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Photosensitivity of the Ni-A state of [NiFe] hydrogenase from *Desulfovibrio vulgaris* Miyazaki F with visible light

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ABSTRACT

[NiFe] hydrogenase catalyzes reversible oxidation of molecular hydrogen. Its active site is constructed of a hetero dinuclear Ni–Fe complex, and the oxidation state of the Ni ion changes according to the redox state of the enzyme. We found that the Ni-A state (an inactive unready, oxidized state) of [NiFe] hydrogenase from *Desulfovibrio vulgaris* Miyazaki F (*Dv*MF) is light sensitive and forms a new state (Ni-AL) with irradiation of visible light. The Fourier transform infrared (FT-IR) bands at 1956, 2084 and 2094 cm^{-1} of the Ni-A state shifted to 1971, 2086 and 2098 cm^{-1} in the Ni-AL state. The *g*-values of $g_x = 2.30$, $g_y = 2.23$ and $g_z = 2.01$ for the signals in the electron paramagnetic resonance (EPR) spectrum of the Ni-A state at room temperature varied for −0.009, +0.012 and +0.010, respectively, upon light irradiation. The light-induced Ni-AL state converted back immediately to the Ni-A state under dark condition at room temperature. These results show that the coordination structure of the Fe site of the Ni-A state of [NiFe] hydrogenase is perturbed significantly by light irradiation with relatively small coordination change at the Ni site.

1. Introduction

Hydrogenases are enzymes which are involved in the energy metabolism of various bacteria, and are considered to control the proton gradient across the cytoplasmic membrane [1,2]. Hydrogenases are classified mainly into three groups according to the metal compositions of their active sites. The groups are [NiFe], [FeFe] and [Fe] hydrogenases, and their active sites consist of a Ni and an Fe ion, two Fe ions and a single Fe ion, respectively [3–7]. [NiFe] hydrogenase from *Desulfovibrio vulgaris* Miyazaki F (*Dv*MF) is attached to the cytoplasmic membrane, and exhibits a heterodimeric structure with a large and small subunit (62.5 and 28.8 kDa) [8,9]. The Ni–Fe active site is buried in the large subunit, and three FeS clusters (one [3Fe–4S] and two [4Fe–4S]) are located at the small subunit [10]. At the Ni–Fe active site, four cysteine Sγ atoms coordinate to the Ni ion, and two of them also coordinate to the Fe ion, constructing bridges between the two metals. In addition, non-protein ligands (one CO and two CN^-) coordinate to the Fe ion [11–13].

Various redox states of the Ni ion in the *Dv*MF enzyme have been identified by spectroscopic studies. Two inactive-oxidized states are known as Ni-A (unready) and Ni-B (ready) states [4,5]. When the Ni-A and Ni-B states are reduced with one electron, they give rise to Ni-SU (EPR-silent) and Ni-SI (EPR-silent) states, respectively [14,15]. The Ni-B state is easily activated within a few minutes in contrast to the Ni-A state, which needs a prolonged activation time [14,16]. The Ni-SI state is further distinguished into two states, Ni-SIr (EPR-silent, inactive ready) and Ni-SIa (EPR-silent, active) states [14,15]. Carbon monoxide (CO)-bound states, such as Ni-CO (EPR-detectable) and Ni-SCO (EPR-silent) states, are also inactive [17–20]. The Ni-SIa state is further reduced to active Ni-C (EPR-detectable) and Ni-R (EPR-silent, fully reduced) states [21–24]. For the *Dv*MF enzyme, detailed structures of the active site have been reported for the Ni-A, Ni-B, H_2-reduced and Ni-SCO states [8,11,25,26]. Diatomic and monoatomic species were detected as a third bridging ligand between the Ni and Fe atoms in the Ni-A and Ni-B states, respectively [11], whereas no clear electron density peak was detected between the Ni and Fe atoms in all three active states (Ni-SIa, Ni-C and Ni-R) (Fig. 1) [25].

