In-situ X-ray Absorption Spectroelectrochemistry for Determination of the Oxidation States and the Local Structure of Metalloprotein Model Compounds

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X-ray absorption spectroscopy combined with an in-situ electrochemical cell has been developed for characterization of unstable, but electrochemically stable metalloprotein model compounds with various oxidation states in solution. The technique has been applied to some dimanganese Schiff-base complexes as a model of Photosystem II and [Ru_2(µ-O)(µ-CH_3COO)_2(bpy)_2L_2] (L=pyridine and 1-methylimidazole) as a model of hemerythrin. It has been found that X-ray absorption near-edge structure (XANES) patterns correlate well with the formal oxidation states of the metal ions and aggregation. The analysis of extended X-ray absorption fine structure (EXAFS) data has revealed characteristic metal-ligand and metal-metal distances within the model compounds with different oxidation states.

Keywords XANES, EXAFS, in-situ electrochemical cell, Photosystem II, hemerythrin

Chemical species formed in redox reactions in solution, which are often too unstable to isolate and characterize by usual chemical synthesis, can be stabilized electrochemically in solution. Many multinuclear metal complexes as models of metalloprotein and metalloenzymes have recently been synthesized, but their unusual high or low oxidation states are often very unstable so that characterization of the species such as structure and electronic state has seldom been made except for cyclic voltammogram of redox reactions in solution. X-ray and neutron diffraction methods have been used to determine the structures of metal complexes in solution, however, the methods require high concentrations (1 M, M=mol dm^-3) of the complexes and thus cannot be applied to dilute solutions usually used for electrochemical investigations. X-ray absorption spectroscopy using intense synchrotron radiation has been proved to be useful in characterizing the local structure (interatomic distance and coordination number) and electronic state of metal complexes in solution, in particular, for dilute solutions (several mM). In the present study, X-ray absorption spectroscopy combined with in-situ electrochemical cell (in-situ X-ray absorption spectroelectrochemistry) has been developed to characterize the chemical species at various oxidation states formed in redox reactions. This technique has been applied to characterize the coordination structure of manganese Schiff-base complexes as a model of Photosystem II and of ruthenium complexes [Ru_2(µ-O)(µ-CH_3COO)_2(bpy)_2L_2] (bpy=2,2'-bipyridine and 1-methylimidazole) as a model of hemerythrin with various oxidation states electrochemically formed in acetonitrile solution.

Experimental

**in-situ Electrochemical Cell**

Two types of in-situ electrochemical cell were made for measurements in both transmission and fluorescence modes. Figure 1 shows an in-situ cell of transmission type. The counter cell, made of glass, is separated from the working cell, made of Teflon, by a No.4 glass filter. The cell windows are made of Mylar and/or Kapton. The optical path length can be varied by inserting appropriate Teflon spacers. All joints are sealed by o-rings. The cell of fluorescence type was similar to that used by Sharpe et al. Cyclic voltammograms were recorded with a potentiostat (Hokuto, HA-501G) with a function generator.
Glassy carbon and platinum electrodes were used as working and counter electrodes, respectively.

**Sample preparation**

Manganese Schiff-base complexes measured are shown in Fig. 2(above). All complexes except for D and E which were prepared electrochemically with the in-situ cell, were prepared according to ref. 3. Solution samples were prepared by dissolving the corresponding complexes into acetonitrile. The manganese concentrations in the sample solutions were 1~3 mM. Electrolysis was performed under nitrogen atmosphere in an acetonitrile solution of 0.1 M tetrabutylammonium perchlorate (Bu$_4$NClO$_4$) with the in-situ cell to prepare complexes D and E. The redox potential was +0.6 V vs SCE for Mn$^{III}$/Mn$^{IV}$. Structure standards used were crystalline powder of Mn(OCOCH$_3$)$_2$.4H$_2$O, Mn(OCOCH$_3$)$_3$.2H$_2$O and MnCl$_2$.4H$_2$O, MnO$_2$ (Wako Pure Chemicals), and Mn foil (Goodfellow).

Ruthenium complexes (1 and 2) are shown in Fig. 2(below). Both complexes were prepared and isolated as PF$_6$- salts according to a procedure in ref. 4. Tetra- butylammonium hexafluorophosphate Bu$_4$NPF$_6$ (Tokyo Kasei Kogyo) was recrystallized from ethanol and used as supporting electrolyte in the electrochemical experiments. Tris-acetylaceto ruthenium(III) and ruthenium(IV) oxide (Wako Pure Chemicals) powder and ruthenium foil (Goodfellow) were used as structure standards. The samples (20 mM) were dissolved in an acetonitrile solution containing 0.5 M Bu$_4$NPF$_6$, and the solutions were deoxygenated by a stream of nitrogen or argon gas. An Ag/AgCl electrode was used as a reference. The redox potentials vs Ag/AgCl for samples were -1.09 ~ -1.16 V for Ru$^{III}$/Ru$^{IV}$, -0.39 ~ -0.55 V for XAFS measurements

