Mechanistic investigation of the formation of H₂ from HCOOH with a dinuclear Ru model complex for formate hydrogen lyase

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ABSTRACT
We report the mechanistic investigation of catalytic H₂ evolution from formic acid in water using a formate-bridged dinuclear Ru complex as a formate hydrogen lyase model. The mechanistic study is based on isotope-labeling experiments involving hydrogen isotope exchange reaction.

1. Introduction
Formate hydrogen lyase (FHL) is an enzyme complex that catalyzes the conversion of HCOOH to evolve CO₂ and H₂ (Eq. 1) [1–3]. This enzyme complex is composed of a formate dehydrogenase (FDH) [4,5] and a [NiFe] hydrogenase ([NiFe]H₂ase) [6–9], and catalyzes oxidation of HCOOH (Eq. 2) and evolution of H₂, respectively (Eq. 3).

\[
\text{HCOOH} \overset{\text{FDH}}{\rightarrow} \text{CO}_2 + 2\text{H}^+ + 2\text{e}^- \quad (2)
\]

\[
2\text{H}^+ + 2\text{e}^- \overset{[\text{NiFe}]\text{H}_2\text{ase}}{\rightarrow} \text{H}_2 \quad (3)
\]

We have previously reported a Ni⁹Ru¹¹ model complex [Ni⁹(X)HC(O)O(H)Ru¹¹(C₆Me₆)] (X = N,N'-dimethyl-3,7-diazanonane-1,9-dithiolato) that can catalyze fast conversion of HCOOH to H₂ and CO₂ [10]...
[turnover frequency \( \text{TOF} = (\text{mol of evolved } \text{H}_2/\text{mol of catalyst}) \text{ per hour} = 857 \text{ h}^{-1} \). This extremely fast reaction rate, however, prevented us from investigating the mechanism of \( \text{H}_2 \) formation.

As part of our efforts to investigate the mechanism of \( \text{H}_2/\text{CO}_2 \) formation from \( \text{HCOOH} \), we now report a dinuclear \( \text{RuI} \) complex \([\text{RuI}_2(\text{CO})_4(\mu-\text{HCOO})_2(\text{DMSO})_2]\) (1) that catalyzes the above reaction at a significantly slower reaction rate (\( \text{TOF} = 13.1 \text{ h}^{-1} \)). Here we disclose the detailed mechanism of \( \text{H}_2 \) formation from \( \text{HCOOH} \) based on isotope-labeling experiments.

2. Experimental details

2.1. Materials and methods

All experiments were carried out under \( \text{N}_2 \) or \( \text{Ar} \) atmosphere by using standard Schlenk techniques and a glovebox. Tetrahydrofuran (THF) was distilled from \( \text{Na/benzophenone} \) under \( \text{N}_2 \) atmosphere prior to use. \( \text{HCOOH} \), \( \text{DCOOOD} \), \( 40\% \text{ NaOD/D}_2\text{O} \), and \( \text{dimethyl sulfoxide} \) (DMSO) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). \( \text{HCOOH} \) and \( \text{DCOOOD} \) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). These materials were used without further purification. Proton nuclear magnetic resonance (\( ^1\text{H} \) NMR) spectra were recorded on a JEOL JNM-AL300 spectrometer (JEOL, Tokyo, Japan) at 25 °C, in which the chemical shifts were referenced to tetramethylsilane (TMS) in chloroform-\( d_1 \) and DMSO in DMSO-\( d_6 \). UV–vis absorption spectra were recorded on a JASCO V–670 UV–Visible–NIR spectrophotometer (the light pass length was 1.0 cm). An IR spectrum was recorded on a Thermo Nicolet NEXUS 870 Fourier transform infrared (FTIR) instrument (Thermo Fisher Scientific, Massachusetts, USA) at 25 °C. Gas chromatographic (GC) analyses were conducted by a Shimadzu GC–8A (He carrier) (Shimadzu, Kyoto, Japan) with activated charcoal at –196 °C (liquid \( \text{N}_2 \)) for quantitative analyses for \( \text{H}_2, \text{HD}, \text{D}_2 \), and \( \text{CO}_2 \) by GC. No CO was observed. No nanoparticles were formed in the catalytic reaction, which was confirmed by dynamic light scattering measurements.

Isotope-labeling experiments for catalytic \( \text{H}_2, \text{HD}, \) and \( \text{D}_2 \) evolution. In a 3.0 mL vial capped with a septum, \( \text{DCOOOD} \) (2.60 mmol) in \( \text{H}_2\text{O} \) (1.0 mL) was added to 1 (1.25 \( \mu \)mol). The \( \text{pH} \) of the resulting solution was adjusted to 1.0–7.0 and the solution was heated at 80 °C for 1 h. The gas above the solution within the vial was sampled with a gas-tight syringe (500 \( \mu \)L) and analyzed for \( \text{H}_2 \) and \( \text{CO}_2 \) by GC. No CO was observed. No nanoparticles were formed in the catalytic reaction, which was confirmed by dynamic light scattering measurements.