The CO and CN^- stretching (ν(CO) and ν(CN^-)) vibrations of the ligands bound to the Fe ion of the Ni-A state of the *Dv*MF enzyme

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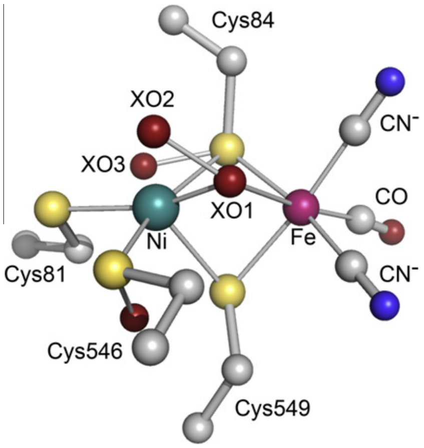

Fig. 1. Structure of the Ni–Fe active site of [NiFe] hydrogenase in the Ni-A state (PDB: 1WUH). A diatomic oxygen ligand (XO1, XO2) is coordinated to the Ni and Fe atoms, and has been suggested as a hydroperoxide (OOH^-) [11,31]. A monatomic oxygen ligand (XO3) is coordinated to the Cys84 Sγ atom.

have been detected at 1956 cm^{-1} (ν(CO)), 2085 and 2094 cm^{-1} (ν(CN^-)), in the FT-IR spectra, whereas those of the Ni-B state were detected at 1955, 2081 and 2090 cm^{-1} [15]. The *g*-values of the Ni(III) ion of the Ni-A and Ni-B states obtained from their EPR spectra at 77K have been reported to be $g_x = 2.32$, $g_y = 2.24$ and $g_z = 2.01$, and $g_x = 2.33$, $g_y = 2.16$ and $g_z = 2.01$, respectively [27]. Photosensitivities of the Ni-C, Ni-SIr and Ni-CO states of [NiFe] hydrogenase have been investigated [17,20,21,28,29]. It has been reported that the Ni-L state, in which the hydride bridging ligand is dissociated from the active site, is produced from the Ni-C state upon illumination with visible light [21,28]. It has also been reported that the bond between the Ni ion and the oxygen bridging ligand in the Ni-SIr state is cleaved upon irradiation with visible light [29]. CO has been reported to photo-dissociate in the Ni-CO state [17,20]. In this study, according to FT-IR and EPR measurements we found that the Ni-A state of [NiFe] hydrogenase from *Dv*MF is also light sensitive.

2. Materials and methods

2.1. Preparation of DvMF [NiFe] hydrogenase and its Ni-A state

*Dv*MF [NiFe] hydrogenase was purified from DvMF cells as reported previously [9,30]. Its Ni-A state was obtained by an addition of Na_2S as described previously [11].

2.2. FT-IR measurements

Infrared spectra of *Dv*MF [NiFe] hydrogenase were measured at room temperature with a FT-IR spectrometer equipped with an MCT detector (FT/IR-6100 V, JASCO Corp.). *Dv*MF [NiFe] hydrogenase in 25 mM Tris–HCl, pH 7.4, were loaded into a gas-tight infrared-transmittance 20 μm cell with CaF_2 windows and a Teflon spacer. After loading the sample to the cell, the cell was placed in the FT-IR spectrometer, which was purged continuously with N_2 gas. Spectral data were collected at 2-cm^{-1} resolution and averaged with 512 scans. The corresponding buffer spectrum was collected as a reference and subtracted from the sample spectra. Light irradiation at 457.9–514.5 nm was performed with an Ar^+ laser (Model 2017, Spectra-Physics) at room temperature. The laser power was 240 mW/cm^2 at the sample point. The FT-IR bands in the spectra were curve fitted using the Igor Pro. ver. 6.0 program (WaveMetrics, Portland). Gaussian curves were assumed for the FT-IR bands of the Ni-A and transient states, and the peak positions of the ν(CO) and ν(CN^-) bands of the Ni-A state were fixed to the frequencies, which have been previously reported [15]. The intensities and bandwidths of the ν(CO) and ν(CN^-) bands of the photo-induced state were fixed to those of the Ni-A state, and the different spectrum between with and without light irradiation was least-square fitted.

2.3. EPR measurements

X-band EPR spectra of *Dv*MF [NiFe] hydrogenase were measured at room temperature with an EPR spectrometer (JES-FA100 N, JEOL). The samples were loaded into an EPR flat cell and irradiated with a 500 W Xenon lamp (SX-UID500XCM, USHIO Inc.) using a cold filter (CLDF-50S, SIGMA KOKI Co., Ltd.). The light power was 1.8 W/cm^2 at the sample point. The experimental conditions were as follows: frequency, 9.416 GHz; microwave power, 2 mW; modulation width, 1 mT; time constant, 0.1 s; integration, 20 times. The positions of the EPR signals were obtained by curve fitting the spectra with a similar method as for the FT-IR spectra using the Igor Pro. ver. 6.0 program.

