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Ultrafast Proteinquake Dynamics in Cytochrome *c*

Chen Zang, Jeffrey A. Stevens, Justin J. Link, Lijun Guo, Lijuan Wang, and Dongping Zhong*

Departments of Physics, Chemistry, and Biochemistry, Programs of Biophysics, Chemical, Physics, and Biochemistry, The Ohio State University, 191 West Woodruff Avenue, Columbus, Ohio, 43210

Received July 22, 2008; E-mail: dongping@mps.ohio-state.edu

Abstract: We report here our systematic studies of the heme dynamics and induced protein conformational relaxations in two redox states of ferric and ferrous cytochrome *c* upon femtosecond excitation. With a wide range of probing wavelengths from the visible to the UV and a site-directed mutation we unambiguously determined that the protein dynamics in the two states are drastically different. For the ferrous state the heme transforms from 6-fold to 5-fold coordination with ultrafast ligand dissociation in less than 100 fs, followed by vibrational cooling within several picoseconds, but then recombining back to its original 6-fold coordination in 7 ps. Such impulsive bond breaking and late rebinding generate proteinquakes and strongly perturb the local heme site and shake global protein conformation, which were found to completely recover in 13 and 42 ps, respectively. For the ferric state the heme however maintains its 6-fold coordination. The dynamics mainly occur at the local site, including ultrafast internal conversion in hundreds of femtoseconds, vibrational cooling on the similar picosecond time scale, and complete ground-state recovery in 10 ps, and no global conformation relaxation was observed.

I. Introduction

Kinetic studies of heme proteins have been playing a critical role on the development of conceptual framework in protein dynamics, such as heterogeneity, substates, energy landscape, and proteinquakes.[1−6] The classic heme−ligand dynamics have been extensively studied in various heme proteins on ultrafast time scales[7−13] and also applied to address important questions of protein folding,[14−20] allosteric regulation,[21−23] and even glass transition.[1,24,25] Although a variety of biophysical techniques have been used to characterize protein motions,[26−38] direct probing of global conformation relaxation has been difficult and only recently was the picosecond-resolved X-ray diffraction on myoglobin relaxation reported.[39−41] In analogy to our recent mapping of hydration dynamics around protein surfaces,[42,43] we can directly map out global protein fluctuations using an optical probe placed one at a time at a desired position with

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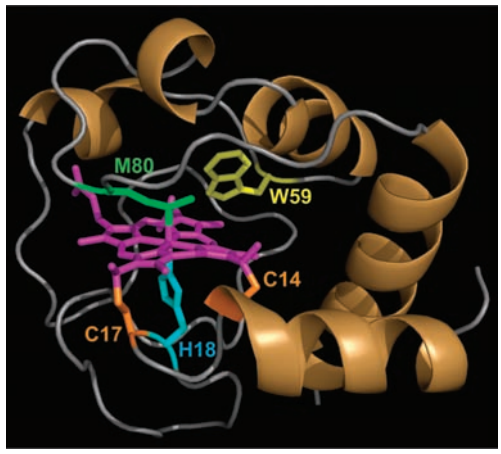

Figure 1. X-ray structure[50] of horse heart cytochrome *c* (PDB: 1HRC) with the prosthetic heme group covalently linked with four residues, M80, H18, C17, and C14. Also shown is the W59 residue as an optical probe for detecting protein conformation dynamics.

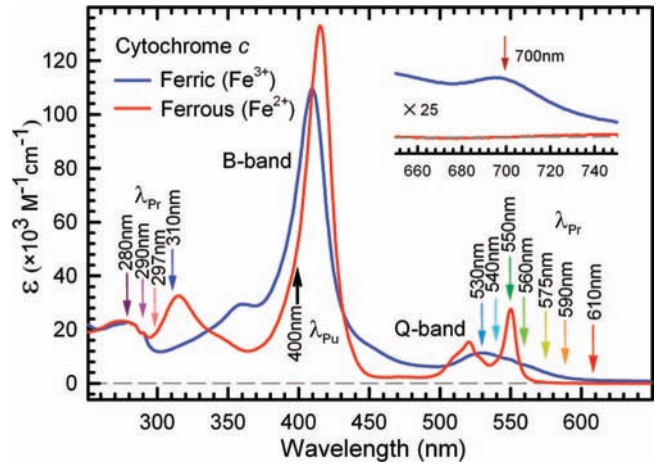

Figure 2. Steady-state absorption spectra of ferric and ferrous cytochrome *c*. Arrows mark two probing regions of visible and UV wavelengths (λ_{pr}) with B-band excitation at 400 nm (λ_{pu}). The inset shows the weak absorption of the heme−M80 charge-transfer state at 695 nm in the ferric state. Note that the UV probing from 297 to 280 nm also detects ground-state tryptophan dynamics.

