Ultrafast Protein Dynamics of Hemoglobin as Studied by Picosecond Time-resolved Raman Spectroscopy

ARTICLE *in* CHEMICAL PHYSICS · MARCH 2012

Impact Factor: 1.65 · DOI: 10.1016/j.chemphys.2011.05.012

2 AUTHORS, INCLUDING:

Masako Nagai
Hosei University
69 PUBLICATIONS **874** CITATIONS

SEE PROFILE

Chemical Physics 396 (2012) 45–52

Contents lists available at ScienceDirect

Chemical Physics

journal homepage: www.elsevier.com/locate/chemphys

Ultrafast protein dynamics of hemoglobin as studied by picosecond time-resolved resonance Raman spectroscopy

Yasuhisa Mizutani [a,*], Masako Nagai [b,c]

[a] Department of Chemistry, Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka 560-0043, Japan
[b] Research Center for Micro-Nano Technology, Hosei University, Tokyo 184-0003, Japan
[c] Department of Frontier Bioscience, Faculty of Engineering, Hosei University, Tokyo 184-8584, Japan

ARTICLE INFO

Article history:
Available online 18 May 2011

Keywords:
Time-resolved spectroscopy
Resonance Raman spectroscopy
Hemoglobin
Protein dynamics
Allostery

ABSTRACT

Time-resolved resonance Raman spectroscopy on human adult hemoglobin (HbA) following ligand photolysis revealed that the frequency of the iron–histidine stretching [ν(Fe–His)] mode exhibited a 2-cm^{-1} downshift with a time constant of about 300 ps, suggesting a structural change in the heme pocket following the ligand photolysis. Low-frequency heme modes suggested that the primary metastable form of HbA has a more disordered orientation of propionates and a less strained environment than the deoxy form. The latter fact is consistent with the experimental observation that the ν(Fe–His) frequency of the metastable form is higher than the deoxy form. The present study shows that HbA adopts a metastable structure within the instrument response time and remains little changed in the subnanosecond to nanosecond time regime. Characteristics of the primary protein response of HbA based on the comparison of the results of HbA with those of the isolated chains and myoglobin are discussed.

1. Introduction

Dynamic properties of proteins are essential for specific biological functions. In numerous biological processes, the ensuing protein structural changes accompanying a reaction at a specific site must spatially extend to global structural changes to achieve a biological function, widely known as allostery [1]. The molecular mechanism of cooperativity in oxygen binding of hemoglobin (Hb) is one of the classical problems in this regard. Human adult hemoglobin A (HbA) has an $\alpha_2\beta_2$ tetramer structure, exhibiting positive cooperativity in oxygen binding. X-ray crystallographic studies have demonstrated the presence of two distinct quaternary structures that correspond to the low-affinity (T or tense) and high-affinity (R or relaxed) states. Typical structures of the T and R states are observed for the complete deoxy and ligated forms, respectively [2]. The cooperative oxygen binding of Hb has been explained in terms of a reversible transition between the two quaternary states upon partial ligation of four hemes [3,4]. The binding of small molecular ligands to the hemes in HbA is a highly localized perturbation. Nonetheless, this localized perturbation initiates a sequence of propagating structural events that culminates in a change of quaternary structure, proceeding from the T state to the R state. The large amplitude motions at the quaternary level, which form a communication link at the subunit interface, are driven by changes of the tertiary structure upon ligation. In this respect, myoglobin (Mb), which is structurally very similar to a subunit of Hb, serves as a model system for the tertiary relaxation processes.

In HbA, the heme is an iron–protoporphyrin IX, in which the Fe^{II} ion is bound to the proximal histidine (His) as the only covalent link to the protein. The heme iron binds diatomic molecules, such as NO, CO, and O_2, at the opposite side of the proximal His. X-ray crystallography provided extensive information about the endpoint equilibrium structures [5,6]. The magnitude of atomic displacements at the heme site that must occur upon ligand binding and release is estimated by examining structures containing the heme with and without bound ligands. The ligated heme has a planar structure in which the low-spin iron atom is in the porphyrin plane. For carbonmonoxy hemoglobin (HbCO), the core size, i.e., the distance between the pyrrole nitrogen atoms and the heme center, is 1.98 Å [6]. Upon ligand dissociation, the iron atom is converted from low to high spin ($S = 0 \rightarrow S = 2$) and moves out of the porphyrin plane by about 0.38 Å [5]. Concomitantly, the porphyrin core size expands to 2.06 Å [5].

Ultrafast absorption studies of Hb show that upon visible excitation of HbCO, the ligand-bound spectrum disappears within 50 fs and the deoxy-like heme spectrum appears with a time constant of about 300 fs [7]. Protein reorganization in response to changes at the heme is expected to occur more slowly. By using CO as the ligand rather than O_2, it is possible to avoid the geminate recombination complicating the reaction dynamics in the picosecond

* Corresponding author. Tel./fax: +81 6 6850 5776.
E-mail address: mztn@chem.sci.osaka-u.ac.jp (Y. Mizutani).

0301-0104/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chemphys.2011.05.012

regime [8]. Thus, the HbCO system is unique in that it gives access to the full range of protein relaxation processes by a single perturbation (ligand dissociation) without complicated side reactions and associated population dynamics. For this reason, an enormous body of work has been accumulated about relaxation processes of HbA, helping to establish general principles related to protein dynamics. These features of HbCO also enabled us to trigger structural changes of the protein optically and to follow the accompanied structural relaxation using optical probes that are sensitive to different aspects of the protein's motion.

