as a model protein; one is wild type (WT HO) and the other is a mutant (KDMY HO) which involves replacements of four residues (K16N, D27A, M31K, Y112F). Their X-ray structures indicated that the 4-vinyl of WT HO seems to have the same environments as that of 2-vinyl of KDMY in crystal. The NMR spectra confirmed the 90° rotation of the heme in solution, and their catalytic activities demonstrated the corresponding differences in the α and δ cleavages of the heme. The resonance Raman spectra of WT HO and KDMY HO are alike regarding major heme skeletal modes, whereas some modes involving heme side chains vary in frequencies and behaviors. To characterize such bands, we reconstituted the two proteins with vinyl- or propionate-deuterated hemes and compared their resonance Raman spectra in their oxidized, reduced, and CO-bound forms. The vinyl associated modes exhibited frequency shifts between the two proteins.

3I0936 ヘムオキシゲナーゼにおける CO 光解離後の構造変化および CO の放出経路

Protein motions and CO migration following CO photolysis in heme oxygenase

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Heme oxygenase (HO) catalyzes the physiological degradation of heme using dioxygen and reducing equivalents to produce biliverdin, ferrous ion, and CO. The HO reaction proceeds without severe product inhibition by CO generated in the second step of the HO reaction, even though CO is known to be a potent inhibitor of HO and other heme proteins. Therefore HO must have an efficient mechanism for discriminating between dioxygen and CO.

To explore protein motions and CO migration in HO following CO photolysis which mimics CO generation during the HO reaction, we collected X-ray diffraction data on the CO-heme-HO complex before and after continuous illumination by CW laser at temperatures below 160 K. The weighted difference Fourier map shows that CO was partially photolyzed from the heme iron, and photodissociated CO had migrated to a hydrophobic cavity, as observed in previous results at 35 K. In contrast to previous results, motions of the distal helix, water, and heme were observed which are the reverse of motions observed in CO binding. Notably, similar motions were absent in dioxygen binding and photolysis. These results imply that the CO-bound form is severely constrained by steric hindrance between the distal helix and CO bound to the heme iron, and that steric hindrance contributes to discrimination between binding of CO and dioxygen. Further, another putative CO trapping site, more polar than previous sites, was found. Combining these studies with previous results, we will discuss CO migration pathways in HO.

3I0948 多様な生物種によるヘムオキシゲナーゼ反応の微調整戦略：オキシヘムの安定化

Strategy of a variety of organisms for the fine-tuning of heme oxygenase reactions: Stabilization of the oxyheme intermediate

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Heme oxygenase (HO) catabolizes regioselective heme-ring opening to produce biliverdin, free iron, and carbon monoxide. In a variety of biological species, the HO reactions participate in recycling of iron, signaling, supplying resources of photoreceptive-molecule production, or generating anti-oxidants. To attain their own requirements for HO, each organism seems to take unique fine-tuning strategy. The HO reaction proceeds successively by consuming oxygen molecules and electrons, so that their constant supply is essential. In addition, formation of the first reactive species, hydroperoxy hemin, requires proton as well as electron transfer to the oxyheme. In different species, HO functions in different milieu, thereby regulated finely and differently by respective active sites. Our HO study is focused on such fine-tuning modes taken in different HOs from different species. In this study, stability of oxyheme, the first converted form of hemin in HO, was compared among the five HO complexes: rat HO1, puffer-fish HO1, cyanobacterial HO1, soybean HO1, and pathogenic-bacterial HO. The auto-oxidation rates of the oxyheme complexes showed unexpectedly big difference, which correlate with pKa of the heme-bound water as well as with g-agisotropy of the hydroxide-coordinated heme. Based on the crystal structures of three heme-HO complexes out of the five, very-well conserved Gly residue in the distal helix, closest to the heme iron, is proposed as the HB donor to stabilize the oxyheme in the heme pockets.

