New Transient Species of Sperm Whale Myoglobin in Photodissociation of Dioxygen from Oxymyoglobin*

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Fumitoshi Sato, Yoshitsugu Shiro, Yoshiro Sakaguchi, Tomohiko Suzuki, Tetsutaro Iizuka, and Hisaharu Hayashi

From the Department of Physics, Faculty of Science and Technology, Keio University, Kohoku, Yokohama 223, Japan, the Institute of Physical and Chemical Research, Wako-shi, Saitama 351 01, Japan, and the Department of Biology, Faculty of Science, Kochi University, Kochi-shi, Kochi 780, Japan

We carried out the flash photolysis of oxy complexes of sperm whale myoglobin, cobalt-substituted sperm whale myoglobin, and Aplysia myoglobin. When the optical absorption spectral changes associated with the O₂ rebinding were monitored on the nanosecond to millisecond time scale, we found that the transient spectra of the O₂ photoproduct of sperm whale myoglobin were significantly different from the static spectra of deoxy form. This was sharply contrasted with the observations that the spectra of the CO photoprod-uct of sperm whale myoglobin and of the O₂ photoproducts of cobalt-substituted sperm whale myoglobin and Aplysia myoglobin are identical to the corresponding spectra of their deoxy forms. These results led us to suggest the presence of a fairly stable transient species in the O₂ photodissociation from the oxy complex of sperm whale myoglobin, which has a protein structure different from the deoxy form. We denoted the O₂ photoproduct to be Mb*. In the time-resolved resonance Raman measurements, the $v_{y_{2}}$-Hia mode of Mb* gave the same value as that of the deoxy form, indicating that the difference in the optical absorption spectra is possibly due to the structural difference at the heme distal side rather than those of the proximal side. The structure of Mb* is discussed in relation to the dynamic motion of myoglobin in the O₂ entry or exit from the heme pocket. Comparing the structural characteristics of several myoglobins employed, we suggested that the formation of Mb* relates to the following two factors: a hydrogen bonding of O₂ with the distal histidine, and the movement of iron upon the ligation of O₂.

It has been of great interest to study a dynamic motion of proteins or enzymes when they play their biological functions. In the cases of hemoproteins such as hemoglobin (Hb) and myoglobin (Mb), their equilibrium (static) structures were revealed by x-ray crystallographic analyses (1, 2). According to Perutz (3, 4), the protein structure of oxyhemoglobin (HbO₂) is significantly different from that of deoxyhemoglobin (deoxygen-Hb), indicating that the protein conformation changes upon the binding of the ligand to heme iron. However, the dynamic motion of the protein structure, which is called dynamic structure, could not be evaluated by the crystallographic models.

One of the most valuable approaches to this problem is a flash photolysis method, in which the ligand binding subsequent to its photodissociation from the iron is monitored by several spectroscopic measurements such as optical absorption (5) and resonance Raman spectra (6, 7). A development of some experimental techniques has allowed us to measure the recombination kinetics in a wide time and temperature range (8-10). In these studies, the CO complexes have been frequently utilized as an analogue of the O₂ complexes, most probably because of a high quantum yield of CO complexes in photoradiation. However, it is more important to examine O₂ complexes of hemoproteins in detail since the O₂ binding is an essential reaction to most hemoproteins including Hb, Mb, cytochrome oxidase, and cytochrome P-450. The difference in the molecular properties of CO and O₂ possibly results in the different interaction with protein moiety. For example, it has been reported for the static structure that the iron-bound O₂ in Mb interacts with the distal histidine through a hydrogen bond (11), whereas the CO does not (12). The difference in the static interaction may effect a dynamic motion of the protein upon the ligand binding.

