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Communication

DFT Analysis of Interligand Vibrations in a Hydroperoxo Complex of Cobalt Bleomycin

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Density functional theory (DFT) has been applied to the analysis of interligand vibrations in two chiral isomers of hydroperoxo complex of cobalt bleomycin (BLM–Co^{III}–OOH, BLM = bleomycin). The DFT-based normal coordinate analysis reveals that $^{16}O/^{18}O$ isotope-sensitive modes associated with the Co–OOH moiety uniquely reflect the chiral organization of ligands around the cobalt atom. This study provides an independent probe of cobalt chirality coordinated to BLM and shows that interligand modes associated with the Co–OOH moiety could be used as a structural marker of the chiral isomers.

While it is generally accepted that the hydroperoxo complex of iron bleomycin (BLM−Fe^{III}−OOH, referred also as "activated" bleomycin) is the key intermediate involved in DNA scission,[1−6] disagreements exist concerning whether the mechanism of the O−OH bond cleavage is homolytic, heterolytic, or involves direct hydrogen abstraction.[4,7,8] The paramagnetic nature of BLM−Fe^{III}−OOH, its high reactivity, and its lack of reliable structural data have been among the factors limiting current understanding of molecular mechanisms for the single- and double-strand DNA scission. Therefore, most of the information concerning activated BLM comes from interpretation of spectroscopic data, computational modeling, or studies with other transition metals.[2,5,6] In particular, cobalt diamagnetic analogues have been considered as excellent structural models of activated bleomycin,[9] capable of cleaving DNA, when the drug bound to DNA is irradiated with UV light. More-stable BLM−Co^{III}−OOH models have been studied by NMR techniques in combination with molecular modeling[10] and resonance Raman (RR) spectroscopy.[11] The RR technique is a particularly useful probe of local environment of the Co−OOH moiety in both unbound and DNA-bound complexes.[11]

An open issue is the chiral organization of the ligands around cobalt relative to the organization around iron. The BLM−Co^{III}−OOH structure determined by 2D NMR[9c] compared to crystallographic studies of BLM−Cu^{II}−Cl bound to a resistance protein[12] has established that the chirality and ligands of BLM−Co^{III}−OOH and BLM−Cu^{II}−Cl are very similar. Modeling of BLM−Fe^{III}−OOH bound to DNA with different chiral organization have shown that only one conformer has the OOH group properly oriented toward DNA scission, while the other has the OOH group far removed, more than 6 Å, from the cleavage center. Further considerations lead to the conclusion that it is unlikely for the BLM−Fe^{III}−OOH complex to adopt a different screw sense, which is observed for BLM−Co^{III} and/or BLM−Cu^{II} complexes.[6]

The purpose of this Communication is to investigate the structure and chirality of BLM−Co^{III}−OOH, emphasizing the information that could be obtained from vibrational spectroscopy coupled with computational modeling. Two structural isomers in the screw sense of the BLM−Co^{III}−OOH complex have been investigated on the basis of the

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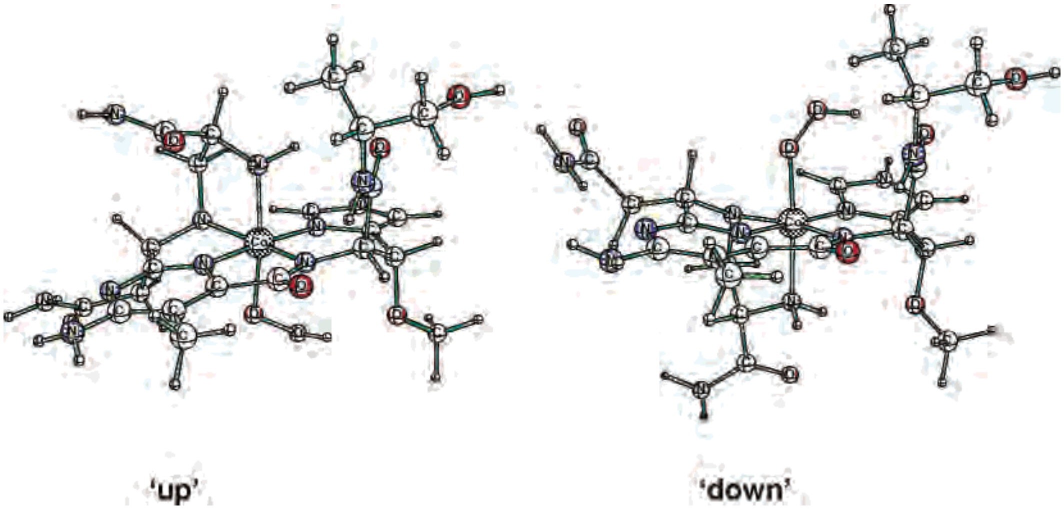

Figure 1. Structural models of BLM$-$Co$^{III}-$OOH corresponding to 'up' and 'down' arrangements obtained at the BP86/6-31G(p) level of theory. The 'down' isomer is referred to the chiral organization of BLM, where the primary amine of the aminoalanine is on the same side as disugar moiety, while 'up' on is opposite.

