II-G Biomolecular Science

Elucidation of a structure-function relationship of metalloproteins is a current subject of this group and for this purpose we treat proteins and model compounds of their active sites. The primary technique used for this project is the stationary and time-resolved resonance Raman spectroscopy excited by visible and UV lasers. The main themes that we want to explore are (1) mechanism of oxygen activation by enzymes, (2) mechanism of active proton translocation and its coupling with electron transfer, (3) coupling mechanism of proton- and electron transfers by quinones in photosynthetic reaction center, (4) higher order protein structures and their dynamics, and (5) reactions of biological NO. In category (1), we have examined a variety of terminal oxidases, cytochrome P450s, and peroxidases, and also treated their enzymatic reaction intermediates by using the mixed flow transient Raman apparatus and the Raman/absorption simultaneous measurement device. For (2) the third generation UV resonance Raman (UVRR) spectrometer was constructed and we are going to use it to the peroxy and ferryl intermediates of cytochrome *c* oxidase and cytochrome *bo*. In (3) we succeeded in observing RR spectra of quinones A and B in bacterial photosynthetic reaction centers for the first time, but we have focused our attention on detecting tyrosine radical for the P intermediate of terminal oxidases. Some positive evidence was obtained for cytochrome *bo*. For (4) we developed a novel technique for UV resonance Raman measurements based on the combination of the first/second order dispersions of gratings and applied it successfully to 235-nm excited RR spectra of several proteins including mutant hemoglobins and myoglobins. Nowadays we can carry out time-resolved UVRR experiments with nanosecond resolution to discuss protein dynamics. With the newly developed third generation UV Raman spectrometer, we have succeeded in isolating the spectrum of tyrosinate in ferric Hb M Iwate, which was protonated in the ferrous state, and that of the deprotonated state of Tyr244 of bovine cytochrome *c* oxidase. For (5) we purified soluble guanylate cyclase from bovine lung and observed its RR spectra. To further investigate it, we are developing an expression system of this protein.

II-G-1 Resonance Raman Investigation of Fe–N–O Structure of Nitrosylheme in Myoglobin and Its Mutants

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[*J. Phys. Chem. B* **103**, 7044 (1999)]

Resonance Raman spectra have been observed for NO adducts of wild-type (WT) sperm whale myoglobin (MbNO) and its H64G, H64L, L29W, V68W, and V68T mutants at neutral and acidic pH. Raman excitation in resonance with the Soret band enabled us to detect the Fe–NO stretching ($\nu_{Fe–NO}$), N–O stretching (ν_{NO}), and Fe–N–O bending (δ_{FeNO}) bands. The $\nu_{Fe–NO}$, δ_{FeNO}, and ν_{NO} bands of WT MbNO at neutral pH were observed at 560, 452, and 1613 cm^{-1}, respectively, and substitution of the distal His64 to Gly or Leu caused an upshift of ν_{NO} to 1631–1635 cm^{-1} but no change in $\nu_{Fe–NO}$. This change in ν_{NO} is considered to be due to the removal of hydrogen bonding between His64 and bound NO. Conversely, substitution of Leu29 with tryptophan (L29W) altered Fe–NO but caused no change in ν_{NO} at neutral pH. This feature resembles that of MbO_2 but distinctly differs from that of MbCO, for which the Fe–CO and C–O stretching frequencies have an inverse linear correlation. The change in $\nu_{Fe–NO}$ for L29W-MbNO is probably caused by tilting of the Fe–N bond from the heme normal on account of steric hindrance from the large indole ring but would not be due to changes in the Fe–N–O bond angle. When pH is lowered to 4, MbNO adopts the five-coordinate structure due to cleavage of the Fe-His bond. Accordingly, the heme maker bands such as ν_3 and ν_{10}, shifted from 1500 and 1636 cm^{-1} at pH 7.4 to 1509 and 1646 cm^{-1} at pH 4 which are in agreement with those of a five-coordinate Fe-protoporphyrin-NO complex in detergent micelles at neutral pH. The $\nu_{Fe–NO}$ and ν_{NO} bands of acidic MbNO were observed at 520 and 1668 cm^{-1} and exhibited no shift when the distal His was replaced by Gly or Leu. The latter observation supports previous X-ray crystallographic, infrared, and resonance Raman studies which show that the distal histidine becomes protonated at pH 4 and swings out into the solvent away from the bound ligand.