The initial rate of the catalytic \( \text{H}_2 \) evolution against the catalyst concentration. In a 3.0 mL vial capped with a septum, a solution of \( \text{HCOOH} \) (2.60 mmol) in \( \text{H}_2\text{O} \) (1.0 mL) was added to 1 (0.63, 1.25, 2.5, or 5.0 mmol). The \( \text{pH} \) of the resulting solution was adjusted to 3.5 and the solution was heated at 80 °C for 300 s. The gas above the solution within the vial was sampled with a gas-tight syringe (500 \( \mu \)L) and analyzed for \( \text{H}_2 \) by GC. The catalytic reaction is first-order against the catalyst concentration.

Reactivity of hydride species 2 toward proton of \( \text{HCOOH} \). In a 3.0 mL vial capped with a septum, 10
equivalents of HCOOH (9.4 μL, 250 μmol) was added into a DMSO (2.0 mL) solution of 2 (vide infra), which was prepared from the reaction of 1 (14 mg, 25 μmol) with HCOONa (1.7 mg, 25 μmol) at 80 °C for 1 h. No H2 gas was formed, as confirmed by GC analysis. The same reaction was conducted using DMSO-d6 (450 μL) instead of DMSO (2.0 mL), which was monitored by 1H NMR spectroscopy. No decrease of the hydride-derived peak of 2 was observed.

H+/D+ exchange of hydride ligand of 2. In an NMR sample tube, a DMSO-d6 solution (400 μL) of 1 (14 mg, 25 μmol) with HCOONa (1.7 mg, 25 μmol) was heated at 80 °C for 1 h to form 2, which was confirmed by 1H NMR spectroscopy. Then, D2O (50 μL) was added into the resulting DMSO-d6 solution under N2 atmosphere. The H+/D+ exchange of hydride ligand of 2 was confirmed by 1H NMR spectroscopy with CH2Br2 as an internal standard to investigate the intensities of hydride- and formate-derived peaks.

X-ray crystallographic analysis of 1. A single crystal of 1 suitable for X-ray analysis was obtained from the diffusion of diethyl ether into its THF solution. Measurements were performed on a Rigaku/MSC Saturn CCD diffractometer (Rigaku, Tokyo, Japan) with confocal monochromated Mo-Kα radiation (λ = 0.7107 Å). Data were collected and processed using the CrystalClear program. All calculations were performed using the CrystalStructure crystallographic software package except for refinement, which was performed using SHELXL–97. Crystallographic data for 1 have been deposited at the Cambridge Crystallographic Data Centre as Supplementary Publication No. CCDC 1556459. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK {fax.: (+44)1223–336–033; e-mail: deposit@ccdc.cam.ac.uk}.

3. Results and discussion

A bis(μ-formate) RuI2 complex, [RuI2(CO)4(μ-HCOO)(DMSO)2] (1) was synthesized from the reaction of [Ru0(CO)12] with HCOOH and DMSO in THF under a N2 atmosphere, which was then characterized by X-ray analysis, and 1H NMR, UV-vis, and IR spectroscopies (Figures 1–4). Both Ru atoms adopt a distorted octahedral geometry in which the Ru ion is ligated by two C(CO), an S(DMSO), two O(μ-formate), and an adjacent Ru atom (Figure 1). Two Ru atoms are tethered by a Ru–Ru bond and two formate ligands. The bond distance of Ru1–Ru2 (2.6654(3) Å) is similar to those of other formate-bridged dinuclear Ru complexes (2.679 and 2.720 Å) [13,14]. The C–O bond distances of each HCOO− ligand (C1–O1 = 1.258(2), C1–O2 = 1.262(2), C2–O3 = 1.263(2), and C2–O4 = 1.259(2) Å) are almost the same, which indicates that this could be explained by the delocalization of a double bond over two C–O bonds. The C–O bond distances of four CO ligands {1.142(2)–1.148(2) Å} are slightly longer than that of free CO (1.128 Å), suggestive of back donation of electron density from the t2g orbital of low-valent RuI to the n*-antibonding orbital of CO.
The IR spectrum of 1 shows the stretching frequencies of CO coordinated to RuI centers at 1950, 1994, and 2041 cm⁻¹ (Figure 2), which are lower than the free CO stretching frequency (2143 cm⁻¹). This weakened CO bond is also caused by the back donation from the low-valent RuI to the CO ligand. The ¹H NMR signals observed in the diamagnetic region revealed that 1 is diamagnetic, which originates from an antiferromagnetic exchange interaction between two RuI centers through metal–metal bonding (Figure 3). The UV-vis absorption spectrum of 1 in H₂O shows a sharp band at 280 nm (8480 M⁻¹ cm⁻¹) and a broad band at 420 nm (550 M⁻¹ cm⁻¹) (Figure 4).
The bis(μ-formate) Ru$_2$ complex 1 can convert to a (hydride)(formate) species 2 with evolution of CO$_2$ in DMSO at 80 °C in the presence of 1 equivalent of HCOONa. β-Hydrogen elimination is expected to be assisted by the metal centers to release CO$_2$ and form the hydride ligand [15]. The $^1$H NMR spectrum of 2 in DMSO-$d_6$ shows hydride- and formate-derived signals at –12.4 and 7.76 ppm, respectively, with the same intensities (Figure 5(a)). The hydride-derived peak is typical of those found in hydride-bridged Ru complexes [16–19]. The dimer structure of 2 with an antiferromagnetic exchange interaction between two Ru$^+$ centers, was suggested by the signals observed in the diamagnetic region of the $^1$H NMR spectrum and its ESR silent character.