Resonance Raman (RR) spectroscopy has provided a considerable amount of information regarding the influence of tertiary and quaternary structures on a few selected vibrational modes of heme. In particular, the iron–proximal histidine stretching [ν(Fe–His)] frequency [9,10] has been shown to couple with the tertiary and quaternary structures and also to be a direct reflection of proximal strain—a major determinant of ligand-binding reactivity. The Fe–His bond is the only linkage between the heme and globin, and it thus functions as the focal point for the forces driving the structural changes. In this regard, time-resolved resonance Raman (TR^3) studies of the ν(Fe–His) mode are the key to elucidating protein dynamics. Resonance Raman studies by Scott and Friedman showed that the temporal evolution of the ν(Fe–His) frequency of HbA subsequent to CO photodissociation was observed in the nanosecond to microsecond regime [11]. An earlier study on Mb [12] reported the change of the ν(Fe–His) band was completed within 30 ps, however, our close examination revealed the temporal downshift of the ν(Fe–His) band with a time constant of about 100 ps [13]. For HbA, resonance Raman spectra of transient species occurring at 25 ± 5 ps were observed using single picosecond pulses [14]. Later, Franzen et al. reported subpicosecond TR^3 spectra of HbA [15,16]. However, temporal evolution of the ν(Fe–His) band in the picosecond region has not been characterized in detail to date. Therefore, precise picosecond time-resolved measurements on the ν(Fe–His) mode are necessary to elucidate the primary protein response of HbA to ligand dissociation. In this study, we closely examined the temporal behavior of TR^3 spectra of HbA and its isolated chains and found that the ν(Fe–His) frequency exhibited a downshift of 2 cm^{-1} with a time constant of about 300 ps, both for HbA and the isolated chains. We will discuss the characteristics of the primary protein response of HbA based on the comparison of the results of HbA with those of the isolated chains and Mb.

2. Experimental

2.1. Sample preparation

HbA was purified from fresh human blood by preparative isoelectric focusing electrophoresis using 5% ampholine (pH 6–9) [17]. Isolated chains of HbA were prepared as follows. HbA was split into chains by adding *p*-chloromercuribenzoate (PMB) [18]. The α-PMB and β-PMB chains in the CO-form were separated by an isoelectric focusing electrophoresis on a Sephadex G-75 super fine gel bed containing 5% ampholine (pH 3.5–9.5). The PMB of α and β chains were removed by adding dithiothreitol (3 mg/ml) in 0.1 M Tris buffer, pH 8.2, and then passed through a Sephadex G-25 column [19].

One milliliter of the sample solution was placed into an airtight NMR tube (10 mm∅). The remaining oxygen in the solution was removed by more than five cycles of degassing and back-filling with CO. Finally, the solution was equilibrated under 1 atm of CO. The samples were reduced with a 10-fold stoichiometric amount of sodium dithionite in a small amount of solution (approximately 10 μL). The sample solution in the cell was continuously spun during the measurements to prevent multiple probing of the same portion of the sample. The sample was replaced with a fresh one every three hours. Sample integrity was confirmed with ultraviolet (UV)-visible absorption spectra after the TR^3 measurements. The samples of the deoxy form were prepared by the same procedure as the CO-bound form preparation, except for back-filling with N_2 and equilibration of the sample under 1 atm of N_2.

2.2. Picosecond time-resolved resonance Raman measurements

The details of the picosecond TR^3 apparatus are described elsewhere [13]. Pump pulses at 540 nm (15 μJ/pulse, 1 kHz) were generated by a home-built OPG/OPA device pumped by the second harmonic of the output of a Ti:sapphire regenerative amplifier (Spitfire, Positive Light) seeded by a picosecond Ti:sapphire oscillator (Tsunami, Spectra Physics). Probe pulses at 442 nm (0.3 μJ/pulse) were generated by stimulated Raman scattering in compressed methane gas excited by the second harmonic. The pump and probe beams were made collinear and coaxial using a dichroic mirror. The polarization of the pump beam was rotated by 55° relative to that of the probe beam to minimize the effects of molecular rotations on the observed kinetics. Both beams were continuously monitored with photodiodes (Hamamatsu Photonics, S2387-1010R) and found to be stable within ±10%. A cross correlation trace of the pump and probe pulses was measured with a 1-mm BBO crystal, which indicated a width of 2.3 ps. The 0.0 ps of delay time (uncertainty < 0.2 ps) was calibrated using sum frequency mixing in the same crystal.

2.3. Data acquisition

The sample solution was placed in a 10-mm∅ NMR tube and spun with a spinning cell device that was designed to minimize the off-center deviation during rotation [14]. The sample was spun at 3400 rpm in the spinning cell configured for 135° backscattering illumination and collection. Spherical and cylindrical lenses were used to focus the pump and probe beams on the sample in line-focusing condition. Laser fluxes of the pump and probe pulses were ~500 and ~7 MW/cm^2, respectively.

The TR^3 data acquisitions were carried out as follows [13]. At each delay time, Raman signals were collected for three 20-s exposures with both the pump and probe beams present in the sample. This was followed by equivalent exposures for pump-only, probe-only, and dark measurements. The transient Raman spectra were obtained by averaging the data for 10 entire cycles for the high frequency spectra and 90 entire cycles for the low frequency spectra. The pump-only spectrum was directly subtracted from the pump-and-probe spectrum, yielding the "probe-with-photolysis" zspectrum. The dark spectrum was directly subtracted from the probe-only spectrum, yielding the "probe-without-photolysis" spectrum (HbCO). The probe-without-photolysis spectrum was subtracted from the probe-with-photolysis spectrum to yield the photoproduct spectrum. The moment analysis needs no assumption about the band shape and, therefore, provides general information even if the band shape is asymmetric. The band intensity and position were calculated in terms of the zero and first moments of the band, respectively.

Raman scattering was collected by a doublet achromat [80-mm focal length (FL), *f*/2] and was imaged onto the 200-μm entrance slit of a single spectrometer (Spex, 500 M) by a doublet achromat (200-mm FL, *f*/5). A dichroic short-pass filter was placed between the lenses to remove the scattered pump beam. A holographic notch filter (Kaiser Optical Systems, HSNF-441.6-1.0) was used to reject the unshifted scattering. A polarization scrambler was placed at the entrance slit to remove the effects of polarization on the spectrograph throughput. The spectrometer was equipped

with a blazed-holographic grating (2400 grooves/mm) that enabled measurements of a spectrum as wide as about 1000 cm^{-1} in the Soret region. The dispersed light was detected by a liquid-nitrogen-cooled CCD detector (Roper Scientific, Spec-10:400B/LN). Raman shifts were calibrated with cyclohexane and carbon tetrachloride. The peak positions of the Raman bands were accurate within ± 2 cm^{-1}.