site-directed mutations. Two potential molecular probes have recently been reported: One is intrinsic amino acid tryptophan (W or Trp) by measurement of UV absorption changes,[44−46] and the other is nitrile-derivatized amino acids by detection of IR absorption changes.[47−49]

Cytochrome *c* (Cyt *c*), a model heme protein, has been widely used to study folding and unfolding[14−20] with novel initiation methods of fast temperature jump,[20] electron transfer between two redox states (ferric Fe^{3+} and ferrous Fe^{2+}),[14] and ligand dissociation under certain denaturation conditions.[16] Figure 1 shows the X-ray structure of horse heart Cyt *c* (PDB:1HRC), an α-globular protein with a prosthetic heme group ligated with a proximal H18 and a distal M80 and covalently linked with C17 and C14 residues.[50] The protein only contains a single tryptophan residue (W59), which is ideal for specifically probing local conformation relaxation. Figure 2 shows the absorption spectra of the protein in two redox states. Two ultrafast studies of the heme dynamics in Cyt *c* have been recently reported.[11,32] One studied ferrous Cyt *c* with excitation at the B-band S_2 state (400 nm) and probing in the visible region (450−650 nm).[11] The other investigated both ferric and ferrous Cyt *c* with the Q-band S_1 state excitation (550 nm) and probing by resonance Raman and transient absorption methods in the B-band region (385−445 nm).[32] Both studies focused on the local heme site and elucidated a series of time scales of heme−ligand dissociation, vibrational cooling, and ground-state recovery. However, the critical protein conformational relaxation induced by the heme dynamics was not yet investigated.

In this work, we systematically examine the heme dynamics and resulting protein conformational relaxations at the B-band excitation with femtosecond resolution. The main goal here is to develop a methodology to determine the time scale(s) of protein conformational relaxation caused by the proteinquake from the epicenter of heme−ligand photolysis. Such proteinquake dynamics have been proposed early in myoglobin[2,51] and recently in photoactive yellow protein[52−54] as well as described in theory.[55,56] With a wide range of probing wavelengths (Figure 2) from the visible (700 nm) to the UV (280 nm) we are able to unequivocally determine the different heme excited-state dynamics in the ferrous and ferric states. By monitoring the absorption changes of ground-state tryptophan (W59) with UV light we can follow protein conformational relaxations. To

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further ascertain the tryptophan detection we mutated W59 to phenylalanine (F) with site-directed mutagenesis. These results not only elucidate the unique heme−ligand dynamics in Cyt *c* but also provide a direct way to probe protein conformational fluctuations triggered by the local heme reaction in widely studied heme proteins.

II. Materials and Methods

A. Sample Preparation. Wild-type horse heart cytochrome *c* was purchased as a lyophilized powder from Sigma with 99% purity or purified using plasmid pJRhrsN, generously provided by Prof. Jon Rumbley (University of Minnesota, Duluth), by following the published procedures with our modification.[57,58] The mutant protein of W59F was designed and purified with the same procedures. For both steady-state and time-resolved experiments the wild-type protein was dissolved in 0.1 M potassium phosphate buffer at pH 6.5. For the mutant we used 25 mM sodium phosphate buffer at pH 7.6. For ferric experiments the sample was used without further purification. Ferrous Cyt *c* was prepared by purging the ferric protein with high-purity nitrogen to remove oxygen and then reducing it with a 1:5 ratio of the protein to sodium dithionite.[59] The protein concentration was maintained to be 400−500 μM, and all femtosecond experiments were carried out at room temperature with a moving 1 mm quartz cuvette to avoid heating and sample degrading. To ensure that there was no change of the protein quality and heme redox states, the absorption spectra of the samples were verified before and after time-resolved experiments.