3. Results

3.1. Changes in the FT-IR spectra of the Ni-A state with light irradiation

The intensities of the ν(CO) band at 1956 cm^{-1} and the ν(CN^-) bands at 2084 and 2094 cm^{-1} in the FT-IR spectrum of the Ni-A state of *Dv*MF [NiFe] hydrogenase decreased with irradiation of

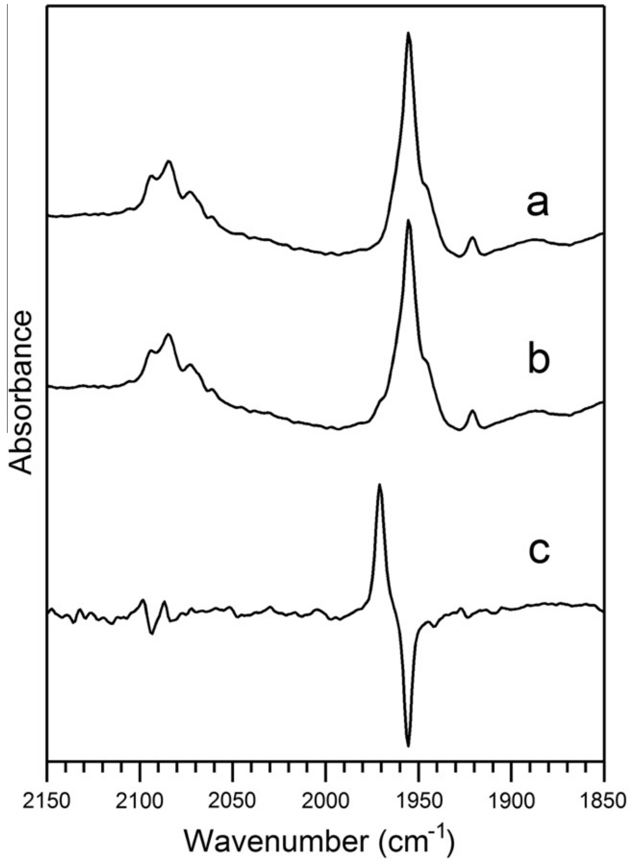

Fig. 2. FT-IR spectra of the Ni-A state of *Dv*MF [NiFe] hydrogenase with and without light irradiation. The spectra of the Ni-A state obtained under (a) dark condition and (b) upon irradiation with an Ar^+ laser (488.0 nm, 240 mW/cm^2 at the sample point) and (c) their difference spectrum (light irradiation minus dark condition) are shown. The intensity of the difference spectrum is expanded by a factor of 10. Experimental conditions: frequency range, 2500 to 1000 cm^{-1}; resolution, 2 cm^{-1}; cell pathlength, 20 μm; integration, 512 scans; temperature, room temperature.