Mn K-(6.5 keV) and Ru K-(22.1 keV) edge XAFS spectra were recorded, respectively, in fluorescence mode at beam line 7C and in transmission mode at beam line 10B in Photon Factory (KEK, Tsukuba, Japan), respectively. In the former beam line monochromatization of X-rays was made with Si(111) crystals, one of which was bent to intensify fluorescence X-rays, whereas in the latter monochromatic radiation was obtained by channel-cut Si(311) crystals. Energy calibrations were made relative to the absorption edges of reference KMnO$_4$ and Ru foil at Mn K and Ru K-edges, respectively. Ion-chambers with flowing appropriate gas were used for detection. For the fluorescence measurement a Lytle detector$^5$ was used. Combinations of gases used before and after a sample were N$_2$ and N$_2$+Ar(15%) at Mn K-edge and Ar and Kr at Ru K-edge.

Data analysis was performed by using standard procedures for background subtraction, spline fitting and removal of self-absorption, and Fourier transformation.$^6$$^7$ Theoretical phase shifts and
Results and Discussion

XANES spectra

The XANES spectra of the manganese complexes in solution are shown in Fig. 3. For monomeric manganese complexes A-D, it has been found that the energy of absorption edge shifts to the higher energy side with increasing oxidation state of Mn. Another interesting finding in Fig. 3 is that the XANES patterns of the dimeric manganese complexes differs from each other, depending on the formal oxidation states of Mn, (III and III) for E, (III and IV) for G, and (IV and IV) for F.

The XANES spectral shape of the ruthenium complexes was practically identical for all samples containing the same moiety of the complexes. However, the absorption edge shifted slightly towards higher energy upon oxidation of the sample, as found for the manganese complexes.

It is concluded that XANES spectra are sensitive to the oxidation state and polymerization of metal complexes and thus could be used to estimate the oxidation state of native metalloproteins and metalloenzymes.

EXAFS of the manganese complexes

Figure 4 shows Fourier transforms (uncorrected for the phase shifts). In the figure, the predominant first peak around 1.5 Å is assigned to the Mn-O/N bonds within the Mn complexes. In addition, the Mn-Cl bond also contributes to the peak for Complexes A-D. The second peak around 2.5 Å probably corresponds to the interactions between the second neighbor C and Mn atoms and a central Mn atom. The structure parameters finally obtained are given in Table 1. The obtained Mn-O/N distances are in good agreement with those expected for Mn$^{III}$ and Mn$^{IV}$. An interesting finding is chloride ion binding to Mn$^{IV}$ in solution. Probably the chloride ion stabilizes the tetravalent state of Mn in solution, which is, on exposing to water vapor, readily reduced to the lower oxidation states. The Mn-Mn distance in the di µ-oxo Mn complexes were determined as 2.82 Å for Mn$^{IV}$Mn$^{III}$ and 2.71 Å for Mn$^{III}$Mn$^{IV}$. In Photosystem II, the Mn-Mn distance has been reported to be 2.72 Å. By comparing this value with the present results, it is most likely that the oxidation states of Mn in the Photosystem II are +4 and/or +3 as a dimeric moiety.

EXAFS of the ruthenium complexes

Figure 5 shows Fourier transforms. The obtained structural parameters are given in Table 2. In Fig. 5 the largest peak around 1.5 Å represents the Ru-O$_{bridge}$, Ru-O$_{acetate}$ and Ru-N$_{ligand}$ bonds, and the second largest peak around 3 Å mostly the Ru-Ru separation. Only in some cases it was possible to resolve the Ru-O$_{bridge}$ distance from the Ru-O$_{acetate}$ and Ru-N$_{ligand}$ distances. The Ru-Ru distances decrease with decreasing formal oxidation state of Ru. This is interpreted as a decreased electrostatic repulsion between the Ru atoms. At the same time the Ru-O$_{bridge}$ distance increases, interpreted as a decreased electrostatic
attraction between the Ru atom and bridging O atoms. The Ru-O\textsubscript{extra} and Ru-N\textsubscript{ligand} distances remained the same, within the error limit of the analysis, and a dependence on the Ru oxidation state was not experimentally observed. The overall change in shape is thus an opening of the Ru-O\textsubscript{bridge}-Ru bond angle.

Conclusions

X-ray absorption spectroscopy combined with an in-situ electrochemical cell has been applied to dilute acetonitrile solutions of monomeric and dimeric manganese Schiff-base and dimeric ruthenium complexes with various oxidation states and has been proved to be very useful for characterization of the local coordination structure of the metal complexes with a series of oxidation states formed along cyclic voltammogram. It has been found that XANES patterns depend on the oxidation state and aggregation of the metal complexes. EXAFS data have revealed the local coordination structure of the metal complexes with uncertainties in interatomic distances of 0.02-0.05 Å.

References