AppA is a transcriptional antirepressor of photosynthesis gene expression in *Rhodobacter sphaeroides* that regulates cellular responses to light and oxygen. It is a blue-light photoreceptor that mediates blue light-induced photosynthesis gene repression. The N-terminus of AppA contains a BLUF (blue light photoreceptor utilizing flavin adenine dinucleotide (FAD)) domain (residues 16−108) and binds to flavins. Absorption of blue light results in a ~10 nm red shift of the UV/vis absorbance spectrum. The lifetime of the red-shifted absorbance spectrum for the light-induced signaling state of AppA is among the longest of all known flavin-based photoreceptors (~1800 s), yet is formed within 1 ns from the singlet-state flavin. Emerging evidence from spectroscopic and mutational studies suggests that light catalyzes flipping of the conserved Q63 side chain ~180 degrees to form a strong hydrogen bond between the Q63 side chain amide and the flavin C4=O, altering the electronic configuration of the flavin isoalloxazine ring and giving rise to the observed ~10 nm red-shift of its UV/vis absorbance spectrum, as well as ~16 cm−1 downshift of the C4=O stretching mode in FTIR spectra. In contradiction to this, the recently reported crystal structure of AppA mutant C20S has been interpreted that in the dark the Q63 side chain is oriented with its carbonyl oxygen near the Y21OH group and its side chain amide close to the flavin O4 (opposite the dark state orientation proposed by Anderson et al.) and that in response to light it flips by ~180 degrees.

In light of this discrepancy, it is very important to determine which model represents the dark state and light-induced signaling state orientation of Q63 side chain for AppA in solution. Here we present NMR data describing changes in the core of the BLUF domain of AppA in response to blue light illumination supporting the model that, in solution, AppA WT undergoes a flip of the Q63 side chain in response to light to form strong hydrogen bonds between the Q63 side chain amide and the flavin C4=O and between the Q63 side chain carbonyl oxygen and Y21 side chain hydroxyl, as proposed by Anderson et al.

The chemical shift position of the FAD H3 proton was previously assigned to the H3 resonance of H44 because it appeared in the HSQC spectrum of AppA (Figure 1, inset). Exchange of the 15N-labeled FAD for unlabeled FAD leaves an identical 1D NMR spectrum (Figure 1a), but because the FAD H3 is attached to 15N, it is not split into a doublet in 1D 1H NMR spectra recorded without decoupling of 15N (Figure 1b), demonstrating the correct assignment.

The difference in chemical shift of the H3 proton between the dark state and the light-induced signaling state is the largest observed change for all resonances in the protein (Δδ ~0.6 ppm). The observed change in chemical shift for the FAD H3 proton resonance can be caused either by a change in the electronic structure of the flavin isoalloxazine ring or by a change in the local chemical environment due to a structural change of the protein. Other photoreceptor proteins such as PYP or LOV domains are known to undergo large structural unfolding transitions in their signaling states, but it is thought that AppA undergoes only a small structural perturbation in response to light. From NOESY spectra in Figure 2, it can be seen that the chemical shifts of dark state NOEs to the flavin H3 proton (S41/β, H44e1, and V75y2) are virtually unchanged in the light. In addition, other resonances in this region of the NOESY spectrum show small chemical shift changes and almost identical NOE patterns. The high degree of similarity of these NOE patterns is consistent with very little overall structural perturbation of the flavin binding core. Therefore, the light-induced change in chemical shift of the FAD H3 proton resonance is most likely caused by changes in the electronic structure of the flavin isoalloxazine ring. Formation of a strong hydrogen bond to the C4=O position in the light-induced signaling state withdraws electron density from the ring structure and deshields the H3 proton and is the most likely cause of the downfield shift observed for the FAD.
structure of AppA WT,11 which shows the conserved Q63 side chain carbonyl oxygen in hydrogen bonding arrangement with the indole NH of conserved W104 (Figure 3a) and does not show any covalent adducts. The solution structure of AppA WT in the dark15 shows that W104 is in the core of the protein, with its indole NH able to hydrogen bond with the Q63 side chain carbonyl oxygen (as in Figure 3a). This orientation is supported by more than 20 separate NOEs (see Supporting Information), demonstrating that, in the dark and in solution, W104 is buried in the core of AppA WT. This dark state orientation of Q63 agrees with available spectroscopic dataa and is independent experimental confirmation of the orientation of the Q63 side chain in the crystal structure of AppA WT in the dark.11

We conclude here, that in the light-induced signaling state of AppA, the structure of the flavin binding core in general is only minimally altered. The observed FAD H3 chemical shift change and NOE patterns provide evidence that the strongest local effect of light-induced signaling state formation is alteration of the electronic structure of the flavin isoalloxazine ring upon formation of a strong hydrogen bond between the flavin C4=O and Q63 side chain amide. Observation of the Y210H resonance in the light confirms formation of a strong hydrogen bond between the Y210H and the Q63 side chain carbonyl group. These conclusions are supported by inspection of available crystal and solution structural data, are consistent with the model proposed by Anderson et al. for orientation of the Q63 side chain,11 and confirm flipping of the Q63 side chain as the likely mechanism of signaling state formation. How this light-induced side chain flip affects the rest of the BLUF domain is currently under study.

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Supporting Information Available: Materials and methods, supplemental results, and discussion. This material is available free of charge via the Internet at http://pubs.acs.org.

References

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