B. Femtosecond Transient-Absorption Method. The transient-absorption measurement configuration has been described in detail elsewhere.[60] Briefly, the femtosecond laser pulse after the Spitfire Ti:Sapphire amplifier (Spectra-Physics) has a temporal width of 110 fs with energy of ~2 mJ per pulse and a repetition rate of 1 kHz. The laser beam is then equally split to pump two optical parametric amplifiers (OPA-800C, Spectra-Physics). The pump pulse at 400 nm was generated by direct doubling of the fundamental 800 nm from the first OPA through a 0.2 mm thick β-barium borate (BBO) crystal. The various probe wavelengths from 500 to 700 nm were generated by mixing of the idler or signal with the fundamental from the second OPA through another BBO crystal (0.2 mm). Certain wavelengths were further doubled by a third BBO crystal (0.2 mm) to generate UV probing wavelengths from 280 to 310 nm. The pump beam polarization was set at the magic angle (54.7°) with respect to the probe beam. The sensitivity of the transient-absorption method can reach 10^{-4}−10^{-5} of absorbance change.

III. Results and Discussion

Figures 3 and 4 show the transient-absorption (TA) dynamics of wild-type ferrous and ferric Cyt *c*, respectively, upon 400 nm excitation and probed by a series of wavelengths from 280 to 700 nm. Since the transients show complex dynamics and also the heme dynamics on the longer time scales often follow the power-law behaviors,[1,2,4,5,10,51] both plots of log *I* vs log *T* and log *I* vs *T* were used to examine nonlinearity and are also shown in the Supporting Information. All transients are best represented using multiple-exponential decay functions, and the final fitting results are also given in the Supporting Information. On such short time scales within a few picoseconds these multiple exponential

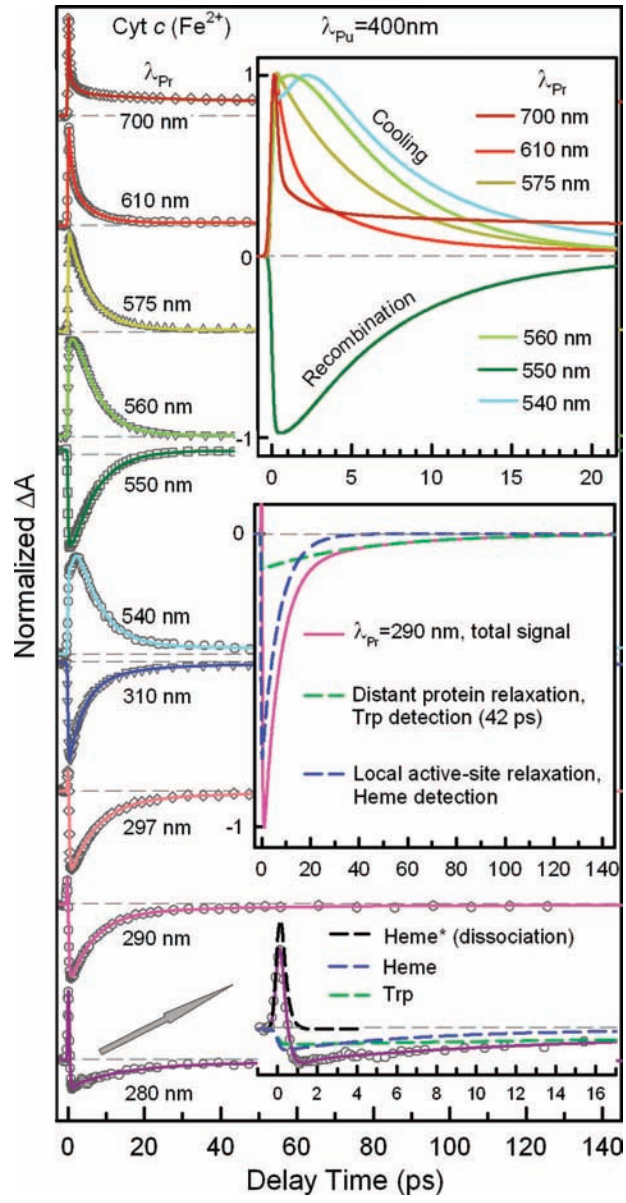

Figure 3. Femtosecond-resolved transient absorption of the ferrous state upon 400 nm excitation probed from UV 280 nm to visible 700 nm. The top inset shows the fitting transients probed by visible wavelengths for comparison. The middle inset gives deconvolution of the 290 nm UV transient into the heme and tryptophan dynamics. The bottom inset shows deconvolution of the 280 nm UV transient into initial excited-heme dynamics, ground-state heme−ligand recombination, and protein conformation relaxation by tryptophan detection. Clearly, all UV transients show an initial ultrafast decay in less than 100 fs and then a negative bleaching and recovery signal.

