness of NO and the Ile-257 protons in the calculated end-on structures in Figure 3 are in agreement with this idea. In contrast, the steric interaction in the side-on geometry is minimal: the removal of Ile-257 from model **1** and reoptimization of NO lead to a slightly increased Cu−N−O angle of 84°.

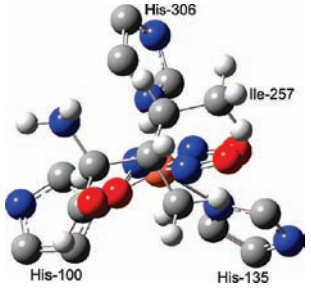

Figure 3. Different energy minima with end-on bound NO in the active site of CuNIR.

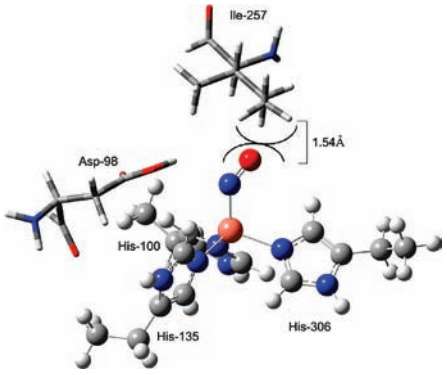

Figure 4. Structure **3**, optimization of NO without Ile-257 and Asp-98. Ile and Asp are shown in tube form in their crystallographic positions. The presence of Ile prevents this intrinsically preferred end-on orientation because of an unfavorable steric interaction.

The removal of Ile-257 and Asp-98 from the active site model **1** in Figure 1 and reoptimization of NO resulted in the end-on structure **3**, 10.8 kcal/mol lower in energy than **1** (Figure 4). *Overall, end-on binding of NO is therefore intrinsically more favorable than side-on coordination, even in the Cu(His)$_3$ motif in the CuNIR active site*. If the obtained NO orientation in **3** is incorporated into the complete active site model **1**, the energy increases to +14 kcal/mol relative to **1** due to severe steric interactions with Ile-257, again emphasizing the directing role of Ile-257 for the NO orientation. In structure **1** and the related structures in Figure 3, the energy gain for end-on binding is reduced from 10.8 to 6−8 kcal/mol (Figure 3), in part reflecting the steric congestion of the CuNIR active site due to Ile-257.

Tocheva et al. speculated that Asp-98 stabilizes the side-on geometry via a hydrogen bond.[3a] Periyasamy et al. also state that Asp-98 is crucial in the formation of the side-on bound complex.[8b] To explore the possible role of this hydrogen bond, we calculated its total energy using structure **1** and formic acid as a model, *resulting in a total hydrogen bond energy of only 3.3 kcal/mol*. This energy is too small to counteract the ∼8 kcal/mol energy gain for the side-on to end-on transition and, hence, this cannot be the main reason for the experimentally observed side-on Cu−NO structure. In addition, because the hydrogen bond length increases only by ∼0.7 Å from side-on to end-on, the total change in the hydrogen bond energy is only 1−2 kcal/mol. The hydrogen bond is therefore *not* the deciding factor for side-on binding. However, this hydrogen bond is key for the generation of the local energy minimum for the side-on structure; the removal

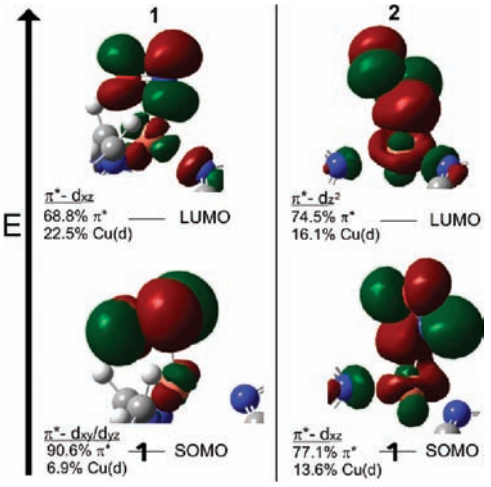

Figure 5. Visualization of the SOMOs and LUMOs of **1** and **2**.

of Asp-98 in **1** and reoptimization of NO, in fact, generate an end-on structure. In this way, *Asp-98 assists in but does not cause the side-on coordination of NO*. Tocheva et al. also proposed that the hydrogen bond with Asp-98 determines the orientation of NO such that its N atom points toward Asp-98.[3a] However, we found that inverting the orientation of NO gave an energy change of −0.80 kcal/mol relative to **1**, with the lowest-energy geometry actually being opposite to Tocheva et al.'s proposed structure. The small energy difference suggests that both orientations could be present in the crystal (see also ref 8). *In summary, the hydrogen bond will not orient NO in the CuNIR active site nor cause the observed side-on binding*.