In the present study, we applied the flash photolysis method to oxy complexes of various myoglobins: sperm whale Mb (SWMb); SWMb, chemically substituting the heme with cobalt-protoporphyrin (CoMb), and myoglobin from the radular muscle of Aplysia kurodai (AplysiaMb). To elucidate the dynamic property of Mb in the O₂-binding reaction, we focused our attention on the optical and the Raman spectral changes on the nanosecond (ns) to millisecond (ms) time range at an ambient temperature. In this time range, the CO complex of SWMb (SWMbCO) yields the deoxy form upon the photoirradiation, as was evidenced by optical absorption and Raman spectral measurements (13, 14). However, we found in the present study that the transient spectrum of SWMb induced by the O₂ photolysis is distinguishable from that of deoxy form. Since Mb consists of a single polypeptide chain, the obtained data were interpreted with relevance to the tertiary structural change caused by the O₂ photodissociation. Our results will provide a new evidence on a dynamic protein structure and a control mechanism of the ligand-binding reaction in hemoproteins.

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**MATERIALS AND METHODS**

The oxy complex of SWMb (Sigma) was prepared by passing a dithionite-reduced protein solution through Sephadex G-25 (Pharmacia LKB Biotechnology Inc.) column equilibrated with O2-saturated 10 mM potassium phosphate buffer at pH 6.5 followed by purification with CM52 (Whatman). Cobalt protoporphyrin and CoMb were prepared and purified according to the methods of Yone-tani et al. (15). The oxy form of CoMb was prepared by pressuring O2 gas in the sample solutions. The oxy complex of AplysiaMb was isolated directly from the radular muscle of *A. kurodai* and was further purified by DEAE-cellulose (Whatman) chromatography according to the standard method (16). The deoxy form of these Mbs were prepared either by reduction of O2 in a sample solution with a small amount of Na2S2O4 or by purging O2 in the solution with bubbling N2. The sample conditions for all flash photolysis measurements were kept at pH 7.0 and 22 °C.

The ns photolysis experiments were performed by using the second harmonic (532 nm) of a Quanta-Ray DCR-1 Nd:YAG laser, producing an excitation flash of 6 mJ with a pulse width of 6 ns. The sample solutions in rectangular cells were exposed to the laser pulse with a path length of 2 mm, which was illuminated by a xenon lamp. The time dependence of the transient absorption intensity was measured by the combination of a monochromator (Nicon G250) and photomultiplier (Hamamatsu Photonics, R636) at each wavelength. The details of the measurement system are reported by Sakaguchi and Hayashi (17).

On the other hand, another laser flash photolysis system (Uniooku) was used from microsecond (μs) to ms experiments. With this system, absorption spectra can be measured with a multichannel photodiode array. Rhodamin 6G (Wako Pure Chemical Ind.) dissolved in methanol was used to produce an excitation flash of 250 mJ with a pulse width of 30 ns and a peak at 591 nm. For this measurement, a path length of the cells was 10 mm, and the sample solutions were kept at 10 μM at 25 °C. The equilibrium spectrum of deoxy minus oxy form was measured with the same spectrophotometers in flash photolysis systems, as well as with Hitachi U-3210 UV-Vis spectroscopy. The wavelengths of the spectrophotometers were calibrated with wavelength standards of length in the mercury lamp.

Resonance Raman spectra were recorded with instrumentation using the spinning cell method described by Kaminaka et al. (18). The excitation source was the 441.6 nm line of a helium-cadmium laser, and the spectrometer step size was 0.25 cm⁻¹. Raman shifts were calibrated with Indene (1200–1700 cm⁻¹) and CCl₄ (150–550 cm⁻¹) and determined with an accuracy of 1 cm⁻¹. All spectral data are presented without smoothing.

