氏　　名	李　　江
学位（専攻分野）	博士（理学）
学 位 記 番 号	総研大甲第1014号
学位授与の日付	平成１８年９月２９日
学位授与の要件	先導科学研究科　光科学専攻 学位規則第６条第１項該当
学 位 論 文 題 目	Resonance Raman Studies on Reaction Mechanism of Photolyases: Structural Characteristics of the Active Site and Photo-repair Mechanism of UV-damaged DNA
論 文 審 査 委 員	主　査　教授　渡辺　正勝 教授　蟻川　謙太郎 特任教授　猿倉　信彦 特任教授　北川　禎三 教授　西野　武士（日本医科大学）

論文内容の要旨

Ultraviolet light radiation in the wavelength range between 260 and 320 nm causes damage to DNA by forming the dimerization of adjacent pyrimidines in the same DNA strand. This covalently linked dimer influences the replication and transcription, and leads to cell death or skin cancer. Most (70-80%) of UV-induced DNA lesions is cyclobutane pyrimidine dimer (CPD) and, a lesser extent (20-30%) is (6-4) photoproduct. These two major types of UV-damaged DNA are repaired under illumination with near-UV/visible light by CPD photolyase and (6-4) photolyase, respectively. The both photolyases contain flavin adenine dinucleotide (FAD) as an essential cofactor for DNA repairing. The structure and catalytic mechanism of CPD photolyase have been extensively studied. However, its detailed structure of the active site containing CPD is uncertain until now. The structure of (6-4) photolyase is less well studied. The genes for this enzyme exhibit a sequence similarity to CPD photolyase, especially in the FAD binding sites. Such high similarity indicates a similar structure and reaction mechanism in two photolyases. Unexpectedly, (6-4) photolyase presents a much lower quantum yield compared to that of CPD photolyase, indicating the differences in structure and reaction mechanism between (6-4) and CPD photolyase. Such precise differences between CPD photolyase and (6–4) photolyase regarding substrate binding and DNA repair needs to be clarified.

To investigate the unclear structure and environment of the active site in (6-4) photolyase, we measured resonance Raman spectra of (6-4) photolyase having neutral semiquinoid and oxidized forms of FAD, which were selectively intensity enhanced by excitations at 568.2 and 488.0 nm, respectively. DFT calculations were carried out for the first time on the neutral semiquinone. The marker band of a neutral semiquinone at 1606 cm^{-1} in H_2O, whose frequency is the lowest among various flavoenzymes, apparently splits into two comparable bands at 1594 and 1608 cm^{-1} in D_2O, and similarly that at 1522 cm^{-1} in H_2O does into three bands at 1456, 1508, and 1536 cm^{-1} in D_2O. This D_2O effect was recognized only after being oxidized once and photoreduced to form a semiquinone again, but not by simple H/D exchange of solvent. Some Raman bands of the oxidized form were observed at significantly low frequencies (1621, 1576 cm^{-1}) and with band splittings (1508/1493, 1346/1320 cm^{-1}). These Raman spectral characteristics indicate strong H-bonding interactions (at N5-H, N1), a fairly hydrophobic environment, and an electron-lacking feature in benzene ring of the FAD cofactor, which seems to specifically control the reactivity of (6-4) photolyase.

To clarify the structure of active site upon substrate binding and the mechanism of DNA repair, we examined the resonance Raman spectra of complexes between damaged DNA and the neutral semiquinoid and oxidized forms of (6-4) and CPD photolyases. The marker band for a neutral semiquinoid flavin and band I of the oxidized flavin, which are derived from the vibrations of the benzene ring of flavin adenine dinucleotide (FAD), were shifted to lower frequencies upon binding of damaged DNA by CPD photolyase but not by (6-4) photolyase, indicating that CPD interacts with the benzene ring of FAD directly but that (6-4) photoproduct does not. Bands II and VII of the oxidized flavin and the 1398/1391 cm^{-1} bands of the neutral semiquinoid flavin, which may reflect the bending of the U-shaped FAD, were altered upon substrate binding, suggesting that CPD and (6-4) photoproduct interact with the adenine ring of FAD. When substrate is bound, there is an upshifted 1528 cm^{-1} band of the neutral semiquinoid flavin in CPD photolyase, indicating a weakened hydrogen bond at N5-H of

FAD, and in (6-4) photolyase, band X is downshifted, indicating a strengthened hydrogen bond at N3-H of FAD. These Raman spectra led us to conclude that the two photolyases have different electron transfer mechanisms as well as different hydrogen bonding environments, which account for the higher redox potential of CPD photolyase.

