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Simulations of two-dimensional infrared and stimulated resonance Raman spectra of photoactive yellow protein

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ABSTRACT

We present simulations of one and two-dimensional infrared (2DIR) and stimulated resonance Raman (SRR) spectra of the dark state (pG) and early red-shifted intermediate (pR) of photoactive yellow protein (PYP). Shifts in the amide I and Glu46 COOH stretching bands distinguish between pG and pR in the IR absorption and 2DIR spectra. The one-dimensional SRR spectra are similar to the spontaneous RR spectra. The two-dimensional SRR spectra show large changes in cross peaks involving the C = O stretch of the two species and are more sensitive to the chromophore structure than 2DIR spectra.

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1. Introduction

The reaction to light in all biological organisms is governed by photoreceptor proteins, which transduce light into a usable chemical signal to initiate light perception in bacteria, plants, and animals [1,2]. The initial events in photoreceptors often involve a cis–trans isomerization in an embedded chromophore followed by a local relaxation occurring on ns timescales. Characterizing the initial events in photoreceptors is challenging because both high structural resolution and ultrafast time resolution are required [3]. Time-resolved vibrational spectroscopy is an ideal probe because it provides excellent temporal and structural resolution [3,4].

Predicting local active site motions in photoreceptors through vibrational spectroscopy could help further the understanding of the initial events in a wide range of photoreceptors existing in bacteria, plants, and animals. Photoactive yellow protein (PYP) is a 125 residue, 14 kD, globular protein with an α/β fold that is proposed to initiate the negative phototactic response in *Halorhodospira halophila* [3,2]. *p*-coumaric acid (pCA), the chromophore within one of PYP's two hydrophobic cores, is covalently bound to Cys69 by a thioester linkage and is stabilized by a hydrogen bond network with Glu46, Glu42, Thr50, and Cys69 [5,3,2].

Upon absorption ($\lambda_{max} = 446$ nm), PYP undergoes a complex photocycle that was initially characterized by UV/visible absorption [6], and subsequently by time-resolved X-ray crystallography [7–11]. In the dark state (pG or I_0), pCA exists in the deprotonated trans form with a hydrogen bond to the amide group of Cys69 [12,5]. After excitation, the chromophore undergoes a fs trans to cis isomerization via a bicycle pedal mechanism that disrupts the hydrogen bond between pCA and Cys69 [3,11]. Within several nanoseconds, relaxation around the active site leads to the formation of the red-shifted intermediate (pR or I_1) [13,8,10,11]. The chromophore is then protonated by Glu46 [13,14], causing a protein quake that partially unfolds it [7,15] on a millisecond timescale to form its putative signaling state (pB or I_2). Finally, in a subsecond process, the pG state is recovered. This involves the deprotonation of pCA by Glu46, the reisomerization of pCA, and the refolding of the protein [3].

The isomerization follows a "volume-conserving" path because the chromophore is embedded within the densely packed hydrophobic core [13,8,11]. The strong hydrogen bonding network and the covalent attachment to Cys69 further constrain the number of such mechanisms [16]. The trans to cis isomerization is achieved via a bicycle pedal mechanism where the rotation about the double bond is concomitant with a rotation about the C–C (=O) bond. This breaks the hydrogen bond between Cys69 and pCA, shifting the pCA carbonyl stretch from 1633 to 1666 cm^{-1} [17]. Following this bicycle pedal mechanism, rotations about the S–C (=O), C^{α}-C^{β}, and C^{β}–S bonds leads to the formation of pR by relieving strain in the active site [13,8,16,10,11].

Both Infrared (IR) and resonance Raman (RR) spectroscopy detect vibrational resonances, but through a different window. In RR, only those vibrations which are coupled to an excited state are observed, which permits the observation of vibrational dynamics of a chromophore within a host. This site selectivity has been used to study photosensors such as rhodopsins and phytochromes. On the other hand, IR spectroscopy observes all vibrational resonances within the pulse bandwidth. The secondary structure of proteins is observed via the amide transitions. However, if a small number

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of vibrational resonances are spectrally isolated (e.g. by isotope labeling), IR spectra may also be used to study local dynamics.

In this paper, we report simulations of IR and RR spectra of the pG and pR states of PYP. Details of the quantum chemical and molecular dynamics simulations are given in Section 2. The simulated IR spectra are presented in Section 3 and compared with experiment. We particularly focus on the IR difference spectrum of the Glu46 COOH stretch. We also present the simulated 2D IR spectra for pG and pR. In Section 4, the simulated spontaneous RR spectra are presented and compared with experiment. The simulated one and two-dimensional stimulated RR spectra are presented as well. We finally conclude and discuss future extensions.

2. Methods

2.1. Molecular dynamics simulations

Molecular dynamics (MD) simulations of the pG and pR states of PYP were performed using the GROMACS software package [18]. The pG initial structure was taken from PDB 1NWZ [9] and the pR initial structure was taken from PDB 1OT9 [10]. The protein was modeled using the Gromos96 united-atom force field [19] and the parameters for PCA were obtained from a previous study [20].

Each structure was solvated in a cubic box of SPC water [21] with a minimum solvation layer of 0.9 nm. This resulted in a box of length 62.7 Å for pG and 63.4 Å for pR. To neutralize the system, six water molecules were replaced with sodium ions. The systems were minimized for 5000 steps using a steepest descent algorithm. The water, ions, and protein hydrogens were then allowed to relax for 250 ps in the NPT ensemble while non-hydrogen protein atoms were harmonically restrained. The systems were equilibrated in the NPT ensemble for 500 ps at 300 K, after which the protein RMSD and total energy remained constant.