**RESULTS**

**Binding Processes in Flash Photolysis of SWMbO₂**—We examined the kinetic features of O2 binding to Mb in the time range of ns to ms by the flash photolysis method. Figs. 1 and 2 show the kinetic traces of O2 photolysis from the oxy complex of SWMb (SWMbO2) in the μs and ns time ranges, respectively. As can be seen in both figures, flash irradiation induces instantaneous changes of absorbances, showing photodissociation of the iron-bound O2. In the μs to ms time range (Fig. 1A), the observed absorbance change after the O2 photolysis is seemingly monophasic, and its semi-logarithmic plot (ln ΔA, versus t) gave a straight line (data not shown). The observed apparent reaction rate constant was linearly dependent on the bulk O2 concentration (Fig. 1B) and is consistent with a bimolecular reaction. According to the pseudo-first order analysis, the association and dissociation rate constants of this reaction at 22 °C were 1.61 × 10⁷ s⁻¹ M⁻¹ and 10 s⁻¹, respectively. By contrast, the kinetic traces in the ns range exhibited a biphasic feature (Fig. 2). The slow phase, which seemed constant in the ns time scale, corresponded to the bimolecular reaction observed in the μs range. On the other hand, the fast phase shows the rebinding process of O2, which still stayed in the protein matrix after the photodissociation (geminate process), since the rate constants of the O2 rebinding were independent of the O2 concentration. The kinetic feature for SWMbO2 was identical to the results reported so far (10, 19, 20).

**Optical Spectral Changes in Flash Photolysis of SWMbO₂**—We examined the spectral change of photoproduct from SWMbO₂ in the μs range. In Fig. 3A, the solid curves are the transient difference spectra of the O2 photoproduct minus the oxy form at the Soret region in the time range of 3–300 μs. The spectrum immediately after irradiation smoothly changed into that of SWMbO₂, with a peak at 437 nm, a trough at 416 nm, and an isosbestic point at 426 nm. Comparison of the spectra with the difference spectrum of deoxy minus oxy forms (dotted line in Fig. 3A) indicated that the μs transient spectrum of the photodissociated form was slightly but significantly distinguishable from that of deoxy-SWMb; an isosbestic...
process, between 0 and 500 ns; and the slow process, which appears constant in this trace. The slow phase firmly relaxes in ps time region, below 400 nm (Fig. 3A).

The spectral discrepancy was also observed in near-UV region upon the laser irradiation, a small but clear change was detectable at 424 nm but not at 426 nm. These results on the ns transient spectrum were also observed in the near-UV region below 400 nm (Fig. 3A).

We also followed the spectral change of SWMb in the ns O₂-rebinding reaction by plotting the magnitudes of the initial absorbance change induced by the laser irradiation against the monitoring wavelength (between 400 and 450 nm). The solid curve in Fig. 4 is the transient difference spectrum of the O₂ photoproduct minus the oxy form at 30 ns after laser irradiation, and the dotted curve is the difference spectrum of deoxy minus oxy forms. The ns transient spectrum was also not superimposable on that of deoxy minus oxy forms but was identical to the μs transient spectrum described above. When we monitored the absorbance changes at both 424 and 426 nm upon the laser irradiation, a small but significant change in absorbance was observed at 424 nm but not at 426 nm (Fig. 3B). This result confirmed the significance of the spectral difference between the O₂ photoproduct and deoxy-SWMb. The spectral discrepancy was also observed in near-UV region below 400 nm (Fig. 3A).

In order to check the instrumental and experimental accuracy of our measurements, we carried out the flash photolysis of SWMbCO from the 3 μs to 5-ms time range with the same measurement system. As shown in Fig. 5, the transient difference spectrum (solid line) after the laser irradiation was completely superimposed on the spectrum of deoxy minus CO forms (dotted line), in which the isosbestic points were observed at 410 and 430 nm. Hence, we conclude that the spectrum of the O₂ photoproduct produced from SWMbO₂ is significantly different from that of deoxy-SWMb and that the O₂ photoproduct is a transient species with a characteristic protein structure and a relatively long lifetime.

