but show enzymatic activity. These mutants show the ligand-induced folding and are considered to be good models for IUP. In order to examine their induced-folding reaction mechanisms, the kinetics of ligand-induced folding are examined with the stopped-flow CD measurement. These mutants can be categorized into two groups in terms of kinetics. One shows significant ligand concentration effect on both the rate constant and the amplitude of burst phase. The other does not show burst phase and the ligand concentration dependence of rate constant is completely different from the former. By the analysis of rate equations, we concluded that the former obeys the scheme 2, while the latter follows the scheme 1. The requisite for the scheme 1 will be discussed.

2SC0903 A Breathing Raman Band Reveals Heme-Globin Interaction

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Electronic states of an iron in hemoproteins are controlled by a ligand field of both an equatorial porphyrin ligand and axial ligands. Enzymic reactions are accomplished on the heme iron but marvelous performance cannot be fully understood without taking account of an additive field to the iron porphyrin, "Protein field". However, it is difficult to elucidate the interaction because of small quantities.

To evaluate subtle interaction in hemoprotein we focused on the strongest Raman band, ν_7 , which is a breathing vibrational mode, in metalloporphyrin resonance Raman (rR) spectra excited near resonance with Soret band. Frequency shift by 5 cm^{-1} is observed for an artificial iron porphyrin (DMDP) and the ^{13}C -DMDP (a methin carbon substituted with ^{13}C) itself, which is called $(\Delta\nu_7)_{\text{intrinsic}}$ and $\Delta\nu_7$ becomes small when the iron porphyrins are incorporated into apo-myoglobin or apo-HRP. $\Delta\nu_7$ becomes even smaller when a certain axial ligand is coordinated in the reconstituted hemoproteins. Since the methin carbon (^{13}C and ^{12}C) is fixed on the opposite side to propionates in the porphyrin, it turns out that a subtle interaction in the interior of the heme pocket increases differences of the frequency shifts, $(\Delta\nu_7)_{\text{intrinsic}} - \Delta\nu_7$. The Raman results are interpreted by the aid of vibrational mode analyses based on DFT calculations.

2SC0913 共鳴ラマン分光法から観たインドールアミンジオキシゲナーゼの酵素/基質/酸素三者複合体における基質-酸素相互作用 Interaction between Substrate and Dioxygen in the Ternary Complex of Indoleamine Dioxygenase as Probed with Resonance Raman Spectroscopy

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Indoleamine dioxygenase (IDO) is a heme enzyme and catalyzes the first reaction in tryptophan (Trp) catabolic pathway in mammals. It incorporates two oxygen atoms from dioxygen into L-Trp and generates N-formylkynurenine. Recent resonance Raman (RR) studies revealed the two reaction intermediates, namely, the oxygenated $(\text{Fe(III)}-\text{O}_2^-)$ and the ferryl-oxo $(\text{Fe(IV)}=\text{O}^{2-})$ intermediates. The presence of the $\text{Fe(IV)}=\text{O}$ heme was unexpected. In the present study, we have examined the interaction between the heme-bound O_2 or CO with Trp, which is bound in the heme pocket, through observation of the vibrational signature of O_2 ($\nu(\text{OO})$ and $\nu(\text{Fe}-\text{O}_2)$) or of CO ($\nu(\text{CO})$, $\nu(\text{Fe}-\text{CO})$ and $\delta(\text{FeCO})$). The oxygenated intermediate of substrate-free IDO exhibited only the $\nu(\text{Fe}-\text{O}_2)$ mode at 569 cm^{-1} , while that of L-Trp-bound IDO exhibited both of the $\nu(\text{Fe}-\text{O}_2)$ mode at 564 cm^{-1} and the $\nu(\text{OO})$ mode at 1138 cm^{-1} . The activation of the RR band of the $\nu(\text{OO})$ mode for the latter case is an indication of a specific interaction between O_2 and L-Trp. On the other hand, CO-bound IDO in the presence of L-Trp, D-Trp and no Trp all exhibited the $\nu(\text{CO})$, the $\nu(\text{Fe}-\text{CO})$ and the $\delta(\text{FeCO})$ modes, but the binding geometries of CO were distinct among three species as judged from the significant difference in the vibrational frequencies. The difference in interaction of the substrate and the ligand will be discussed based on the RR data.

2SC0933 ヘムシャペロン蛋白質のヘム輸送機構 Heme transfer mechanism of heme chaperone protein

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c-type cytochromes are electron transfer proteins that are essential for the life of almost all organisms. They characteristically have covalently-bound heme *via* thioether bonds to two cysteines in the protein. In Gram-negative bacteria, biogenesis of c-type cytochrome is conducted by a multiprotein complex system known as a cytochrome *c* maturation (Ccm) system. This system consists of 8 gene products (*ccmA-ccmH*). CcmE, which binds heme and delivers it to apocytochrome *c* (apo Cyt *c*), is called as a heme chaperone. To clarify the heme chaperone mechanism, spectroscopic properties of CcmE were investigated.