3. Results

3.1. Raman spectral change of the heme

Fig. 1 shows the TR^3 difference spectra of photodissociated Hb in the 1100–1700 cm^{-1} region for various delay times of the probe pulse with respect to the pump pulse. In these spectra, the contribution of unreacted species has been subtracted. The TR^3 spectrum for a 4-ps delay contains only the bands arising from the in-plane vibrations of heme at 1353 (ν_4), 1469 (ν_3), 1562 (ν_2), and 1621 (ν_{10}) cm^{-1} [20,21]. These bands exhibited narrowing and frequency upshifts in the first few picoseconds, but no further changes occurred after that. These changes can be ascribed to vibrational cooling [22]. The TR^3 spectra at a few picoseconds resemble the spectrum of deoxyHb, indicating that the photodissociated heme has mostly relaxed to the domed structure within the instrument response time (~2 ps).

Close comparison of the spectra revealed that the ν_4 band of the transient species at 1000 ps is 3 cm^{-1} lower in frequency than that of the stable deoxy form. In addition to the ν_4 band, the two core size marker bands, ν_2 and ν_3, are also found to be downshifted relative to the deoxy form. These features are in contrast to those of Mb in which there is a barely detectable shift between the corresponding species. These results are consistent with the results reported by Friedman et al. [23] and Dasgupta and Spiro [24]. These frequency differences were reported in nanosecond TR^3 spectra for the ν_4 band and the ν_2 and ν_3 bands. The frequencies of the ν_2 and ν_3 bands are well known to be sensitive to the core size of the porphyrin ring [25–28]. This demonstrates that the initial expansion of the core size is instantaneous upon CO photodissociation.

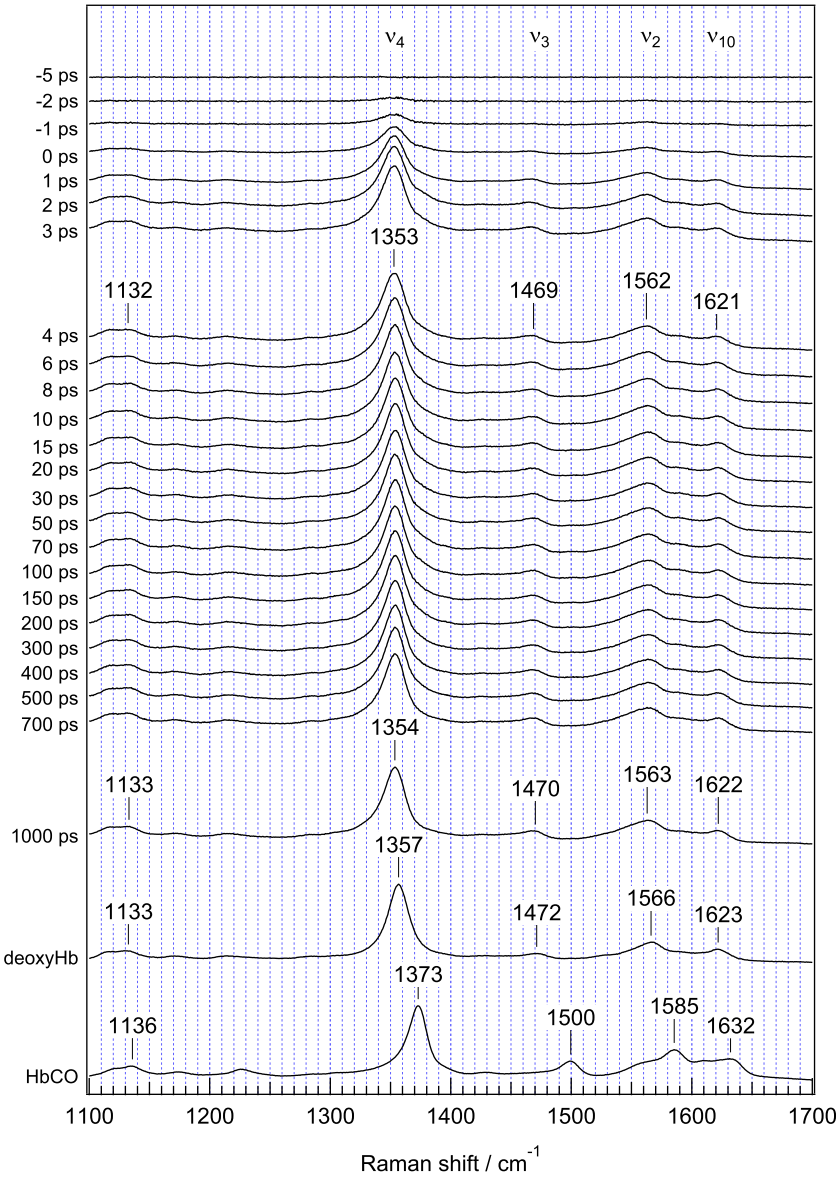

Fig. 1. Time-resolved resonance Raman spectra of photodissociated HbCO in the 1100–1700-cm^{-1} region. Spectra of the equilibrium states of deoxyHb and HbCO are depicted at the bottom for comparison.

Fig. 2 shows the TR^3 spectrum in the 180–850-cm^{-1} region for delay times between –5 and 1000 ps. The bands at 231, 305, 365, and 673 cm^{-1} in the spectrum for a 1000-ps delay are assigned to the vibrations of the heme. The large peak at 231 cm^{-1} arises from the stretching mode of the covalent bond between the heme iron and the N_ε of proximal His, ν(Fe–His) [29]. The peak at 305 cm^{-1} is an out-of-plane mode (γ_7; methine wagging) [30]. The peak at 365 cm^{-1} is a substituent mode, $\delta(C_\beta C_c C_d)$, involving deformation of the propionate methylene groups [30]. The peak at 673 cm^{-1} is an in-plane mode (ν_7; breathing-like mode of the porphyrin inner ring). Similar to the spectra in the high-frequency region, RR bands exhibited appreciable frequency upshifts in the first few picoseconds, but no further significant changes occurred after that.

We observed frequency differences between the transient species and the deoxy form in the ν_2, ν_3, and ν_4 bands as described above. There were also some distinct differences in the Raman bands in the low frequency region between the transient species and the deoxy form. First, the band at 341 cm^{-1} in deoxyHb was absent in the photoproduct. Second, the band position of the γ_7 mode of the photoproduct differed from that of the deoxy form. These results suggest the heme structure is not fully relaxed within a nanosecond for Hb, consistent with the nanosecond TR^3 measurements reported by Friedman and coworkers [11]. This feature is in contrast to the case of Mb, where frequencies of the heme modes relax almost instantaneously to those of the deoxy form. In the case of Mb, the transient spectra at 1 ns and the deoxy form closely resemble each other [13].