decays reasonably represent excited-state dynamics, vibrational cooling, and ground-state recovery (see below), although these ultrafast dynamics may be nonexponential. Clearly, both states in Figures 3 and 4 show quite different dynamical patterns. With the UV probing (310−280 nm) we expect to detect the initial excited-state dynamics, and the TA signals for the two states do show drastic differences. The ferrous state shows an initial ultrafast decay and then a dominant negative signal of bleaching and recovery. The ferric state exhibits nearly all positive TA signals, simply reflecting the dynamics of excited states. In the visible region (700−530 nm) the ferrous Cyt *c* simply shows either positive or negative signals while for the ferric state the transients

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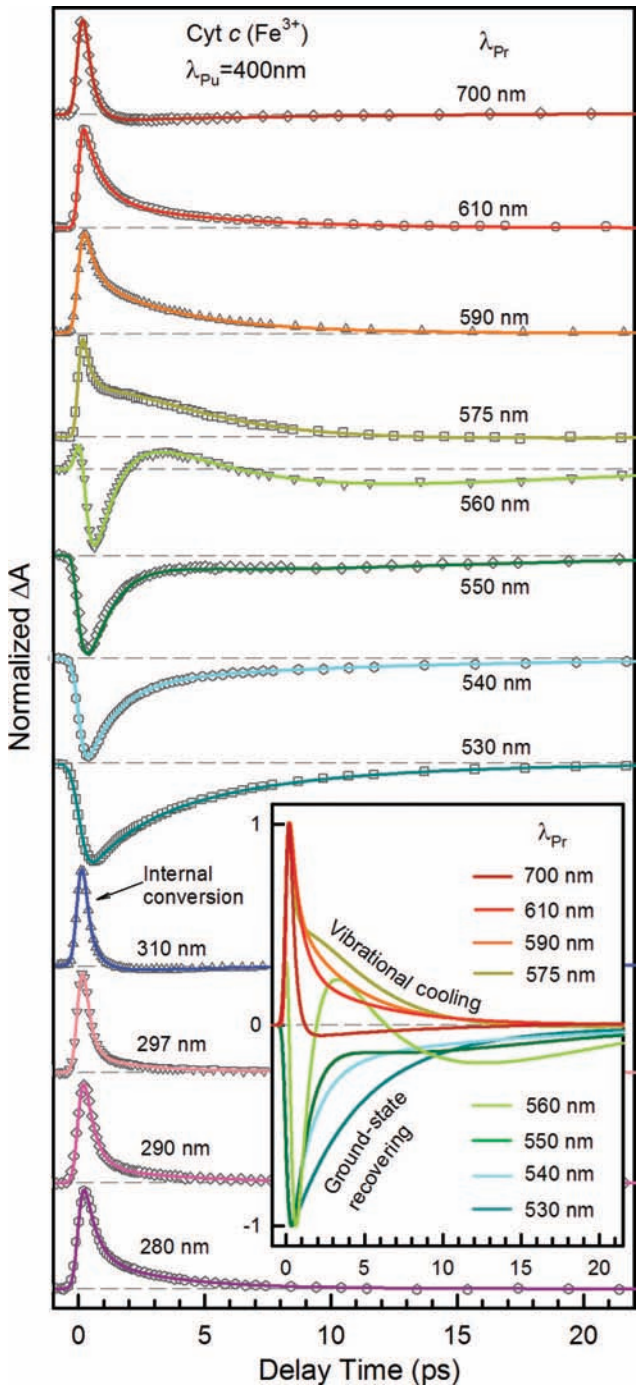

Figure 4. Femtosecond-resolved transient absorption of the ferric state upon 400 nm excitation probed from UV 280 nm to visible 700 nm. The inset shows the fitting transients probed by visible wavelengths for comparison. Clearly, all UV transients (310−280 nm) show positive decay dynamics, drastically different from those in Figure 3 for the ferrous state, while in the visible region (700−530 nm) transients are convoluted by a systematic mixture of decay and formation dynamics.

show complex TA signals with mixed positive decay and negative formation dynamics. Clearly, the two redox forms of Cyt *c* have distinct excited-state dynamics and different protein responses.

