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Kinetic studies of electron transfer on photoinduced hydrogen evolution with hydrogenase

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Abstract

The kinetic properties of the elementary processes of electron transfer on photoinduced hydrogen evolution with hydrogenase, using cytochrome c_3 and methyl viologen as electron carriers, were studied. The electron transfer from reduced methyl viologen to cytochrome c_3 was monophasic and proceeded with a rate constant of 1.5×10^7 mol^{-1} dm^3 s^{-1}. The rate constants for hydrogenase reduction with reduced cytochrome c_3 were determined to be $k_2 = 154$ s^{-1} and $K_m = 5.4 \times 10^{-7}$ mol dm^{-3}, and were more efficient than that of reduced methyl viologen. These results indicate that cytochrome c_3 reduction with reduced methyl viologen occurs, followed by rapid electron transfer from reduced cytochrome c_3 to hydrogenase.

Keywords: Cytochrome c_3; Electron transfer; Hydrogenase; Photoinduced hydrogen evolution

1. Introduction

Photoinduced hydrogen evolution from water has been studied extensively using a system composed of four components: electron donor, photosensitizer, electron carrier and catalyst [1]. The photoexcited triplet state of the photosensitizer, such as zinc(II) tetraphenylporphyrin tetrasulphonate (ZnTPPS), reduces the electron carrier with the concomitant oxidation of the electron donor. The reduced electron carrier reacts with the catalyst, such as hydrogenase, so that hydrogen is evolved. Although methyl viologen is often used as the electron carrier in such a system, cytochrome c_3 may be a better electron carrier than methyl viologen for photoinduced hydrogen evolution, as cytochrome c_3 is the natural electron carrier of hydrogenase from *Desulphovibrio vulgaris* (Miyazaki). As reported previously, efficient photoinduced hydrogen evolution has been observed for a system with hydrogenase (Hase), using methyl viologen (MV) and cytochrome c_3 (Cyt. c_3) as electron carriers (Scheme 1) [2].

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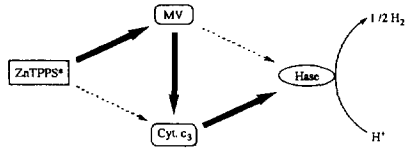

Scheme 1.

In this scheme, the photoexcited triplet state of ZnTPPS reacts more easily with methyl viologen than cytochrome c_3, and reduced methyl viologen predominantly transfers electrons to cytochrome c_3. Reduced cytochrome c_3 reacts with hydrogenase and hydrogen is evolved.

For more detailed information on photoinduced hydrogen evolution, it is necessary to obtain kinetic data on the elementary processes of photoinduced hydrogen evolution. In this investigation, kinetic studies of the elementary processes of electron transfer reactions in this photoinduced hydrogen evolution system were carried out. Data on the electron transfer between reduced cytochrome c_3 and hydrogenase were compared with those of reduced methyl viologen, and the electron transfer pathway was discussed.

2. Experimental details

Hydrogenase and cytochrome c_3 from *D. vulgaris* (Miyazaki) were purified according to the literature [2,3]. Protein concentrations were determined using the following molar absorption coefficients: for cytochrome c_3, $\epsilon = 110$ mmol^{-1} dm^3 cm^{-1} at the reduced α-band maximum (552 nm); for hydrogenase, $\epsilon = 47$ mmol^{-1} dm^3 cm^{-1} at 400 nm. Sample solutions were prepared in 25 mmol dm^{-3} Tris–HCl buffer (Tris, tris(hydroxymethyl)aminomethane) (pH 7.4) and deaerated by repeated freeze–pump–thaw cycles. The con-

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centration of dithionite was determined by anaerobic spectrophotometric titration against ferricyanide monitored at 420 nm [4]. Sample solutions were transferred to a stopped-flow spectrophotometer by means of a gas-tight syringe. Stopped-flow measurements were carried out using an Otsuka Electronical Inc. RA-415 stopped-flow apparatus operated under anaerobic conditions at 23 °C.