3.2. Raman spectral change of the Fe–His stretching mode

The ν(Fe–His) band was observed at 233 cm^{-1} at 4 ps. The band gradually downshifts in this time window. The band position was calculated in terms of the first moment of the band as described previously [13]. The band positions of Hb are plotted against time in Fig. 3. The ν(Fe–His) band showed a small downshift in the 100-ps time range. The downshift of the ν(Fe–His) band of HbA can be well described by single-exponential kinetics, providing a time

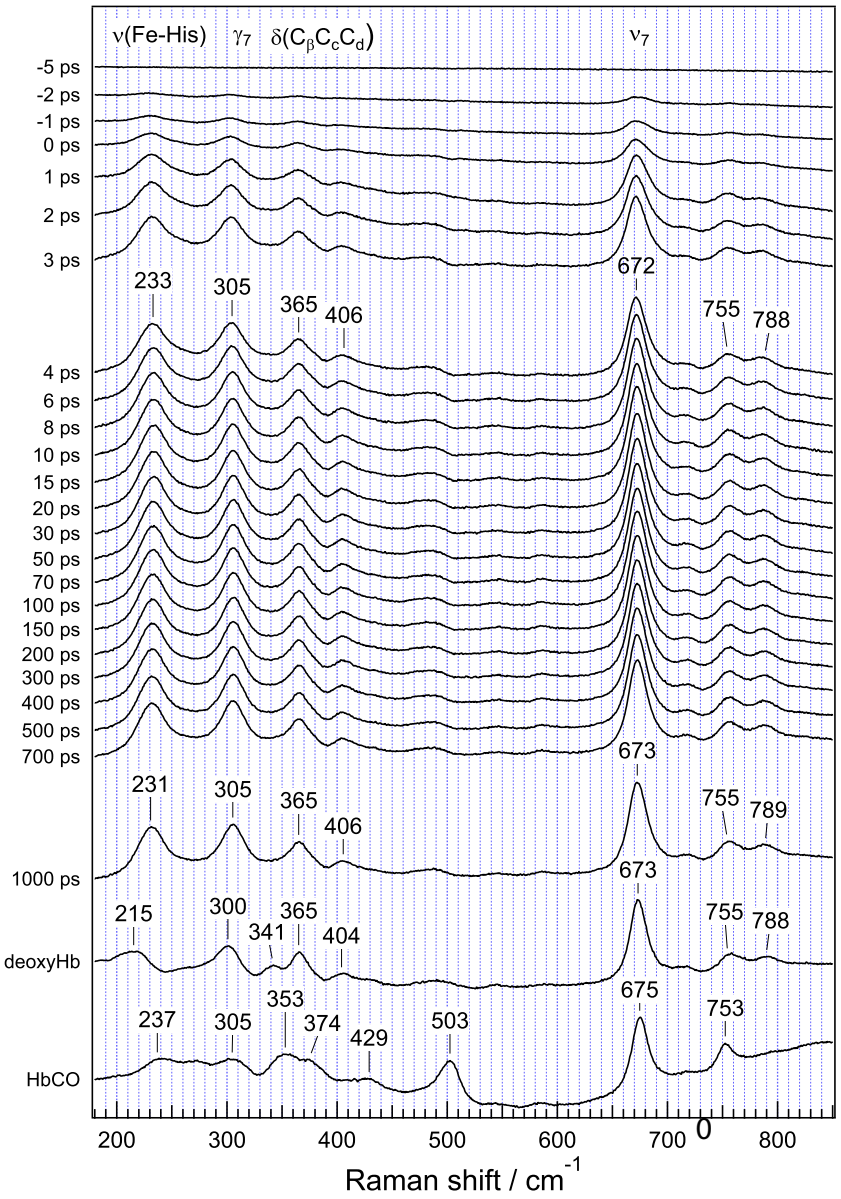

Fig. 2. Time-resolved resonance Raman spectra of photodissociated HbCO in the 150–850-cm^{-1} region. Spectra of the equilibrium states of deoxyHb and HbCO are depicted at the bottom for comparison.

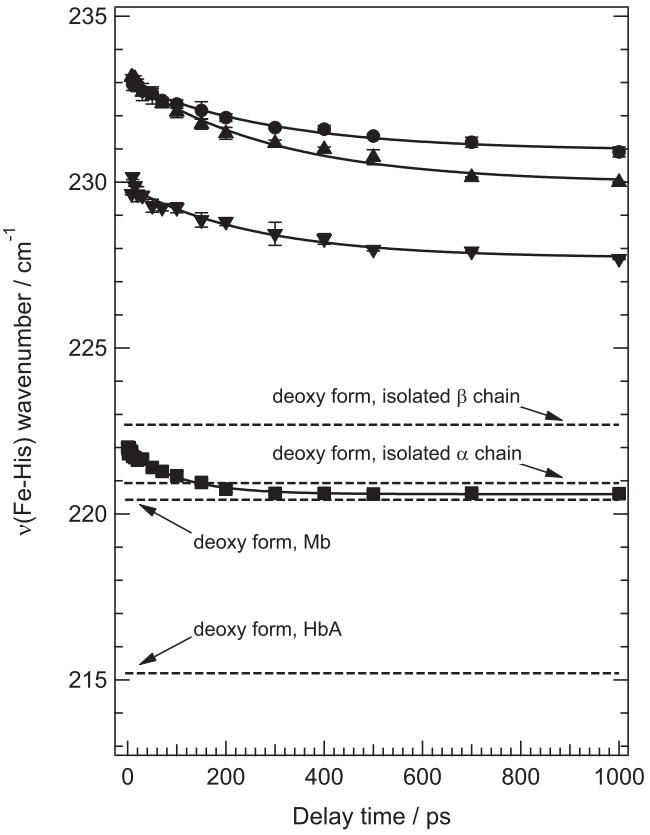

Fig. 3. Temporal changes of the ν(Fe–His) frequencies of HbA (circles) and the isolated α (triangles) and β chains (inverted triangles). The temporal shift of the ν(Fe–His) band was fitted using a single exponential function, yielding time constants of 284 ± 38, 256 ± 35 and 298 ± 42 ps, for HbA and the isolated α and β chains, respectively. The data for Mb (squares) were taken from the previous work (time constant, 106 ± 14 ps) [13]. The broken lines show the band positions of the equilibrium deligated form (i.e., the deoxy form).