A. Photochemistry of Ferrous Cyt *c*, Impulsive Proteinquake Formation, and Global Conformation Relaxation. As shown in Figure 3, in the UV region the transients show an ultrafast initial positive decay in ∼100 fs and then suddenly change to a negative signal. This dynamic pattern clearly shows a photochemical reaction of heme−ligand dissociation. The ultrafast sign switching from positive to negative signals results from the transformation from 6-fold to 5-fold coordination of the ferrous state. The initial positive signal results from the absorption difference of ground-state bleaching and excited-state formation of 6-fold coordination ferrous state. After heme−ligand dissociation the heme transforms from 6-fold to 5-fold coordination and the negative signal mainly depends on their absorption difference, indicating that the 5-fold coordination ferrous Cyt *c* has weaker absorption. This observation is completely different from that in ferric Cyt *c* (Figure 4 and see below), which shows all positive signals in the UV region, thus clearly distinguishing two different excited-state dynamics in the two states. The observation of ligand dissociation is consistent with recent studies using visible light probing by Negrerie et al.[32] at the Q-band excitation and Wang et al.[11] at the B-band excitation, which indicated that photodissociation of heme−M80 occurs in about 40 fs.

The impulsive heme−ligand bond breakage in less than 100 fs instantaneously generates significant structural perturbation, resulting in an ultrafast proteinquake formation[2,51] at the local heme site which immediately spreads outward and induces protein conformation fluctuation. At 310 nm we observed ground-state heme formation in 4 (76%) and 13 ps (23%). This cascaded formation dynamics represents vibrational cooling in a few picoseconds and then complete ground-state heme−M80 recombination and recovery in 13 ps (also see visible-light probing below). Significantly, in the region of 297−280 nm besides formation of the signal of heme−M80 in 7 ps we observed an extra long-time formation dynamics in ∼40 ps. This long-time signal was not observed at 310 nm nor at any visible wavelengths (see below). This signal is from the dynamic changes of ground-state W59 absorption. Because tryptophan has nearly no absorption at wavelengths longer than 300 nm we did not observe this long-time component in any transients probed at longer wavelengths than 300 nm. Below 300 nm we observed this long-time component at all wavelengths we probed, at 297, 295, 290, 285, and 280 nm. This result is significant and shows that the protein conformation recovery induced by the impulsive proteinquake is ultrafast and only takes ∼40 ps, a time scale of only about three times longer than the local heme relaxation (13 ps).

The conformation relaxation of myoglobin, induced by heme−CO dissociation, has been extensively studied by various methods.[1−13,61−63] Relaxation occurs on multiple time scales and is very nonexponential and heterogeneous. The initial proteinquake caused by dissociation is not very strong, but the perturbation continues from CO diffusion inside the protein and its escape from site to site. Recently, an ultrafast substate conformation switching was observed to occur even in 50 ps.[63] However, using tryptophan as a local probe and with more than 15 mutations we recently did not observe significant conformation relaxation of myoglobin after photolysis in an attempt to map out global relaxation, as also confirmed by others.[64] Clearly, the results depend on the probe locations and extent of perturbation. In Cyt *c* the situation is different. The residue M80

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is part of the protein and covalently linked to the backbone of a close loop. The impulsive dissociation significantly shakes the protein, and the formed quake is strong enough to cause the noticeable conformation fluctuation. The probe W59 is linked to a distant loop but points inside the hydrophobic region and even forms a hydrogen bond with a propionate of the heme. Around W59 several residues, for example, L35, T40, L64, Y67, and I75, from different helices and loops are in proximity. Significantly, we observed the complete heme recovery in 13 ps after the full recombination in 7 ps, but the absorption recovery of W59, representing relaxation of its neighbor protein environment/conformation, only needs about 40 ps. Comparing with myoglobin, this relaxation process is ultrafast. One of the main reasons is because the heme−M80 recombination occurs ultrafast in 7 ps while in myoglobin the dissociated CO molecule does not recombine back and causes continuous local conformation perturbations over wide time scales.

It should be pointed out that the detected negative signal at 290 nm has two contributions. The main signal (85−90% of the total negative amplitude) is from the absorption difference of 6-fold and 5-fold coordination ferrous states. The molar extinction coefficient is about 15 000 M^{-1} cm^{-1} at 290 nm for the 6-fold coordination ferrous state,[65] and our negative signal shows that the extinction coefficient for the 5-fold one is smaller, consistent with the trend observed for myoglobin.[66] The other part of the signal (10−15%) is from the ground-state absorption change of tryptophan in different protein conformations, before and after dissociation, a process of the Stark effect. The molar extinction coefficient of tryptophan is about 3770 M^{-1} cm^{-1} at 290 nm.[67] Thus, the observed negative signal (10−15%) indicates that the tryptophan absorption after dissociation shifts to the blue side, resulting in a decrease of tryptophan absorption at 290 nm. Using the extinction coefficient difference of several thousands for 6-fold and 5-fold coordination ferrous myoglobin at 290 nm[66] the observed 10−15% signal indicates that the extinction coefficient change of tryptophan is about several hundreds, which means that the tryptophan absorption spectrum shifts to the blue side in less than 1 nm.