II-G-2 Novel Iron Porphyrin-Alkanethiolate Complex with Intramolecular NH···S Hydrogen Bond: Synthesis, Spectroscopy, and Reactivity

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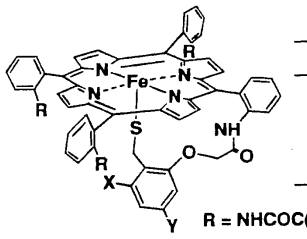
[*J. Am. Chem. Soc.* **121**, 11571 (1999)]

Among heme enzymes, cytochrome P450 and NO synthase (NOS) have strong oxidizing ability and unusual structure, in that their heme irons have thiolate coordination. We report here a novel iron porphyrin-alkanethiolate complex with an intramolecular NH···S hydrogen bond that we synthesized in order to examine the influence of the NH···S hydrogen bond on catalytic oxidation. Complex 1 (see Figure 1) was designed to form an NH···S hydrogen bond by introducing amide NH in the vicinity of the thiolate, while complexes 2 and 3 were designed not to form an NH···S hydrogen bond by replacing amide NH with N-methyl or by introducing acetamide in a position apart from the sulfur atom.

Complexes 1-3 were characterized by FAB MS, IR, EPR, electronic absorption spectroscopy, resonance Raman spectroscopy, and X-ray crystal structure

analysis. The absorption spectra of the ferrous-CO complexes of 1-3 exhibited typical hyperporphyrin spectra for a thiolate-ligated iron(II) porphyrin-CO complex. The Soret band of the ferrous-CO complex of 1 (456 nm, which arises from a transition between the lone pair p orbital of the thiolate and the e_g orbital of heme) was considerably blue-shifted compared to that of the other complexes, indicating electron deficiency of thiolate in complex 1 arising from the NH···S hydrogen bond.



	X	Y
1	-NHCOCH$_3$	H
2	-N(CH$_3$)COCH$_3$	H
3	H	-NHCOCH$_3$
SR	H	H

Figure 1. Structures of complexes 1–3 and **SR**.

II-G-3 Mechanism of the Anionic Cyclopolymerization of Bis(dimethylvinylsilyl)methane

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[*Macromolecules* **32**, 1362 (1999)]

The driving force of the complete cyclization in the anionic cyclopolymerization of bis(dimethylvinylsilyl)-methane with *n*-BuLi/TMEDA in hexane is clarified with resonance Raman and ^{1}H NMR measurements. Vinyl groups coordinating to the lithium cation are detected in both measurements of the polymerization mixture at –70 °C, and they, at least some part of them, are shown to be the vinyl groups in uncyclized end units. Disappearance of these species from the resonance Raman spectrum at –20 °C indicates that the cyclization proceeds fast and is accelerated by the coordination of the second vinyl group in the uncyclized end unit. This is the first case that the interaction between the vinyl group in an uncyclized end unit and the counterion was found in ionic cyclopolymenzation.

II-G-4 Synthesis and Characterization of Novel Alkylperoxo Mononuclear Iron(III) Complexes with a Tripod: Pyridylamine Ligand: A Model for Peroxo Intermediates in Reactions Catalyzed by Non-Heme Iron Enzymes

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[*Inorg. Chem.* **38**, 3592 (1999)]

Previously, by the use of a tripodal pyridylamine ligand, tris-(6-neopentylanaino-2-pyridylmethyl)amine (TNPA), we first succeeded in the preparation of [Fe(tnpa)(OH)(PhCOO)]ClO_4 as a model complex for an active form of soybean lipoxygenase-1, in which stable formation of the hydroxo-iron(III) complex was accomplished by intramolecular hydrogen bonds. We also achieved the isolation of [Cu(bppa)(OOH)]ClO_4 having Cu(II)-OOH species by employing a similar tripodal pyridylamine ligand, bis(6-pivalamido-2-pyridylmethyl)(2-pyridylmethyl)amine (BP-PA). Here, in order to understand the coordination environment of peroxo intermediates in reactions catalyzed by non-heme iron enzymes, we have tried to synthesize stable alkylperoxo mono-nuclear iron(III) complexes using BPPA ligand and to examaine the physicochemical properties.

