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

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## Introduction

The active site of $H_2$-decomposing enzyme, [NiFe]-hydrogenase, has a Ni-Fe binuclear center, in which the nickel ion ($Ni^{n+}$) is surrounded by four Cys sulfur atoms from two Cys-$X_2$-Cys sequence and bound to an iron ion ($Fe^{2+}$) 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 ($L_1$) as ligands, we found that the $L_1$ reacts with $Ni^{2+}$ in the ratio of $Ni^{2+}/L_1$ = 1/2 to form $NiS_4$ compounds (UV-vis.). The solution composed of $Ni^{2+}/L_1$ = ½ showed an inflection point at 0.9~1.0 eq. in the titration of $Fe^{II}(CH_3COOH)_2$, suggestive of the formation of a $L_1/Ni^{2+}/Fe^{2+}$ = 2/1/1 compound. On the other hand, we also found from a titration experiment using a square-planar $NiS_4$ complex $[Ni(dpmt)_4]^{2-}$ (Hdpmt = $Ph_2CHSH$) that it reacts with $[Fe^{II}Br(CN)_2(CO)_3]^-$ to form a $Ni^{2+}/Fe^{2+}$ = 1/1 compound.

In this XAFS experiment, we studied the coordination spheres of Ni and Fe to discuss whether or not the $Ni^{2+}$ ions in the Cys-containing and alkanethiolate systems possess proximal $Fe^{2+}$ bridged with two sulfur atoms like the active sites of [NiFe]-hydrogenases.

## Experimental

All synthetic and sampling procedures were carried out in Ar atmosphere ($O_2$ < 0.05 ppm). The peptide ligand Cys-Pro-Leu-Cys ($L_1$) was prepared by Boc-liquid-phase method. Polyethylene pellet and DMF solution samples of $Ni^{2+}/L_1$ = 1/2 (**1**) and $Ni^{2+}/Fe^{2+}/L_1$ = 1/1/2 (**2**) were prepared for XAFS spectrum analyses. Polyethylene pellets of $[Ni(dpmt)_4]^{2-}/[Fe^{II}Br(CN)_2(CO)_3]^-$ = 1/1 (**3**) and $[Ni(dpmt)_4]^{2-}$ (**4**) were prepared by the same way.

The Ni K-edge XAFS spectra of **1**~**4** were observed at 298 K in transmission mode using the EXAFS facilities of beam line BL-10B of Photon Factory in the High energy accelerator research organization (KEK). Si(311) cm were calibrated using the 8333.8 eV K-edge Ni foil. Data were analyzed by using REX2000 and EXAFSH combined with FEFF 8.10 [2].

## Results and Discussion

From the XAFS spectrum analyses of **1**, it was suggested that $Ni^{2+}$ has a tetrahedral $NiS_2O_2$ coordination, in which the oxygen was supposed coming from DMF. The force constant of Ni-S bond ($\nu_{Ni\text{-}S}$ = 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 (!$_{Ni\text{-}S}$ = 330 $cm^{-1}$) [3]. Addition of $Fe^{2+}$ to the solution for sample **1** did not change the kind and number of directly-coordinating atoms ($S_2$) on the $Ni^{2+}$, as well as the coordination geometry ($NiS_2O_2$); however, showed the existence of a proximal $Fe^{2+}$ (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.

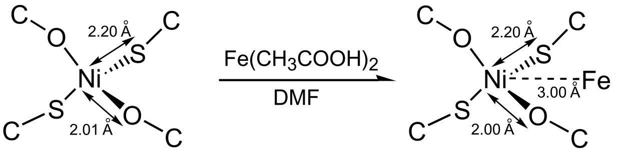

Fig. 1. Proposed coordination geometries of $Ni^{2+}$/Cys-Pro-Leu-Cys = 1/2 (left; sample **1**) and $Fe^{2+}/Ni^{2+}$/Cys-Pro-Leu-Cys =1/1/2 (light; sample **2**).

Comparison of the XANES spectra of samples **3** and **4** suggested that the coordination geometry of $Ni^{2+}$ 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).

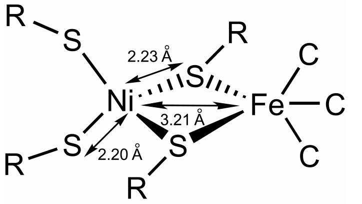

Fig. 2. The structure of the coordination sphere in sample **3** estimated from EXAFS analyses.

## References

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