

Peptide Models for [NiFe] hydrogenase Active Site. Search of Protonated [Ni(Cys-S)₄] and Ni-Fe Compounds

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Introduction

[NiFe] hydrogenase is an enzyme that catalyzes the reversible oxidation of H₂. The active center consists of one nickel atom and one iron atom, which are bridged by two cysteinic thiolates and one unidentified ligand forming S₂Ni(μ-S₂)(μ-X)Fe core[1]. Many synthetic efforts have been made to reproduce the core structure using alkylthiolate, arylthiolate, and dithiocarbamates [2]. Differently from these efforts, we carried out the synthesis of Ni complexes of Cys-Pro-Leu-Cys (**1**) considering that the Ni ions of the active centers are bound to Cys-X-Y-Cys sequences in [NiFe] hydrogenases. In the course of this synthetic approach, we found that Ni ion and **1** react by the stoichiometry of Ni²⁺/1 = 1/1, 1/1.5, and 1/2 in the presence of DIEA, and that the isolated compound obtained from Ni²⁺/1 = 1/2 will take NiS₄ Sq. Pl. geometry. We also found that the organic layer shows different spectroscopic (UV-vis. and NMR) behavior depending on the acidity/basicity of the aqueous solution used for washing, suggestive of that [Ni(CPLC)₂]²⁻ changes the coordination geometry and number depending on the pH of the aqueous solution. In order to clarify the structural feature of this change in [Ni(CPLC)₂]²⁻, we carried out XAFS studies.

Experimental

All synthetic and sampling procedures were carried out in Ar atmosphere (O₂ < 0.1 ppm). The peptide ligand **1** was prepared by Boc-liquid-phase method. Several types of samples were prepared for the Ni²⁺/1/DIEA system in CH₃CN: (a) without washed but different in the stoichiometry, (b) Ni²⁺/1/DIEA = 1/2/4 washed by 7% citric acid solution and Ni²⁺/1/DIEA = 1/2/4 washed by 10% NaHCO₃ solution.

The Ni K-edge XAFS spectra of a-b were observed in pellets and solutions at 298 K in transmission mode using the EXAFS facilities of beam line BL-7C and BL-9C of Photon Factory in the High Energy Accelerator Research Organization (KEK). Si(311) monochromator was used. Energy was calibrated by using the K-edge of Ni foil (8333.8 eV). Data were analyzed by using REX2000 combined with FEFF 8.10 [3]. Low temperature measurements were also performed for [Ni(CPLC)₂]²⁻/[Fe(CO)₃(CN)₂Br] system to survey the evidence of Ni-S-Fe bridging structure.

Results and Discussion

XANES analysis for a-b revealed that the Ni²⁺ ion largely changes its coordination geometry depending on the stoichiometry and the washing conditions. The Ni²⁺

ion in Ni²⁺/1 = 1/1 unwashed solution showed a Td geometry giving a NiS₂O(N)₂ coordination sphere with r(Ni-S) = 2.186 Å and r(Ni-O(N)) = 2.017 Å in RDF. The O atom is expected to come from the water molecules of [Ni(H₂O)₆](BF₄)₂, the Ni source. The Ni²⁺ ion in Ni²⁺/1 = 1/1.5 unwashed solution gave a NiS₃O(N) coordination sphere with r(Ni-S) = 2.099 Å and r(Ni-O(N)) = 1.799 Å. This O atom is also expected to come from [Ni(H₂O)₆](BF₄)₂. The peak at R = 3.03 Å found in the RDF of Ni²⁺/1 = 1/1.5 was assigned to Ni-Ni connected via S [4]. Thus, the species in Ni²⁺/1 = 1/1.5 will take S₃Ni(μ-O)NiS₃ bridging structure. On the other hand, the unwashed solution of Ni²⁺/1 = 1/2 showed a Sq. Pl. NiS₄ geometry giving r(Ni-S) = 2.12 Å. This sample showed a broad peak at R ~ 3.75 Å. This peak was assigned to the 4-leg multiple scattering S ↔ Ni ↔ S generally observed in a Sq. Pl. structure. All these results were consistent with those estimated from the XANES spectra.

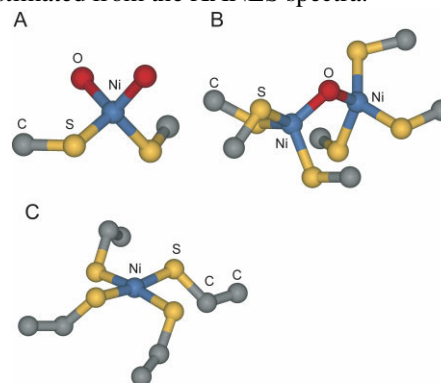


Figure Proposed structures of Ni/CPLC complex prepared for FEFF calculation. A: Ni²⁺/CPLC = 1/1 unwashed, B: Ni²⁺/CPLC = 1/1.5 unwashed and C: Ni²⁺/CPLC = 1/2 unwashed sample.

References

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