ELSEVIER

Journal of Molecular Catalysis A: Chemical 96 (1995) 329–333

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

Effect of Hg(II) on hydrogenase activity from *Desulfovibrio vulgaris* (Miyazaki)

Toshiaki Kamachi, Seiichi Uno, Tomohiro Hiraishi, Ichiro Okura *

Department of Bioengineering, Tokyo Institute of Technology, Nagatsuta, Yokohama 227, Japan

Received 24 August 1994; accepted 8 November 1994

Abstract

Effect of Hg(II) on the intact hydrogenase activity from *Desulfovibrio vulgaris* (Miyazaki) was studied kinetically. When the hydrogenase was incubated with Hg(II), the hydrogen evolution rate was strongly inhibited. From the absorption spectra of the iron–sulfur cluster(s) and the EPR spectra of [3Fe–4S] cluster and Ni center, reaction mechanism is discussed.

Keywords: *Desulfovibrio vulgaris*; Hydrogenase; Inhibition; Mercury ion

1. Introduction

The hydrogenase from *Desulfovibrio vulgaris* (Miyazaki) has been purified, and the catalytic and the molecular properties have been studied extensively [1–5].

Effect of Hg(II) has been reported only for the tryptic digested hydrogenase and a displacement mechanism of iron ions of the iron–sulfur clusters by mercury ions has been proposed [10]. The tryptic digested hydrogenase, however, is partially hydrolyzed [11], and such hydrogenase may not reflect the properties of the native one. In this study, we hope to describe the effect of Hg(II) on the intact hydrogenase and to discuss the inhibition mechanism.

2. Experimental

2.1. Cultivation of bacteria

Desulfovibrio vulgaris (Miyazaki) was grown anaerobically at 30°C according to the literature [8], and stored at −80°C before use.

2.2. Purification of hydrogenase

The hydrogenase was solubilized from membrane fraction using non-ionic detergent Triton X-100 and was purified to homogeneity as reported previously [11].

2.3. Hydrogenase assay

The hydrogenase activity was measured by the hydrogen evolution rate in the presence of dithionite-reduced methyl viologen in a 5 ml test tube sealed with Septa at 30°C. The reaction mixture

* Corresponding author.

SSDI 1381-1169(94)00057-3

contained 200 μmol of Tris–HCl buffer (pH 7.4), 12 μmol of methyl viologen and 80 μmol of dithionite. One unit of activity was defined as 1 μmol H_2 evolved per min.

2.4. Protein assay

Protein concentration was determined by modified Lowry protein assay [9] with bovine serum albumin as a standard.

2.5. Absorption spectra

Optical spectra were measured by Shimadzu UV-265 spectrophotometer using a cell of 1 cm path length.

2.6. Inhibition of hydrogenase with Hg(II)

The hydrogenase was incubated with 1 mmol dm^{-3} Hg(II) for 4 days at 4°C. This Hg(II) inhibited hydrogenase was applied to Sephadex G-25 gel chromatography to remove excess of Hg(II) followed by ultrafiltration with PM-30 membrane (Amicon Co.) to concentrate the solution. The concentrated hydrogenase solution was deaerated and was frozen in an EPR tube at liquid nitrogen temperature to apply EPR measurement.

2.7. EPR measurement

EPR spectra were recorded with JEOL FE3XG spectrophotometer equipped with variable temperature cryostat, Air Products Heli-tran LTR-3-110.

3. Results and discussion

3.1. Inhibition of hydrogenase activity by Hg(II)

The sample of the hydrogenase as isolated was incubated at 4°C in the presence of Hg(II), and the hydrogen evolution rate from reduced methyl viologen was measured. Effect of incubation time is shown in Fig. 1. Hydrogenase activity decreased with incubation time with Hg(II). When 0.1 mmol dm^{-3} of Hg(II) was used, the hydrogenase

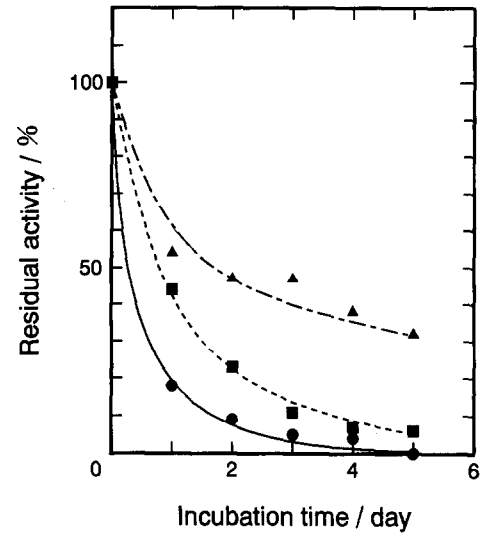

Fig. 1. Effect of incubation time on hydrogenase activity. The hydrogenase was incubated with 1 mmol dm^{-3} (●), 0.5 mmol dm^{-3} (■) or 0.1 mmol dm^{-3} (▲) of Hg(II) at 4°C in 25 mmol dm^{-3} Tris–HCl buffer, pH 7.4.

