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Backbone Modification of Retinal Induces Protein-like Excited State Dynamics in Solution

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Supporting Information

ABSTRACT: The drastically different reactivity of the retinal chromophore in solution compared to the protein environment is poorly understood. Here, we show that the addition of a methyl group to the C=C backbone of all-*trans* retinal protonated Schiff base accelerates the electronic decay in solution making it comparable to the proton pump bacteriorhodopsin. Contrary to the notion that reaction speed and efficiency are linked, we observe a concomitant 50% reduction in the isomerization yield. Our results demonstrate that minimal synthetic engineering of potential energy surfaces based on theoretical predictions can induce drastic changes in electronic dynamics toward those observed in an evolution-optimized protein pocket.

Double bond *cis*–*trans* isomerizations are found ubiquitously in nature as the first step in a variety of light-induced processes.[1] A prominent example is the proton pump bacteriorhodopsin (bR) found in the archaeon *Halobacterium halobium*, which converts light energy into chemical energy.[2] This photosynthetic system is triggered by the fast, efficient, and selective all-*trans* to 13-*cis* isomerization of a protein-bound retinal protonated Schiff base (RPSB) chromophore. Isomerization of the chromophore in solution is almost an order of magnitude slower (~4 vs 0.5 ps),[3,4] exhibits a lower quantum yield (0.16 vs 0.64),[5,6] and prefers the 11-*cis* over the 13-*cis* product. The correlation between ultrafast dynamics and high reaction efficiency is not exclusive to retinal but is a common feature of highly efficient light-induced biological processes.[7,8] Understanding and eventually controlling the reactivity and dynamics of RPSBs has thus become an intense field of study both from an experimental and theoretical perspective.[9−17] Attempts to modify the reactivity of RPSBs in solution, including changes to the solvent,[18−20] backbone structure[21−23] and introduction of the opsin shift,[24] have failed to show any appreciable effects on the excited-state dynamics or quantum yield (QY) compared to the reactivity in the protein environment. Here, we demonstrate that addition of a methyl group to the retinal backbone results in protein-like photophysics for all-*trans* RPSB in solution while reducing the photoisomerization yield.

Rather than attempting to mimic the effect of the protein pocket by changing the environment of the chromophore, we opted for a straightforward modification of the retinal backbone: the addition of a methyl group to the C_{10} position as depicted in Figure 1. Such a modification is expected to reduce the excited to ground electronic state energy gap after relaxation out of the Franck−Condon region due to inductive stabilization of the partial positive charge along the retinal backbone. While such a modification will have a minor effect on the ground state, the bond order inversion expected upon population of the first excited electronic state[25] places a partial positive charge at the 10-position, which is effectively stabilized by the presence of the methyl group. At the same time, this modification has the advantage of leaving the absorption spectrum unchanged (see Supporting Information), thus, leading to an overall minimal perturbation of the native system. The addition and removal of methyl groups to the retinal backbone has been an active field of study to investigate steric effects within the bR and rhodopsin protein pockets.[26−28] Direct comparison with the results presented herein is difficult due to the different environment, use of other isomers, and the lack of any information on reaction dynamics in the previous works. Here, we focus solely on the reactive properties of native and modified all-*trans* protonated Schiff bases in solution both from a photochemical and photophysical perspective.

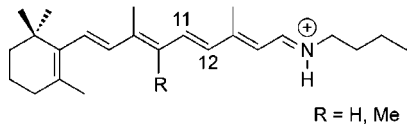

Figure 1. Native and methylated all-*trans* retinal protonated Schiff bases.

We performed ultrafast pump−probe measurements on native and all-*trans*-10-methyl RPSB in methanol to investigate the consequences of our structural modification on the excited-state dynamics. Pump pulses of 25 fs duration centered at 500 nm ensured population of the first excited state, S_1. A differential absorption map for unmodified all-*trans* RPSB is shown in Figure 2A. Here, we focus on the probe wavelength region between 600 and 900 nm, which is free of any ground-state signatures. Immediately following time zero, we observed a dual-peaked stimulated emission (SE) band at 690 and 800 nm that decayed biexponentially at 810 nm with 2 and 7 ps time constants in agreement with previous results.[29] The overall

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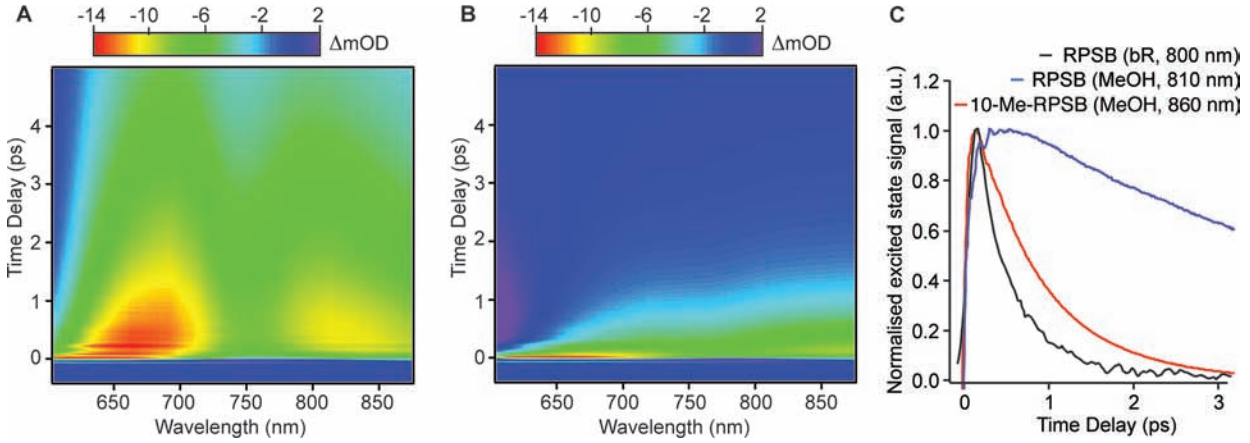