3. Results and discussion

When cytochrome c_3 was mixed anaerobically with various concentrations of dithionite-reduced methyl viologen, monophasic reaction traces were observed at 552 nm as shown in Fig. 1. Fig. 1(a) shows the time dependence of the reduction of 0.5 μmol dm^{-3} cytochrome c_3 with 0.25 μmol dm^{-3} of dithionite-reduced methyl viologen. The reaction was completed within 300 ms. As the reduced methyl viologen concentration increases, the rate of cytochrome c_3 reduction increases as shown in Fig. 1(b). These results indicate that cytochrome c_3 is reduced by reduced methyl viologen and excess dithionite. Fig. 2 shows the dependence of the pseudo-first-order rate constants of electron transfer between reduced methyl viologen and cytochrome c_3 on the concentration of dithionite-reduced methyl viologen. Such a plot can be fitted to the following relationship

$$k_{app} = k_1[MV^+] + k_2 \quad (1)$$

where k_{app} is the apparent rate constant, k_1 is the rate constant of cytochrome c_3 reduction with reduced methyl viologen, k_2 is the rate constant of cytochrome c_3 reduction with excess dithionite and $[MV^+]$ is the concentration of dithionite-reduced methyl viologen. The slope of the k_{app} vs. $[MV^+]$ plot gives $k_1 = 1.5 \times 10^7$ mol^{-1} dm^3 s^{-1} and the intercept at $[MV^+] = 0$ gives the rate constant of cytochrome c_3 reduction with excess dithionite as $k_2 = 8.3$ s^{-1}.

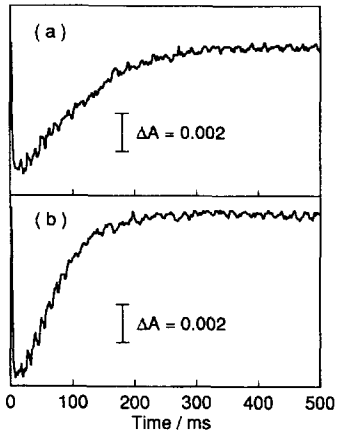


Fig. 1. Kinetic traces of cytochrome c_3 reduction with reduced methyl viologen (the sample solution contained dithionite (0.24 μmol dm^{-3}) and cytochrome c_3 (0.5 μmol dm^{-3}) in 25 mmol dm^{-3} Tris–HCl buffer (pH 7.4)): (a) 0.25 μmol dm^{-3} reduced methyl viologen; (b) 1.5 μmol dm^{-3} reduced methyl viologen. Both traces were monitored at 552 nm.

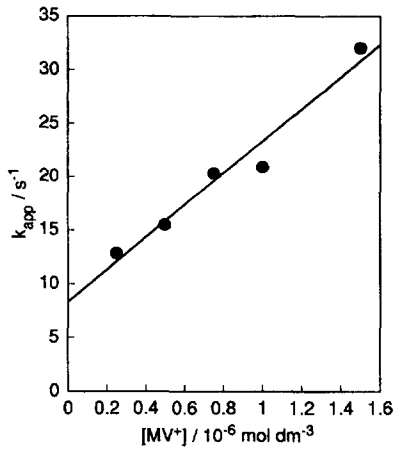


Fig. 2. Dependence of the pseudo-first-order rate constants of cytochrome c_3 reduction with reduced methyl viologen on the reduced methyl viologen concentration.

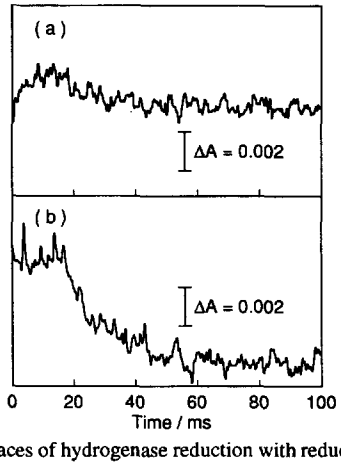


Fig. 3. Kinetic traces of hydrogenase reduction with reduced cytochrome c_3 (the sample solution contained dithionite (0.24 μmol dm^{-3}) and cytochrome c_3 (0.5 μmol dm^{-3}) in 25 mmol dm^{-3} Tris–HCl buffer (pH 7.4)): (a) 0.26 μmol dm^{-3} hydrogenase; (b) 1.5 μmol dm^{-3} hydrogenase. Both traces were monitored at 552 nm.