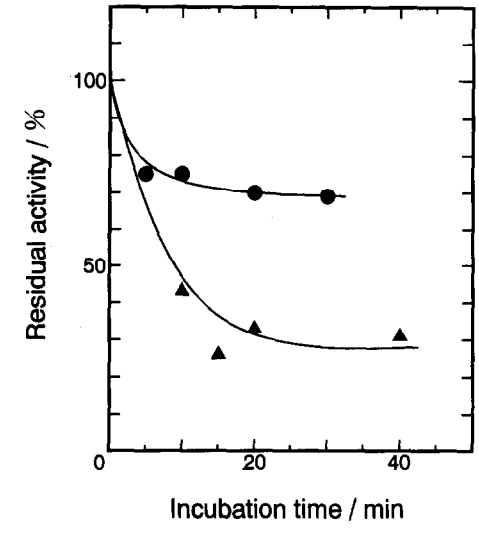

Fig. 2. Effect of incubation time on hydrogenase activity. The Triton X-100 solubilized hydrogenase (●) and tryptic digested hydrogenase (▲) were incubated with 10 mmol dm^{-3} Hg(II) in 25 mmol dm^{-3} Tris–HCl buffer, pH 7.4 and their relative activities were measured at the time indicated.

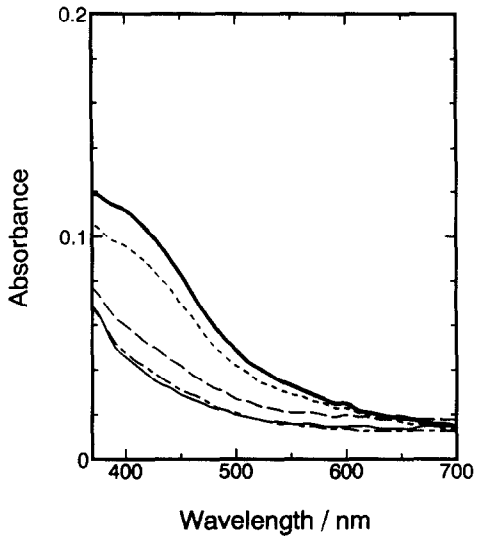

Fig. 3. Effect of Hg(II) on the absorption spectrum. The hydrogenase was incubated with 1 mmol dm^{-3} Hg(II) at 4°C in 25 mmol dm^{-3} Tris–HCl buffer, pH 7.4 and their absorption spectrum were measured at 0 min (——), 5 min (– – – –), 10 min (— — —), 100 min (– · – · –), 200 min (——).

became inactive for 5 days' incubation. The inhibition of hydrogenase with Hg(II) was irreversible since no activity was detected after removal of excess Hg(II) by gel filtration with Sephadex G-50. In the absence of Hg(II), the hydrogenase was stable at least 10 days at 4°C. These results are almost the same as in the case with the tryptic digested hydrogenase [8].

Fig. 2 shows a comparison of the activities of Triton X-100 solubilized, native, hydrogenase and tryptic digested one against Hg(II). The Triton X-100 solubilized hydrogenase is much more stable against Hg(II) than tryptic digested one. In Triton X-100 solubilized hydrogenase, another 2 kDa polypeptide is left [11] compared with tryptic digested one, and the polypeptide may play an important role to disturb the attack of Hg(II) against the active site of the hydrogenase.

3.2. *Effect of Hg(II) on the absorption spectrum*

Fig. 3 shows the time dependence of optical absorption spectra of the hydrogenase treated with 1 mmol dm^{-3} Hg(II). The absorption band at 400 nm decreased with incubation time. This indicates the destruction of iron–sulfur clusters in the hydrogenase with Hg(II) treatment. The absorption change at 400 nm and the hydrogenase activities during the incubation of hydrogenase with Hg(II) are shown in Fig. 4. The absorption band at 400 nm decreased coincidentally with the loss of hydrogenase activity.