**Time-resolved Resonance Raman Spectra of SWMbO₂ and SWMbCO**—In order to get further insight into the protein structure of SWMb after the photodissociation of O₂ from its oxy form, we measured the time-resolved resonance Raman spectra. In Fig. 6 is illustrated the high (1200 - 1700 cm⁻¹) and the low frequency (150 - 550 cm⁻¹) regions of the resonance Raman spectrum of SWMb after the O₂ photolysis, which is compared with those of deoxy-SWMb and the CO photoprodut. A helium-cadmium laser (441.6 nm) with a power of 55 milliwatts was employed as a light source of both the O₂ photodissociation and the Raman excitation. Since the diameters of the cell and the laser beam were about 2 cm and 100 μm, respectively, and the spinning velocity of the cell was 1600 rpm, we estimated that we can observe the spectra of SWMbCO at 68 μs after the O₂ dissociation. In the high frequency region (Fig. 6A), the ν₉ band of the oxidation state marker of the O₂ photoproduct was observed.
Optical Absorption Spectral Change of O$_2$ Rebinding to CoMb—We also followed the optical absorption spectral change of CoMb in the rebinding of the photodissociated O$_2$ on the μs (Fig. 7A) and the ns (Fig. 7B) time range. In this complex, O$_2$ reversibly binds to the Co$^{2+}$ to form a stable oxy form. The O$_2$-rebinding reaction to CoMb after the photodissociation of the oxy form exhibited a biphasic feature on this time region$^2$ (21). The μs difference spectra (10 μs ~ 1 ms) showed uniform changes with an isosbestic point at 413 nm, the peak at 401 nm, and the trough at 427 nm (Fig. 7A). The spectra obtained after the laser irradiation were indistinguishable from the difference spectrum of deoxy minus oxy forms of CoMb. The ns transient spectra (open circle in Fig. 7B), which were obtained by plotting the magnitudes of the absorbance change at 10 ns after the laser photolysis against the monitoring wavelength, have a pattern identical to that of the μs transient spectrum (Fig. 7A) as well as that of deoxy CoMb (dotted line in Fig. 7B). Although the reaction is kinetically composed of two distinct phases, only one deoxy-like compound was spectrophotometrically observed, implying that any intermediates in both ns and μs time range are spectrally indistinguishable from deoxy-CoMb.

**Optical Absorption Spectral Changes in the O$_2$ Photodissociation from AplysiaMb—**AplysiaMb, which is a monomeric hemoprotein, is similar to SWMb in molecular size but differs in other respects. Compared with SWMb, one of the remarkable structural characteristics of AplysiaMb is that the valine residue is located at the heme distal side instead of the histidine of SWMb (22, 23). In Fig. 8 are illustrated the difference spectral changes of AplysiaMbO$_2$ at the Soret region of the CoMb. The ns transient spectra (open circle in Fig. 7B), which were obtained by plotting the magnitudes of the absorbance change at 10 ns after the laser photolysis against the monitoring wavelength, have a pattern identical to that of the μs transient spectrum (Fig. 7A) as well as that of deoxy CoMb (dotted line in Fig. 7B). Although the reaction is kinetically composed of two distinct phases, only one deoxy-like compound was spectrophotometrically observed, implying that any intermediates in both ns and μs time range are spectrally indistinguishable from deoxy-CoMb.

**Fig. 4.** Nanosecond transient difference spectrum of O$_2$ photoproduct minus SWMbO$_2$ between 400 and 450 nm. The μs kinetics after flash photolysis of SWMbO$_2$ was followed between 400 and 450 nm, and the absorbance changes at 30 ns after the laser irradiation were plotted against the monitoring wavelength (solid curve). The dotted curve indicates the difference spectrum of the deoxy form minus SWMbO$_2$. The μs transient spectrum was not superimposable on the spectrum of deoxy minus oxy SWMb but was almost identical to the μs transient spectra in Fig. 3. Other experimental conditions were the same as in Fig. 1.

**Fig. 5.** Microsecond transient difference spectra of CO photoproduct minus SWMbCO between 380 and 460 nm. The solid curves are the spectra measured at 3 and 500 μs, 1, 2, 3, and 5 ms after giving laser irradiation to 10 μM SWMbCO in 0.1 M potassium phosphate buffer at pH 7.0, 20°C. The dashed curve indicates the difference spectrum of deoxy-SWMb minus SWMbCO. The isosbestic points at 410 and 450 nm for the CO photoproduct minus CO spectra were coincident with those for deoxy minus oxy spectra. Other experimental conditions were the same as in Fig. 3.

