Ni and Fe XAFS studies on the Core Site Models of Hydrogenases

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Introduction
The active site of H₂-decomposing enzyme, [NiFe]-hydrogenase, has a Ni-Fe binuclear center, in which the nickel ion (Ni²⁺) is surrounded by four Cys sulfur atoms from two Cys-X₂-Cys sequence and bound to an iron ion (Fe²⁺) having CO, CN, or SO via two bridging sulfur atoms [1].

In our titration study for this active site using a Cys-containing tetra-peptide Cys-Pro-Leu-Cys (L₁) as ligands, we found that the L₁ reacts with Ni²⁺ in the ratio of Ni²⁺/L₁ = 1/2 to form NiS₄ compounds (UV-vis.). The solution composed of Ni²⁺/L₁ = ½ showed an inflection point at 0.9~1.0 eq. in the titration of Fe II (CH₃COOH)₂, suggestive of the formation of a L₁/Ni²⁺/Fe²⁺ = 2/1/1 compound. On the other hand, we also found from a titration experiment using a square-planar NiS₄ complex [Ni(dpmt)₄]²⁻ (Hdpmt = Ph₂CHSH) that it reacts with [Fe II Br(CN)₂(CO)₃]⁻ to form a Ni²⁺/Fe²⁺ = 1/1 compound.

In this XAFS experiment, we studied the coordination spheres of Ni and Fe to discuss whether or not the Ni²⁺ ions in the Cys-containing and alkanethiolate systems possess proximal Fe²⁺ bridged with two sulfur atoms like the active sites of [NiFe]-hydrogenases.

Experimental
All synthetic and sampling procedures were carried out in Ar atmosphere (O₂ < 0.05 ppm). The peptide ligand Cys-Pro-Leu-Cys (L₁) was prepared by Boc-liquid-phase method. Polyethylene pellet and DMF solution samples of Ni²⁺/L₁ = 1/2 (1) and Ni²⁺/Fe²⁺/L₁ = 1/1/2 (2) were prepared for XAFS spectrum analyses. Polyethylene pellets of [Ni(dpmt)₄]⁺/[Fe(CN)₆]³⁻ (Hdpmt = Ph₂CHSH) that it reacts with [Fe(CN)₆]³⁻ to form a Ni²⁺/Fe²⁺ = 1/1 compound.

In this XAFS experiment, we studied the coordination spheres of Ni and Fe to discuss whether or not the Ni²⁺ ions in the Cys-containing and alkanethiolate systems possess proximal Fe²⁺ bridged with two sulfur atoms like the active sites of [NiFe]-hydrogenases.

Results and Discussion
From the XAFS spectrum analyses of 1, it was suggested that Ni²⁺ has a tetrahedral NiS₂O₂ coordination, in which the oxygen was supposed coming from DMF. The force constant of Ni-S bond (νNi-S = 348 cm⁻¹) was derived from the temperature dependence of the Deby-Waller like factor of sample 1. Its value was close to that of previously reported compound (νNi-S = 330 cm⁻¹) [3]. Addition of Fe²⁺ to the solution for sample 1 did not change the kind and number of directly-coordinating atoms (S₂) on the Ni²⁺, as well as the coordination geometry (NiS₂O₂); however, showed the existence of a proximal Fe²⁺ (3.0 Å) (Fig. 1). FEFF simulation based on the structure shown in Fig. 1 gave the bond lengths of Ni-S = 2.200 Å and Ni-O = 2.000 Å.

Comparison of the XANES spectra of samples 3 and 4 suggested that the coordination geometry of Ni²⁺ changed from square planar (sample 4) to tetrahedral (sample 3); on the other hand, the EXAFS analyses demonstrated a multiple-scattering peak originating from square-planar geometry for sample 4, whereas not for sample 3. The analyses for sample 3 strongly indicated the existence of a Ni-S-Fe bridging structure just same as the active sites of [NiFe]-hydrogenases, but the distance of Ni-Fe in sample 3 was 3.205 Å, longer than that in the latter (Fig. 2).

Fig. 1. Proposed coordination geometries of Ni²⁺/Cys-Pro-Leu-Cys = 1/2 (left; sample 1) and Fe²⁺/Ni²⁺/Cys-Pro-Leu-Cys =1/1/2 (right; sample 2).

Fig. 2. The structure of the coordination sphere in sample 3 estimated from EXAFS analyses.

References
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