3I1010 Ultraviolet Resonance Raman Study on Indoleamine 2, 3-Dioxygenase

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Indoleamine 2, 3-Dioxygenase (IDO) catalyzes incorporation reaction of two oxygen atoms into L-tryptophan (Trp), generating N-formylkynurenine, which is in the major catabolic pathway of Trp in mammals. Visible resonance Raman (RR) studies identified a key intermediate of ferryl-oxo (Fe=O) species. This suggested that two oxygen atoms were transferred to substrate one by one. The Fe=O species seemed to lie in the compound II oxidation level and a question arose whether such an intermediate could perform oxo-transfer reaction, since no example had been reported. Such a reaction might be possible if the substrate intermediate is unusually reactive. In the present study, we have adopted ultraviolet (UV) RR spectroscopy to characterize the substrate bound to IDO, since the technique selectively enhances vibrational spectra of aromatic molecules. UVR scattering was excited at 229 nm for the ferrous state of IDO. UVR spectrum of substrate-free IDO was primarily contributed by Trp and Tyr residues. In the spectrum of the substrate-bound IDO, intensity of the W3 band of Trp was increased. This was ascribed either to bound-Trp or Trp residue in IDO. In the latter case, binding of Trp induced structural change of intrinsic Trp in IDO. In order to distinguish these possibilities, we utilized indole-2-¹³C- and indole-D₅-labeled Trp. The results showed that bound-Trp and not the intrinsic Trp exhibited the intensity change. The present study provides a new method to study the structure of the bound-substrate to IDO.

3I1022 An Intermediate Conformation of Cytochrome Oxidase in the Ligand-Free State Identified by Time-Resolved Resonance Raman Spectroscopy

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Cytochrome *c* oxidase (CcO) reduces dioxygen to water by heme *a*₃ and drives proton pump by heme *a*. In the present study, we applied green-pump and violet-probe time-resolved resonance Raman (TRRR) spectroscopy to monitor the dynamics of hemes after CO-photolysis with a time resolution of 10 ns. The green and violet light were used for CO-photolysis and resonance Raman excitation, respectively. TRRR spectra after CO-photolysis were measured at delay time = 10 ns - 5 ms. The Fe-His stretching mode was located at 221 wavenumbers at delay time between 10 ns and 100 ns, and then decreased exponentially with time with a time constant of 600 ns and at 5 ms reached 215 wavenumbers, which was the same value for equilibrium ligand-free form. This behavior was similar to that reported earlier. In this study, we found that intensity of the same mode decreased to two thirds at 100 ns relative to that at 10 ns and remained constant up to 5 ms, but was significantly smaller than the intensity for equilibrium ligand-free form. Results indicate that an intermediate conformation exists after ligand dissociation at delay time = 0.002 to 5 ms. This conformation might have a significantly smaller affinity to dioxygen compared with the conformation seen at 10 - 100 ns as judged from the Fe-His stretching frequency. The affinity control should be important to adjust the timing of dioxygen binding appropriately relative to the timing of proton loading for an efficient proton pumping.

3I1034 ポンプ・プローブレーザーと同期したパルスフローシステムの開発とそれを用いたタンパク質の時間分解赤外分光解析

Time-Resolved IR Analyses of Proteins Using a Novel Pulse Flow System Synchronized with Pump/Probe Lasers

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IR spectroscopy is a method to analyze structures and reactivities of functional groups, and powerful to reveal protein reaction mechanisms. This spectroscopy, however, has the crucial shortcoming that the application to aqueous solution is practically impossible in most cases because of strong IR absorption of water, which has prevented time-resolved (TR) IR measurements using a flow cell. Recently, we developed a new pump-probe TRIR spectrometer based on the femtosecond IR technology and using a spinning cell. Our spectrometer can detect a 30 μ OD protein signal even in the presence of $\sim$ 2 OD water background in 50 sec of data accumulation, and is promising for flow-flash TRIR measurements. Here, we developed a novel flow system that can be coupled