Having ruled out the hydrogen bond causing the side-on binding, we investigated the effect of the histidine orientation on the binding mode. Starting from structure **3** and using the initial side-on (from **1**, **1-His**) or optimized end-on orientation of NO (**3-His**), we optimized the histidines with the CuNO units frozen, leading to an energy gain of 9.6 and 9.3 kcal/mol, respectively, for the side-on and end-on orientations. Interestingly, both cases produce the same histidine movement (see the Supporting Information, Figure S15). *This demonstrates that the histidine orientation in CuNIR does not discriminate between side-on and end-on binding*.

Finally, optimizing NO and the histidines in structure **1** while keeping the other atoms fixed causes an energy gain of 16.1 kcal/mol, giving the end-on structure **4**. This energy difference can be incrementally calculated from the previous results, using (i) the energy difference between end-on and side-on binding of NO (−8.4 kcal/mol), (ii) the energy gain from the histidine movement (−9.3 kcal/mol), and (iii) the loss of hydrogen bonding (about +1 kcal/mol). Adding up these numbers, we predict an energy of −16.7 kcal/mol for structure **4** relative to **1**, close to the calculated energy difference. Hence, the values i−iii represent incremental energy changes in the CuNIR active site.

The DFT results allow us to also analyze the electronic structures in the different binding modes of NO. The side-on structure exhibits a spin density profile similar to those presented in refs 4 and 8b. The singly occupied molecular orbital (SOMO) of the complex shown in Figure 5, left, has 91% π* character with a 7% metal d admixture, forming a δ-type bond. The lowest unoccupied molecular orbital (LUMO) represents a classic π-backbond between Cu and NO. In the end-on structure, the SOMO has 77% π* and 14% Cu d character (cf. Figure 5, right), forming a π bond. The LUMO corresponds to a somewhat unusual π-backbond mediated by d_{z^2}, which differs from the model complexes.[2b] This interesting difference may cause the discrepancy in the Cu−N−O angles, where the predicted end-on Cu−N−O angle in CuNIR of 134° is distinctly smaller than that observed in the model complexes (160−175°).[2] In both the side-on and end-on cases, the electronic structure is clearly of the Cu(I)−NO(radical) type rather than a spin-coupled Cu(II)−NO^- system, in agreement with refs 4 and 8b. The weaker Cu−NO bond in the side-on case is due to a reduction in backbonding, caused by the weak orbital overlap of the δ bond. In this way, the side-on structure mediates an overall weaker Cu−NO backbond and, hence, a lower binding energy of NO.

In summary, using DFT calculations, we were able to determine (i) that the Cu−NO side-on structure observed in CuNIR corresponds to a local minimum while (ii) the end-on structure is 6−8 kcal/mol more stable, (iii) that Ile-257 determines the orientation of NO in the CuNIR active site, (iv) that the hydrogen bond is only worth about 3 kcal/mol, which assists in stabilizing the side-on form, and finally (v) that the histidine movement is similar for the side-on and end-on geometries. Therefore, our results point toward Ile-257 being the predominate amino acid to affect the side-on binding as compared with the literature stating Asp-98 as the major contributor.[3a,8]

On the basis of these results, we believe that the side-on structure found in the protein is largely due to steric interactions with Ile-257. This destabilizes the end-on structure relative to the side-on structure. In addition, Figure 3 only shows a static picture with a "frozen" Ile-257; however, in the protein, the dynamics of Ile motion and internal vibrations must be considered. Under these conditions, the effective space demand of Ile-257 will further increase. This likely causes the observed side-on geometry in the crystal structure, where the overall orientation of the protein side chains must therefore be strongly restricted. Correspondingly, a small change in the conformation in solution that slightly reorients Ile-257 would then allow NO to bind end-on as observed in solution for CuNIR and the known model complexes.[2b,5] This is due to the fact that, intrinsically, the end-on structure is always energetically favored. More insight into the dynamics of the CuNIR active site will require molecular dynamics simulations of crystalline CuNIR.

Acknowledgment. We thank the Dow Corning Corp. for financial support of this research.

Supporting Information Available: Computational details and tables of optimized Cartesian coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.