Ultrafast structural dynamics in BLUF domains: transient infrared spectroscopy of AppA and its mutants

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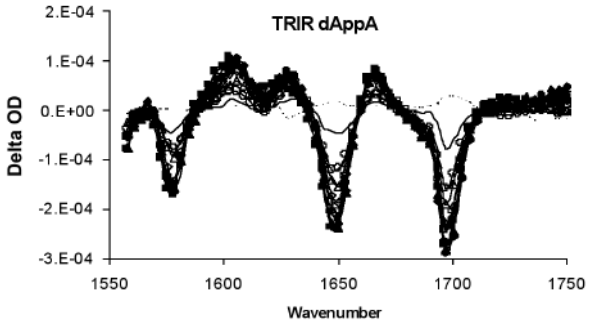
In this paper we will describe ultrafast vibrational studies of the dynamics underlying the operation of blue light sensing using FAD (flavin adenine dinucleotide) (BLUF) proteins. The BLUF domain of AppA, a transcriptional antirepressor from the photosynthetic bacterium *Rhodobacter sphaeroides* will be studied as an example. Under low light conditions, the dark state of AppA binds to PpsR forming an AppA-PpsR$_2$ complex that is unable to bind DNA. Upon blue light photoexcitation, AppA dissociates from PpsR, allowing formation of the repression-competent PpsR tetramer which binds to DNA and inhibits gene transcription.

AppA is an unusual photoactive protein. In PYP and the rhodopsin family electronic excitation is followed by a fast structural change in the excited state which acts as the trigger for formation of the signaling state of the protein. In contrast for FAD in AppA there is no apparent excited state structure change. All that is seen is a 10 nm red shift in the absorption spectrum between dark (dAppA) and light (lAppA) adapted states. Structural studies point to changes in the interaction between FAD and the protein, probably through modified H-bonding interactions.[1] Recently we have shown that time resolved IR spectroscopy is a powerful tool in investigating ultrafast structural changes in proteins.[2] Here we apply this method to dAppA, lAppA and two mutants. Some typical transient IR data are shown in the figure. All transients appear on a sub-picosecond timescale. Many can be assigned to FAD, though modified compared to the spectrum in solution.[3] Through mutagenesis we identify one transient which is uniquely a marker for the capability to form the signaling state.

An example of the experimental data is shown in the figure. Both light and dark adapted forms and photoactive and inactive mutants of AppA have been investigated. The complete data set is described elsewhere.[4] The transient absorption marker mode for proteins capable of forming the signalling state on photon absorption was identified. This transient, which is formed promptly on excitation, is suggested to arise from an H-bond reorganisation in the protein rather than a chromophore mode. It was proposed that electronic excitation of FAD leads to changes in the proton affinity of atoms involved in H-bonding (N5, N3, C2=O). This in turn results in the breaking or disruption in the network of residues involving residues linked to the chromophore through H-bonds (Q63, N45 and H44). The



TRIR spectra of dark adapted AppA recorded 1 (filled squares) 5 (filled diamonds) 10 (filled triangles) 20 (filled circle), 50 (open square) 100 (open diamond) 300 (open triangle) 500 (open circle) and 2000 ps (line) after excitation. Note that data for 1,5,10 ps are overlapped.

mutant data confirm the significance of this 1666 cm^{-1} mode and suggest a role for Q63. A more definite assignment will be possible by 13C labelling of the Q in AppA. We have exploited such methods in studies of green fluorescent protein dynamics,[2] and similar experiments for AppA are planned.

A number of other changes in the vibrational spectroscopy between FAD in solution and bound in AppA were studied.[4] Broadly these measurements are consistent with existing steady state experiments. In addition the TRIR spectra suggest that irradiation leads to a degree of inhomogeneity in the light adapted AppA structure, particularly around the C4=O bond. Interestingly none of the spectra revealed the growth of new vibrational modes, suggesting that further changes associated with signalling state formation take place outside the temporal or energy window of these experiments. We plan to extend these ranges in future experiments.

Ground state recovery kinetics were also recorded. Delayed ground state recovery for dAppA in the first few picoseconds was tentatively assigned to relaxation in the upper state. The very rapid quenching of lAppA compared to dAppA was suggested to arise from enhanced electron transfer quenching of the isoalloxazine ring by an adjacent amino acid, induced through structural changes in the light adapted form.

In summary the first ultrafast transient IR studies of the BLUF domain have been reported and novel information on the mechanism of the primary steps in the blue light sensing response was identified.

References

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