THE JOURNAL OF PHYSICAL CHEMISTRY B
A JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Perspective

Impact of Ultrafast Electric Field Changes on Photoreceptor Protein Dynamics

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Cite This: *J. Phys. Chem. B* 2022, 126, 581−587

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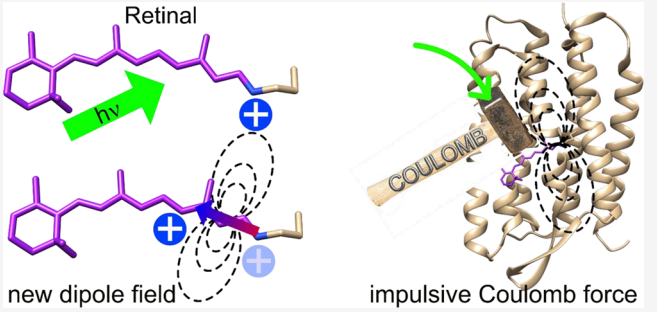
ABSTRACT: Studies on photoreceptors provide a wealth of information on cofactor and protein dynamics on the microsecond to seconds time-scale. Up to now, ultrafast dynamics addresses mainly the cofactor or chromophore, but ultrafast protein dynamics are poorly understood. Increasing evidence show that protein responses can occur even faster than the cofactor dynamics. The causal reason for the ultrafast protein response cannot be explained by the localized cofactor excitation or its excited-state decay, alone. We propose a Coulomb interaction mechanism started by a shock wave and stabilized by a dipole moment change at least partially responsible for coherent oscillations in proteins, protonation changes, water dislocations, and protein changes prior to and beyond chromophore's excited-state decay. Photoexcitation changes the electron density distribution of the chromophore within a few femtoseconds: The Coulomb shock wave affects polar groups, hydrogen bonds, and protein bound water molecules. The process occurs on a time-scale even faster than excited-state decay of the chromophore. We discuss studies on selected photoreceptors in light of this mechanism and its impact on a detailed understanding of protein dynamics.



INTRODUCTION

Proteins are the central workhorses of cells and fulfill a variety of functions. They are essential for metabolic reactions, signaling, and transporting molecules. Bacteriorhodopsin (bR) from *Halobacterium salinarium* is a prime example of a well-studied protein.[1−3] A bundle of seven transmembrane α-helices connect the cytoplasmatic with the extracellular side encasing a hydrogen-bonded network (HBN) across the protein (see Figure 1). In the center of the protein, the retinal molecule (purple region Figure 1c,d) is covalently bound to amino acid residue lysine 216 and dissected the HBN in two parts.[4] The retinal molecule (chromophore) changes the properties of the protein to a purple photoreceptor absorbing visible light at an absorption maximum of 568 nm. Upon light absorption, bR is promoted to its electronic excited-state bR* initiating a photocycle on a millisecond time-scale. The decay of bR* is accompanied by an isomerization of the all-*trans* retinal to a 13-*cis* retinal on a subpicosecond time-scale.[5] In contrast to the rapid isomerization process, the ultrafast protein response is barely known. On a microsecond (μs) to millisecond (ms) time-scale, the protonated Schiff base transfers its proton to D85 depicted in Figure 1b (green arrow 1), and a proton is released from the protonated water network (cyan area in Figure 1c,d) stabilized by E204 to the water on the extracellular side (yellow arrow 2 in Figure 1b).[6] The protein bound water molecules in the HBNs around E204 and D85, and the arginine R82, are part of the proton release site of bR. Deprotonation of the 13-*cis* retinal chromophore shifts the color of bR to yellow (M-state).[7] Subsequently, the chromophore is reprotonated by D96 (red arrow 3 in Figure 1a,b) on the cytoplasmatic side of the chromophore followed by reprotonation of D96 from the cytoplasmic surface (purple arrow 4 in Figure 1a). Retinal isomerization from 13-*cis* to all-*trans* induces the proton transfer from D85 to the protonated water network around E204 (cyan arrow 5). These processes describe the essential proton transfer steps during the bR photocycle triggered by light absorption (first arrow): bR $\xrightarrow{h\nu}$ bR* → K→ L → M → N → O → bR. The photocycle passes through various intermediates, e.g., K, L, M, etc., assigned by visible absorption changes, and recovers the initial bR ground state within 100 ms. Vibrational studies in the infrared (IR) spectral range show detailed protein changes giving rise to many more substates.[8] The high-resolution crystal structure displays regions of protein bound water molecules (cyan areas in Figure 1) stabilized by hydrogen bonds with

