by over 10,000-fold, suggesting significant stabilization of the bound waters. X-ray structure of the Leu-mutant at 1.6 Å resolution revealed that Leu side chain blocks water passage through the gate in either substrate-free or -bound forms. These results support our proposal. Here, Asp297 was mutated to less bulky Ala to produce a space corresponding to that would be generated by conformational change of Asp297 side chain occurring during the water exclusion. This mutation did not change significantly the camphor binding affinity, indicating normal water exclusion. X-ray structure of the Ala-mutant at 1.6 Å resolution demonstrated that Phe87, contacting with Asp297 and Tyr96 in the wild type protein, changes its side chain conformation in a way to generate a space for camphor passage. Bound camphor forms a hydrogen bond with Tyr96. The present results suggest that conformational change of Phe87 to allow camphor passage induces that of Asp297, which facilitates the gate opening.

3P-077 インドールアミン 2、3 ジオキシゲナーゼの酸素同位体感受性共鳴ラマンバンドの検出

Detection of Two Oxygen-Isotope Sensitive Resonance Raman Bands for Human Indoleamine 2,3-Dioxygenase

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Indoleamine 2,3-dioxygenase (IDO), found in mammals, catalyzes direct incorporation of two oxygen atoms into L-tryptophan in kynurenine pathway in which this reaction is the initial and the rate-limiting step. IDO is a soluble enzyme containing a heme B as its reaction center. Sugimoto *et al.* discussed the mechanism of dioxygenase in terms of the crystal structure of recombinant human IDO (rhIDO) (1). No structural information about the catalytic intermediate of any IDO has been reported. Resonance Raman (RR) spectroscopy is one of the most powerful tools to study the structure of the active site of heme containing enzymes. The RR spectra of rhIDO with and without substrate have been reported (2) yet in the static forms. Here, we report the RR spectra of rhIDO in oxygenated and oxo forms. The RR spectrum of oxygenated rhIDO exhibits the oxidation state marker band (ν_4) at 1375 cm^{-1} which is higher compared to that of the ferric form at 1371 cm^{-1} . The Fe-O stretching mode for the oxygenated rhIDO is found at 569 cm^{-1} in $^{16}\text{O}_2$ -bound form, which has been confirmed by an isotopic shift to 541 cm^{-1} in $^{18}\text{O}_2$ -bound form at pH 8.0. When 2 mM L-tryptophan is added to 10 μM of oxygenated rhIDO, the ν_4 band is found at 1375 cm^{-1} , whereas the Fe-O stretching mode is found at 798 cm^{-1} for $^{16}\text{O}_2$ -rhIDO, shifting to at 762 cm^{-1} for $^{18}\text{O}_2$ -rhIDO. According to the region of the oxygen-isotope sensitive RR band, the state of the heme can be concluded to an oxo form. This species has absorption maxima at 410, 544, 577 and 593 nm compared to those found in oxygenated rhIDO at 413, 542 and 577 nm.

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3P-078 結晶中の完全酸化型ウシ心筋チトクロム酸化酵素の酸素還元中心の解析

Characterization of the O_2 reduction site of crystalline fully oxidized bovine heart cytochrome *c* oxidase.

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It has been well established that bovine heart cytochrome *c* oxidase shows two types the fully oxidized forms under nonturnover conditions, namely, fast and slow forms, characterized by cyanide binding rate and the Soret band position and that the fast form is enzymatically active. The physiological significance of the slow form is still not clear. Bovine heart cytochrome *c* oxidase purified with our procedure for preparing crystals for X-ray diffraction experiments shows the Soret band at 423 nm and reacts with cyanide showing the half life time of the order of one second, indicating clearly that our enzyme preparation is in the fast form. However, the crystalline enzyme showed two peaks at 428 nm and 411 nm. Both band positions are clearly different from those of the slow and fast forms. The Soret band splitting are likely to be induced by strong protein-protein contacts in the crystal lattice which stabilize part of the multiple conformers of the enzyme in the solution or by the anisotropy in relative positions of the two hemes in the crystals.

In order to examine the possibilities, the crystals were dissolved in a neutral buffer containing decylmaltoside, to give its Soret band at 417 nm which is close to the Soret band position of the slow form. The Soret band gradually returned towards the position of the Soret band before crystallization. The results suggest that the dissolved enzyme preparation is not in the slow form. Or, the species with its Soret band at 417 nm seems to be an enzyme species which transiently appears. Extensive examinations of factors affecting the absorption spectral changes after dissolving the crystals are under way, in order for characterization of the O_2 reduction site of the bovine cytochrome *c* oxidase in the crystal lattice.