**Fig. 6.** Resonance Raman spectra of deoxy-SWMb, SWMbCO, and SWMbO$_2$ in the ν$_s$ (A) and ν$_{RE,LO}$ (B) band regions. Helium-cadmium lasers at 441.6 nm were employed as a light source for the ligand (CO, O$_2$) photodissociation and the Raman excitation. The CO and O$_2$ photoproducts of SWMb were observed with a spinning cell. The spectral slit width and time constant were 7.0 cm$^{-1}$ and 3.2, respectively. The position of the ν$_s$ and ν$_{RE,LO}$ bands for the O$_2$ photoproduct (1355, 220 cm$^{-1}$, respectively) is identical to that of deoxy form and the CO photoproduct (1356, 220 cm$^{-1}$, respectively).

**DISCUSSION**

In the present study, we examined the kinetic and spectral properties of Mbs after the photolysis of O$_2$ from heme iron in the time range between ns and ms. For SWMb, we observed two (fast and slow) phases for the O$_2$-rebinding reaction in this time range. The O$_2$ concentration dependence of the rebinding rate revealed that the slow phase in the μs range is

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Transient spectra were similar to each other (Figs. 3 and 4) in the ns time region is a geminate reaction. This kinetic feature of the O₂ binding to SWMb was identical to those following two-step model: the association of O₂ from and to the heme iron of SWMb by the reaction scheme for the dissociation and can describe the reaction.

We reported previously by many investigators (10, 20, 24). We have observed that the difference spectrum of the O₂ photoproduct minus oxy-CoMb at 10 ns after the laser irradiation; the dashed line shows that of deoxy- minus oxy-CoMb. The isosbestic points at 431 nm were identical for the O₂ photoproduct minus oxy form, and deoxy minus oxy forms. Other experimental conditions were the same as in Fig. 1.

A bimolecular reaction of O₂ and SWMb, whereas the fast one in the ns time region is a geminate reaction. This kinetic feature of the O₂ binding to SWMb was identical to those reported previously by many investigators (10, 20, 24). We can describe the reaction scheme for the dissociation and association of O₂ from and to the heme iron of SWMb by the following two-step model:

\[
\text{SWMbO}_2 \rightarrow [\text{SWMb} \cdots \text{O}_2] \rightarrow \text{deoxy-SWMb} + \text{O}_2
\]

where \([\text{SWMb} \cdots \text{O}_2]\) denotes the ns geminate pair.

Since we are interested in the protein conformation of SWMb during these reactions, we have carefully followed the absorption spectral changes after the O₂ photolysis from SWMbO₂. We found that the difference spectrum of the O₂ photoproduct minus SWMbO₂ is significantly distinguishable from that of deoxy minus oxy forms and that the ns and μs transient spectra were similar to each other (Figs. 3 and 4) in spite of the different location of the dissociated O₂. We also observed that the difference spectrum of the O₂ photoproduct of Mbs from other sources such as horse heart or bovine heart was distinguishable from that of the deoxy form, where the position of the isosbestic point differs by 2 nm between the photoproduct and deoxy form. Furthermore, the O₂ photoproducts from artificial SWMbs that contain 2,4-modified heme such as meso-, deuter-, diacetyldeutero-hemes also afforded the spectra distinguishable from those of their deoxy forms (data not shown). These findings suggest that the optical absorption spectrum of the O₂ photoproduct of SWMb is different from that of its deoxy form. By contrast, the transient spectra of SWMbCO (Fig. 5), CoMbO₂ (Fig. 7), and AplysiaMbO₂ (Fig. 8) after the ligand photolysis were indistinguishable from those of the corresponding deoxy forms. We concluded that the O₂ photodissociation from SWMbO₂ yields a reliably stable transient species that has a subtly different protein conformation from deoxy-SWMb. The protein structure of the oxy form may not be fully relaxed to the deoxy form even at a few hundred μs after the O₂ photodissociation. Based on these findings, we denote the transient species to be myoglobin star (Mb*) and rewrite Equation 1 as:

\[
\text{SWMbO}_2 \rightarrow [\text{Mb}^* \cdots \text{O}_2] \rightarrow \text{Mb}^* + \text{O}_2
\]

\[
(= \text{deoxy-SWMb} + \text{O}_2)
\]

