Characterization of the optically excited state of a bis (µ-oxo)-dicopper(III) species mimicking the hemocyanin and tyrosinase active sites

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Abstract. Optical excited molecules play an increasingly important role in research at light sources. Here we compare two approaches to structurally characterize such states, pumped-XAS and an innovative combination of EXAFS spectroscopy and resonant Raman scattering. The later combination allows to study efficiently charge-transfer complexes in their ground and excited state. The design of the experimental setups for pumped-XAS and resonant Raman scattering at different temperatures as well as results obtained are presented. We receive two-fold information on the structural and electronic properties of both states elucidating the alterations upon induced charge transfer in the Cu₂O₂-core of a system mimicking the active site of tyrosinase and hemocyanin.

1. Introduction

The availability of new photon-sources with pulse lengths in the fs-regime will permit fascinating research on triggerable compounds [1]. The relaxation of excited states into the ground state will provide new insights into the dynamics of the systems under study. Of particular interest to the molecular biological and bioinorganic communities will be the dynamics of metal-donor interactions. Excited states of biomolecules can be made available to the structural and spectroscopic analysis by a variety of strategies, ranging from optical pumping to stabilization of chemical mimics. Here, we focus on a system mimicking the Cu₂O₂-core in hemocyanin and tyrosinas. Hemocyanin serves in many arthropods as an oxygen carrier, similar to hemoglobin in vertebrates. The enzyme tyrosinase is present in plant and animal tissue and shares the dinuclear copper binding site as well as most of the structural features of hemocyanin. In fact, many hemocyanins even exhibit tyrosinase activity [2]. Tyrosinase catalyses the oxidation of phenols, required for the production of melanin and other pigments. This phenol oxidase activity is of considerable interest for industrial applications [3].
Figure 1. Potential Oxo-form (O-state, left) and Peroxo-form (P-state, center) of complexes modelling hemocyanin and tyrosinase active sites. Formation of $[\text{Cu}_2\text{L}_2(\mu-\text{O})_2]\text{I}_2$ (right) upon oxidation of $[\text{Cu(L)}\text{I}]$ with dioxygen at 25 °C monitored by the absorption intensity for $\lambda_{\text{max}}$ (366 nm).

In both metalloproteins molecular oxygen is binding to two copper ions in the side-on peroxo mode (P-state) [4]. Each of these copper ions is stabilised by three histidine groups. Complexes modelling these features have the potential to serve as highly specific oxidation catalysts [3]. In these complexes the biomimetic donor ligand has the function to stabilize the complex after reaction with molecular oxygen either in the P-or O-state: with a peroxo-dianion or two $\mu$-oxo donors bridging the Cu ions, respectively (Figure 1). Typically, these systems are stable at cryogenic temperatures only [5].

Recently, we presented the first system stabilizing the O-state at room temperature [6]. It is formed by the reaction of molecular oxygen with the Cu(I)-complex, $\text{Cu(L)}\text{I}$ with $\text{L}=(\text{B(TMPip)G}_2\text{p})$ [7] within several hours as visualized by the increasing absorption at 366 nm (Figure 1, right). In its ground state the complex comprises an O-state as shown by XAFS data, collected at 20 K in fluorescence mode at beamline D2 (EMBL Hamburg, DESY, Germany) [6]: A Cu-dimer with terminal N-donors at 2.00(4) Å, two bridging $\mu$-oxo donors at 1.93(1) Å and a Cu-Cu distance of 2.84(1) Å could be identified in the EXAFS. Such systems typically can be optically excited by UV-light [5]. Here, we compare strategies to characterize this excited state either by optical-pumping or by resonance Raman spectroscopy.