The resonance Raman spectra of a MeCN solution containing the complex **2** which were measured at room temperature by using 600 nm laser excitation revealed strong resonance-enhanced Raman features at 873, 838, 629, and 469 cm^{-1}, while that of **3** exhibited the features at 878, 838, 639, 548, and 493 cm^{-1}. The Raman features normally observed at ca. 800 cm^{-1} are in the range characteristic of ν(O–O) vibrations of peroxide species and are insensitive upon the addition of $H_2{}^{18}O$. Since these vibrational data were in agreement with those observed for the terminal η^1-alkylperoxo species obtained from the reaction of Fe(II)–(6–Me_3TPA) complexes with alkylperoxides, we deduced that the alkylperoxo moiety is retained on the iron(III) ion in an end-on fashion and the intense absorption bands near 585 and 613 nm for complexes **2** and **3**, respectively, are thus assignable to the alkylperoxo-to-iron(III) charge transfer transition.

II-G-5 Interactions of Phosphatidylinositol 3-Kinase Src Homology 3 Domain with Its Ligand Peptide Studied by Absorption, Circular Dichroism, and UV Resonance Raman Spectroscopies

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[*Biopolymers (Biospectroscopy)* **57**, 208 (2000)]

Absorption, circular dichroism (CD), and UV resonance Raman (UVRR) spectroscopies were applied to selectively examine the environmental and structural changes of Trp and Tyr residues in the phosphatidylinositol 3-kinase (PI3K) SH3 domain induced by ligand association. Comparison of the spectra of PI3K SH3 in the presence or absence of its ligand peptide RLP1 (RKLPPRPSK) indicated that RLP1 binding changed the environment of Trp55 of the SH3 to be more hydrophilic and its H bonding weaker and that of Tyr residues to be more hydrophobic. The D21N mutant (Asp21 → Asn) of the SH3 yielded a UV CD distinct from that of the wild type, and its spectral changes induced by RLP1 binding were smaller and different from those of the wild type in absorption, CD, and UVRR spectra, suggesting that the mutation of conserved Asp21 affected the conformation of the

ligand binding cleft and thus might lead to the decrease in the ligand affinity. These data provide direct evidence for the occurrence of environmental and structural changes of PI3K SH3 by the association of a ligand and the D21N mutation.

II-G-6 Resonance Raman Studies of Oxo Intermediates in the Reaction of Pulsed Cytochrome *bo* with Hydrogen Peroxide

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[*Biochemistry* **39**, 6669 (2000)]

Cytochrome *bo* from *Escherichia coli*, a member of the heme-copper terminal oxidase superfamily, physiologically catalyzes reduction of O_2 by quinols and simultaneously translocates protons across the cytoplasmic membrane. The reaction of its ferric pulsed form with hydrogen peroxide was investigated with steady-state resonance Raman spectroscopy using a home-made microcirculating system. Three oxygen-isotope-sensitive Raman bands were observed at 805/X, 783/753, and (767)/730 cm^{-1} for intermediates derived from $H_2{}^{16}O_2/H_2{}^{18}O_2$. The experiments using $H_2{}^{16}O^{18}O$ yielded no new bands, indicating that all the bands arose from the Fe=O stretching ($\nu_{Fe=O}$) mode. Among them, the intensity of the 805/X cm^{-1} pair increased at higher pH and the species giving rise to this band seemed to correspond to the P intermediate of bovine cytochrome *c* oxidase (CcO) on the basis of the reported fact that the P intermediate of cytochrome *bo* appeared prior to the formation of the F species at higher pH. For this intermediate a Raman band assignable to the C–O stretching mode of a tyrosyl radical was deduced at 1489 cm^{-1} from difference spectra. This suggests that the P intermediate of cytochrome *bo* contains an Fe^{IV}=O heme and a tyrosyl radical like compound I of prostaglandin H synthase. The 783/753-cm^{-1} pair, which was dominant at neutral pH and close to the $\nu_{Fe=O}$ frequency of the oxoferryl intermediate of CcO, presumably arises from the F intermediate. On the contrary, the (767)/730-cm^{-1} species has no counterpart in CcO. Its presence may support the branched reaction scheme proposed previously for O_2 reduction by cytochrome *bo*.