constant of 284 ± 38 ps, although the kinetics involve several steps of relaxation. Similar to Mb, frequency shift of this mode was observed for Hb in the picosecond regime. However, the rate of the shift for HbA was three times slower than that of Mb. For HbA, although frequency shifts of the ν(Fe–His) mode in nanoseconds to microseconds have been reported [13], the temporal behavior in the picosecond regime has remained unclear. The high-precision measurements in this study revealed the presence of the relaxation process of Hb in the 100-ps time range. The ~300-ps process for the ν(Fe–His) band is the fastest structural response reported so far for the Fe–His linkage of the HbA photoproduct.

3.3. Spectral changes of Hb isolated chains

Distinct differences were observed in picosecond TR^3 spectra of the photolyzed species of HbA and Mb. The transient spectra of Hb showed differences in the γ_7, ν_8 and ν(Fe–His) bands from the deoxy spectra, while those of Mb did not. To determine whether the reduced response in Mb is related to its being monomeric and/or noncooperative, we measured a similar set of spectra from the isolated subunits of HbA. Figs. 4 and 5 show TR^3 spectra of the isolated α and β chains of HbA, respectively. Both isolated β chains (tetrameric and noncooperative) and isolated α chains (equilibrium between monomer and dimer; noncooperative) exhibited similar trends to Hb, i.e., weak intensity of the ν_8 band at 1 ns, and higher frequency positions of the ν(Fe–His) and γ_7 bands compared to those of the deoxy form. In the high frequency region, the downshifts of the ν_2, ν_3 and ν_4 bands were observed similar to the intact HbA (see Supporting information).

Although the structures of Mb and isolated chains of HbA closely resemble each other and ligand binding properties of the isolated chains are similar to those of R-state Hb or Mb [31], the dynamics in the protein structure are distinctly different. Fig. 3 shows temporal changes of the band position of the ν(Fe–His) bands of the isolated chains in addition to those for Hb and Mb [13]. For both isolated chains, a 2-cm^{-1} frequency shift for the ν(Fe–His) band with a time constant of ~300 ps was observed. Although the absolute values of the band position of the α chain were shifted to the lower frequency, the rate and magnitude of the frequency shift were similar to Hb and the β chain. There are large differences between the band positions of the transient species at 1 ns and the corresponding deoxy form (broken lines), which is in contrast to the case of Mb. On the other hand, characteristics of HbA were observed for the isolated chains. This result demonstrates the fact that the subunits of HbA behave like isolated chains on the picosecond time scale. Slow dynamics of deligation-induced changes for HbA compared to Mb are inherent to its subunits.

4. Discussion

4.1. Structural relaxation of the heme pocket

The present study revealed picosecond dynamics of the heme and heme pocket of Hb, demonstrating that Hb exhibits a frequency shift of ν(Fe–His) in the picosecond time regime. The frequency shift was not observed for the ν(Fe-Im) band of the model compound without protein matrix, hemin-2-methyl-imidazole complex [13], indicating that the structural change seen for HbA is not a characteristic of the heme–histidine (or imidazole) unit and that it is associated with protein relaxation.

Kitagawa and coworkers [9,32,33] showed that RR spectra of ligand-free ferrous forms of heme proteins with an axial ligand of His contain a band in the region between 200 and 250 cm^{-1} assignable to the ν(Fe–His) mode. The intensity and wavenumber of this band depends strongly on the tertiary and quaternary structures of the respective heme protein [9,10]. The ν(Fe–His) bond in Mb and Hb is the sole covalent linkage between the heme and the protein. Consequently, this mode is a good indicator of heme protein tertiary and quaternary structure. Bangcharoenpaurpong et al. [34] proposed that the origin of the intensity of the ν(Fe–His) mode results from orbital overlap between the σ^* orbital of the Fe–His bond and the π^* orbital of the porphyrin ring, which is small in the planar structure but becomes large in the domed structure.

It is well recognized that the proximal Fe–His linkage in heme proteins plays a pivotal role in communicating protein structural changes to the functional heme group, thus affecting its biological properties [35]. When the ligand-free ferrous form of Hb changes its quaternary structure from the R to the T state, the ν(Fe–His) band shifts to lower frequencies. In deoxyHb within the quaternary T state the wavenumber of the band maximum is at 216 cm^{-1}, whereas the R state of the modified deoxy NES-des(Arg141 α)Hb exhibits a Raman band at a wavenumber of 221 cm^{-1} [36]. Matsukawa et al. observed a definite correlation between the ν(Fe–His) wavenumber and the first Adair constant K_1, which is related to the Gibbs energy of the first oxygenation step [37]. This finding was explained in terms of Perutz's strain model [2]. Hence, the ν(Fe–His) band became a prominent marker band in the investigation of the relationship between structure and function of this class of proteins.

The temporal changes of the ν(Fe–His) band of HbA have been studied by TR^3 spectroscopy. In the low-frequency region of the