All simulations were performed using cubic periodic boundary conditions. Long-range electrostatic interactions were calculated using the particle mesh Ewald method [22,23] with a short range cutoff of 9 Å. Van der Waals interactions were computed using a cutoff of 14 Å. For NPT equilibration, the temperature and pressure were controlled via weak coupling to an external bath [24] with a temperature coupling time constant of 1 ps, pressure coupling time constant of 0.5 ps and isothermal compressibility of 4.5×10^{-5} bar^{-1}. The temperature of the NVT simulations was controlled using a Nose–Hoover thermostat [25,26] with a 0.1 ps damping coefficient. The LINCS [18] and SETTLE algorithms [27] were used to constrain bond lengths, which permitted a 2 fs timestep.

2.2. Electronic structure calculations

Electronic structure calculations (Section 4) were performed using the Gaussian09 software package [28]. We used the PBE0 functional [29–32] and the 6-311++G** basis set using a polarizable continuum model with conductor like solvations [33,34] to simulate the active site within an aqueous environment.

3. IR spectra

The IR spectra of PYP were simulated using an effective vibrational Hamiltonian which can be recast using the bosonic creation ($b_i^\dagger$) and annihilation (b_i) operators [35–37]:

$$H(t) = \hbar\sum_i \omega_i(t) b_i^\dagger b_i + \hbar\sum_{ij} J_{ij}(t) b_i^\dagger b_j + \frac{\hbar}{2}\sum_i \Delta_i b_i^\dagger b_i^\dagger b_i b_i \qquad (1)$$

ω_i and Δ_i are the fundamental frequency and the anharmonicity of mode i respectively, and J_{ij} is the coupling between modes i and j. The interaction of the vibrational system with the field is given by:

$$H'(t) = \sum_i \boldsymbol{\mu}_i(t)\cdot\boldsymbol{E}(t)\left(b_i + b_i^\dagger\right) \qquad (2)$$

In the Hamiltonian, we include 126 modes in the carbonyl stretching region: 124 amide I modes, the Glu46 COOH side chain stretching mode, and the pCA C=O stretch. We have used electrostatic DFT maps from the literature to evaluate the fluctuating parameters in $H(t)$ and $H'(t)$ for the amide I and COOH vibrations. For the amide I vibrations, the electrostatic DFT map of [38] was used to evaluate $\omega(t)$ while the transition dipole was fixed to the gas phase value [39] and the anharmonicity was fixed to the measured value of -16 cm^{-1} [40]. The Glu46 carboxylic acid vibration was modeled using the electrostatic DFT map in reference [41]. We have further constructed a new electrostatic DFT map for the pCA C=O stretch. Details are given in the supporting information. J_{ij} was given by the transition–dipole coupling model. The nearest-neighbor coupling between amide I modes was given by the Torii and Tatsumi dihedral angles map [42].

3.1. IR absorption

All simulations were performed in the inhomogeneous limit by averaging over 2000 configurations taken from 4 ns MD simulations. The field-free frequencies of the amide-I modes and the Glu46 COOH mode were set to 1681 cm^{-1} and 1745 cm^{-1}, respectively, to match experimental results [43]. Likewise, a shift of -75 cm^{-1} was applied to the cis and trans pCA C=O stretching modes to match experimental results [17]. Note that the same shift was applied to each isomer. The homogeneous vibrational dephasing was fixed to 5.5 cm^{-1} for all transitions.

The simulated spectrum of pG is shown in Fig. 2. It compares well with experiment. The spectrum is dominated by the amide I

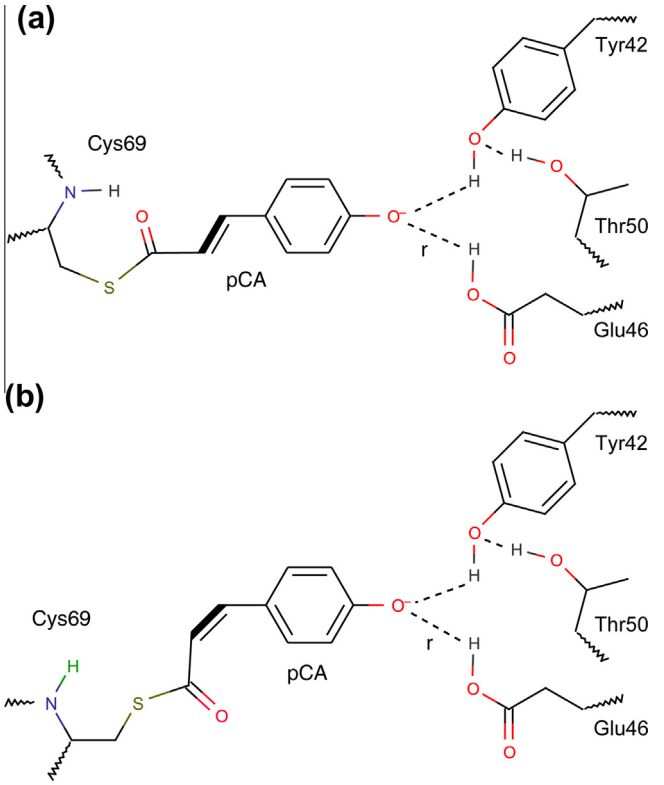

Fig. 1. Active site structure of PYP in the (a) pG and (b) pR states. Hydrogen bonds are marked in dotted lines and the Glu46(H)-pCA (O–) hydrogen bond is labeled as r. The trans to cis isomerization occurs about the pCA C=C bond and a concomitant rotation about the C–C (=O) bond breaks the hydrogen bond between pCA and Cys69.

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