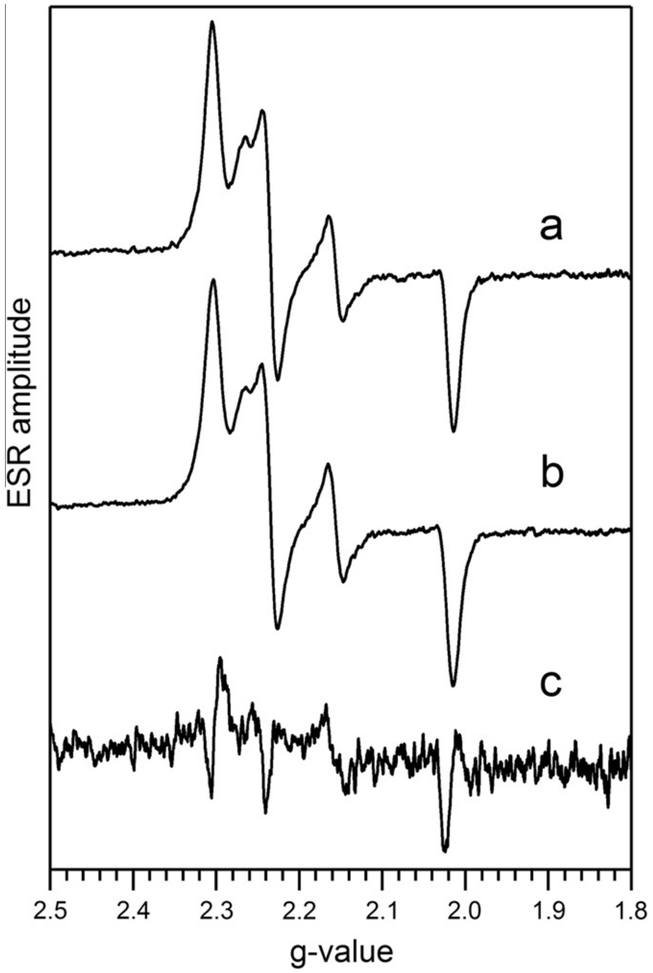

Fig. 3. EPR spectra of the Ni-A state of *Dv*MF [NiFe] hydrogenase with and without light irradiation. EPR spectra of the Ni-A state obtained under (a) dark condition and (b) upon irradiation with light from a Xe lamp (1.8 W/cm^2) and (c) their difference spectrum (light irradiation minus dark condition) are shown. The intensity of the difference spectrum is expanded by a factor of 5. Experimental conditions: frequency, 9.416 GHz; microwave power, 2 mW; modulate amplitude, 1 mT; integration, 20 scans; temperature, room temperature.

Table 1
Vibrational frequencies of the diatomic ligands at the active site of the Ni-A, Ni-AL and Ni-B states of *Dv*MF [NiFe] hydrogenase.

State	ν(CO) (cm^{-1})	ν(CN$^-$) (cm^{-1})
Ni-A	1956	2084, 2094
Ni-AL	1971	2086, 2098
Ni-B	1955	2081, 2090

Data of the Ni-A and Ni-AL states were obtained in this study, whereas those for the Ni-B state are from Ref. [15].

Table 2
The *g*-values of the Ni-A, Ni-AL and Ni-B states of *Dv*MF [NiFe] hydrogenase.

State	g_x	g_y	g_z
Ni-A	2.30	2.23	2.01
Ni-AL	(−0.009)	(+0.012)	(+0.010)
Ni-B	2.33	2.16	2.01

Data of the Ni-B state are measured at room temperature with the as-isolated *Dv*MF [NiFe] hydrogenase. The *g*-values of the Ni-AL state are show as differences from those of the Ni-A state.

an Ar$^+$ laser light at 488.0 nm at room temperature, generating new bands at 1971, 2086 and 2098 cm^{-1} (Fig. 2). We assigned the new band at 1971 cm^{-1} to the ν(CO) mode of a photo-induced state (Ni-AL), due to its relatively strong intensity and close frequency to those of the ν(CO) mode of other [NiFe] hydrogenase states [15,20,28,29]. We assigned similarly the other 2086 and 2098 cm^{-1} bands to the ν(CN$^-$) modes.

The photo-induced FT-IR bands disappeared immediately under dark condition at room temperature, and the FT-IR spectrum converted back to that of the Ni-A state (Fig. S1). When the Ni-A state was irradiated with the laser light again, the new bands at 1971, 2086 and 2098 cm^{-1} were generated again (Fig. S1), showing that the photo-induced reaction is reproducible. The intensity of the Ni-AL state did not depend much on the wavelength between 457.9 and 514.5 nm (Fig. S2). However, no significant change was observed in the FT-IR spectra of the Ni-B state upon light irradiation under the same conditions.

3.2. Changes in the EPR spectra of the Ni-A state with light irradiation

The EPR signals of the Ni-A state of *Dv*MF [NiFe] hydrogenase were detected at $g_x = 2.30$, $g_y = 2.23$ and $g_z = 2.01$ when measured at room temperature under dark condition (Fig. 3 and Table 2). However, the *g*-values of the Ni-A state were shifted about $\Delta g_x = -0.009$, $\Delta g_y = +0.012$ and $\Delta g_z = +0.010$ with light irradiation, and the difference EPR spectrum between the spectra of the Ni-A state with and without white light irradiation exhibited new peaks. The shifts in the difference spectrum disappeared immediately under dark condition, although the peaks were observed repeatedly in the difference spectra between the spectra with and without light irradiation.

4. Discussion

According to FT-IR and EPR measurements (Figs. 2 and 3 and Table 1), we found that a new photo-induced Ni-AL state is generated upon light irradiation of the Ni-A state of *Dv*MF [NiFe] hydrogenase. The FT-IR bands of the Ni-AL state were observed at 1971, 2086 and 2098 cm^{-1}, which were shifted from those of the Ni-A state (Fig. 2). The new ν(CO) band exhibited the highest wavenumber among the corresponding bands of various redox states of [NiFe] hydrogenase reported so far. The high wavenumber indicates that the electron donation from the Fe ion to the π^* orbitals of its bound CO is relatively weak in the Ni-AL state, presumably due to the low electron density of the Fe ion. The low electron density of the Fe ion may be due to decrease in electron donation from the ligands to the Fe ion.