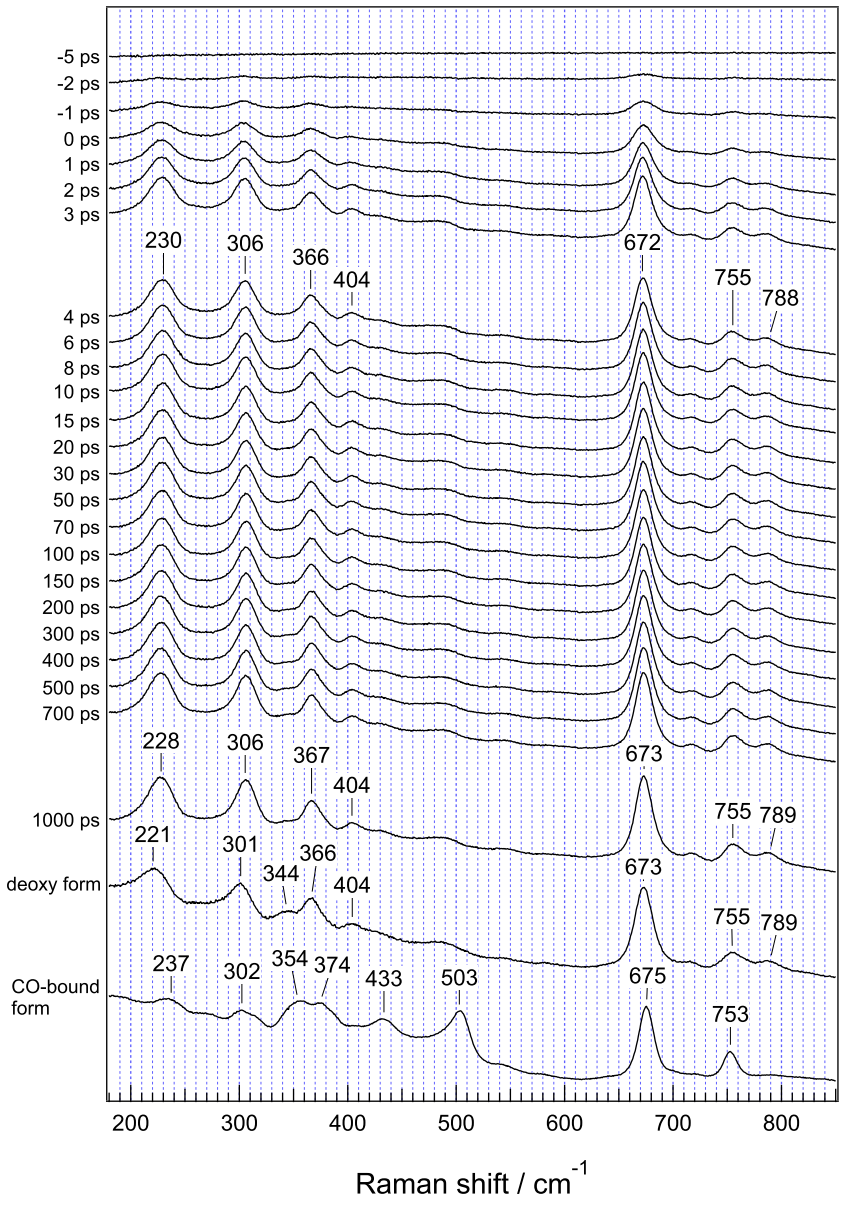

Fig. 4. Time-resolved resonance Raman spectra of the photodissociated form of the carbonmonoxy isolated α chain in the 150–850-cm^{-1} region. Spectra of the equilibrium states of the deoxy and CO-bound forms are depicted at the bottom for comparison.

RR spectrum, it has been shown that the ν(Fe–His) RR band is absent from HbCO, but appears at a shifted position of 223–228 cm^{-1} in photolyzed (R state) HbCO as measured at 30 ps and 10 ns, later relaxing to the equilibrium (T state) deoxy position of 210–216 cm^{-1} on the 100 ns to microsecond time scale [11]. Although single-color transient Raman measurements [14] and TR^3 measurements [15,16] were carried out for the protein dynamics in the picosecond region, no detailed characterization of temporal behavior in the picosecond region has been done. The high-precision measurements in this study revealed the presence of the relaxation process of HbA in the 100-ps time range.

The difference in the ν(Fe–His) frequencies of T and R of Hb forms was attributed to a difference in the azimuthal angle [34]. Peterson et al. measured the ν(Fe–His) frequencies of several mutants of sperm whale Mb and observed a correlation between the ν(Fe–His) frequency and heme proximal-His geometry [38]. They attributed changes in the ν(Fe–His) frequency to changes in the azimuthal angle of the His imidazole ring driven by a change in steric factors. Recently, Samuni et al. reported the ν(Fe–His) frequencies of truncated Hbs were higher than those of Mb [39]. The difference in the frequency was attributed to the difference in azimuthal angle. Thus, it is highly likely that the frequency shift of the ν(Fe–His) mode observed in this study is due to the change in the azimuthal angle in the structural relaxation following the CO dissociation.

To date, the frequency shift of the ν(Fe–His) band has been studied for several proteins that exhibit a globin fold. Comparable sizes of the frequency shift of the ν(Fe–His) band were observed for Mb and HemAT [40], in which the sensor domain exhibits a globin fold. On the other hand, no frequency shifts were observed for neuroglobin [41] and cytoglobin [41]. Interestingly, this trend has a correlation with the coordination structure of the heme in the deoxygenated ferrous form. Hb, Mb and HemAT have penta-coordinated structures, while neuroglobin [41] and cytoglobin [41] have hexa-coordinated structures in the deoxygenated ferrous forms. The 2-cm^{-1} downshift of the ν(Fe–His) band in the picosecond region seems to be characteristic of a globin-folded protein with penta-coordinated structure.

