Transition from Ni-B to Ni-A State in [NiFe]-Hydrogenase from *D. vulgaris* Miyazaki F and Their Structural Characterization

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Hydrogenases catalyze oxidoreduction of molecular hydrogen and thus have a potential use for a new energy source. [NiFe]-hydrogenase possesses two oxidized states, Ni-A (inactive) and Ni-B (active). The active site of [NiFe]-hydrogenase in the oxidized state consists of Ni and Fe atoms coordinated with four cysteinyl sulfur and four non-protein ligands. The non-protein ligands are two CN^- groups and one CO bound to the Fe atom and a bridging ligand between the Ni and Fe atoms. The bridging ligand disappears by reduction of the enzyme. We have shown that CO coordinates to the Ni atom of the enzyme and the Ni and Sγ(Cys546) atoms are most flexible, which suggested that these two atoms play a key role during the initial H_2-binding process [1]. In this study, we have controlled the conversion between the two states, Ni-A and Ni-B, and clarified the difference on the basis of their three-dimensional structures.

By addition of Na_2S to the Ni-B state of [NiFe]-hydrogenase from *D. vulgaris* Miyazaki F under strictly anaerobic conditions, $[Fe_3S_4]^+$ in the enzyme was reduced first. After the reduction, the Ni-B EPR signals converted to new EPR signals at g = 2.00, 2.14, and 2.29, which converted to the Ni-A signals by addition of oxygen. Crystal structures of Ni-A and Ni-B are solved at high resolution. The shape and size of the electron densities show that Ni-B possesses a monatomic non-protein bridging ligand at the Ni-Fe active site, whereas Ni-A has a diatomic bridging ligand. The terminal atom of the bridging species of Ni-A occupies a similar position as C of the exogenous CO in the CO complex (inhibited state). Sγ of Cys546 has a modified atomic species (X546) in both Ni-A and Ni-B. In Ni-A, Sγ of Cys84 is also modified (X84), and X546 is shifted towards the Ni atom. The essential features of the enzyme structure at the resting state and the transition mechanism from Ni-B to Ni-A are proposed.

[1] H. Ogata, Y. Mizoguchi, N. Mizuno, K. Miki, S. Adachi, N. Yasuoka, T. Yagi, O. Yamauchi, S. Hirota, Y. Higuchi, J. Am. Chem. Soc. 124 (2002) 11628-11635.