It is known that heme is non-covalently bound to CcmE (*b*-type CcmE), and

then, a covalent bond is formed between heme and CcmE, which is called as holo CcmE. Resonance Raman spectroscopy showed that *b*-type CcmE is bound heme through His130 and His147, but one of His is replaced with Tyr134 in holo CcmE. Although Holo CcmE has been considered to be essential for heme transfer to apo Cyt *c*, analysis of fluorescence quenching indicates that *b*-type CcmE, not holo CcmE, interacts with apo Cyt *c*. When *b*-type CcmE reacted with apo Cyt *c*, Soret band diminished gradually, but it was much slower than that observed when holo CcmE was reacted with apo Cyt *c*. This suggests that heme in *b*-type CcmE is buried though the complex formation with apo Cyt *c*, which repressed the degradation of heme by the reducing reagent. We propose a new heme chaperone mechanism that *b*-type heme can transfer heme to apo Cyt *c* before it is converted to holo CcmE.

2SC0953 原子レベル構造で考えるシトクロム *c'* の NO 結合機構 Understanding of Nitric Oxide-Binding Mechanism of Cytochrome *c'* Based on Atomic Resolution Structures

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Cytochrome *c'* (cyt *c'*) are found in the periplasmic space of a number of photosynthetic, nitrogen-fixing and denitrifying bacteria. In denitrifiers, they have been proposed to have a role in mediating nitric oxide (NO) transfer and protecting the organism from the potentially toxic levels of NO. The ligand-binding property by this protein is anomalous when compared to other hemoproteins except soluble guanylate cyclase (sGC) of mammal. Interestingly, NO forms 5-coordinate heme adducts with both proteins. In the previously reported structure of NO-bound cyt *c'*, NO disrupts His-Fe bond and binds to the proximal face of the heme, giving a 5-coordinate species. However, we thought that this structure had some problems. The electron density map close to the heme iron had "Y" shape, which is likely to be corresponding to a molecule containing three non-hydrogen atoms. The authors interpreted that NO was binding in two alternative bent conformations, each with half occupancy. This model has been believed and accepted for last decade. However, there was no evidence that the electron density was from NO.

Recently, we independently determined crystal structures for NO-bound cyt *c'* in addition to the ferric, the ferrous and the CO-bound forms at higher resolution ($\sim 0.92\text{ \AA}$). Furthermore, we collected the single crystal absorption spectra to ensure what was the state of the iron center in these crystals. In this conference, we will report new structural aspects and NO-binding mechanism of cyt *c'* based on our atomic resolution structures.

2SC1013 Theoretical analysis for structures and reaction mechanisms of atypical cytochrome P450

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Thromboxane synthase (TXAS) and prostacyclin synthase (PGIS), members of cytochrome P450 super family, catalyze isomerization reactions from prostaglandin H_2 (PGH_2) to thromboxane A_2 (TXA_2) and prostacyclin (PGI_2), respectively. TXA_2 and PGI_2 play a key role in cardiovascular and pulmonary diseases. The details of the invisible isomerization processes of TXA_2 and PGI_2 biosyntheses remain obscure due to the experimental difficulties. In order to clarify the unusual catalytic role, we have investigated model reaction mechanisms for the isomerization from PGH_2 to TXA_2 and PGI_2 catalyzed by cytochrome P450 using the unrestricted B3LYP density functional level of theory. Based on our results, we propose new reaction mechanisms of TXA_2 and PGI_2 biosyntheses. Our results show that an iron-containing catalyst is essential to facilitate TXA_2 and PGI_2 formations, and suggest a possibility that the reactions proceed not only through a Fe(IV) -alkoxy radical intermediate but also through a Fe(III) -porphyrin π -cation radical intermediate, which had not been considered in studies of TXA_2 and PGI_2 biosyntheses in the past. The one-electron transfers coupled with proton transfer and C-O bond formation were observed in both the PGI_2 and TXA_2 biosyntheses, respectively. Finally, we will present results by MD simulation of the PGH_2 -human PGIS complex.

2SC1033 金属タンパク質における X 線損傷 Radiation Damage in Redox Proteins

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Manganese complexes play critical roles in the metabolism of oxygen that is important in many biological systems. Among these systems, one of the most important is the photo-induced oxidation of water to dioxygen by the photosynthetic protein complex, Photosystem II (PS II). This reaction is catalyzed by a Mn_4Ca cluster located in the PS II complex. Single crystals of PS II isolated from thermophile have been studied by X-ray diffraction up to a resolution of $2.9\text{ \AA}$ (1). However, the limited resolution is short of what is