Umezawa X-ray structure[13] of the copper analogue. Both structures were simplified, retaining the metal binding site of BLM intact, in a similar way as was employed in previous theoretical studies on iron BLM complexes.[14] The structures have been fully optimized using two different functionals, B3LYP and BP86 with the 6-31G(d) basis set as is summarized in the Supporting Information (see Table ST1). The results are consistent, and both B3LYP and BP86 functionals predict that the 'down' conformer is energetically more stable by 1.44 and 2.01 kcal/mol, respectively. While optimized structures are very similar regardless of the theory level applied, the computed O$-$O stretching in BLM$-$Co$^{III}-$OOH models differ noticeably and depend on the functional applied. Specifically, employing the BP86/6-31G(d), the ν(O$-$O) vibration was predicted at 871/870 cm^{-1} for up/down isomers respectively, while for the B3LYP/6-31G(d) the prediction was at 942/934 cm^{-1}. To further investigate this difference in applied functionals, test calculations have been performed for H_2O_2 molecule for which the O$-$O stretch band is experimentally determined at 877 cm^{-1}.[15] It appears that B3LYP functional noticeably overestimates the ν(O$-$O) stretch in H_2O_2 ($\sim$954 cm^{-1}) in comparison to experiment, while BP86 functional is in a much closer range (902 cm^{-1}), which may require only a minor scaling of computed frequencies. Therefore, further analysis of the BLM$-$Co$^{III}-$OOH complexes was based on the vibrational force field corresponding to BP86/6-31G(d) level of theory (see Figure 1 above and Supporting Information for details).

The truncated nature of structural isomers representing the BLM$-$Co$^{III}-$OOH complex (Figure 1) does not permit a direct comparison of simulated Raman spectra with experiment; however, the difference spectra employing the ^{16}O/^{18}O isotope labeling in Co$-$OOH moiety simulated for both 'up' and 'down' conformers are directly comparable with experiment. Such comparison is legitimate because the structural simplifications introduced to computational modeling do not affect the environment of the ligands directly associated with the metal center. Figure 2 summarizes results of calculations for both up and down isomers along with the experimental difference spectrum reproduced from ref 11. To compute these spectra, the Cartesian force constants were transformed to natural internal coordinates and the resulting secular vibrational equation was solved for Co$-^{16}$O^{16}OH and Co$-^{18}$O^{18}OH isotope labeling.

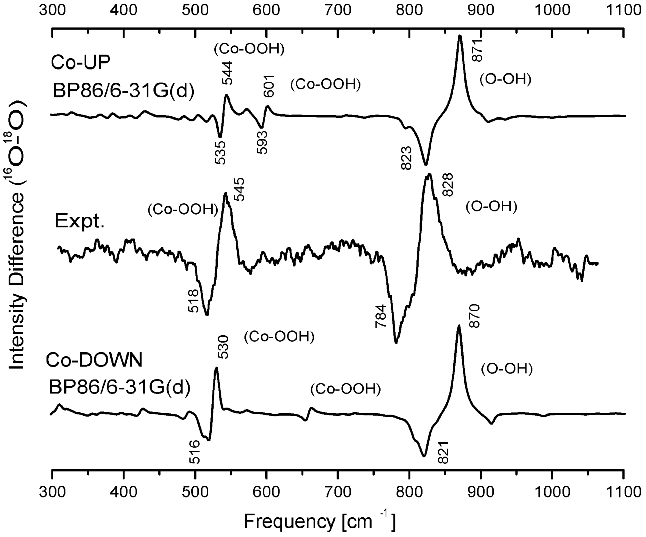

Figure 2. Comparison of experimental Raman difference ^{16}O/^{18}O spectrum (reproduced from ref 11 with permission) with simulated employing 'up' and 'down' structural models from Figure 1. The computed spectra are reported without frequency scaling. It appears that some of the peaks (e.g., ν(Co$-$OOH) stretch for ^{18}O) are not shifted with respect to experiment, while others (e.g., ν(O$-$OH) stretch) show a large shift. Most likely the basis set truncation, the incomplete treatment of correlation energy, and the anharmonicity effects are responsible for different accuracy.

The DFT-based normal coordinate analysis reveals the presence of three isotope-sensitive modes corresponding to O^{16} → O^{18} substitution in the 500$-$900 cm^{-1} spectral range.