Received: September 15, 2021
Revised: December 15, 2021
Published: January 13, 2022

https://doi.org/10.1021/acs.jpcb.1c08131
J. Phys. Chem. B 2022, 126, 581−587

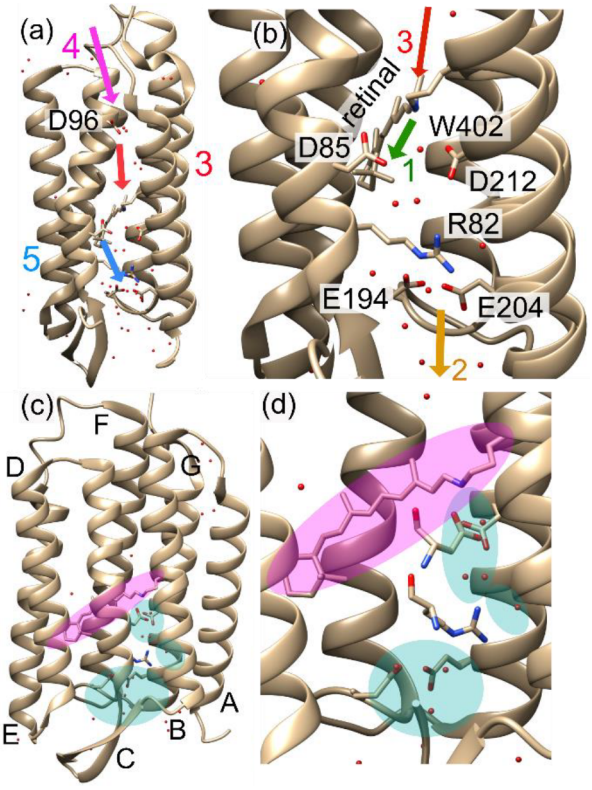


Figure 1. Ground-state structure of bacteriorhodopsin at 1.3 Å resolution (5ZIM.pdb selection A): top cytoplasmatic side, bottom extracellular side; numbers and arrows show proton pathways. Purple area: chromophore. Cyan area: H-bonded water networks. (a) Side view without helix B. (b) Zoomed area of panel a. From top to bottom: chromophore, D85 (left), water W402 (middle), and D212 (right); R82, E194 (left) and E204 (right). (c) Side view with all helices A−G. (d) Zoomed area of panel c without helix C.

glutamic acids, aspartic acids, and arginine groups.[4] These hydrogen-bonded water networks are able to stabilize a proton and can subsequently act as a proton acceptor and donor during the photocycle.[6]

Hydrogen bonds are weak but very important interactions in proteins. It is a local type of bonding involving a proton donor and a proton acceptor, such as protein bound water and polar and ionic amino acid side groups. Water and some amino acid side groups, e.g., aspartic and glutamic acids, can act as proton donor and proton acceptor groups at the same time. A chain of these groups can form hydrogen-bonded networks (HBN) or hydrogen-bonded networks containing several protein bound water molecules (HBWN). These networks can extend over the entire protein, facilitating ion transport. Thus, Coulomb interactions may affect distant groups connected via HBNs or HBWNs. Since the binding energies of hydrogen bonds are in the range of kT, they are prone to dynamic changes at physiologic conditions.[9] Thus, HBNs and HBWNs are unique areas able to be reshaped upon pH changes, electric field changes, ion concentration changes, protonation changes, conformational changes, substrate binding, and substrate release. HBNs and HBWNs rapidly propagate those changes through the protein. Protein bound water molecules are central for connecting polar groups in HBNs and act as sources of electric fields as well as reacting on electric fields on ultrafast time-scales. Moreover, they participate directly in transient proton loaded HBWNs inside proteins that are important for ion transport. Therefore, hydrogen bonds, HBNs, and HBWNs are key players for protein function and protein interaction.[6,8,10,11]