II-G-7 A New Measurement System for UV Resonance Raman Spectra of Large Proteins and Its Application to Cytochrome *c* Oxidase

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[*J. Phys. Chem. B* **104**, 10765 (2000)]

A new type of ultraviolet resonance Raman (UVRR) measurement system suitable to a limited amount of large protein samples is proposed and the results from its application to bovine cytochrome *c* oxidase (CcO) is presented. To minimize the sample damage caused by high-flux UV laser illumination and to reject visible fluorescence from the sample, frequency-doubling of a mode-locked Ar^+ ion laser and a solar blind multi-channel detector were employed, respectively. A new spinning cell was designed so that the sample solution could be stirred during spinning of the cell. Combination of all these devices resulted in successful observation of high quality UVRR spectra of CcO excited at 244 nm. The RR bands of tryptophan- and tyrosine residues dominated the observed spectra, while an extra band appeared at 1656 cm^{-1}. The frequency of the extra band as well as those of all other bands were unaltered by the redox change of metal centers and ligand binding to heme a_3. Deprotonation of a tyrosine residue(s) with a low pK_a value was detected for the resting state at pH 9.1. Examination of all possible assignments led us to conclude that the extra band arose from the linoleoyl side chain of phospholipids and its intensity suggested the presence of 21 linoleoyl groups per CcO molecule.

II-G-8 An Approach to the O_2 Activating Mononuclear Non-heme Fe Enzymes: Structural Characterization of Fe(II)-Acetato Complex and Formation of Alkylperoxoiron(III) Species with the Highly Hindered Hydrotris(3-tert-butyl-5-isopropyl-1-pyrazolyl)borate

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[*Inorg. Chim. Acta* **297**, 162 (2000)]

Structural characterization of an F(II)-acetato complex and attempts to synthesize mononuclear Fe(III) dioxygen complexes bearing the highly sterically demanding $Tp^{tBu,iPr}$ (= hydrotris(3-tert-butyl-5-isopropyl-1-pyrazolyl)borate) ligand have been investigated. X-ray crystallography reveals that the acetato complex consists of the distorted square pyramidal Fe(II) center as found for the previously reported O_2-reactive Tp^{iPr2} derivative. In contrast to the less hindered Tp^{iPr2}, (= hydrotris(3,5-diiso-propyl-1-pyrazolyl)borate) complexes, oxidative addition of O_2 to the coordinatively unsaturated Fe(II) centers of the acetato and a hydroxo complexes with $Tp^{tBu,iPr}$ has never been observed in any conditions. Reaction of the ferrous hydroxo complex with ROOH (R = H, alkyl) results in the formation of the thermally unstable intermediates. Especially, the Fe(III)-alkylperoxo complex is characterized by UV-Vis, ESR and resonance Raman spectroscopy. The extremely bulky $Tp^{tBu,iPr}$ ligand hinders the approach of the exogenous O_2 molecule to the Fe(II) centers but stabilizes the unstable Fe(III)alkylperoxo intermediate enough to be detected.

II-G-9 Structures of Reaction Intermediates of Bovine Cytochrome *c* Oxidase Probed by Time-Resolved Vibrational Spectroscopy

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[*J. Inorg. Biochem.* **82**, 9 (2000)]