The EPR signals of the Ni-A state of *Dv*MF [NiFe] hydrogenase shifted slightly with light irradiation (Fig. 3). The intensities of the EPR signals of the Ni-A state at *g*-values 2.30, 2.23 and 2.01 decreased, and new signals generated with changes in the *g*-values of −0.009, +0.012 and +0.010, respectively, from those of the Ni-A state (Fig. 3 and Table 2). Since the differences in the *g*-values between the Ni-A and Ni-AL states were relatively small, the conformation of the ligands and electronic state around the Ni ion in the Ni-AL state may not differ significantly from those of the Ni-A state, although those around the Fe ion may differ largely according to the FT-IR results.

Four Sγ atoms of four cysteine residues (Cys81, Cys84, Cys546, and Cys549) and an oxygen atom (XO1, the oxygen atom which is directly bridging the Ni and Fe atoms, see Fig. 1) of the diatomic bridging ligand are coordinated to the Ni atom in the Ni-A state of *Dv*MF [NiFe] hydrogenase, exhibiting a five-coordinate square pyramidal ligand field (Fig. 1). The ν(CO) frequency of the Ni-AL state was significantly higher than that of the Ni-A state. Therefore, the XO1 atom of the oxygen bridging ligand may suffer conformational changes with less electron donation to the Fe ion, since this

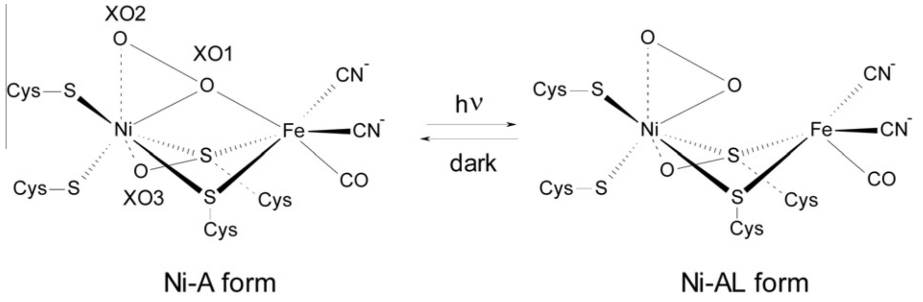

Fig. 4. Schematic view of the putative structural change of the Ni-A state upon light irradiation.

ligand is located in an opposite position to the CO ligand against the Fe ion. However, the conformation around the Ni ion between the Ni-A and Ni-AL states may not change significantly, since the EPR signals of the Ni-A and Ni-AL states were similar. The XO2 (the oxygen atom which is not directly bridging the Ni and Fe atoms, see Fig. 1) and XO3 atoms (the oxygen atom of the modified Sγ of Cys84) are closely located to the Ni atom (Ni-XO2, 2.06 Å; Ni-XO3, 1.83 Å), and the interactions between the Ni ion and these oxygen atoms may stabilize the structure around the Ni ion.

A putative scheme for the photo-reaction of the Ni-A state is presented in Fig. 4. The ν(CO) FT-IR frequency of the CO ligand of the Fe ion in the Ni-AL state was largely shifted from that in the Ni-A state, whereas the differences in the EPR signals were relatively small. These results suggest that the difference in the electronic state around the Fe ion is large between the Ni-AL and Ni-A states, but that around the Ni ion is relatively small. From the ν(CO) frequency in the FT-IR spectra, a decrease in the electron donation from the Fe-ligands to the Fe ion was indicated in the Ni-AL state compared to the Ni-A state. Therefore, the Fe–XO1 bond may cleave in the Ni-AL state, with the conformation around the Ni ion not changing significantly. Under dark conditions, the XO1 atom may rebind to the Fe ion and the light-induced Ni-AL state may immediately convert back to the Ni-A state at room temperature. To our knowledge, this is the first report on light sensitivity of the Ni-A state of [NiFe] hydrogenase. Light sensitivity has been reported in many other states of [NiFe] hydrogenase [20,21,28,29], and its wide light sensitivity may be important for the hydrogenase activity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.10.136.

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