Heme Doming in Ferric Cytochrome c: Femtosecond X-ray Absorption and X-ray Emission Studies

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Abstract: The photoinduced dynamics of ferric Cytochrome c was investigated by ultrafast non-resonant X-ray emission (XES) and X-Ray Absorption (XAS) spectroscopies, and a cascade through high spin states accompanied by heme doming are observed for the first time.

1. Introduction

Cytochrome c (Cyt c) is a heme protein found in the mitochondrial membrane and responsible for electron shuttling between complexes II and IV in the electron transport chain [1]. Its active centre consists of a six-coordinated iron porphyrin complex, the heme group, with a histidine (His18) as a proximal ligand that links the heme to the protein peptide chain and a methionine (met80) as a distal ligand (Figure 1a). It exists in both the ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms, and the redox process between the two oxidation states is central to its function. Upon photoexcitation, ferrous heme proteins such as Haemoglobins, Myoglobins and Cyt c undergo distal ligand dissociation, which is followed by the population of anti-bonding Fe d-orbitals, resulting on a high spin excited state and heme doming. The doming process consists of an out-of-plane motion of the Fe atom of the penta-coordinated porphyrin, and its subsequent relaxation back to the planar hexacoordinated ground state are key steps in the respiratory function. [2] In ferric proteins, however, optical spectroscopy experiments, such as transient absorption and time-resolved Raman show no dissociation of the distal ligand, and the relaxation processes have been suggested to proceed entirely via vibrational cooling back to the ground state, [3–5]. Time-resolved X-ray spectroscopy techniques such as X-ray absorption near edge structure (XANES) and non-resonant X-ray emission (XES) are ideally suited to tackle the issue of spin and structural changes in heme proteins, and are in addition, element-selective so that the central Fe atom can be interrogated. In this work, we employ both fs-XANES and fs-XES to elucidate the photoinduced dynamics of ferric Cyt c and we show that, contrary to previously proposed, the relaxation is almost entirely an electronic cascade through spin states, which leads to doming of the hexa-coordinated excited state.

2. Results and Discussion

Ultrafast X-ray spectroscopy techniques require short, ultrabright x-ray pulses, which are only available at Free Electron Laser (FEL) facilities. The experiments presented in this work were carried out at SwissFEL (fs-XANES and fs-XES) and European XFEL (fs-XES). A 4 mM Cyt c solution, delivered by a liquid jet, was photoexcited into the Soret band of the porphyrin (Figure 1b) by sub-80fs pump pulses in the region of 350-400 nm. The XANES was recorded by scanning the monochromatized X-ray beam across the Fe K-edge (7.12 keV) while the XES was obtained by spectrally dispersing the fluorescence of the sample, irradiated by an 8 keV pink beam, with a von Hamos spectrometer to obtain both the Fe Kα and Kβ emission lines. Both sets of measurements were repeated for different pump-probe delays in order to capture the full relaxation pathway of the system and its corresponding kinetic traces. A schematic of the setup is shown in Figure 1c.

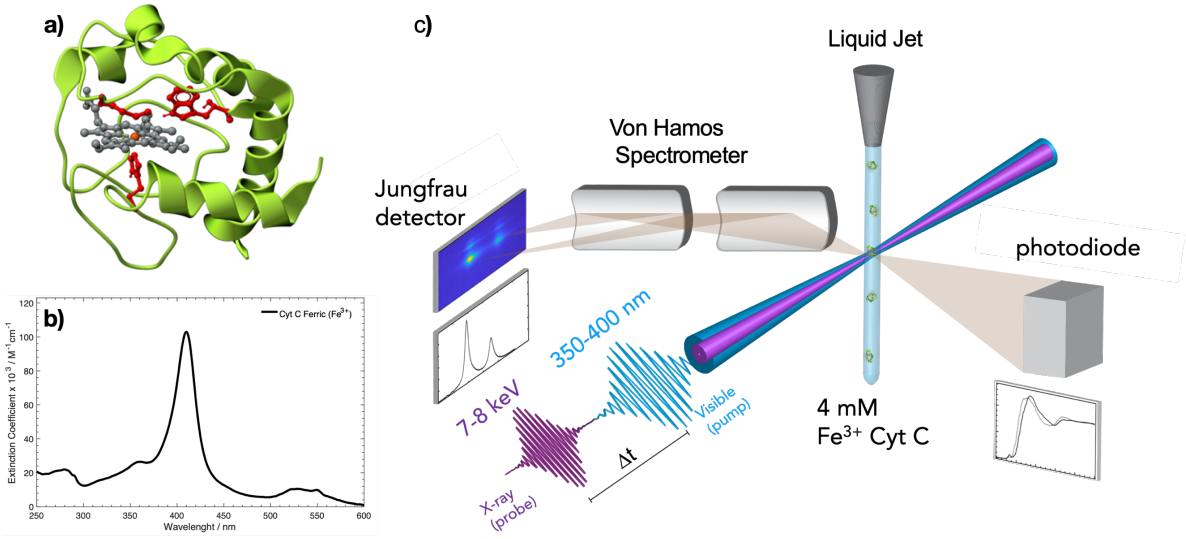

Figure 1: a) Structure of Cytochrome c (pdb: 1HRC). b) UV-Vis spectrum of ferric Cyt c c) Experimental setup for ultrafast X-ray emission (XES) and X-ray absorption (XANES) spectroscopy measurements at SwissFEL and European XFEL.