As for the CO complexes of hemoproteins, several photoproducts different from their deoxy forms have been reported so far. First, Gibson (25) applied the ms flash photolysis method to HbCO and observed a photoproduct having an absorption spectrum distinguishable from that of deoxy-Hb. This photoproduct has been called hemoglobin star (Hb*) and has been defined as the Hb without external ligand but with a protein conformation of ligated state. Since Gibson, many investigators have been interested in the photochemical intermediate species and have tried to characterize them by various methods of molecular spectroscopies (26–30). Since the conversion of the iron spin state from the ferrous low spin to high spin states is so fast, the differences in the spectra have been predominantly attributed to the transient state of the heme and/or the protein part in the relaxation from ligated to deligated states (26–28). In the case of Hb, either the relaxation of the bond between the iron and the proximal histidyl imidazole (Fe-His) or that of the porphyrin core skeleton occurs within a few μs time scale, as was manifested.
by the time-resolved measurements of both resonance Raman (29) and visible spectra (30). On the other hand, the CO photodissociation from SWMbCO also gives the transient and unrelaxed photoproduction (14, 26–28). According to Petrich et al. (14), the Fe-His bond and porphyrin core of SWMb are converted to the deoxy form within several ns after the CO photodissociation from the heme iron; the conversion is much faster than is the case for Hb.

In spite of the comprehensive studies for transient species of hemoproteins in their ligand binding, the structural changes of Mb upon the O₂ photodissociation or the subsequent recombination have been rarely reported. The reaction with O₂ is essential for Mb. In addition, it is significant to reveal the dynamic structure of Mb in its O₂-binding reaction. In this context, the detection of Mb* in the O₂ photodissociation is important. Since the optical spectrum of Mb* is different from that of deoxy-SWMb, we expected the structural differences of the heme and/or the protein part interacting with the heme. As stated above, the transition of the structural and electronic state of the heme from the ligated to the de-ligated forms is too short (less than several nanoseconds) to contribute to the optical spectrum. Indeed, the time-resolved resonance Raman measurements showed that the electronic structure of the heme and the structure of the porphyrin skeleton are indistinguishable between Mb* and deoxy-SWMb, as was evidenced by the position of the \( \nu_1 \) band (1356 cm⁻¹). In addition to this observation, the \( \nu_{Fe-His} \) band of Mb* is also identical to that of the deoxy form, indicating that the heme and the Fe-His bond of Mb* are fully converted to the deoxy structure after the O₂ photodissociation. At this point, the structure of Mb* is very characteristic, compared with the transient species of hemoproteins reported so far, in which the Fe-His bond is unrelaxed after the ligand dissociation. Our present results indicate that the heme iron of Mb* is in a ferrous high spin state and locates out of plane of the porphyrin. Therefore, we concluded that a subtle difference in the protein part, probably at the heme distal side, may cause the difference in the optical absorption spectrum between Mb* and deoxy-SWMb.

