Photoinduced structural relaxation and electron transfer in azurins labeled with Re(I) carbonyl-diimines

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Introduction

Proteins labeled with photo- and redox-active metal complexes are promising emerging materials for constructing molecular devices. Their investigation also brings a wealth of information and understanding of how natural systems (enzymes, photosynthetic reaction center, etc.) function. *Pseudomonas aeruginosa* azurins are ideal systems for investigations of protein dynamics associated with long-range ET reactions. They provide a structurally well characterized scaffold where metal-containing sensitizers can be attached at defined positions, allowing for definitive investigations of electron tunneling through a folded polypeptide. Labeling protein surfaces with $Re^{I}(CO)_3(N,N)$(imidazole) (N,N - α-diimine) opens the way for studies of relaxation dynamics in the region around one of the redox centers under the same conditions that normally trigger ET. Owing to the high sensitivity of CO stretching vibrations to structural perturbations and changes in electron-density distributions, it is possible to follow relaxation of an electronically excited Re^{I} label using time-resolved IR (TRIR) spectroscopy.

Discussion

We have concentrated on five different azurin mutants (Fig. 1), where the position of the surface histidine residue (HisX) has been modified. These azurins were subsequently labeled at the specific surface positions X by the $[Re(CO)_3(NN)]^+$ (N,N = 1,10-phenanthroline (phen) for X = 83, 109, 124, 126; N,N = 4,7-dimethyl-1,10-phen (dmp) for X = 107) chromophore, which is also a strong excited-state oxidant. The complex $[Re^{I}(CO)_3(phen)(im)]^+$ (Re(im)) is used as a model for the azurin labeling site.

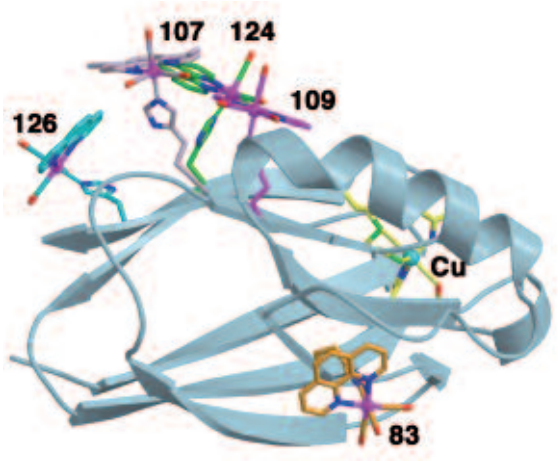

Figure 1. The five *Pseudomonas aeruginosa* azurins labeled with $Re^{I}(CO)_3(NN)$(HisX)AzCu at different surface positions (NN = phen, dmp).

TRIR studies of bands due to CO stretching vibrations, ν(CO), show that excitation of the $Re(CO)_3(NN)$ chromophore in all five azurins and the free sensitizer Re(im) with 400 nm, ~150 fs laser pulses partially depletes the ground-state population. The conversion to an excited state in each case is manifested by the negative bleach and positive transient bands, which emerge in the TRIR spectra within the instrument time resolution. The excited-state ν(CO) IR spectral pattern of Re(im) and the Re-azurins is characteristic of a Re→phen ^{3}MLCT excited state: all ν(CO) bands are shifted to higher energies relative to the ground state.

In-depth analysis of the TRIR data reveals that optical excitation of the Re center can trigger two kinds of processes: dynamic structural motions (relaxation) and long-range electron transfer.

Dynamic relaxation

The dynamic structural motions of the azurin series were studied with the copper centre in its oxidation state II, preventing long-range excited-state electron transfer.

Intramolecular vibrational redistribution, vibrational relaxation, and solvent relaxation are complete during the first few tens of ps, being only slightly protein-dependent. However, Re-azurins are highly dynamic in the metallolabel region on time scales of ~10^2 picoseconds up to a few nanoseconds. The protein relaxation dynamics are manifested in TRIR by time-dependent shifts of ν(CO) bands to higher energies (Fig. 2), whose kinetics are Re-binding-site specific, Fig. 3.

The responsible molecular motions were identified as peptide and side-chain torsions and Re-label displacements and reorientation that optimize electrostatic and coulombic interactions between the excited label and the azurin.[1]

Long range ET

Excitation of Re-azurins that contain a reducing Cu(I) center triggers Cu(I)→*Re long-range ET that normally occurs with μs and or slower lifetimes. However, if tryptophan (W) is present next to the Re label, as is the case of Re(H124)(W122)AzCu(I), the

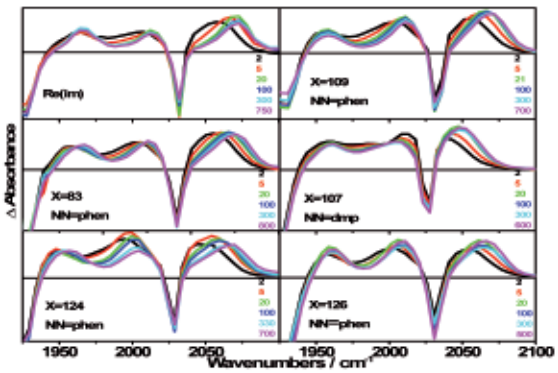

Figure 2. Difference time-resolved IR spectra of Re(im) and the five Re-azurins in KP$_i$ (D_2O, pD ~ 7.1) buffer measured at selected time delays after 400 nm, ~150 fs excitation.