A group of important membrane photoreceptors are ion pumps and channels, such as bR, channelrhodopsin-1 in *Chlamydomonas augustae* (*Ca*ChR1), and other rhodopsins. They exhibit HBNs and HBWNs within the protein connecting both sides of the protein, forming the route for ion transport across the membrane.[11−14] These hydrogen-bonded substructures are highly anisotropic and labile. Upon light absorption by the retinal chromophore, an ultrafast isomerization process on a subpicosecond time-scale occurs resulting in a reorientation of the chromophore's Schiff base proton, altering its accessibility from one protein side to the other. The chromophore excitation followed by its photoisomerization gives rise to a driving force between the extracellular and cytoplasmatic side of the protein, resulting in a cascade of various protonation changes leading to net ion transport through the membrane protein.

In rhodopsins, the energy of the absorbed photon of about 20 000 cm^{-1} drives the conformational change of the chromophore and heats the chromophore and its direct surroundings on a picosecond time-scale. The retinal isomerization introduces an ultrafast steric change in the protein. Similarly, femtosecond X-ray experiments in myoglobin tracked the ultrafast release of CO from the heme group inducing steric alterations in the heme binding pocket. This process is accompanied by collective movements of the protein (protein quake) dissipating the excess energy throughout the protein.[15,16] Simulations interpreted this phenomenon as a pressure wave propagating in a highly anisotropic and asynchronous manner across the protein and solvent shell, thereby establishing long-living structural changes.[15,17] Since most femtosecond X-ray experiments are performed with light intensities giving rise to multiphoton processes,[18] it is unclear whether and to what extent protein quake-like processes are connected with single-photon processes which are expected in nature.[19] Recent femtosecond X-ray experiments on bR and on phytochrome do not report protein quake-like processes.[19,20] Nevertheless, various femtosecond X-ray experiments demonstrate far-reaching anisotropic structural changes within the protein on a picosecond time-scale together with damped coherent motions of the water molecules and protein side chains.[15,17,19] Apart from the light induced conformational change of the chromophore, the knowledge on other ultrafast processes steering the subsequent protein reaction cascade is mainly unknown. Most consistent with all experimental studies is the participation and altering of hydrogen bonds and HBNs in protein structural changes. Thus, interactions of HBNs are promising mechanisms for steering the biological function of the protein on all time-scales.

Motivated by the question of how the protein arrangement controls the reaction and biological function in detail, investigations on various photoreceptors were performed.

Locked Chromophores and Ultrafast Protein Changes. Experiments were designed to separate the impact of the chromophore isomerization on the protein dynamics. Locked chromophores were introduced into proteins, with nearly identical optical properties as the original chromophore but without the potential to isomerize. A comparison of wild-type (wt) bR and bR with locked chromophores (locked bR) shows nearly identical dynamical features on early time-scales, but no photoproduct formation and a photoreaction halted on a subnanosecond time-scale for locked bR.[21,22] Time-resolved IR and Raman investigations demonstrated an ultrafast protein