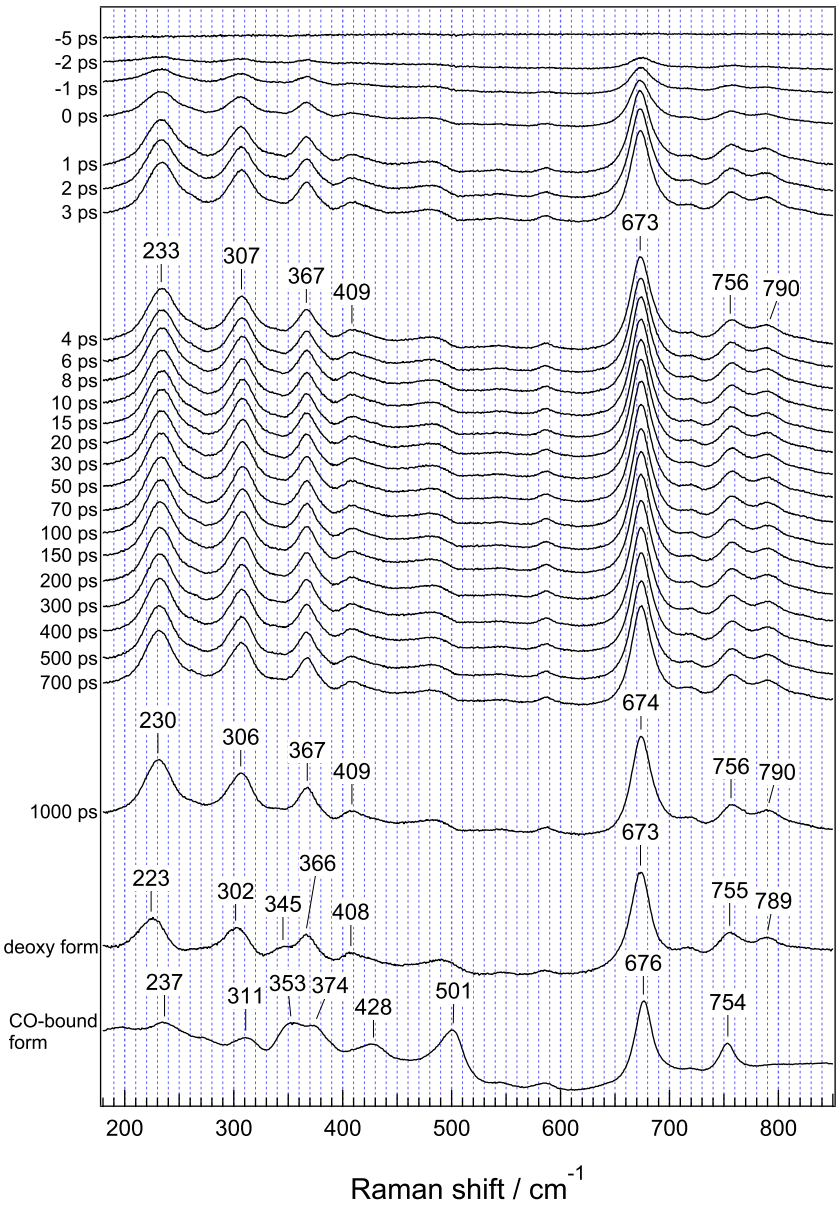

Fig. 5. Time-resolved resonance Raman spectra of the photodissociated form of the carbonmonoxy isolated β chain in the 150–850-cm^{-1} region. Spectra of the equilibrium states of the deoxy and CO-bound forms are depicted at the bottom for comparison.

4.2. Rate of structural relaxation of the heme pocket

Rates of the changes of the heme and ν(Fe–His) modes in the TR^3 spectra reflect those of the conformational changes of the protein. Although the sizes of the frequency shifts of the ν(Fe–His) band were similar between Hb and Mb, the time constants of the frequency shift of HbA was about three times slower than that of Mb. This indicates that the structural relaxation of the heme pocket of HbA is slower than that of Mb. The differences from Mb were also observed for the γ_7 and ν_8 bands in the TR^3 spectra of HbA. Features of these bands for the transient species at 1000 ps and the deoxy state for Mb closely resembled each other, while they were substantially different for HbA. These differences in HbA were observed in the nanosecond TR^3 studies [11,42], which showed that HbA forms a metastable structure in the nanosecond region, which was not observed for Mb [12,22]. The present study shows that the metastable form appears within the instrument response time after CO dissociation. This suggests that heme doming is an ultrafast process, as noted in earlier studies by Franzen et al. [15,16]. Interestingly, the isolated subunits exhibited similar temporal behaviors of their RR bands to those of HbA, but were completely different from those of Mb. In solution, isolated α and β chains are supposed to form monomers/dimers and tetramers, respectively. They do not show cooperative interactions in oxygen binding. Because the features of the γ_7 and ν_8 bands of both isolated chains are very similar to that of Hb, the difference in the dynamics between Hb and Mb is not due to intersubunit interactions, such as tetramer formation and cooperativity, but due to the inherent characters of the Hb subunits.

The observed differences in the ν_8 and γ_7 bands between the transient species and the deoxy form provide insight into the structure of the metastable form in the picosecond time region. The ν_8 is the in-plane stretching mode of the iron and pyrrole nitrogens. It has been suggested that the intensity of this band is correlated with disorder in the orientation of the propionate groups [38]. As this disorder increases, the ν_8 band intensity decreases. For example, the ν_8 band of hemoglobin appears when the heme propionates are motionally restricted in a trehalose glass [43]. The heme-γ_7-mode

band at approximately 300 cm^{-1} has been shown to be sensitive to proximal strain with lower frequencies, which is indicative of increased proximal strain [44]. Thus, it is suggested that the primary metastable form of HbA has a more disordered orientation of propionate groups and a less strained environment than the deoxy form. The latter fact is consistent with the experimental observation that the ν(Fe–His) frequency of the metastable form is higher than the deoxy form. The present study showed that HbA adopts this structure within the instrument response time and remains little changed in the subnanosecond to the nanosecond time regime.

5. Conclusions

In this study, we closely examined the temporal behavior of TR^3 spectra of HbA and its isolated chains and found that the ν(Fe–His) frequency exhibited a downshift of 2 cm^{-1} with a time constant of about 300 ps for both HbA and the isolated chains. The ~300-ps process for the Fe–His linkage is the fastest structural response reported so far for the Fe–His linkage of the HbA photoproduct. The 2-cm^{-1} downshift of the ν(Fe–His) band in the picosecond region seems characteristic of a globin-folded protein with penta-coordinated structure. We discussed characteristics of the primary protein response of HbA based on the comparison of the results of HbA with those of the isolated chains and Mb. It is suggested that the primary metastable form of HbA has a more disordered orientation of propionate groups and a less strained environment than the deoxy form. The latter fact is consistent with the experimental observation that the ν(Fe–His) frequency of the metastable form is higher than the deoxy form. The present study showed that HbA adopts the metastable structure within the instrument response time and remains little changed in the subnanosecond to the nanosecond time regime.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research (B) (Grant No. 17350009) to YM from the Japan Society for the Promotion of Science and by a Grant-in-Aid for Scientific Research on the Priority Area "Molecular Science for Supra Functional Systems" (Grant No. 19056013) to YM from the Ministry of Education, Science, Sports and Culture of Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemphys.2011.05.012.