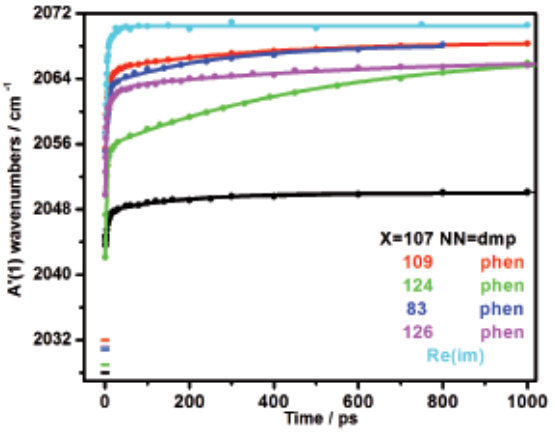

Figure 3. Time dependences of the peak energies of the A'(1) ν(CO) band of the five Re-azurins containing Cu(II).

ET is strongly accelerated (at least 100-times). This is clearly documented in TRIR (Fig. 4) by the appearance and kinetics of the down-shifted IR bands due to intermediates containing reduced $Re^{I}(CO)_3(phen^{\bullet-})$ unit (marked "ET" in Fig. 4).

The dramatic ET acceleration originates in multistep tunneling (hopping), whereby the ~20 Å ET path is split in two steps: W122→*Re and Cu(I)→$W122^{\bullet+}$.[2] This ET mechanism and the individual step rate constants are shown in Fig. 5. Migration of the electron from Cu(I) via $(W122)^{\bullet+}$ is complete in less than 50 ns and charge recombination proceeds on the microsecond time scale.

Conclusions

Importantly, we have shown that time-resolved IR spectroscopy of Re(I) carbonyl-diimine complexes is a powerful probe of dynamical responses of molecular units at or around protein surfaces.

Acceleration of long-range ET by multistep tunneling seems to be a general principle, applicable to designing new systems capable of ultrafast photoinduced charge separation.

We conclude that azurin ps- and ns-dynamics must be taken into account when interpreting ET kinetics. The structural relaxation dynamics and early stages of photoinduced ET occur together and, as was found herein, on similar timescales. This opens the possibility that these two kinds of processes are dynamicaly coupled, affecting each other.

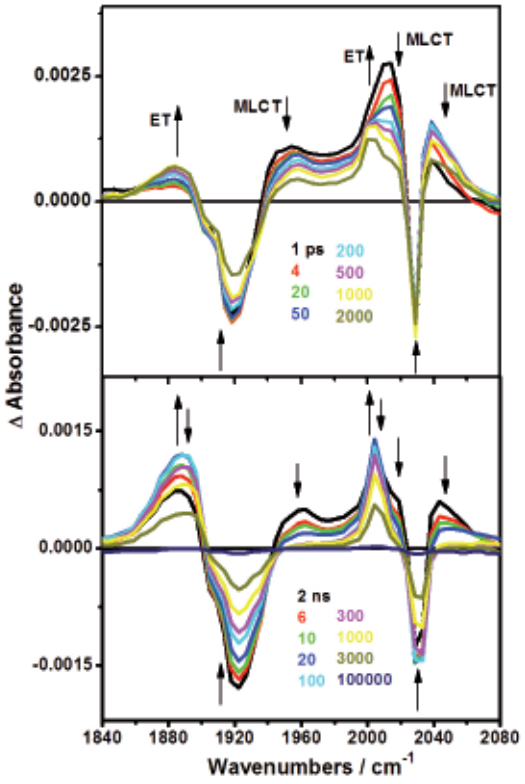

Figure 4. TRIR spectra of Re(H124)(W122)AzCu(I) measured on a ps (top) and ns (bottom) timescale.

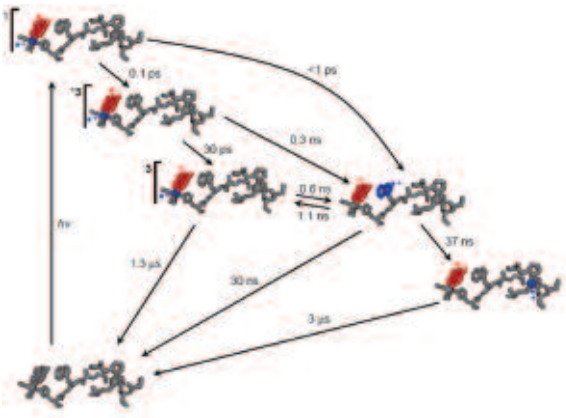

Figure 5. Mechanism of photoinduced electron transfer in Re(H124)(W122)AzCu(I).

Acknowledgements

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References

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2. C. Shih, A. K. Museth, M. Abrahamsson, A. M. Blanco-Rodriguez, A. J. Di Bilio, J. Sudhamsu, B. R. Crane, K. L. Ronayne, M. Towrie, A. Vlcek, J. H. Richards, J. R. Winkler, and H. B. Gray, *Science*, **320**, 1760-1762 (2008).