Structures of reaction intermediates of bovine cytochrome *c* oxidase (CcO) in the reactions of its fully reduced form with O_2 and fully oxidized form with H_2O_2 were investigated with time-resolved resonance Raman (RR) and infrared spectroscopy. Six oxygen-associated RR bands were observed for the reaction of CcO with O_2. The isotope shifts for an asymmetrically labeled dioxygen, $^{16}O^{18}O$, has established that the primary intermediate of cytochrome a_3 is an end-on type dioxygen adduct and the subsequent intermediate (P) is an oxoiron species with Fe=O stretch ($\nu_{Fe=O}$) at 804/764 cm^{-1} for $^{16}O_2/^{18}O_2$ derivatives, although it had been long postulated to be a peroxy species. The P intermediate is converted to the F intermediate with $\nu_{Fe=O}$ at 785/751 cm^{-1} and then to a ferric hydroxy species with ν_{Fe-OH} at 450/425 cm^{-1} (443/417 cm^{-1} in D_2O). The rate of reaction from P to F intermediates is significantly slower in D_2O than in H_2O. The reaction of oxidized CcO with H_2O_2 yields the same oxygen isotope-sensitive bands as those of P and F, indicating the identity of intermediates. Time-resolved infrared spectroscopy revealed that deprotonation of carboxylic acid side chain takes place upon deligation of a ligand from heme a_3. UVRR spectrum gave a prominent band due to *cis* C=C stretch of phospholipids tightly bound to purified CcO.

II-G-10 Heme Structure of Hemoglobin M Iwate [α87(F8)His → Tyr]: A UV and Visible Resonance Raman Study

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[*Biochemistry* **39**, 13093 (2000)]

Heme structures of a natural mutant hemoglobin (Hb), Hb M Iwate [α87(F8)His → Tyr], and protonation of its F8-Tyr were examined with the 244-nm excited UV resonance Raman (UVRR) and the 406.7- and 441.6-nm excited visible resonance Raman (RR) spectroscopy. It was clarified from the UVRR bands at 1605 and 1166 cm^{-1} characteristic of tyrosinate that the tyrosine (F8) of the abnormal subunit in Hb M Iwate adopts a deprotonated form. UV Raman bands of other Tyr residues indicated that the protein takes the T quaternary structure even in the *met*-form. Although both hemes of α and β subunits in *met*Hb A takes a six-coordinate (6c) high-spin structure, the 406.7-nm excited RR spectrum of *met*Hb M Iwate indicated that the abnormal α subunit adopts a 5c high-spin structure. The present results and our previous observation of the $\nu_{Fe-O(tyrosine)}$ Raman band (*Biochemistry* **28**, 2418, (1989)) have proved that F8-tyrosinate is covalently bound to Fe(III)-heme in the α subunit of Hb M Iwate. As a result, peripheral groups of porphyrin ring, especially the vinyl and the propionate side chains, were so strongly influenced that the RR spectrum in the low frequency region excited at 406.7 nm is distinctly changed from the normal pattern. When Hb M Iwate was fully reduced, the characteristic UVRR bands of tyrosinate disappeared and the Raman bands of tyrosine at 1620 (Y8a), 1207 (Y7a), and 1177 cm^{-1} (Y9a) increased in intensity. Coordination of distal His(E7) to the Fe(II)-heme in the reduced α subunit of Hb M Iwate was proved by the observation of the ν_{Fe-His} RR band in the 441.6-nm excited RR spectrum at the same frequency as that of its isolated α chain. The effects of the distal-His coordination on the heme appeared as distortion of the peripheral groups of heme. Possible mechanism for the formation of Fe(III)-tyrosinate bond in Hb M Iwate is discussed.

II-G-11 Model Complexes for the Active Form of Galactose Oxidase. Physicochemical Properties of Cu(II)- and Zn(II)-Phenoxyl Radical Complexes

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[*Inorg. Chem.* **39**, 3708 (2000)]

One-electron oxidations of the Cu(II)- and Zn(II)-phenolate complexes of ligand 1H afford relatively stable phenoxyl radical complexes, which exhibit very characteristic UV-NIR features similar to those exhibited by the active forms of the native enzymes. Comparison of the spectroscopic characteristics (UV-vis and ESR) of the Cu(II) and Zn(II) complexes of $1^{\bullet}$ to those of the corresponding complexes of $2^{\bullet}$ indicates that the methylthio group of $1^{\bullet}$ exerts an electron-sharing conjugative effect, thus stabilizing the radical form of the cofactor, as has been demonstrated in model studies of the metal-free radicals. Such an important role for the thioether group (electron-sharing conjugative effect) has also been predicted by ab initio theory and demonstrated by high-frequency ESR studies of model radicals. It should be noted, however, that such an electronic effect of an alkylthio group is not always observed in other model systems, suggesting that the molecular geometry of a complex is also very important to the enhancement of this effect. The smaller ε values for the NIR features of the model complexes as compared to those for the active forms of the native enzymes may indicate a strong contribution from $Tyr272^{\bullet} \rightarrow Tyr495^{-}$ interligand charge transfer in addition to intramolecular charge transfer from the benzene ring to the alkylthio group in the phenoxyl radical group itself in the enzymatic systems.