We have confirmed that the hydride ligand of 2 has a protic character rather than hydridic character based on the following investigations. We observed 2 being unreactive toward proton of HCOOH, i.e. dihydrogen gas was not formed via protonation of the hydride ligand, which was confirmed by $^1$H NMR spectroscopy and GC analysis. Then, we observed an H$^+$/D$^+$ exchange of the hydride ligand of 2, as confirmed by $^1$H NMR spectroscopy (Figure 5). The intensity of hydride-derived peak of 2 only decreased by the addition of D$_2$O into DMSO-$d_6$ solution of 2 (Figure 5), meaning that the hydride ligand underwent the H$^+$/D$^+$ exchange with D$^+$. Complex 1 is a precursor to catalyze the pH-dependent conversion of HCOOH to H$_2$ and CO$_2$ in water at pH 1.0–7.0 (Figures 6–10 and Table 1). H$_2$ and CO$_2$ gases were detected by GC. No nanoparticles were formed in the catalytic reaction, which was confirmed by dynamic light scattering measurements. Figure 6 shows the time-dependent profile of turnover numbers (TONs, mol of H$_2$ evolved/mol of 1) of H$_2$ evolution in the reaction of 1 with an excess amount of HCOOH in water at 80 °C. The pH-dependent TON shows a maximum around pH 3.5 (Figure 7). We investigated the dependence of initial rate for H$_2$ production against the concentration of 1 (0.63–5.0 mM). This linear correlation clearly indicates that the catalytic reaction is first-order against the catalyst concentration (Figure 8).

![Figure 9](image)

**Figure 9.** A proposed reaction mechanism for the conversion of HCOOH to H$_2$ and CO$_2$ catalyzed by dinuclear Ru complexes in H$_2$O.

![Figure 10](image)

**Figure 10.** A proposed reaction mechanism for the hydrogen isotope exchange reaction in the conversion of HCOOD to H$_2$, HD, D$_2$, and CO$_2$ in D$_2$O catalyzed by dinuclear Ru complexes (entry 3 of Table 1).
Table 1. Dehydrogenation of HCOOH, DCOOH, HCOOD, or DCOOD in H2O or D2O by using 1 as a precursor

| Entry | Substrate | Solvent | TONa | Ratio of H2: HD: D2 (%) |
|-------|-----------|---------|------|------------------------|
| 1     | HCOOH     | H2O     | 13.1 | H2: 100, HD: 0, D2: 0   |
| 2     | DCOOH     | H2O     | 5.2  | H2: 7, HD: 93, D2: 0    |
| 3     | HCOOD+    | D2O     | 6.7  | H2: 1, HD: 91, D2: 8    |
| 4     | DCOOD+    | D2O     | 2.4  | H2: 0, HD: 2, D2: 98    |

aReaction conditions: 1 (1.25 mM) and HCOOH (2.60 M), DCOOH (2.60 M), or DCOOD (2.60 M) in H2O or D2O at 80 °C at pH or pD 3.5.

4. Conclusions

Isotope-labeling experiments suggest that the formation of dihydrogen gas from formic acid takes place via H+/D+ exchange of the protic hydride ligands and reductive elimination from the protic hydride ligands of the dinuclear Ru complex in H2O/D2O.

Disclosure statement

No potential conflict of interest was reported by the authors.

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