To further ensure that the long-time component is from the tryptophan signal, not from the heme dynamics, we performed the mutation studies of W59F to remove the single W59. Figure 5 shows the results probed at 290 nm for both the wild type and the mutant. Cleary, the long-time component in ∼40 ps is completely absent in the mutant, but the transient still has the initial ultrafast positive signal of dissociation in ∼100 fs and the negative signal of heme−M80 recovery in 7 ps. Similarly, we did not observe any long-time component in ∼40 ps at other UV wavelengths. This mutation control experiment unambiguously proves that the observed 40 ps dynamics in the wild-type protein is from W59, a ground-state dynamics reflecting the conformation changes induced by the strong perturbation of the heme−M80 dissociation. Therefore, W59 has enough sensitivity to probe the conformation changes caused by the proteinquake, unlike in myoglobin where the perturbation is weaker and the probe may not be at desired positions.

In the visible region (Figure 3) we observed systematic decay and formation signals but all faster than 7 ps. As shown in Figure 2, above 560 nm, there is nearly no ground-state absorption, and thus, the positive decay signals are dominant. For 700, 640

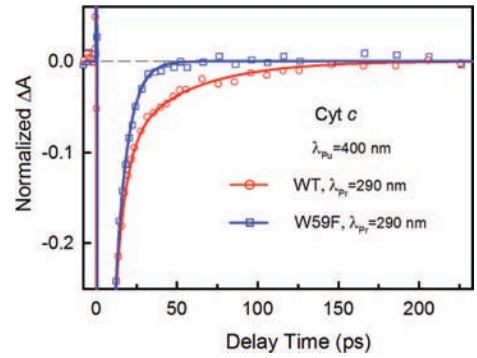

Figure 5. Femtosecond-resolved, normalized transient absorption signals of the wild-type and mutant (W59F) Cyt *c* probed at 290 nm. Here we focus on the long-time components. In the mutant, the long-time component is clearly absent, while in the wild type, the long-time component in ∼42 ps is apparent, which is from the ground-state absorption changes of W59 due to the conformation fluctuations caused by the proteinquakes.

(not shown), and 610 nm we observed two ultrafast decay components of 0.18−0.78 and 4.5−5 ps besides a minor nanosecond component (<5%, no heme−M80 dissociation or recombination). At 575 nm we only observed a single-exponential decay of 5.7 ps. At 560 nm we observed a rise component in 1.8 ps and a decay component of 5.5 ps. At 550 nm around the Q-band absorption peak we observed a cascaded ground-state formation signal in 1.1 and 6.7 ps. Finally, at 540 nm, due to weaker ground-state absorption, we observed one rise component in 1.0 ps and two dominant decay components again in 0.56 and 6.3 ps.

With combination of UV and visible probing, the dynamics of the ferrous state of Cyt *c* is clear. Figure 6 illustrates the dynamical processes involved in the ferrous state of Cyt *c* upon heme excitation. The photodissociation of heme−M80 is the dominant channel, and the bond breakage occurs in less than 100 fs. After ligand dissociation it seems that the 5-fold coordination ferrous state is formed in the vibrationally hot ground state, although formation in the electronically excited state could be possible. The cascaded vibrational cooling occurs within 7 ps, accompanying heme−M80 recombination. The complete relaxation of the local heme site takes 13 ps. A series of proteinquake formations by initial impulsive bond breakage in less than 100 fs and subsequent local environment cooling and the heme−M80 rebinding within 7 ps all induce significant protein conformation changes. These collective structural relaxations take 42 ps to completely dissipate energy to the tryptophan environment and have the conformation fully recovered.