Figure 2a shows the ground and excited state XANES spectra at a pump-probe delay of 500 fs. The edge shift towards lower energies observed in the excited state can generally represent a change in oxidation state, electronic configuration, molecular structure or, more frequently, a combination of these effects. Comparing the excited state spectrum to that of ferrous Cyt c, recorded by Mara *et al* at the LCLS, [9] we observe a remarkable resemblance between the two data sets. This strongly indicates that the main contributor to the XANES transient features at 500 fs is the doming of the heme plane, despite the lack of distal ligand dissociation in ferric system. Figure 2b shows the Fe Kα emission spectra for different time delays, where it is possible to observe a clear broadening of the FHWM upon photoexcitation. Such a broadening is the fingerprint of an increase in the number of unpaired electrons in a metal-centered state (i.e. high spin states) as demonstrated by Kawai *et al*. [10] These sets of results are nicely complementary and paint a clear picture of the relaxation dynamics of ferric Cyt c, which occurs via higher spin states of the metal leading to heme doming, which are observed in a ferric heme protein for the first time.

The kinetics of the relaxation of both XANES and XES transient features are identical, and are described by a biexponetial decay back to the ground state with time constants of ~600 fs and ~8 ps. This time constanst also match with great accuracy those obtained by optical transient absorption [5] and fluorescense up-conversion [11] experiments. The biexponential behaviour represents a cascade via an intermediate (S=3/2) spin state state after excitation of the porphyrin, via the ultrafast decay of its Q-state, whiche then decays in ca. 600 fs to the high spin state (S=5/2). The latter returns to the ground state (S=1/2) in ca. 8 ps. The evidence of doming, which is one of the most important evolution-preserved deformations in heme proteins, in the case of ferric Cyt c calls for its possible involvement in the electron transfer function of this protein.

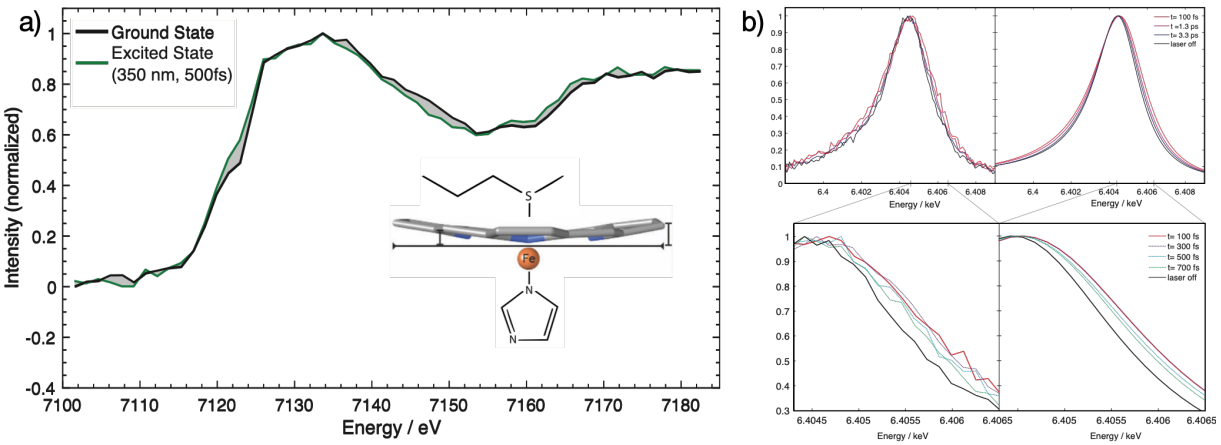

Figure 2: a) XANES spectra of Cyt c in the ground state (black) and excited state at 500 fs (green). The difference between the spectra is highlighted in the shaded gray area. b) Normalized Fe Kα1 XES spectra (left) and fit (right) for the ground state (black) and multiple time delays after excitation. The zoomed traces (bottom) show the increase of the FHWM as a function of pump-probe delay.

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3. References

[1] R. A. Scott and A. G. Mauk, *Cytochrome c: A Multidisciplinary Approach* (Univ Science Books, 1996).
[2] M. F. Perutz, A. Wilkinson, M. Paoli, and G. Dodson, Annu. Rev. Biophys. Biomol. Struct. **27**, 1 (1998).
[3] V. Karunakaran, ChemPhysChem **16**, 3974 (2015).
[4] M. Negrerie, S. Cianetti, M. H. Vos, J.-L. Martin, and S. G. Kruglik, J. Phys. Chem. B **110**, 12766 (2006).
[5] C. Consani, O. Bräm, F. van Mourik, A. Cannizzo, and M. Chergui, Chem. Phys. **396**, 108 (2012).
[6] J. Helbing, L. Bonacina, R. Pietri, J. Bredenbeck, P. Hamm, F. van Mourik, F. Chaussard, A. Gonzalez-Gonzalez, M. Chergui, and C. Ramos-Alvarez, Biophys. J. **87**, 1881 (2004).
[7] J. Helbing, Chem. Phys. **396**, 17 (2012).
[8] C. Consani, G. Auböck, O. Bräm, F. van Mourik, and M. Chergui, J. Chem. Phys. **140**, 025103 (2014).
[9] M. W. Mara, R. G. Hadt, M. E. Reinhard, T. Kroll, H. Lim, R. W. Hartsock, R. Alonso-Mori, M. Chollet, J. M. Glownia, S. Nelson, D. Sokaras, K. Kunnus, K. O. Hodgson, B. Hedman, U. Bergmann, K. J. Gaffney, and E. I. Solomon, Science **356**, 1276 (2017).
[10] J. Kawai, C. Suzuki, H. Adachi, T. Konishi, and Y. Gohshi, Phys. Rev. B **50**, 11347 (1994).
[11] O. Bräm, C. Consani, A. Cannizzo, and M. Chergui, J. Phys. Chem. B **115**, 13723 (2011).