https://doi.org/10.1021/acs.jpcb.1c08131
J. Phys. Chem. B 2022, 126, 581−587

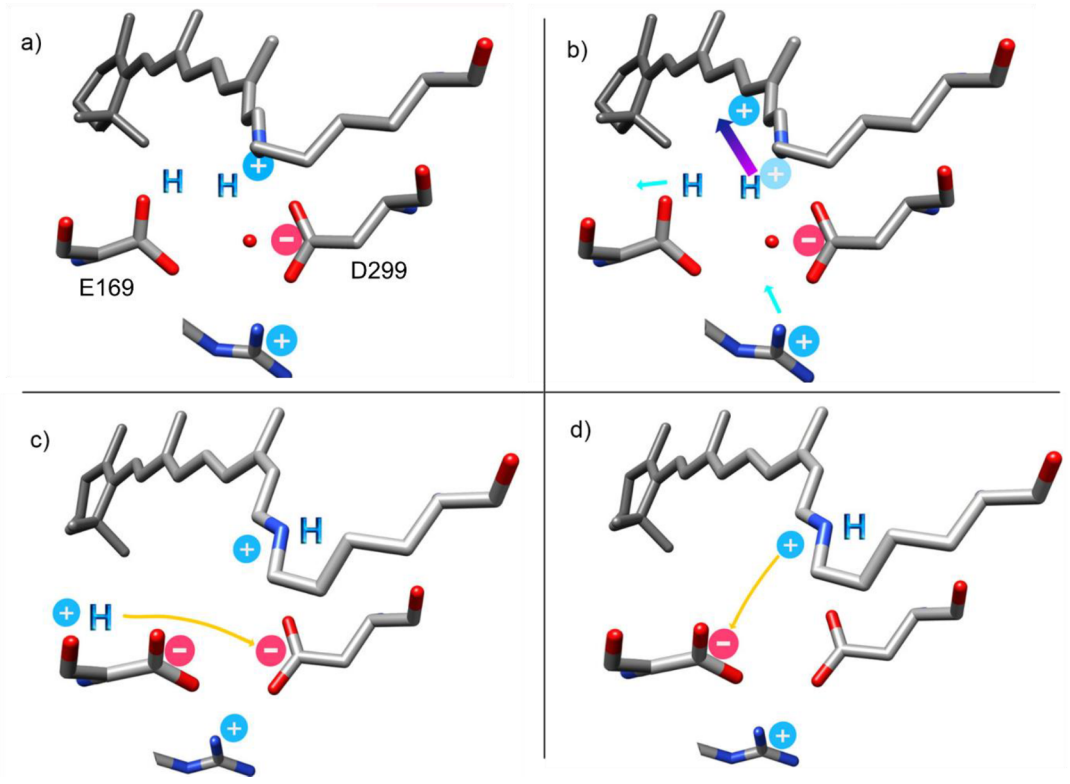


Figure 2. Ultrafast protein dynamics in *Ca*ChR1 upon photoexcitation: (a) protonation states of the counterion prior excitation; (b) electron density translocation along the retinal chromophore, concomitant deprotonation of Glu169 and protonation of the Glu169 backbone CO group; (c) isomerized retinal with 13-*cis* geometry, deprotonation of Glu169 backbone CO group, deprotonated carbonyl group of Glu169, and protonation of Asp299 with a time constant of 16 ps (yellow arrow), formation of early P1; (d) transition from P1 to P2 on the tens of microseconds time-scale accompanied by deprotonation of retinal and protonation of Glu169 (yellow arrow). Model from 1DZE.pdb.

response upon chromophore excitation in wt and locked bR generating long-living signals. For bR, a significant increase of the chromophore's dipole moment in the electronic excited was reported.[23] Thus, Gross et al. stated, "The altered dipole can couple to all charges, dipoles, or polarizable groups in adequate distance from the chromophore, e.g., protein bound water molecules, specific amino acid residues, or even to the dipole of an entire α-helix."[21] In this study, the perturbation of the protein persisted much longer than the electronic excited state of bR, demanding additional processes for the protein response.[21]

Studies on channelrhodopsin-2 from *Chlamydomonas reinhardtii* show that retinal isomerization does not only influence the side chains and HBN in the vicinity of the chromophore but also transfers energy to the protein backbone. These changes persist much longer than the excited-state lifetime, suggesting an efficient vibrational energy transfer from the chromophore to the protein.[24]

If one considers the structural change of the chromophore, i.e., the isomerization together with the chromophore relaxation processes in rhodopsins, as the only relevant interaction for the photoreaction, the driving force could be condensed to a local steric perturbation of the protein propagating across the protein by a pressure wave.[17] This contradicts ultrafast protein responses with locked chromophores and protein responses in systems without pressure wave evolution.[19−21]

Separating ultrafast processes on a femtosecond time-scale is difficult in IR studies with system responses of about 300 fs. Ultrafast visible experiments can provide system responses below 50 fs and are thus better suited to separate dynamic processes in time, but they are only sensitive to the chromophore and the amino acid side groups of tryptophan and tyrosine. These two amino acids are typically not involved in changes of protonation states but are sensitive to local electric fields and can be used to monitor ultrafast electric field changes inside the protein.