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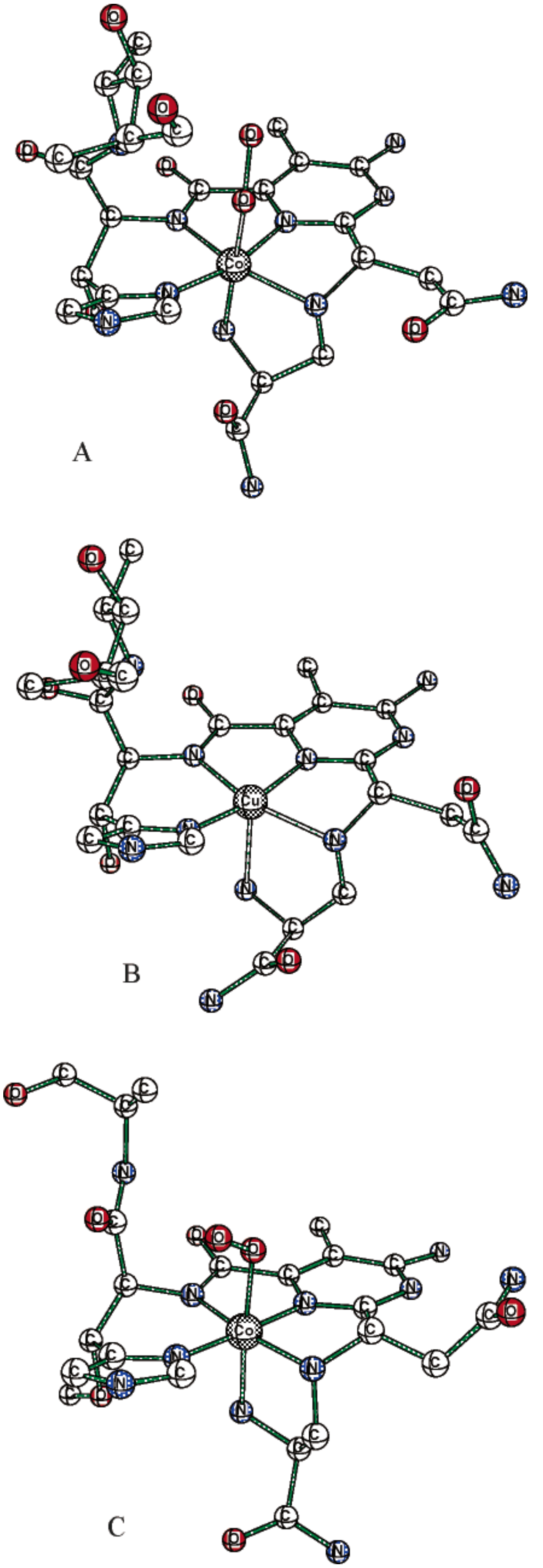

Figure 3. (A) Cobalt coordination domain determined by 2D NMR spectroscopy and (B) copper domain corresponding to Cl−Cu(II)BLM bound to resistance protein. The Cartesian coordinates corresponding to (A) were extracted from PDB file 1GJ2 (ref 9c), while that in (B) corresponds to 1JIF (ref 12). Lower panel (C) represents the 'down' isomer of BLM−Co^{III}−OOH obtained in the present work.

These are described as the ν(O−OH) stretch, the τ(Co−OOH) torsion, and ν(Co−OOH) stretch. The stretch observed at 828 cm^{-1} exhibits 44 cm^{-1} isotope shifts. The unscaled DFT frequencies for both isomers predicted at 871 ('up') or 870 cm^{-1} ('down') with an isotope shift equal to 48 or 49 cm^{-1}, respectively, agree well with experiment (Figure 2). The mode composition shows that no mixing with other vibrations takes place for ν(O−OH) and the contribution from the O−O stretch is dominant and equal to 90%. This mode is entirely localized, and is almost like an ideal harmonic oscillator for the O−O vibration. The situation with the τ(Co−OOH) and ν(Co−OOH) vibrations is quite different. Although the ν(Co−OOH) mode is described as the cobalt−oxygen stretch, the actual contribution is only in the range of 20−30%. Moreover, both ν(Co−OOH) and τ(Co−OOH) modes are inherently coupled due to the presence of an off-diagonal force constant responsible for mixing. The coupling between them can be readily explained by analysis of the Co−OOH moiety that shows that once the Co−O is stretched this structural change has to be accompanied by the Co−OOH torsion (Figure 1). The coupling between them and the extent of mixing depend on the chiral organization of ligands around the cobalt atom that modulate the position of the τ(Co−OOH) band. This leads to the stronger coupling for the 'up' isomer than for the 'down' analogue. The strength of the coupling is clearly pronounced in the difference Raman spectra (Figure 2). The torsion vibration should not be observed in the Raman spectrum and its appearance in the case of the 'up' conformer at 601 cm^{-1} is attributed to the mixing with stretch located at 544 cm^{-1}. In the case of the 'down' isomer, the coupling and extent of mixing is much smaller. Consequently, the torsion is hardly visible in the difference spectrum of 'down' isomer.

The DFT-based analysis of interligand vibrations shows that only the 'down' model of BLM−Co^{III}−OOH can be considered as a valid structural isomer. Direct comparison with simulated Raman data indicates that only the spectrum with two marked isotope-sensitive vibrations matches experimental data. Moreover, comparison with two other structural models, one obtained from 2D NMR analysis[9c] and the second from X-ray,[12] shows that the DFT-based 'down' structural model is fully consistent with the local metal environment reported in previous studies (Figure 3).

Thus, the present study provides an independent probe of cobalt chirality coordinated to BLM, which further can be extended for study with different metals, most importantly with iron.

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Supporting Information Available: One table and five figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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