2. Material and Methods

$[\text{Cu}_2\text{L}_2(\mu-\text{O})_2]\text{I}_2$ has been synthesised as described before [6]. For Raman and EXAFS measurements [8], the following Cu(I) solution was prepared for subsequent oxygenation: 0.05 mmol of $\text{B(TMPip)G}_2\text{p}$ (33 mg) in 5 mL of THF were added under stirring to a solution of 0.05 mmol of $\text{CuI}$ (9 mg) in 5 mL of THF. The resulting concentration is 5 mmol/L, which corresponds to 3.1 g Cu/L.

For optical excitation a custom designed UV-LED source with a 1 mm focal spot has been installed at beamline C (HASYLAB, DESY, Hamburg, Germany). This source is based on a NSSU100A LED (NICHIA, Tokyo, Japan) with optics comprising 3 UV-transparent lenses ($f=9\text{mm}$, $f=100\text{mm}$ and $f=99\text{mm}$)

Figure 2. Spectral distribution of the light emitted by our UV source (left). Setup for the XAS experiments on optical excited solution of $[\text{Cu}_2\text{L}_2(\mu-\text{O})_2]\text{I}_2$ with optics of the UV-source (black), multi-element fluorescence detector, safety shield (Kapton, orange), and UV-excited sample in an Al-sample holder with Mylar windows. (center: in direction of the x-ray beam, right: top view).
Figure 3. Axially symmetrical sketch of the custom-made cuvette for temperature dependent measurements (left). Setup for the temperature dependent Raman experiments on solution of [Cu$_2$L$_2$(µ-O)$_2$]I$_2$ (center: picture of the cuvette, right: top view on the cryostat).

The maximum wavelength at 365nm is in line with the Raman active transition [6]. The resulting total power is 14.5mW, of which 70% are localized in the spot. Cuvettes were sealed with Mylar windows, because it is transparent at this wavelength. Kapton, in contrast, strongly absorbs this UV-light and thus has been used for safety precautions (Figure 2).

Resonant Raman experiments were performed using a custom made UV resonance Raman spectrometer with a completely reflective optics and a cw-laser system (Kr$^+$, 351nm, power at spectrometer entrance optics: 9.6mW) being located in a cleanroom [9]. Because all other compounds mimicking the O-state are unstable at room temperature we compared the quality of Raman spectra collected at different temperatures. A custom-build cuvette was mounted within a liquid helium cryostat (KONTI cryostat Spectro 6111, Cryovac). A Suprasil window defines the sample volume of 200 µl. The solution was inserted through a small hole that was closed by a screw afterwards (Figure 3).

3. Results and Discussion

The ground state of [Cu$_2$L$_2$(µ-O)$_2$]I$_2$ has been characterized by Cu-K edge fluorescence XAS at 20 K (Figure 4, left). To characterize the excited state transmission and fluorescence signals were monitored at 8990 eV upon optical excitation at room temperature. Whereas the transmission increases immediately the fluorescence follows with a delay of a few seconds (Figure 4, center). The reasons for these observations are enigmatic: The UV-light might heat up the sample causing lower absorption of x-rays in the 1mm thick cuvette. Then a small percentage of the Cu-complex precipitated prior to the experiment dissolves at elevated temperatures. Thus, after switching off the UV light the transmission decreases below the starting point. Other differences in the XANES with and without UV-light illumination could not be identified for a variety of concentrations indicating a too low UV-photon intensity. This is in line with the lifetime of the excited state, which is estimated to $\Delta t \approx h/\Delta E \approx h/0.2$ eV $\approx$ 20 fs based on the width of the resonance Raman peaks. At the given UV intensity $\sim$10$^{16}$ photons are generated per second; considering the absorption probability of $\sim$40% in 1mm solution only a small fraction of the 10$^{15}$ Cu ions elucidated by the 1 x 1 mm$^2$ x-ray beam are optically excited when the pulsed synchrotron beam hits the sample. Therefore, the characterization of optical excitation states by XAS might only be feasible with the help of a synchronized, pulsed UV-source. Even in this case the total power absorbed by the sample might cause similar artifacts.