Electric Field Changes in Proteins. The impact of transient electric field changes on tryptophan residues interacting with the excited chromophore was demonstrated by Schenkl et al. in bR.[23] Two tryptophan residues in bR experience a spectral shift upon excitonic coupling with the retinal chromophore as a function of excited-state dipole moment strength.[23] Thus, significant dipole moment changes within some femtoseconds were tracked around the chromophore of bR. In a similar way, femtosecond stimulated Raman experiments on tryptophan and tyrosine were performed to track the response of these protein residues upon photoexcitation of bacteriorhodopsin. The authors concluded that the first event in the photocycle of bR is the ultrafast photoinduced change of the protein environment. This process would be faster than retinal isomerization.[25] Until now, the importance of electric fields in proteins for intermolecular interactions and ultrafast changes was not so often considered. A powerful method to determine the impact of quasistatic electric fields on protein residues is to measure the vibrational Stark effect. Using or introducing vibrational probes, such as carbonyl or nitrile groups, allows for measuring absolute electric fields or electric field changes.[26] Conformational changes in the protein modulate local electric fields reflecting altered intermolecular interactions.[27]

https://doi.org/10.1021/acs.jpcb.1c08131
J. Phys. Chem. B 2022, 126, 581−587

Ultrafast Water Dislocations. In solution, it is well-known that an ultrafast change in solute charge distribution induces an ultrafast change of the aqueous solvent within 50 fs. This includes rotational motion of water molecules.[28] Inside the protein, water molecules are mainly part of HBNs, and such protein bound water molecules are important for dynamic proton exchange. Furthermore, it was shown that electronic excitations are able to dislocate water molecules in proteins. Claesson et al.[20] performed femtosecond X-ray crystallographic data of a bacterial phytochrome at delay times of 1 ps after photoexcitation at different excitation intensities. They observed the dislocation of the conserved pyrrole water next to the chromophore upon photoexcitation of the tetrapyrrole.[20] Similarly, in bR the position of the water molecule W402 between D85 and D212 is shifted upon photoexcitation within 100 fs.[19,29] In both cases, the water molecule in close vicinity to the protonated chromophore is relocated in the electronic excited state before the isomerization process takes place. The causal reason for the significant ultrafast dislocation is unclear.

Ultrafast Protein Protonation Changes. Similar to water molecule responses, protons can respond in proteins on a femtosecond time-scale. Recently, we were able to demonstrate ultrafast protein protonation changes prior to the conformational change of the chromophore.[30] In *Ca*ChR1, the protonated Schiff base of the retinal chromophore interacts with a counterion consisting of protonated Glu169, deprotonated Asp299, and water molecules. The electrostatic interaction between these groups stabilizes the conformation of the counterion around the Schiff base (see Figure 2a). Upon photoexcitation, the partial positive charge is moved along the retinal backbone, E169 deprotonates, and the carbonyl backbone vibration of E169 is changed (Figure 2b). The backbone dynamics are assigned to a transient protonation of the E169 carbonyl group.[30] Deprotonation of E169 occurs prior to the isomerization process. With the decay of the electronic excited state, all-*trans* retinal isomerizes with a time constant of 100 fs and adopts the 13-*cis* conformation.[31,32] Subsequently, D299 is protonated with a time constant of 16 ps, and the carbonyl backbone vibration of E169 is deprotonated with a slightly shifted frequency compared to the parent state.