B. Photophysics of Ferric Cyt *c* and Ultrafast Energy Dissipation. As shown in Figure 4 in the UV region we basically observed all positive signals, totally different from those for the ferrous state (Figure 3), with two systematic decays for all transients from 310 to 280 nm with two time constants of 0.14−0.46 and 0.51−4 ps, reflecting gradual relaxation from initial higher to cascaded lower energy states detected by longer to shorter wavelengths. These results strongly suggest that the excited Cyt *c* do not proceed to heme−ligand dissociation and the observed dynamics represent excited-state cascade relaxation. Otherwise, dissociation would result in instantaneous transformation from 6-fold to 5-fold coordination and cause sudden absorption changes (see above for the ferrous state). At 310 nm we also observed a small negative formation signal in about 6 ps, but the absence of formation signals in other UV wavelengths (297−280 nm) indicates dominant excited-state

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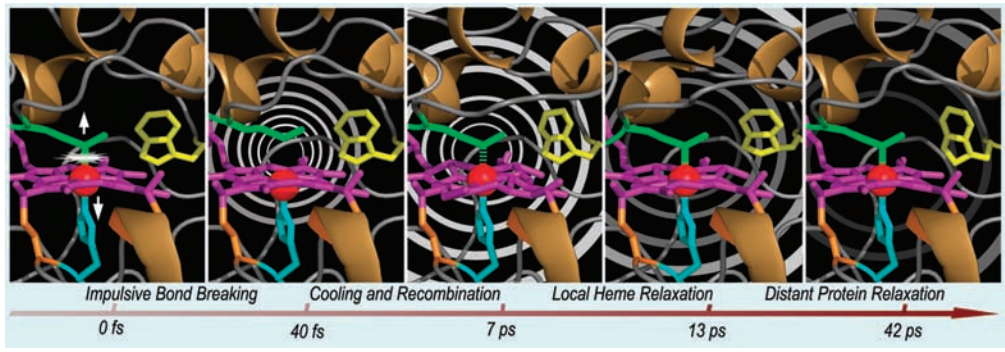

Figure 6. Schematic representation of a series of snapshots of cascaded heme and protein dynamics for the ferrous state upon excitation at $t = 0$. The impulsive bond breakage of heme−M80 occurs in about 40 fs, resulting in proteinquake formation, represented by a series of concentric circles. At about 7 ps, the residue M80 rebinds back to the central iron of the heme after a series of vibrational cooling, including local structural relaxation. During this time the heme is highly distorted and the proteinquakes spread outward and shake global conformation. At 13 ps the proteinquakes at the local heme site are fading away and the heme environment is fully relaxed. However, the proteinquakes around the probe Trp take 42 ps to disappear and the perturbed protein conformation fully recovers on the time scale of about three times longer than the local heme recovery.

absorption and a similar time scale of ~4 ps for ground-state recovery. It should be emphasized here that we did not observe any long-time signal ($\tau \geq 20$ ps) at 297−280 nm by Trp-absorption change, which also indicates no heme−ligand dissociation and no significant conformation perturbation. Negrerie et al.[32] recently reported extensive studies of the ferric Cyt *c* upon the Q-band (S_1 state) excitation by both time-resolved TA and resonance Raman detection. By observation of the absent strong B-band absorption at 390−400 nm for 5-fold coordination ferric Cyt *c* and of different Raman spectra between ferric and ferrous states they concluded that photolysis in ferric Cyt *c* does not occur and the heme−M80 bond is not broken. Such a conclusion is directly shown here by our UV probing. The observation of no heme−M80 dissociation and no long-time conformation relaxation further indicates that the energy dissipation in the ferric state is ultrafast and mainly occurs at the local heme site. There are no significant structural changes in the local heme site of ferric Cyt *c*, and thus, no noticeable protein conformational change occurs. Thus, we did not observe any dynamics of W59 in the ferric state. Therefore, if we do not observe W59 ground-state dynamics it indicates no significant structural perturbation and no heme−M80 dissociation.

In the visible region (700−530 nm) we observed a series of nonexponential cascaded vibrational cooling, conformation relaxation, and final ground-state recovering. From 700 to 590 nm we basically observed two decay components in hundreds of femtoseconds (0.3−0.7 ps) and a few picoseconds (3−5 ps), mainly reflecting hot ground-state cooling. At 700 nm we also observed a small negative formation signal in about 6 ps. As shown in Figure 2 such formation was probed by a weak absorption band of heme−M80 charge transfer.[68] For the other visible wavelengths of 575−530 nm because the Q-band absorption becomes evident (Figure 2) we observed systematic evolution of decay and formation signals: At 575 nm two decays (0.38 and 4.7 ps) and one main formation (0.77 ps); at 560 nm two decays (0.14 and 4.9 ps) and two formations (1.2 and 9.2 ps); at 550 nm one decay (3.6 ps) and two formations (1.4 and 9.1 ps); and finally at 540 and 530 nm two formation components of 1.3−4 and ~11 ps.