3.3. *Effect of Hg(II) on EPR signals*

The effect of Hg(II) on the hydrogenase was studied by EPR method to focus the iron–sulfur center(s) and Ni center. Fig. 5 shows the low temperature EPR spectra of the hydrogenase. The intact hydrogenase as isolated shows a typical [3Fe–4S] EPR signal at low temperatures below 30 K as shown in Fig. 5-a. On treatment of hydrogenase with Hg(II) as mentioned in Experimental, the low temperature EPR signal generated from [3Fe–4S] cluster was eliminated and new signal was generated as shown in Fig. 5-b.

At high temperature, the intact hydrogenase shows typical EPR spectra generated from Ni center as shown in Fig. 6-a. The high temperature

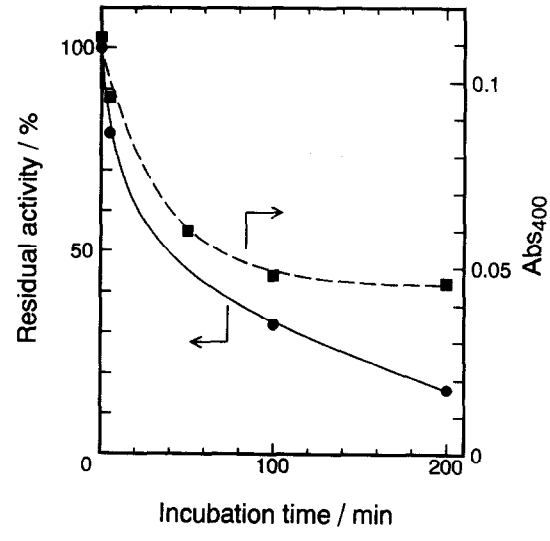

Fig. 4. Relationship between the absorbance at 400 nm and residual activity. The absorbance at 400 nm (■) and the residual activity (●) of the hydrogenase were plotted against the incubation of the hydrogenase with 1 mmol dm^{-3} of Hg(II) in Tris–HCl buffer, pH 7.4.

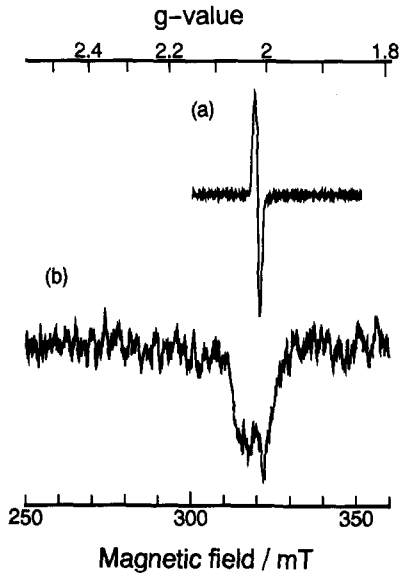

Fig. 5. Low temperature EPR spectrum of *D. vulgaris* (Miyazaki) hydrogenase. (a) intact hydrogenase; temp. 8.8 K; microwave power 0.1 mW; microwave frequency 9.031 GHz; modulation amplitude 1 G. (b) Hg(II) inhibited hydrogenase; temp. 8.7 K; microwave power 1 mW; Microwave frequency 8.998 GHz; modulation amplitude 2.5 G.

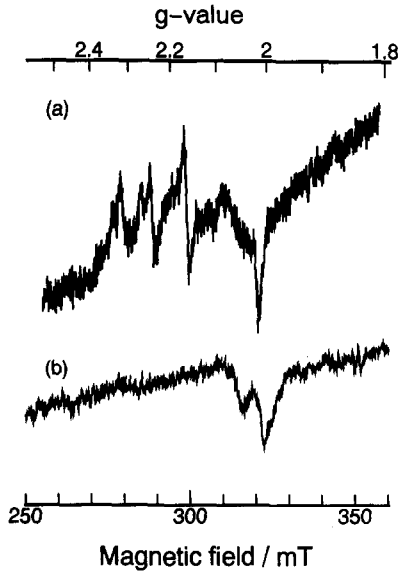

Fig. 6. High temperature EPR spectrum of *D. vulgaris* (Miyazaki) hydrogenase. (a) intact hydrogenase; temp. 113.5 K; microwave power 10 mW; microwave frequency 9.030 GHz; modulation amplitude 10 G. (b) Hg(II) inhibited hydrogenase; temp. 118.2 K; microwave power 10 mW; Microwave frequency 8.996 GHz; modulation amplitude 5 G.