This proton transfer to D299 depicted in Figure 2c reflects protein dynamics significantly after the isomerization process.[7,30] The established protonation pattern paves the way for the proton transfer from the retinal to Glu169 (Figure 2d) in the transition of the long-living intermediates P1 to P2.[33,34] These observations demonstrate processes steering protein dynamics both prior to and beyond chromophore isomerization.

Collective Coherent Motions in Proteins. Photoisomerization processes were believed to be localized to the chromophore and its directly interacting groups. Thus, it was expected that ultrafast processes are local, and the impact of the isomerization process propagates through the protein via steric interaction and diffusional processes on a slow time-scale. Until now, there was no mechanism regarding how an isomerization process could propagate across the protein on a time-scale of a few picoseconds. Coherent oscillations in the visible spectral region were observed for protein side groups or ligands directly connected to the chromophore.[35] Recently, coherent oscillations of protein groups and water molecules were observed by femtosecond X-ray experiments upon photoexcitation, but their origin remained unclear.[15,16,19] These oscillations involve several amino acids and water molecules throughout the protein on a time-scale of a few hundred femtoseconds. In particular, water molecules and residues participating in the proton release site, a HBWN, toward the extracellular side show strong coherent contributions in bR.[19] The residues and water molecules in the proton release site of bR are neither bound to the chromophore by covalent or a direct strong hydrogen bond, nor involved in its electronic excitation (see Figure 1). Thus, a direct coupling to the chromophore is negligible, and vibrational energy relaxation processes are too slow for a subpicosecond response of these groups.[36] Interactions between these groups are expected to be dominated by collisions. This is reflected by ultrafast visible investigations on bR that show no signs of coherent protein vibrations.[37] To date, there is no conclusive evidence for the origin of such coherent oscillations. Hence, the structural change of the chromophore upon electronic excitation cannot be the causal reason for coherent protein vibrations throughout the protein.

Another interesting aspect regarding coherent oscillations is the proton tunneling process between residues. In green fluorescent protein, a proton tunneling process at pH 8 was reported for the hydroxyl group of serine (S205) with a pK_a of ∼16.[38] The serine group is part of an HBN composed of water molecules and uncharged amino acid residues. The distance between proton donor and acceptor was shown to be essential for an effective proton transfer.[39] Coherent vibrations along the HBN could modulate the donor−acceptor distance enhancing the tunneling probability of the proton.[40,41] Thus, coherent oscillations could directly support protein conformational changes.

Impulsive Electric Field Changes Drive Ultrafast Protein Responses. Here, we propose a new aspect of the reaction mechanism that is at least partially responsible for coherent oscillations in proteins, protonation changes, water dislocations, and protein structural changes both prior to and beyond the excited-state lifetime.

Photoexcitation creates a superposition of the electronic ground and excited states that dephases within a few femtoseconds and populates the electronic excited state. This process is connected with an electronic transition dipole moment (TDM, in the dipole approximation) fixed within the chromophore. Accompanied with the change of the wave functions is a change of electron density distribution at the chromophore. This is described by the change of the dipole moment from the ground to the electronic excited state. The dipole moment change can be very strong for charge transfer states as in bR, where a dipole moment change of about 12 debeye was observed.[23] The change of the dipole moment and electron density distribution is set by the electronic excitation and remains for the lifetime of the electronic excited state. Concomitant with the change of electron density, the electric field is changed. The impulsive electric field change at the chromophore can induce a Coulomb force shock wave on its surroundings, affecting polar groups instantaneously. It is like a "Coulomb-Hammer" hitting the chromophore, changing the electric field and unleashing Coulomb forces impulsively. The time-scale of the impulsive electric field change can be as fast as the light absorption of about two femtoseconds and corresponding to a frequency range >10 000 cm^{-1}. This spectral range covers all fundamental vibrations of the protein enabling structural changes throughout the protein. Water is known as the fastest solvent, and a solvent response faster than 50 fs dominates aqueous solvation dynamics.[28] The efficient shielding effect of water solvation shells is absent inside proteins,[9,28,42,43]