Combining these results from UV to visible probing the photophysics of ferric Cyt *c* is clear. The internal conversion from the B band to the Q band and the ground state is ultrafast, occurring in hundreds of femtoseconds (<250 fs) detected mainly in the UV region. The internal conversion from S_1 to the ground state occurs on a similar time scale.[32] The resulting molecules are formed in the highly vibrationally excited ground state, and a series of energy dissipation follows: initial intramolecular and subsequent intermolecular vibrational redistributions and then local conformation relaxation. The vibrational energy dissipation is highly nonequilibrium with multiple exponential decays but all on ultrafast time scales. The longest decay time of cooling processes is 4.9 ps observed at 560 nm. The dissipated energy into the surrounding protein causes local conformation fluctuation, and such conformational relaxation inversely perturbs ferric heme Q-band absorption.[69] The complete conformational relaxation takes ~11 ps as detected at 530 nm by the full recovery of ground-state ferric Cyt *c*.

The observed time scales of 4.9 ps cooling and 11 ps recovery with the B-band excitation are a little longer than those of ~4 ps TA decay and ~7 ps temperature decay observed by Negrerie et al.[32] at the Q-band excitation. Our results are also in good agreement with recent MD-simulation results observed by Bu and Straub[70] on vibrational energy relaxation of heme cooling upon 420 nm excitation. They observed biphasic distributions of 1.5 (60%) and 10.1 ps (40%), corresponding to two dissipation mechanisms: The fast component is due to the energy transfer via the coupling between heme and collective motions of the protein mainly through covalent bonds with M80, H18, C14, and C17 residues and through hydrogen bonds with Y48, T49, N52, and T78 residues and neighboring water molecules. The slow phase is due to energy transfer via nonbonded collisional contacts with nearby heme-pocket residues such as R38, T40, G41, and F46 and solvent. The observed multiple cascade relaxation in a few picoseconds here seems more complex than the simulations, and the observed longest 11 ps dynamics reflects the total relaxation of the local heme pocket.

IV. Conclusion

We reported here our critical use of UV probing of the local heme dynamics and induced global conformation relaxation in the ferric and ferrous states in cytochrome *c* upon heme excitation. The two redox states show drastically different heme

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and protein dynamics, which must be due to the different number of electrons in the d orbitals of the central iron, resulting in different energetics, coupling and dissociative or bound character of excited-state potentials. In the ferrous state the dissociative channel is dominant and occurs in less than 100 fs, faster than the internal conversion rate, which is observed in the ferric state in hundreds of femtoseconds. Both 5-fold coordination ferrous and 6-fold coordination ferric states were mainly formed in the vibrationally hot ground state, and the cooling processes were observed within 7 ps. However, full recovery of the local heme environment takes a little longer: 11 ps for ferric and 13 ps for ferrous states.

One significant observation by UV probing is direct probing of conformation relaxation caused by proteinquakes which are created by impulsive local heme−ligand dissociation in the ferrous state. Such cascade dynamics from the local heme site to distant protein conformation are completed in 42 ps, faster than expected. Unlike in other heme proteins such as myoglobin, dissociation of carbon monoxide from the heme site causes continuously weak structural perturbation without any prompt rebinding, leading to protein relaxation on multiple time scales.[71,72] With violent proteinquakes the conformation relaxation could occur on much longer time scales (nanoseconds to microseconds) and proteins could even unfold.[14,38] Here, the ultrafast proteinquake dynamics must be due to the prompt bond-breaking and bond-remaking processes in 7 ps as well as the local structural integrity with another three covalent connections between the protein and the heme group. Using the powerful UV probing of ground-state tryptophan absorption perturbation, as proved here by mutation work and also shown in recent studies of retinal charge translocation in bacteriorhodopsin[44] and electron transfer in photosynthesis,[46] we can now directly map the protein conformation dynamics after the shaking by proteinquakes with global site-directed mutations.

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Supporting Information Available: Different kinetic-model fittings of the transients, the fitting parameters of Figures 3 and 4, complete ref 4. This material is available free of charge via the Internet at http://pubs.acs.org.

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