MLCT Relaxation of $[Re^{I}(CO)_3(phen)(HisX)]^+$ (X = 83, 109) Pseudomonas aeruginosa Azurins

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Introduction

Polypeptide and solvent dynamics are important concepts in the study of proteins and their elucidation is crucial to obtain a complete understanding of the behaviour of these biological species.

We have set to study the ultrafast relaxation dynamics of a metal to ligand charge transfer (MLCT) excited state of $[Re^{I}(CO)_3(phen)(im)]^+$ (phen= 1,10-phenathroline; im = imidazole) and two metal-derivatised azurines, $Re^{I}(CO)_3(phen)(His83)Az$ and $Re^{I}(CO)_3(phen)(His109)Az$, (Figure 1) in D_2O by means of picosecond time resolved infrared spectroscopy (TRIR).

Figure 1. Structure of $Re^{I}(CO)_3(phen)(His83)Az$. The sensitizer produces only minor changes in the structure of the folded polypeptide.

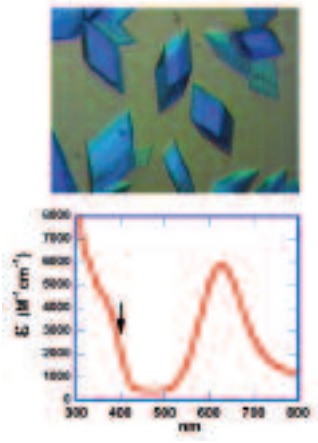


Figure 2. Crystals of $Re^{I}(CO)_3(phen)(109His)$-AzCu(II) (upper panel) and the UV-visible absorption spectrum of an aqueous solution of the same protein (lower panel). Arrow in the lower panel marks the TRIR excitation wavelength.

Discussion

Excitation with a 400 nm laser populates the singlet Re→phen MLCT state (Fig. 2) in each of the three samples, which then undergoes intersystem crossing to the lowest triplet state. The TRIR spectra, shown in Figure 3, consist of two negative bands, a sharp one at ~2030 cm^{-1} and a broad one at ~1925 cm^{-1}, corresponding to the A'(1) and the overlapping A'(2) and A'' CO vibrations, and the three positive bands corresponding to those vibrations in the excited state. The shift of the CO vibrations to higher frequency is indicative of the MLCT character of the excited state. [1,2]

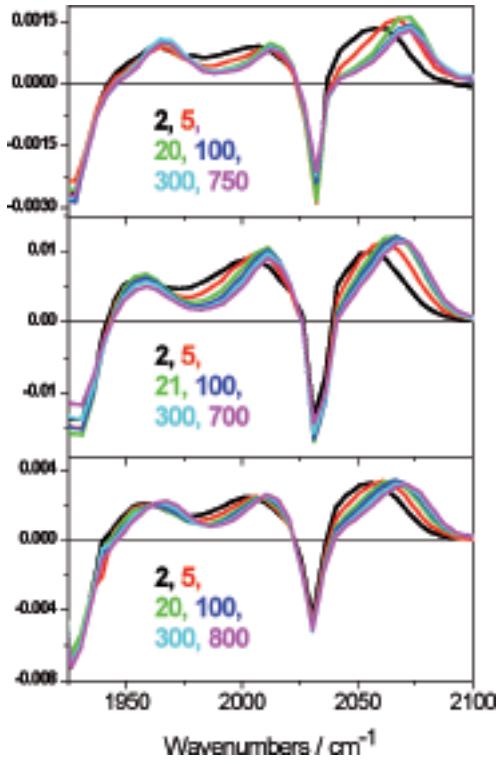


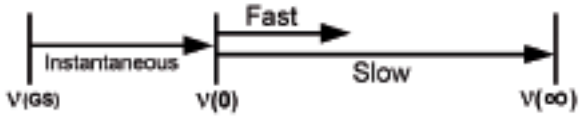
Figure 3. Difference TRIR spectra of $[Re^{I}(CO)_3(phen)(im)]^+$ (upper panel), $Re^{I}(CO)_3(phen)(His83)AzCu(II)$ (middle panel), and $Re^{I}(CO)_3(phen)(His109)AzCu(II)$ (lower panel) at various time delays in the ps time-scale. Separation between experimental points is 4-5 cm^{-1}.

The dynamics of this state were studied by measuring the shift of the excited state TRIR band position with time. The excited state bands shift to higher frequency and narrow as can be seen on Figure 3. Table 1 shows an overview of the dynamic results. The character of the MLCT state of the Re chromophore and its environment remains the same in the three samples. The excited state dynamics of the Re-proteins, however, are very different

than those of $[Re^{I}(CO)_3(phen)(im)]^+$ as can be seen in Figure 4. The total shift is comparable in both the protein samples and the $[Re^{I}(CO)_3(phen)(im)]^+$ which indicates that the charge redistribution of the chromophore is about the same in D_2O as in the protein environment.