II-G-12 Characterization of Imidazolate-Bridged Cu(II)-Zn(II) Heterodinuclear and Cu(II)-Cu(II) Homodinuclear Hydroperoxo Complexes as Reaction Intermediate Models of Cu, Zn-SOD

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[*Chem. Commun.* 1051 (2000)]

Imidazolate-bridged Cu(II)-Zn(II) heterodinuclear and Cu(II)-Cu(II) homodinuclear hydroperoxo complexes are generated in the reactions between imidazolate-bridged heterodinuclear homodinuclear complexes and H_2O_2 in the presence of triethylamine base and characterized spectroscopically as reaction intermediate models of Cu, Zn-SOD.

II-H Fast Dynamics of Photoproducts in Solution Phases

Picosecond time-resolved resonance Raman (ps-TR^3) spectroscopy is a promising technique to investigate ultrafast structural changes of molecules. However, this technique has not been used as widely as nanosecond TR^3 spectroscopy, mainly due to the lack of light source which has suitable repetition rates of pulses and wavelength tunability. In order to obtain qualified TR^3 spectra, first we need two independently tunable light sources for pump and probe pulses. Second, the repetition rate should be higher than kilohertz to keep a moderate average laser power without making the photon density of probe pulse too high. We succeeded in developing light sources for ps-TR^3 spectroscopy having wide tunability and kHz repetition, and applied them to study fast dynamics of photo-excited molecules. For carbonmonoxy myoglobin (MbCO), vibrational relaxation with the time constant of 1.9 ps was observed for CO-photodissociated heme. For Ni-octaethylporphyrin in benzene, differeces in rise times of population in vibrationally excited levels among various modes were observed in the anti-Stokes spectra for the first time. This technique has been applied to identify the trans ligand of CO in the CO-bound transcriptional factor, Coo A.

On the other hand, we have constructed a nanosecond temperature-jump apparatus using a water absorption in near infrared. The new apparatus based on a Nd:YAG laser was combined with a time-resolved Raman measurement system and applied successfully to explore thermal unfolding of ribonuclease A.

II-H-1 Saturation Raman Spectroscopy as a tool for Studying the Excited States of Complex Organic Molecules: Application to Nickel Octaethylporphyrin

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[*Asian J. Phys.* **17**, 365 (1998)]

The nanosecond saturation resonance Raman (RR) technique has been reviewed and its peculiarities have been examined on the basis of a well-known molecular system, nickel octaethylporphyrin [Ni(OEP)] in solution. The results of mathematical treatment of saturation RR spectra of Ni(OEP) in weakly coordinating pyridine solvent suggest that the quantum yield of photogeneration of the six-coordinate Ni(OEP)-(pyridine)$_2$ species is low, with the rate of complexation process being about one tenth of the rate of excitation deactivation within the manifold of four-coordinate species.

II-H-2 Construction of Novel Nanosecond Temperature Jump Apparatuses Applicable to Raman Measurements and Direct Observation of Transient Temperature

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[*Appl. Spectrosc.* in press (2000)]