References

[1] J. Monod, J. Wyman, J.-P. Changeux, J. Mol. Biol. 12 (1965) 88.
[2] M.F. Perutz, Nature 228 (1970) 726.
[3] M.F. Perutz, Annu. Rev. Biochem. 48 (1979) 327.
[4] M.F. Perutz, G. Fermi, B. Luisi, B. Shaanan, R.C. Liddington, Acc. Chem. Res. 20 (1987) 309.
[5] G. Fermi, M.F. Perutz, B. Shaanan, R. Fourme, J. Mol. Biol. 175 (1984) 159.
[6] J.M. Baldwin, J. Mol. Biol. 136 (1980) 103.
[7] J.W. Petrich, J.L. Martin, Chem. Phys. 131 (1989) 31.
[8] J. Friedman, T. Scott, G. Fisanick, Simon, E. Findsen, M. Ondrias, V. Macdonald, Science 229 (1985) 187.
[9] T. Kitagawa, The heme protein structure and the iron histidine stretching mode, in: T.G. Spiro (Ed.), Biological Application on Raman Spectroscopy, John Wiley and Sons, New York, 1988, pp. 97–131.
[10] D.L. Rousseau, J.M. Friedman, Transient and cryogenic studies of photodissociated hemoglobin and myoglobin, in: T.G. Spiro (Ed.), Biological Application on Raman Spectroscopy, John Wiley and Sons, New York, 1988, pp. 133–216.
[11] T.W. Scott, J.M. Friedman, J. Am. Chem. Soc. 106 (1984) 5677.
[12] E.W. Findsen, T.W. Scott, M.R. Chance, J.M. Friedman, M.R. Ondrias, J. Am. Chem. Soc. 107 (1985) 3355.
[13] Y. Mizutani, T. Kitagawa, J. Phys. Chem. B 105 (2001) 10992.
[14] E.W. Findsen, J.M. Friedman, M.R. Ondrias, S.R. Simon, Science 229 (1985) 661.
[15] S. Franzen, B. Bohn, C. Poyart, J.L. Martin, Biochemistry 34 (1995) 1224.
[16] S. Franzen, J.C. Lambry, B. Bohn, C. Poyart, J.L. Martin, Nat. Struct. Mol. Biol. 1 (1994) 230.
[17] M. Nagai, S. Kaminaka, Y. Ohba, Y. Nagai, Y. Mizutani, T. Kitagawa, J. Biol. Chem. 270 (1995) 1636.
[18] E. Bucci, C. Fronticelli, J. Biol. Chem. 240 (1965) PC551.
[19] M. Nagai, M. Aki, R. Li, Y. Jin, H. Sakai, S. Nagatomo, T. Kitagawa, Biochemistry 39 (2000) 13093.
[20] M. Abe, T. Kitagawa, Y. Kyogoku, J. Chem. Phys. 69 (1978) 4526.
[21] X.Y. Li, R.S. Czernuszewicz, J.R. Kincaid, P. Stein, T.G. Spiro, J. Phys. Chem. 94 (1990) 47.
[22] Y. Mizutani, T. Kitagawa, Science 278 (1997) 443.
[23] J.M. Friedman, R.A. Stepnoski, M. Stavola, M.R. Ondrias, R.L. Cone, Biochemistry 21 (1982) 2022.
[24] S. Dasgupta, T.G. Spiro, Biochemistry 25 (1986) 5941.
[25] S. Choi, T.G. Spiro, K.C. Langry, K.M. Smith, D.L. Budd, G.N. La Mar, J. Am. Chem. Soc. 104 (1982) 4345.
[26] W.A. Oertling, A. Salehi, Y.C. Chung, G.E. Leroi, C.K. Chang, G.T. Babcock, J. Phys. Chem. 91 (1987) 5887.
[27] L.D. Spaulding, C.C. Chang, N.-T. Yu, R.H. Felton, J. Am. Chem. Soc. 97 (1975) 2517.
[28] T.G. Spiro, J.D. Stong, P. Stein, J. Am. Chem. Soc. 101 (1979) 2648.
[29] T. Kitagawa, K. Nagai, M. Tsubaki, FEBS Lett. 104 (1979) 376.
[30] S. Hu, K.M. Smith, T.G. Spiro, J. Am. Chem. Soc. 118 (1996) 12638.
[31] E. Antonini, M. Brunori, Hemoglobin and Myoglobin and Their Reactions with Ligands, Elsevier North-Holland Publishing, Amsterdam, 1971.
[32] T. Kitagawa, K. Nagai, M. Tsubaki, FEBS Lett. 104 (1979) 376.
[33] H. Hori, T. Kitagawa, J. Am. Chem. Soc. 102 (1980) 3608.
[34] O. Bangcharoenpaurpong, K.T. Schomacker, P.M. Champion, J. Am. Chem. Soc. 106 (1984) 5688.
[35] B.R. Gelin, A.W. Lee, M. Karplus, J. Mol. Biol. 171 (1983) 489.
[36] K. Nagai, T. Kitagawa, Proc. Natl. Acad. Sci. USA 77 (1980) 2033.
[37] S. Matsukawa, K. Mawatari, Y. Yoneyama, T. Kitagawa, J. Am. Chem. Soc. 107 (1985) 1108.
[38] E.S. Peterson, J.M. Friedman, E.Y.T. Chien, S.G. Sligar, Biochemistry 37 (1998) 12301.
[39] U. Samuni, Y. Ouellet, M. Guertin, J.M. Friedman, S.-R. Yeh, J. Am. Chem. Soc. 126 (2004) 2682.
[40] H. Yoshimura, S. Yoshioka, Y. Mizutani, S. Aono, Biochem. Biophys. Res. Commun. 357 (2007) 1053.
[41] H. Sawai, M. Makino, Y. Mizutani, T. Ohta, H. Sugimoto, T. Uno, N. Kawada, K. Yoshizato, T. Kitagawa, Y. Shiro, Biochemistry 44 (2005) 13257.
[42] M.R. Ondrias, J.M. Friedman, D.L. Rousseau, Science 220 (1983) 615.
[43] D.S. Gottfried, E.S. Peterson, A.G. Sheikh, J. Wang, M. Yang, J.M. Friedman, J. Phys. Chem. 100 (1996) 12034.
[44] E. Podstawka, C. Rajani, J.R. Kincaid, L.M. Proniewicz, Biopolymers 57 (2000) 201.