EPR signal also changed on treatment of hydrogenase with Hg(II) as shown in Fig. 6-b.

These data indicate that either the iron atom(s) in the cluster and/or the nickel atoms may exchange for Hg ions or Hg(II) may interact magnetically with the above center(s).

3.4. EPR signal of [3Fe–4S] cluster on Hg(II) treatment

The hydrogenase was incubated with 1 mmol dm^{-3} of Hg(II) at 4°C, and excess Hg(II) was also removed from the hydrogenase samples by using gel filtration. The results are shown in Fig. 7. The intensities of the double integrated EPR signal of the [3Fe–4S] cluster decreased with incubation time simultaneously with the decrease of the hydrogenase activity. These results support the inhibition of the hydrogenase with Hg(II) was caused by the destruction of [3Fe–4S] cluster.

The effect of Hg(II) was also studied with the hydrogenase from *Desulfovibrio gigas* [6]. Though the metal contents, subunit structure or prosthetic groups of *D. gigas* hydrogenase were very similar to those of *D. vulgaris* (Miyazaki),

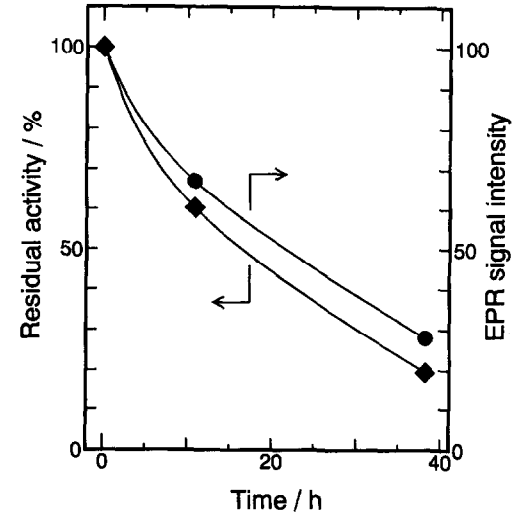

Fig. 7. Time dependence of EPR signal intensity and hydrogen evolution activity of *D. vulgaris* (Miyazaki) hydrogenase. The residual activity (◆) and EPR signal with $g=2.015$ intensity (●) after treatment with Hg(II) were plotted against the incubation time.

the relation between the EPR intensity and the hydrogenase activity is different. The EPR signal of the [3Fe–4S] center from *D. gigas* hydrogenase decreased prior to the decrease of the hydrogen uptake activity. But the decrease of the EPR signal amplitude of the [3Fe–4S] cluster from Hg(II) inhibited *D. vulgaris* (Miyazaki) hydrogenase was coincident with the decrease of the hydrogen evolution. Though the reason is not clarified yet, the inhibition mechanism may be different from that of *D. gigas* hydrogenase.

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research on Priority Area 'Organic Electrochemistry' (No. 06226226) from the Ministry of Education, Science and Culture, Japan.

References

[1] Y. Higuchi, N. Yasuoka, M. Kakudo, Y. Katsube, T. Yagi and H. Inokuchi, J. Biol. Chem., 262 (1987) 2823.
[2] H.M. Deckers, F.R. Wilson and G. Voordouw, J. Gen. Microbiol., 136 (1990) 2021.
[3] M. Asso, B. Guigliarelli, T. Yagi and P. Bertrand, Biochim. Biophys. Acta, 1122 (1992) 50.
[4] T. Yagi, K. Kimura, H. Daidoji, F. Sakai, S. Tamura and N. Inokuchi, J. Biochem., 79 (1976) 661.
[5] C.E. Hatchikian, M. Bruschi and J. LeGall, Biochem. Biophys. Res. Commun., 82 (1978) 451.
[6] V.M. Fernandez, M.L. Rua, P. Reyes, R. Cammack and E.C. Hatchikian, Eur. J. Biochem., 185 (1989) 449.
[7] T.F. Fagan and S.G. Mayhew, Biochem. J., 293 (1993) 237.
[8] T. Yagi, K. Kimura, H. Daidoji, F. Sakai, S. Tamura and H. Inokuchi, J. Biochem., 79 (1976) 661.
[9] A. Bensadoun and D. Weinstein, Anal. Biochem., 70 (1976) 241.
[10] I. Okura, K. Nakamura and S. Nakamura, J. Mol. Catal., 6 (1979) 311.
[11] T. Kamachi, S. Uno, T. Hiraishi, and I. Okura, J. Mol. Catal., 95 (1995) 93.