Figure 2. Ultrafast dynamics of native and all-*trans*-10-methyl retinal protonated Schiff base (RPSB). (A) Differential transient absorption map of native RPSB in MeOH following excitation by a 25 fs pulse centered at 500 nm. (B) Corresponding map for all-*trans*-10-methyl RPSB. (C) Temporal dynamics taken from fluorescence upconversion measurements on bacteriorhodopsin (bR)[31] and (A) and (B), respectively.

appearance of the SE band is attributed to an excited state absorption band at 760 nm.[30]

The dynamics for all-*trans*-10-methyl RPSB under identical conditions differ drastically as can be seen in Figure 2B. The excited state lifetime is reduced by almost an order of magnitude to 0.7 ps. The SE peaks are shifted from 700 to 710 nm and 810 to 860 nm. A direct comparison of the excited-state dynamics is shown in Figure 2C. We have also included the excited state decay associated with the all-*trans* to 13-*cis* isomerization in bacteriorhodopsin measured by fluorescence upconversion for comparison.[31]

Close inspection of the results depicted in Figure 2 reveals several differences between the native and modified RPSB dynamics beyond the reduction of the excited state lifetime. For native RPSB, a clear relaxation that is complete within 500 fs is observed that results in a 170 fs rise time of the SE signature at 810 nm as shown in Figure 2C. This behavior is in contrast to the near-instantaneous rise of the SE band in the modified RPSB. In addition, native RPSB exhibits clear oscillations with a 100 fs period during the initial relaxation which appear absent for the modified system.

To assess the effect of the accelerated excited state decay on the photochemistry, we determined the photoisomerization yields for the all-*trans* to 11-*cis* reaction using ^{1}H NMR to be 0.16 ± 0.03 and 0.09 ± 0.01 for native and all-*trans*-10-methyl RPSB, respectively (see Supporting Information).[32] No other isomers, including the 13-*cis* product generated by bacteriorhodopsin, were detectable within our experimental error at the low photoconversion used in our experiments. Thus, in contrast to the notion that reaction speed and efficiency are correlated, our modified RPSB exhibited a reversed effect.

Our results can be rationalized using the simple model depicted in Figure 3. Absorption of a photon is followed by rapid relaxation along high-frequency coordinates including C−C and C=C stretching modes with minimal displacement along the isomerization coordinate.[25,33] This relaxation results in a fast and considerable lowering of the S_0−S_1 energy gap as evidenced by the SE band in the near-infrared. The lack of changes to the SE spectrum throughout excited state decay points toward a constant molecular structure at a local minimum.[33] A barrier on the excited state prevents the system from rapidly reaching the ground electronic state via a conical intersection (CI) which results in the observed picosecond-

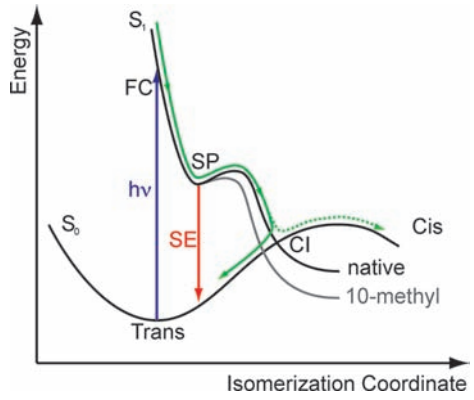

Figure 3. Schematic of the potential energy surfaces involved in all-*trans* RPSB photochemical dynamics. FC, Franck−Condon point; SP, stationary point; SE, stimulated emission; CI, conical intersection.

decay. The surface crossing occurs largely on the reactant side, leading to a low overall reaction yield. Without detailed theoretical investigations, however, we cannot exclude alternative mechanisms based on a modification of the hydrogen-out-of-plane frequencies in the isomerizing region that have been shown to be instrumental in determining the isomerization efficiency for rhodopsin.[34]

Addition of the methyl-group to the retinal backbone causes several changes to the observed spectra and dynamics. The competing S_1−S_X excited state absorption band is shifted to lower energy resulting in a slightly altered peak appearance of the SE band. Nevertheless, the width and position of the SE band remains very similar to native all-*trans* RPSB suggesting comparable stationary points for both species. Such behavior is expected if the major initial structural changes involve backbone stretching coordinates.[35] Subsequent accelerated excited state decay as observed in bacteriorhodopsin demands a lower barrier toward the CI.

The observed lower reaction yield in combination with the inductive effect of the methyl substituent points toward an even earlier surface crossing resulting in a reduced probability of generating the *cis*-product. The lower barrier and QY may also be attributed to a lowered backbone vibrational frequency near the 11,12 C=C bond, although further experimental work and detailed theoretical investigations will be necessary to reveal the exact nature of the effect.

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Our work clearly demonstrates that RPSBs in solution can indeed exhibit vibronic dynamics of the same speed as in the protein environment. It also strongly suggests that reaction yield and speed are not correlated in retinal photochemistry, in agreement with recent studies of an artificial photochemical switch.[36] Nevertheless, it reemphasizes the importance of the bR protein pocket in activating a reactive channel toward the 13-*cis* product which appears completely inactive in solution.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectroscopic data for all compounds. UV/VIS absorption spectra and details of QY calculation. Details of ultrafast pump−probe experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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