https://doi.org/10.1021/acs.jpcb.1c08131
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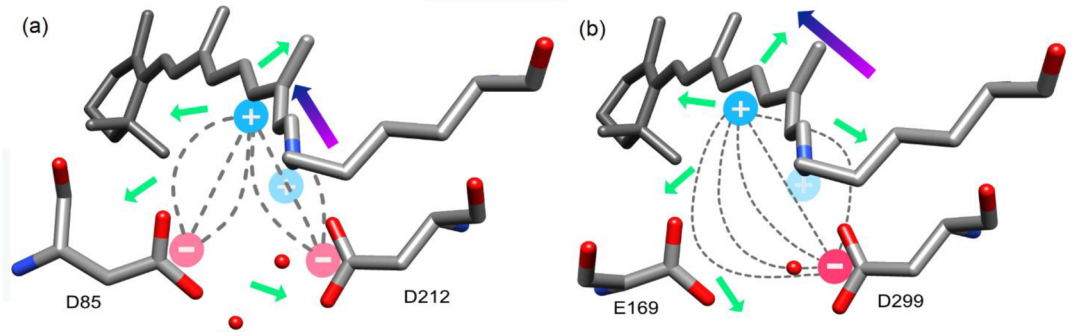

Figure 3. Impulsive electric field changes upon photoexcitation. A partial positive charge is translocated along the retinal backbone (blue arrow); the new charge position is in blue, and the initial position is in light blue. A new electric field (dashed lines) is established with a new Coulomb force differing in direction (green arrows) and strength. (a) Model of bR with counterion D85 and D212 and protein bound water molecules (red balls). (b) Model of *Ca*ChR1 with counterion E169 and D299 and protein bound water molecule (red ball). Deprotonated carboxylic acids indicated with a red circle and a minus sign. Structural models based on bR crystal structure 5ZIM.pdb.

and protein bound water molecules and HBNs in anisotropic substructures are able to respond ultrafast.

The impulsive Coulomb force or electric field change, induced by the new dipole moment in the excited state, could be the cause for *coherent protein oscillations* of charged residues, water molecules, and HBNs as observed in femtosecond X-ray experiments.[15,19] The electric field jump within a few femtoseconds creates a changed electric field around the chromophore (see Figure 3a) and an instantaneous force on polar groups triggering a directed movement with a defined phase. The movement inside the protein is reflected by normal modes with a coherent phase and anisotropic direction. In bR, coherent oscillations were reported for groups participating in the proton release site.[19] The proton release site consists of protein bound water molecules and HBNs (see Figure 1d).

Moreover, *ultrafast protein changes* in *Ca*ChR1 independent of the isomerization process can be explained by an ultrafast dipole moment change in the electronic excited state: As depicted in Figure 3b, photoexcitation translocates a fraction of the positive charge along the retinal chromophore toward the β-ionone ring.[23] This sudden relocation of charges strongly distorts the electrostatic equilibrium and alters the Coulomb forces around the chromophore (see Figure 3b), resulting in a concomitant deprotonation of Glu169 and a change of its protein backbone carbonyl group (Figure 2b). Since neither an overlap of the retinal excited-state wave function to the carboxylic group Glu169 or to the protein backbone exists, nor a steric interaction gives rise to the observed changes on this time-scale, the structural changes of the chromophore in the excited state including isomerization cannot be the causal reason for the protonation dynamics.[30] Thus, we propose the ultrafast altered electric field change to be responsible for these *ultrafast protonation changes*.

Ultrafast water dislocations were observed in femtosecond X-ray experiments in bR and phytochrome.[19,20,29] In both systems, the position of the water molecule close to the chromophore is significantly changed prior to photoisomerization. Since water molecules can respond on a time-scale of about 20 fs on a change of charge distribution,[28] it seems plausible that the causal reason for the dislocation of water molecules is the altered electric field.