Two types of nanosecond temperature jump (T-jump) apparatuses applicable to time-resolved Raman measurements were constructed. T-jump was achieved by direct heating of water using near infrared (NIR) pulses at 1.89 μm in one type and at 1.56 μm in the other. The two NIR pulses were generated through stimulated Raman scattering (SRS) of H_2 or D_2 excited by the fundamental line of a Q-switched Nd:YAG laser, in which a single pass configuration with H_2 was sufficient for 1.89-μm pulses but a seeding-amplification configuration with D_2 was necessary for 1.56-μm pulses. The seeding-amplification configuration brought about significant improvements in conversion efficiency, pulse-to-pulse stability, and beam quality. These apparatuses were applied to transient Raman measurements of MoO_4^{2-} solution and transient temperatures of the heated volume were determined from ratios of anti-Stokes to Stokes Raman intensities. Temporal behaviors of the temperature of heated volume upon illumination of nanosecond heat pulses at 1.89-μm or 1.56-μm were explored and its applicability to studies on the primary process of thermal reactions was examined. It became clear that the continuation time of raised temperature is determined only by replacement of sample in the case of thick sample and by both thermal transfer and sample replacement in a case of thin sample, while thermal diffusion is not effective for both samples.

II-H-3 Identification of Histidine 77 as the Axial Heme Ligand of Carbonmonoxy CooA by Picosecond Time-Resolved Resonance Raman Spectroscopy

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[*Biochemistry* **39**, 12747 (2000)]

The heme proximal ligand of carbonmonoxy CooA, a CO-sensing transcriptional activator, in the CO-bound form was identified to be His77 by using picosecond time-resolved resonance Raman spectroscopy. On the basis of the inverse correlation between Fe–CO and C–O stretching frequencies, we proposed previously that His77 is the axial ligand trans to CO (Uchida *et al.*, *J. Biol. Chem.* **273**, 19988), whereas later a possibility of displacement of His77 by CO with retention of another unidentified axial ligand was reported (Vogel *et al.*, *Biochemistry* **38**, 2679). Although our previous resonance Raman study failed to detect the Fe–His stretching [ν(Fe–His)] mode of CO-photodissociated CooA of the carbonmonoxy adduct due to the rapid recombination, application of picosecond time-resolved resonance Raman technique enabled us to observe a new intense line assignable to ν(Fe–His) at 211 cm^{-1} immediately after photolysis, while it became nondiscernible after 100-ps delay. The low ν(Fe–His) frequency of photodissociated CooA indicates the presence of some strain in the Fe–His bond in CO-bound CooA. This and the rapid recombination of CO characterize the heme-pocket of CooA. The 211 cm^{-1} band was completely absent in the spectrum of the CO-photodissociated form of His77-substituted mutant but the Fe-Im stretching band was observed in the presence of exogenous imidazole (Im). Thus, we conclude that His77 is the axial ligand of CO-bound CooA and CO displaces the axial ligand *trans* to His77 with retention of ligated His77 to activate CooA as the transcriptional activator.

II-H-4 A Role of Solvent in Vibrational Energy Relaxation of Metalloporphyrins

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The formation of a vibrationally excited photoproduct of metalloporphyrins upon (π,π*) excitation and its subsequent vibrational energy relaxation were monitored by picosecond time-resolved resonance Raman spectroscopy. Stokes Raman bands due to a photoproduct of nickel octaethylporphyrin (NiOEP) instantaneously appeared upon the photoexcitation. Their intensities decayed with a time constant of ~300 ps, which indicates electronic relaxation from the (*d*,*d*) excited state (B_{1g}) to the ground state (A_{1g}), being consistent with the results of transient absorption measurements by Holten and coworkers. Anti-Stokes ν_4 and ν_7 bands for vibrationally excited (*d*,*d*) state of NiOEP decayed with time constants of ~10 and ~300 ps. The former is ascribed to vibrational relaxation, while the latter corresponds to the electronic relaxation from the (d,d) excited state to the electronic ground state. While the rise of anti-Stokes ν_4 intensity was instrument-limited, the rise of anti-Stokes ν_7 intensity was delayed by 2.6 ± 0.5 ps, which indicates that intramolecular vibrational energy redistribution has not been completed in subpicosecond time regime. To study a mechanism of intermolecular energy transfer, solvent dependence of the time constants of anti-Stokes kinetics was investigated using various solvents. No significant solvent dependence of the rise and decay constants was observed for NiOEP. For an iron porphyrin, we observed two phases in intermolecular energy transfer. The fast phase was insensitive to solvent and the slow phase depended on solvents. A model of classical thermal diffusion qualitatively reproduced this behavior. For solute-solvent energy transfer process, low-frequency modes of proteins seem to be less important.