It has been postulated for Mb that the distal histidine acts as a barrier to ligand entry \( \Omega_1 \) and exit from the heme pocket (2, 31). The distal histidyl imidazole of SWMbO₂ is locked tightly in the position close to the bound O₂ by the hydrogen bond (32). Recent x-ray crystallographic study of the ethyl isocyanide complex of SWMb (33) has shown that the distal imidazole of this complex located out of the pocket toward the solvent phase is visualized on 45% of the refined occupancy. Moreover, in the case of phenyl-SWMb, which is generated by the reaction of ferric SWMb with phenyl hydrazine, the distal histidine substantially swings up and away from the iron atom toward the protein surface (34). On the basis of these x-ray crystallographic data, the action of the distal histidine in the O₂ exit from the heme pocket has been considered as follows. In the crystal structure of SWMbO₂ (32), the packing of the side chain groups, including the distal histidine imidazole, is so dense that the O₂ cannot move from the heme pocket to the protein surface (closed conformation). When O₂ escapes from the heme distal side after the photodissociation, the distal histidine should rotate about its C-C₀ bond toward the surface of the protein to create an opening large enough for the O₂ escape (open conformation). After the O₂ escape, the distal histidyl imidazole would fix at the position of the deoxy-SWMb. Since Mb* is detectable subsequent to the dissociation of O₂ from the heme iron, it is likely speculated that the distal histidine of Mb* is in a transient state between oxy and deoxy forms. Indeed, the rate constants \( k_{on} \) and \( k_{off} \) for the O₂ binding to Mb* are similar to those obtained from the reaction of deoxy SWMb with O₂ by the temperature jump and the flow-flash experiments (19, 35). This observation appears to show that Mb* is not necessarily peculiar in the O₂ photolysis reaction but that O₂ binds to deoxy-SWMb via Mb* as a transient state of the open conformation.

We must note that Mb* is detectable for the O₂ photodissociation from SWMbO₂, horse heart MbO₂, bovine heart MbO₂, and heme-modified SWMbCO but not for SWMbCO, CoMbO₂, and AplysiaMbO₂. These results allowed us to suggest that whether Mb* is detectable or not depends on the difference in interaction of the iron-bound ligand with the surrounding peptide resulting from different tertiary structure among Mbs and from different physiochemical properties between O₂ and CO. Then, we point out two structural factors that relate to the Mb* formation. First is the hydrogen bond of the iron-bound O₂ with the distal imidazole. Comparing the structures of SWMbO₂ with that of its CO forms, the iron-bound O₂ interacts with the distal histidyl imidazole through a hydrogen bonding (11), whereas the iron-bound CO does not (12). Therefore, the distal histidine of SWMbO₂ is more tightly fixed in its position than that of MbCO. In the case for AplysiaMbO₂, which lacks the distal histidine (displacement by valine), the hydrogen bonding with the iron-bound O₂ is not available (22, 23). Second, we focus on the iron displacement of deoxy-Mb. The iron of deoxy-SWMb places out of plane of the porphyrin by 0.6 Å (36), whereas the iron is in plane for the oxy form (32). According to Perutz’s model (3), the iron movement upon the O₂ dissociation serves as a trigger to induce the conformational change of the protein from the oxy to the deoxy forms. By contrast, the magnitude of the Co²⁺ displacement in deoxy-CoMb has been supposed to be smaller (0.1 – 0.3 Å) than that in SWMb (37–39). Thereby, the dynamic change of the protein structure associated with the O₂ photodissociation is possibly different between CoMb and SWMb. SWMb, together with horse heart Mb, bovine heart Mb, and Mb, having the 2,4-side chain-modified heme, contains these two factors: a cleavage of the hydrogen bond of the metal-bound O₂ with the distal histidine, and the metal movement from the heme plane by 0.6 Å. On the other hand, in the case of SWMbCO, AplysiaMbO₂, and CoMbO₂, the structural change occurs by a single factor: the iron movement in AplysiaMbO₂ and SWMbCO, or the hydrogen bond cleavage in CoMbO₂. We speculated that these two structural factors relate to the Mb* stabilization after the dissociation of the ligand. In order to make more clear the structural control of the dynamic structure of Mb, we are now studying the geminate reaction with O₂ in the ns time range, where the O₂ rebinding is dynamically controlled by the dynamic motion of the protein.

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New transient species of sperm whale myoglobin in photodissociation of dioxygen from oxymyoglobin.
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