3P-079 化学修飾ヘモグロビン酸素結合機能の蛋白濃度依存性

Tetramer-dimer equilibrium of chemically modified hemoglobin as studied by protein concentration-dependences of the oxygen equilibrium curve

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Vertebrate hemoglobin is in an equilibrium between the $\alpha_2\beta_2$ tetramer and the $\alpha\beta$ dimers. This equilibrium is shifted toward dimers upon dilution of the Hb solution and also upon oxygenation when the several intersubunit salt-bridges formed in deoxyHb are ruptured. The chemical modification of the sulfhydryl group of cystein-93(F9) β with *N*-ethylmaleimide (NEM) causes the breakage of the salt-bridge in deoxyHb between the C-terminal histidine imidazole group and the γ -carboxyl group of aspartate-94(FG4) of the same β chain. This breakage will, in turn, reduce the stability of another salt-bridge between the β chain C-terminal carboxyl group and the ε -amino group of lysine-40(C5) of the α chain of the $\alpha_1\beta_2$ pair. Thus, it is expected that this modification will enhance the dissociation of Hb tetramer into dimers. In the present study, we measured oxygen equilibrium curves for native Hb and NEM-treated Hb in a wide range of protein concentration (1 to 1000 μM on a heme basis) and analyzed them to estimate the tetramer-to-dimer dissociation constants. The results indicated that the modification with NEM does not enhance the dissociation into dimers, contrary to the expectation.

3P-080 シトクロム *c* におけるシトクロム *c* 酸化酵素との電子伝達反応に重要なアミノ酸残基の検討

Identification of amino acid residues responsible for the electron transfer reaction with cytochrome *c* oxidase in cytochrome *c*.

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Cytochrome *c* (Cyt *c*), one of the heme proteins in the mitochondrial respiratory chain, transfers electrons between cytochrome *b*₁ and cytochrome *c* oxidase (CcO). While the electron transfer (ET) to CcO is the crucial step for the four-electron reduction of molecular oxygen to water molecules, the detailed regulation mechanism for the ET reaction has not yet been fully understood. Recently, we have measured the ^1H - ^{15}N HSQC spectra for Cyt *c* in the absence and presence of CcO, showing that some Lys and Ile residues are involved in the binding site of Cyt *c* with CcO and suggesting that the specific electrostatic and hydrophobic interactions play essential roles in the formation of the ET complex. To get further insights into the interactions between Cyt *c* and CcO, we focused on some Lys and Ile residues located on the proposed binding site and prepared four Lys mutants (K7,8L, K13L, K79L, K86,87L) and three Ile mutants (I9A, I11A, I81A). Michaelis-Menten analysis for the ET reactions from Cyt *c* to CcO revealed that all of the Lys mutants have large K_M (1.9 - 10.9 μM), compared to wild-type Cyt *c* (0.92 μM) and the negative control mutant K39L (0.57 μM), while the mutations of Ile resulted in the minor perturbation of K_M . The prominent increases in K_M for K13L (10.9 μM) and K86,87L (5.6 μM) clearly indicate that these Lys residues are key residues for the ET complex formation and the electrostatic interactions mediated by these Lys residues would fix the relative positions of heme and the electron entry site of CcO to facilitate the ET reaction from Cyt *c* to CcO.

3P-081 コンピュータシミュレーションによる ABC トランスポーター・Sav1866 の薬剤排出機構の解明

Computer simulation reveals the drug transport mechanism of ABC transporter Sav1866

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The ATP-binding cassette (ABC) transporters are integral membrane proteins which couple the energy of ATP hydrolysis with the translocation of chemically diverse substrates across the lipid bilayers of cellular membranes. ABC transporters are generally classed as either exporters or importers. For example, human ABC exporters, such as ABCB1 and ABCC1, contribute to multidrug resistance of cancer cells through the extrusion of cytotoxic compounds. Despite the various studies, however, a detailed transport system has remained elusive. Sav1866, a *S. aureus* homolog of human ABCB1, is one of the structure-determined ABC transporters. In this study, we applied atomic-level MD simulation and anisotropic network model (ANM) calculation to Sav1866 in order to understand its drug transport mechanism. From the results of ANM calculation, we found that Sav1866 potentially has some characteristic motions including an opening/closing motion in the transmembrane domain that could form a pathway for transporting a trapped drug. The principal component analysis for the MD trajectories also showed that such a motion is a dominant one in the ATP-