This work revealed that the FAD in (6-4) photolyase is characterized by an electron localized structure, and binds to the protein in a fairly hydrophobic and strong hydrogen bonding environment. Specially, a stronger H-bonding at N5-H of FAD was identified for (6-4) photolyase, which may result in the low quantum yield for DNA-repair of this enzyme. Besides, UV-damaged DNA contacts the benzene ring of FAD only in CPD photolyase and the adenine ring of FAD in both photolyases. These structures indicate that the electron transfer during DNA-repair between isoalloxazine and UV-damaged DNA in CPD photolyase is direct, whereas that in (6-4) photolyase is not direct and bridged by adenine.

論文の審査結果の要旨

本論文は2種のDNA光修復酵素の構造と機能の関係を可視光共鳴ラマン分光と密度汎関数法を用いた理論計算で調べた結果で、英文104ページ4章から成るものである。

本論文の新しい結果は、第2章の(6-4)Photolyase中のフラビンラジカルの共鳴ラマンバンドの帰属等を含む物理化学的研究（J. Phys. Chem.に掲載）と、第3章のCPD Photolyaseと(6-4)Photolyaseのフラビン環境の違いと機能的性質の違いを関係づけようとした生化学的な研究（J. Biol. Chem. に掲載）である。

第1章は光損傷DNAの構造と生物に用意された修復システム、その蛋白を共鳴ラマン分光で調べたこれまでの研究、及びこれから更にラマン分光で研究する意義についての背景を記述したものである。光損傷DNAには主としてCyclobutadine Pyrimidine Dimer(CPD)を含むものと、(6-4)光産物と呼ばれるPyrimidine dimerを含むものがあり、各々にCPD Photolyaseと(6-4)Photolyaseが特異的に修復作用をするが、後者の量子収率が前者に比して極端に低い。両酵素ともFAD型のフラビンを補酵素としてもち、フラビンからの光励起電子移動が修復反応の最初の過程である。両者の反応の違いとフラビンの置かれている環境の違いを説明する事が本論文の主目的である。

第2章は(6-4)Photolyaseに関するもので、大腸菌でこの酵素（遺伝子は植物用来のものを使用）を大量に調製するために発現系に色々工夫した。その結果、ラマン分光に使える高純度の酵素が十分な量得られた。チミン8量体$(dT)_8$に紫外光照射で光損傷DNAモデルを用意し、それを基質として酵素活性を調べた。共鳴ラマン散乱は568.2 nmと488.0 nmで励起した。共鳴ラマン効果により、前者でフラビンラジカル状態の、後者でフラビン酸化形の共鳴ラマンスペクトルが選択的に得られた。ラジカル状態の振動帰属がこれまでにないので、密度汎関数法（DFT）で分子振動を計算し、観測ラマンバンドの振動モードを帰属した。酵素を単にD_2O溶液に溶かした場合と、D_2O中で酸化還元をした場合とでスペクトルに違いのある事に気付き、前者をN_3-D、N_5-H形、後者をN_3-D、N_5-D形と考えると計算結果と合う事から、そのように帰属すると共に、ラジカル状態ではN_5-Hが疎水環境に置かれ非常に強い水素結合を形成している事が本酵素の特徴である事を初めて指摘した。

CPD Photolyaseも大腸菌で発現し、精製した。その共鳴ラマンスペクトルを(6-4)Photolyaseのものと比較すると、基質を結合した時のスペクトルの振舞いに相違が見られた。即ちバンドIというベンゼン環部分に由来する振動がCPD Photolyaseでは基質結合で低波数シフトするが、(6-4)Photolyaseではシフトしなかった。この事から、基質とFADとの直接相互作用がCPD Pholyaseではあり、相互作用点がベンゼン環部分であるが、(6-4)Photolyaseでは直接の相互作用はなく、電子移動がアデニン環を介して起こると考えられ、反応機構のこの違いが量子収率の違いを生み出していると説明された。

このように本論文はDNA光修復酵素のフラビン補酵素の性質や反応性との関係を調べた新規な研究として高く評価され、審査員全員が一致して、合格であると結論した。