氏名	目　　喜　直
学位（専攻分野）	博士（理学）
学位記番号	総研大甲第３６号
学位授与の日付	平成４年　９月２８日
学位授与の要件	数物科学研究科　機能分子科学専攻 学位規則第４条第１項該当
学位論文題目	Studies on the Protein Dynamics of Photodissociated Carbonmonoxy Myoglobin and Hemoglobin by Time-Resolved Resonance Raman Spectroscopy
論文審査委員	主　査　教　授　田　中　晃　二 教　授　北　川　禎　三 助教授　冨　宅　喜代一 助教授　大　峯　　巖 飯　塚　哲太郎（理化学研究所）

論 文 内 容 の 要 旨

Myoglobin (Mb) that is monomer and Hemoglobin (Hb) that is tetramer are one of the most basic hemoproteins. Hb has a physiological function as an oxygen carrier in blood, and Mb can store oxygen in muscle. Both of Mb and a subunit of Hb consist of one heme (iron protoporphyrin IX) and a single polypeptide chain. Mb and Hb bind carbonmonoxide (CO) at the oxygen binding site. Carbonmonoxy myoglobin (MbCO) and carbonmonoxy hemoglobin (HbCO) are photodissociable upon laser illumination, reversibly.

According to the x-ray data of crystalline Mb, there is no pathway for the ligand entry from the solvent to the heme pocket. However, in some of derivative Mb another structure was also detected, in which distal histidine swings out toward the solvent and a pathway for the ligand entry is open. This structure is called "open" form, and the other structure that has no pathway is called "closed" form. When the ligand enters into the heme pocket, it is expected that the protein around the heme undergoes the conformational change. On the other hand, IR and Raman spectroscopy showed that in acidic MbCO, the C-O(ν_{C-O}) and Fe-CO(ν_{Fe-CO}) stretching vibrational modes are located at different frequencies from these of neutral MbCO. In the present Raman experiment two ν_{Fe-CO} bands were identified at around 490 cm^{-1} and 510 cm^{-1} for MbCO at pH 4.5, while only one peak was observed at 510 cm^{-1} for MbCO at neutral pH. The value pH 4.5 happens to be equal to the pK_a value of the distal histidine and thus, half of the distal histidine is protonated at pH 4.5. Therefore, it was inferred that the protonated distal histidine repelled

lysine–45 in the vicinity of the heme distal side, and the protein conformation changed to the "open" form. Thus, the higher and lower frequency of ν_{Fe-CO} correspond to the "closed" and "open" form, respectively. The ν_{Fe-CO} frequency is sensitive to the Fe–C–O angle. According to the XANES of MbCO solution at neutral pH, the Fe–C–O bent angle is 150 . From a simple normal–coordinate calculation for isolated three–body oscillators, the ν_{Fe-CO} frequencies for the bent angle of 160 and 180 were estimated at 500 cm^{-1} and 490 cm^{-1}, respectively. It is assumed that the Fe–C–O angle of the "open" form is linear and that of "closed" form is bent.

If the photodissociated ligand recombines to the "open" form and relaxes to the "closed" form gradually at neutral pH, the ν_{Fe-CO} should appear around 490 cm^{-1} first and shift to 505 cm^{-1}. In this study, the nanosecond time–resolved resonance Raman (TR^3) spectra were observed in order to catch the transient species of MbCO on the recombination reaction. TR^3 spectroscopy is a powerful technique to analyze the dynamical conformation in short time scale, and information obtained by it is very basic and important in studies of the relationship between physiological functions and protein dynamics.

MbCO at acidic pH (4.5), containing equal amounts of the "closed" and the "open" forms, were measured by the TR^3 system. If the assumption above is true, the recombination rate of the "open" form should be faster than that of the "closed" form. Indeed, the temporal behaviors of the ν_{Fe-CO} bands of the "open" and "closed" forms were different. The ν_{Fe-CO} band of the "open" form recovered faster than the "closed" form. However, at neutral pH, there were no transient bands around 490 cm^{-1} in all time range observed (–20 ns – 1 ms). It suggests either that the transient "open" form is absent or that the structural is change too fast to be detected (Chapter II).

In the acidic pH, the recombination kinetics may be affected by pH effect. It is desirable to measure both of "open" and "closed" forms under

identical conditions including pH and temperatures. The human abnormal hemoglobins, "Boston" and "Saskatoon" are best models for this purpose, since abnormal hemoglobins contain two types subunits in one molecule, that is normal chains and abnormal chains. In the abnormal chain the distal histidine is replaced by tyrosine. In "Boston", the α–chain is abnormal, and in "Saskatoon" the β–chain is abnormal. Their ν_{Fe-CO} bands arise around 490 and 505 cm^{-1}, corresponding to the "open" and "closed" forms, respectively (Chapter III).