The kinetics of the band position shift were found to be biexponential, with the typical instantaneous shift from the ground state position being followed by the dynamic fast and slow components, Scheme 1and equation (1).

$$\nu(t) = \nu(\infty) - A_f\exp(-t/\tau_f) - A_s\exp(-t/\tau_s) \quad (1)$$



Scheme 1. Dynamic behavior of the position of the A'(1) ν(CO) band after excitation. The ν(CO) band undergoes an "instantaneous" shift upon excitation from the ground-state position ν(GS) to ν(0), which is followed by a dynamic shift to the final position ν(∞). The dynamic shift occurs with two kinetic components, denoted "slow" and "fast"

Table 1. Dynamic Shift Parameters for the A'(1) ν(CO) band of Re-Azs and $[Re(im)(CO)_3(phen)]^+$.

	Re(His83)	Re(His109)	Re(im)(phen)
νGS, cm^{-1}	2030.5	2033	2032
νES(∞),cm^{-1}	2068.1	2067.9	2071
Total shift [a]	38	35	39
Inst. shift [b]	18	14	9
Dyn. shift	20	21	30
A_s, cm^{-1}	5.4±0.3	4.1±0.2	5.3±1.4
A_f, cm^{-1}	14.3±0.6	16.5±0.5	24.9±1.4
τ_s, ps	267±44	227±32	9.5±2
τ_f, ps	4.3±0.3	3.9±0.2	2.1±0.2
A_s/A_f	0.38	0.25	0.21
% A_s	27.4	19.9	17.6

a Defined as νES(∞) - νGS; in cm^{-1}
b Defined as νES(0) - νGS = nES(∞) - A_f - A_s - νGS; in cm^{-1}
c Defined as A_f + A_s; in cm^{-1}

The instantaneous shift arises from the change of electron density upon excitation at the ground-state geometry of the excited chromophore and its environment. A polar environment is arranged to optimize electrostatic interactions with the ground-state chromophore. Excitation changes the electron density distribution of the chromophore from $Re^{I}(CO)_3(phen)$ to $Re^{II}(CO)_3(phen^{-})$ and, hence, the magnitude and orientation of the chromophore dipole moment. The environment dipoles are oriented against this change and limit the extent of the charge transfer from $Re(CO)_3$ to the phen ligand. This effect will be weaker in less polar media. A drop in the instantaneous shift can be seen between $[Re(CO)_3(phen)(im)]^+$ in D_2O to Re-azurins which is caused by lower polarity of the azurin binding site and smaller exposure of the Re chromophore to the solvent. These effects allow for larger electron density redistribution.

The fast component contains information on vibrational cooling and D_2O reorientation during solvation. This type of shift has been observed in similar rhenium tricarbonyl complexes. The component is longer in the protein samples than in the $[Re(CO)_3(phen)(im)]^+$ which indicates a slower cooling process and slower movement of D_2O around the protein than in bulk solvent.

The slow component reports on the extent of structural response of the environment, which includes the azurin binding site and the surface D_2O, to the change of the charge distribution within the Re chromophore upon MLCT excitation. In Figure 4 it is quite obvious that the rate of the slow component is much slower in the azurines. This is due to interactions between the chromophore and amino acids in the protein binding site which slow the chromophore rotation. This component also includes contributions from the reorientation of D_2O, which is slower when it is water bound.

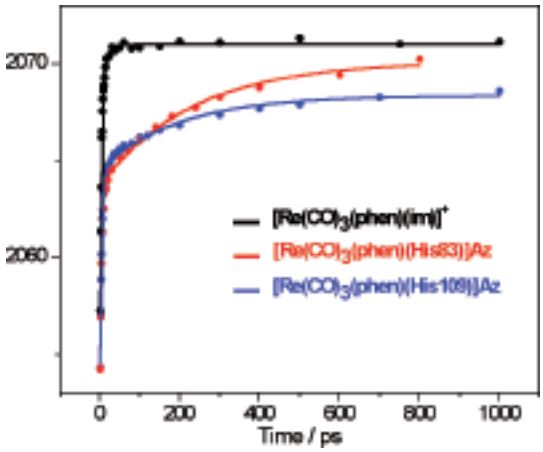


Figure 4. Biexponential fits (solid lines) of the time dependences of the positions of excited-state A'(1) ν(CO) bands of $Re^{I}(CO)_3(phen)(HisX)Az$ (X = 83, 109) and $[Re^{I}(CO)_3(phen)(im)]^+$

Conclusions

It was found that the character of the electronically excited state of the Re-azurins $Re^{I}(CO)_3(phen)(His109)Az$ and $Re^{I}(CO)_3(phen)(His83)Az$ and of the free $[Re^{I}(CO)_3(phen)(im)]^+$ after full relaxation (thermal equilibration) of the Re chromophore and its environment are the same. However the relaxation dynamics are much slower when the $Re(CO)_3(phen)(im)$ unit is bound to the azurin, as compared with D_2O solution. This effect is attributed to conformational changes of the azurin binding site and reorientation of surface-bound D_2O molecules, which are much slower than reorientation of bulk solvent molecules. It follows that protein-bound Re-carbonyl-polypyridyl complexes can be used as probes of the binding-site dynamics, reporting on the dynamic responses of the Re-protein link, the surface D_2O molecules and the protein binding site.

References

1. M. W. George, F. P. A. Johnson, J. R. Westwell, P. M. Hodges, and J. J. Turner, *J. Chem. Soc. Dalton Trans.*, 1993, 2977
2. M. Busby, P. Matousek, M. Towrie, A. J. Di Bilio, H. B. Gray, and A. J. Vlcek, *Inorg. Chem.*, 2004, **43**, 4994