Ultrafast protein changes during the electronic excited state are only significant if they cause a change in the protein on a longer time-scale. In bR, the backbone perturbations initiated upon photoexcitation persist on the 100 ps time-scale.[21] In *Ca*ChR1, the initial deprotonation of E169 leads to protonation of D299 on a longer time-scale, relevant for further downstream processes.[30] Thus, ultrafast protein changes, faster than isomerization, are significant for protein changes on a longer time-scale. The driving force is presumably the altered Coulomb force inducing transient electrostatic changes propagating in time and space in the protein.[10,21]

CONCLUSION

The existence of ultrafast electric field changes in proteins was demonstrated, and the first experiments suggest that they may be the origin of coherent protein oscillations, ultrafast protonation processes, ultrafast water dislocations, and ultrafast protein dynamics. Strong support for these processes comes from femtosecond X-ray experiments providing detailed structural information on the atomic level of the proteins. The data support ultrafast dynamics along hydrogen-bonded networks extending across the protein and at polar groups sensitive to electric field changes.[19,29,38] These observations are consistent with femtosecond visible, IR, and Raman studies demonstrating electrostatic interactions,[23,25] protonation changes,[30] and protein structural changes.[21,24] Visible studies are sensitive to the local environment of the chromophore and show higher time resolution compared to femtosecond X-ray and femtosecond IR studies. IR experiments are very sensitive to hydrogen bonds and proton loaded water networks and can easily be performed in a single-photon excitation regime, which is relevant to understand function at physiological conditions. Since femtosecond X-ray experiments have complementary sensitivity to femtosecond electronic and IR spectroscopic methods, a joint analysis of all methods should further enlighten the largely unexplored ultrafast protein reaction processes. Further IR investigations with improved time resolution should demonstrate coherent protein vibrations in proteins, e.g., bR, corroborating the existence of coherent protein vibrations in a linear excitation regime.

The trigger of the biological relevant protein dynamics is the conformational change of the chromophore, or the excitation process, or both. Here, we want to stress the importance of the excitation process inducing an impulsive electric field change affecting polar groups in an impulsive way. This impulsive process can induce coherent oscillations of protein groups via Coulomb interactions. The electric field jump, induced by the change of dipole moment, triggers protein dynamics during the electronic excited state and can induce persisting changes longer than the time-scale of the activation process of the

https://doi.org/10.1021/acs.jpcb.1c08131
J. Phys. Chem. B 2022, 126, 581−587

chromophore. Moreover, interactions via Coulomb forces are ultrafast, far-reaching, collective, and anisotropic along interacting groups. Concerted protonation changes and reorganization of hydrogen-bonded networks can initiate a cascade of slower and far-reaching processes, starting at individual groups and extending up to helix changes. These processes are promising candidates to steer the protein dynamics and conformational changes to its biological relevant function. A detailed understanding of those interactions from femtoseconds to seconds and from single protonation states to secondary structure changes is mandatory to design and copy processes from nature to artificial systems.

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Author Contributions

The manuscript was written by K.H.

Notes

The author declares no competing financial interest.

Biography

Karsten Heyne received his Ph.D. in physics from Freie Universität Berlin in 2001, and after working at the Max Born Institute for Nonlinear Optics and Short Pulse Spectroscopy in Berlin, he has been a professor for ultrafast protein dynamics in the physics department of Freie Universität Berlin, since 2004. His research interests currently focus on high-resolution and ultrafast electronic and vibrational spectroscopy on biologically relevant molecules and proteins to characterize and steer fundamental processes of life.

ACKNOWLEDGMENTS

The work was supported by the Deutsche Forschungsgemeinschaft (through Grants CRC 1078 "Protonation Dynamics in Protein Function", Project B07). The author thanks J. Luis Pérez Lustres for valuable discussion. The crystal structures 5ZIM.pdb and 1DZE.pdb of bacteriorhodopsin were used to create the structural models for Figures 1−3 with the software Chimera 1.14 and Affinity Designer.

ABBREVIATIONS

bR, bacteriorhodopsin; *Ca*ChR1, channelrhodopsin-1; HBN, hydrogen-bonded network; HBWN, hydrogen-bonded water network

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