Some human MbCO mutants whose distal His was replaced by various amino acids residues through site–directed mutagenesis were also examined at neutral pH in order to make clarity the role of the distal histidine on the rebinding reaction. Some of them have the ν_{Fe-CO} band around 490 cm^{-1} and the others have it around 510 cm^{-1} (Chapter IV). The results from the experiments on these Hbs and Mbs also showed that MbCO with the lower ν_{Fe-CO} band recovered faster than that with higher band. Transient bands observed the time range between 100–1000 ns were significantly broad. The transient "open" form was not detected in all time range for the species with the "closed" equilibrium structure. These results suggested that the ligand entered to the heme pocket through a pathway which was created by a conformational change in the distal side, but the so–called "open" form was never produced during the recombination reaction. The Fe–C–O angle in a transient form seems to be slightly perturbed around the equilibrium angle and it has appreciable distributions.

論文の審査結果の要旨

目喜直君の博士論文 "Studies on the Protein Dynamics of Photo-dissociated Carbonmonoxy Myoglobin and Hemoglobin by Time-Resolved Resonance Raman Spectroscopy" は５章にまとめられている。

１章は本研究の背景の説明に当てられている。そこではミオグロビン（Ｍｂ）とヘモグロビン（Ｈｂ）の一般的性質ならびにヘム近傍のヒスチヂンの空間的位置により第６配位子のヘム鉄への配位が閉ざされているクローズ状態と経路が開かれているオープン状態が存在し、クローズ状態とオープン状態のＣＯ付加体（ＭｂＣＯ）のＦｅ-ＣＯ伸縮振動（ν_{Fe-CO}）が５１０ｃｍ$^{-1}$と４９０ｃｍ$^{-1}$付近に帰属されており、クローズ状態でのヘム鉄へのＣＯの配位はオープン状態を経て進行するためにオープン状態でのＣＯ結合生成に比べて遅いことが提案されていることを述べている。

２章では同君が組み立てた時間分解共鳴ラマン測定装置の概要が説明されている。１０ナノ秒の時間分解能を有する本装置をウマＭｂＣＯに適用した。ＭｂＣＯのν_{Fe-CO}はヘム近傍のヒスチヂンのプロトン化の影響を受けて、ｐＨ４.５では５１０と４９０ｃｍ$^{-1}$に２本観測され、ｐＨ８.０では５１０ｃｍ$^{-1}$のみであることを利用して、光解離後のＣＯのヘム鉄への再結合過程を時間分解的に追跡し、４９０ｃｍ$^{-1}$のラマン線の回復は５１０ｃｍ$^{-1}$のラマン線に比べて圧倒的に速く、また５１０ｃｍ$^{-1}$バンドの再生過程には４９０ｃｍ$^{-1}$が全く含まれないことを見いだし、クローズ状態でのＭｂへのＣＯ結合過程にはオープン状態を経由しないことを明らかにしている。

３章ではプロトン濃度によらず２本のν_{Fe-CO}（５０７と４９０ｃｍ$^{-1}$）を示す２種の異常ＨｂＣＯを用い、光解離後のヘム鉄へのＣＯ再結合過程を測定し、それぞれのラマン線は波数を変化させずに元のＨｂＣＯを１ｍｓで再生することから、クローズ型と考えられている５０７ｃｍ$^{-1}$のＦｅ-ＣＯ結合生成はＨｂにおいてもオープン型を経由せずに進行することを明らかにしている。

４章ではＨｉｓ-６４を置換した６種類の人工変位株ヒトＭｂＣＯを調べた。そこでは疎水基に置換したものがν_{Fe-CO}＝４９０ｃｍ$^{-1}$に、親水基にしたもの

が５１０ｃｍ$^{-1}$付近に現れることを明らかにし、光解離したＣＯのヘム鉄への再結合過程では低波数側のバンドが速く回復することを見いだしている。また、これらの再結合過程は２段階で進行するが、その原因は従来提唱されていたオープン状態ではなく、たん白の外に出ずに戻るものの寄与が大きくなることを指摘している。

５章ではＭｂ中のトリプトファンをＵＶ照射により光励起させた系と可視光によりヘムを励起した系でのヘム鉄とO_2およびＣＯの光解離および再結合過程をＦｅ-ヒスチジンの伸縮振動の時間分解ラマンスペクトルにより検討している。O_2およびＣＯの光解離挙動に関してはかなり異なる点もあるが、トリプトファンからヘム鉄へ効率よくエネルギー移動が起こっていることを見いだしている。

上記内容はＭｂＣＯおよびＨｂＣＯの光解離と再結合過程をｎｓからｍｓの時間領域にわたる時間分解共鳴ラマンスペクトルを用いて測定し、クローズ状態の再結合過程はオープン状態を経由しないこと、ならびにＦｅ-ＣＯ伸縮振動の波数はヘム近傍のアミノ酸の疎水性に依存していることを明らかにしており、理学博士論文として十分値するものと判断される。