The electron transfer rates of dithionite-reduced cytochrome c_3 to hydrogenase were measured with the stopped-flow apparatus under anaerobic conditions. When 0.5 μmol dm^{-3} of dithionite-reduced cytochrome c_3 was mixed with various concentrations of hydrogenase, monophasic absorbance changes were observed at 552 nm as shown in Fig. 3. The dependence of the hydrogenase reduction rate on the hydrogenase concentration shows a hyperbolic character as illustrated in Fig. 4. This result indicates that reduced cytochrome c_3 and hydrogenase interact specifically via complex formation as shown below

$$\mathrm{Cyt.c_{3red} + Hase_{ox} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Cyt.c_{3red} - - Hase_{ox}] \xrightarrow{k_2}}$$

$$\mathrm{Cyt.c_{3ox} + Hase_{red}}$$

With the rapid equilibrium assumption [5], k_{app} is related to the individual constants as

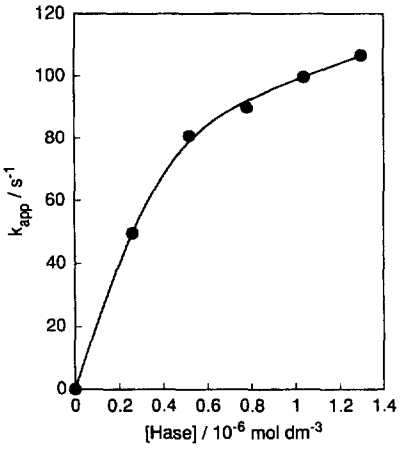


Fig. 4. Dependence of the pseudo-first-order rate constants of hydrogenase reduction with reduced cytochrome c_3 on the hydrogenase concentration.

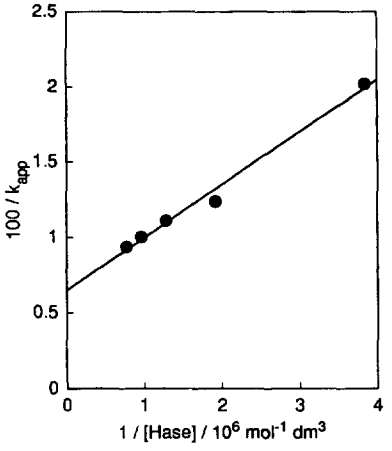
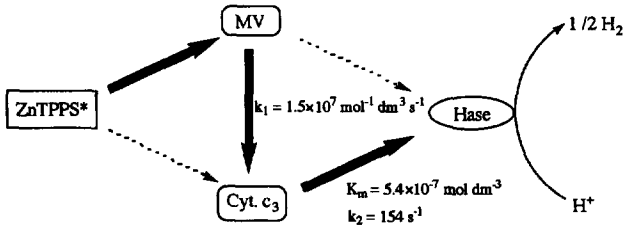


Fig. 5. Double reciprocal plot of the pseudo-first-order rate constants of hydrogenase reduction vs. the hydrogenase concentration.

$$\frac{1}{k_{app}} = \frac{1}{k_2} + \frac{k_{-1}}{k_1 k_2} \frac{1}{[\text{Hase}]} \quad (2)$$

The double reciprocal plot of k_{app} vs. [Hase] is shown in Fig. 5. The rate constants were determined from this plot as follows: $k_2 = 154\ s^{-1}$ and $K_m = k_{-1}/k_1 = 5.4 \times 10^{-7}$ mol dm^{-3}. This result indicates that the second-order rate of electron transfer from reduced cytochrome c_3 to hydrogenase, k_2/K_m, is close to a diffusion-controlled rate ($k_q = 10^9$ mol^{-1} dm^3 s^{-1} [6]).

When 0.5 μmol dm^{-3} of dithionite-reduced methyl viologen was mixed with 0.5 μmol dm^{-3} of hydrogenase, a monophasic absorbance change was observed at 600 nm (data not shown). The apparent first-order rate of hydrogenase reduction with reduced methyl viologen was much lower than that of reduced cytochrome c_3. From the kinetic analysis, the cytochrome c_3 reduction with reduced methyl viologen may be the rate-limiting step in photoinduced hydrogen evolution. These results indicate that photoinduced hydrogen evolution using methyl viologen and cytochrome c_3 proceeds efficiently as shown by the bold arrows in Scheme 2.



Scheme 2.

Acknowledgements

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References

[1] S. Aono, I. Okura and A. Yamada, *J. Phys. Chem., 89* (1985) 1593.
[2] T. Kamachi, T. Hiraishi and I. Okura, *Chem. Lett.*, (1995) 33.
[3] T. Yagi, K. Kimura, H. Daidoji, F. Sakai, S. Tamura and H. Inokuchi, *J. Biochem., 79* (1976) 661.
[4] C. Creutz and N. Sutin, *Proc. Natl. Acad. Sci. USA, 70* (1973) 1701.
[5] S. Strickland, G. Palmer and V. Massey, *J. Biol. Chem., 250* (1975) 4048.
[6] C. Capeillère-Blandin, *Eur. J. Biochem., 128* (1982) 533.