Thus, we looked into a more elegant and instrumentally less demanding approach to elucidate the structural changes induced upon charge-transfer. Resonance Raman does not require full occupancy of the excited state, because only excited molecules contribute. Thus experiments can be performed at lower laser intensities. The Raman spectrum of [Cu$_2$L$_2$(µ-O)$_2$]I$_2$ exhibits strong vibrational excitations that can be assigned to the bending vibration of the copper-oxygen core at 114.3 cm$^{-1}$ and its integer multiples. For a given local symmetry the structural change is calculated from the Huang-Rhys parameter given by the intensity decrease in the Raman spectra [5, 6]. As reported in [6] the copper-oxygen distance increases by +0.01 Å and the oxygen-oxygen distance decreases by -0.13 Å.
Figure 4. left: EXAFS and corresponding FT plus XANES; center: Normalized transmission and fluorescence at 8990 eV; right: Raman spectra of [Cu₂L₂(µ-O)₂]I₂ and THF at 350.7 nm. For data at 293 K and 77 K 10 minutes were given to reach stable temperature. Data around the melting point of THF (165 K) were taken while cooling down (144K/145K). Offset was added for better visualization.

So far, the limited stability of such complexes restricted measurements to cryogenic temperatures. [Cu₂L₂(µ-O)₂]I₂ enables a direct comparison of cryogenic and room temperature conditions. Below the freezing point of the solvent THF (165 K) additional contributions become apart (green curve). These peaks can be assigned to THF (dotted blue line)(Figure 4). Due to the huge relative difference in intensity and similar energy transfers no difference spectra were calculated to avoid artifacts. The induced structural changes show no temperature dependence.

This combination of resonant Raman spectroscopy and X-ray absorption spectroscopy gives direct access to structural and electronic (not shown) parameters of optical excited systems. Whenever the local symmetry of the system allows such an approach, it is experimentally by far less demanding than pumped XAS experiments. Moreover, in future resonance Raman experiments at copper M-edges at free electron laser sources such as FLASH (DESY, Hamburg, Germany) will provide new experimental tools to go even beyond the characterization presented here.

References
[1] Bressler C, Milne C, Pham VT, Elnahhas A, van der Veen RM, Gawelda W, Johnson S, Beaud P, Grölimund D, Kaiser M, Borca CN, Ingold G, Abela R and Chergui M 2009 Science 323 489
Schweigert IV and Mukamel S 2008 J. Chem. Phys. 128 184307
Paulsen H, Grünsteudel H, Meyer-Klaucke W, Gerdan M, Grünsteudel HF, Chumakov AI, Rüffer R, Winkler H, Toftlund H and Trautwein AX 2001 Europ. Phys. J. B 23 463
Decker H, Schweikardt T, Nillius D, Salzbrunn U, Jaenicke E, Tuczek F 2007 Gene 398 183
van Eldik, R and Reedijk J, Advances in inorganic chemistry – homogeneous biomimetic oxidation catalysis, 1 ed., vol. 58, Academic Press, 2006
[4] Magnus KA, Hazes B, Ton-That H, Bonaventura C, Bonaventura J and Hol WG, 1994 Proteins 19 302
[5] Henson MJ, Mukherjee P, Root DE, Stack TDP and Solomon EI 1999 J. Am. Chem. Soc. 121 10332
[6] Herres-Pawlis S, Binder S, Eich A, Haase R, Schulz B, Wellenreuther G, Henkel G, Rübhausen M and Meyer-Klaucke W 2009 Chemistry – A Europ. J. 15 8678
[7] Herres-Pawlis S, Neuba A, Seewald O, Seshadri T, Egold H, Flörke U and Henkel G 2005 Eur. J. Org. Chem. 22 4879
[8] Ascone I, Meyer-Klaucke W and Murphy L 2003 J Synchrotron Radiat. 10 16
[9] Schulz B, Bäckström J, Budelmann D, Maaser R, Rübhausen M, Klein MV, Schoeffel E, Mihill A and Yoon S 2005 Rev. Scient. Instr. 76 073107