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Photocatalytic Conversion of Lactic Acid to Malic Acid through Pyruvic Acid in the Presence of Malic Enzyme and Semiconductor Photocatalysts

Hiroshi Inoue, Mikio Yamachika and Hiroshi Yoneyama*
Department of Applied Chemistry, Faculty of Engineering, Osaka University, Yamada-oka 2-1, Suita, Osaka 565, Japan

The photocatalytic fixation of CO_2 in pyruvic acid to yield malic acid has been achieved in TiO_2 microcrystal and CdS particle suspensions using malic enzyme as the catalyst, methyl viologen as the electron mediator and either 2-mercaptoethanol or lactic acid as the hole scavenger. The evaluation of the rate-determining step in the photocatalytic fixation reaction was made at the TiO_2 microcrystal for the case of 2-mercaptoethanol as the hole scavenger. The use of lactic acid as the hole scavenger resulted in the selective photoproduction of pyruvic acid at the CdS photocatalyst, a fraction of which was then converted into malic acid by reductive CO_2 fixation at the malic enzyme. However, it has been found that lactic acid causes a decrease in the catalytic activities of the enzyme, the degree depending on the relative concentrations of malic enzyme and lactic acid.

Recently fairly intensive attention has been paid to the electrochemical[1–14] and photochemical[15–21] fixation of CO_2 in a variety of organic molecules under mild conditions. Willner *et al.*[19,20] succeeded in the photochemical fixation of CO_2 molecules in oxoglutaric acid and pyruvic acid in the presence of isocitrate dehydrogenase and malic enzyme (ME), respectively, using ruthenium trisbipyridine and metalloporphyrins as photosensitizers.

It was reported from our laboratory[13] that electrolysis of CO_2-saturated TRIS buffer containing oxoglutaric acid, isocitrate dehydrogenase and methyl viologen (MV^{2+}) as an electron mediator yielded isocitric acid with a current efficiency approaching 100%. Later, this electrochemical fixation reaction was applied to a photocatalytic reaction system using CdS as a photocatalyst.[21] Electrochemical fixation of CO_2 in pyruvic acid using ME was also achieved at a current efficiency approaching 100%.[14] However, as in the photochemical fixation reactions reported by Willner *et al.*,[19,20] both ferredoxin–$NADP^+$–reductase (FNR) and $NADP^+$ were essential besides MV^{2+} to fix CO_2 in pyruvic acid, which is different from the case of electrochemical fixation of CO_2 in oxoglutaric acid where both FNR and $NADP^+$ could be eliminated.

In the present study, we have investigated the photochemical fixation of CO_2 in pyruvic acid using photogenerated electrons in semiconductor photocatalysts. Since irradiation of semiconductor photocatalysts with light of energy greater than its bandgaps generates positive holes as well as electrons, it is important to design the overall photocatalytic reaction system taking into consideration how the photogenerated holes will be used. We first used 2-mercaptoethanol as a highly reactive hole scavenger in order to investigate how easily CO_2 fixation in pyruvic acid occurs with the consumption of photogenerated electrons. Since the oxidation product, possibly bis(2-hydroxyethyl)disulfide,[22] does not have a wide utility, it is desirable to produce more useful compounds in the photocatalytic reactions.

Harada *et al.*[23] found that latic acid is oxidized to pyruvic acid with the evolution of H_2 at Pt-loaded semiconductor photocatalysts such as CdS and TiO_2. It is expected that if lactic acid is used in the place of 2-mercaptoethanol, malic acid will be produced as a result of the enzymatic fixation of CO_2 in pyruvic acid, which is produced from photooxidation of lactic acid. The postulated reaction schemes for that case are shown in Fig. 1. The Gibbs free energy change estimated for the net reaction is $+57.7$ kJ mol^{-1}. The use of MV^{2+} as an electron mediator sets energy requirements for the semiconductor photocatalysts such that the potential of the conduction band edge of the catalysts should be negative enough to reduce MV^{2+}. In this regard, CdS macrocrystalline powder and TiO_2 microcrystals fixed in the interlayer spacings of sodium montmorillonite,[24] which is represented in this paper as TiO_2/clay, were used as the photocatalysts.

Experimental

ME (from chicken liver, EC 1.1.1.40) and FNR (from spinach leaves, EC 1.18.1.2) were used as received from Sigma. According to information from the dealer, the former was subjected to successive purification procedures involving extraction, fractionation in ammonium sulfate solution and gel filtration, and then stored in 2.9 mol dm^{-3} ammonium sulfate solution (pH 6.0) containing 10 mmol dm^{-3} potassium phosphate, 0.5 mmol dm^{-3} 2-mercaptoethanol, 10 mmol dm^{-3} manganese chloride and 3 mmol dm^{-3} Na_4EDTA. $NADP^+$ and NADPH were used as received from Oriental Yeast. Sodium pyruvate, sodium lactate, 2-mercaptoethanol, magnesium chloride (Wako Pure), CdS of 99.99% purity (Mitsuwa Chemicals) and MV^{2+} (Tokyo Kasei Kogyo Ltd. Co.) were used as received. The crystal structure of CdS was wurtzite and its specific surface area was 23.1 m^2 g^{-1}, as described previously.[21] TiO_2 microcrystals were prepared in the interlayer spacings of sodium montmorillonite.

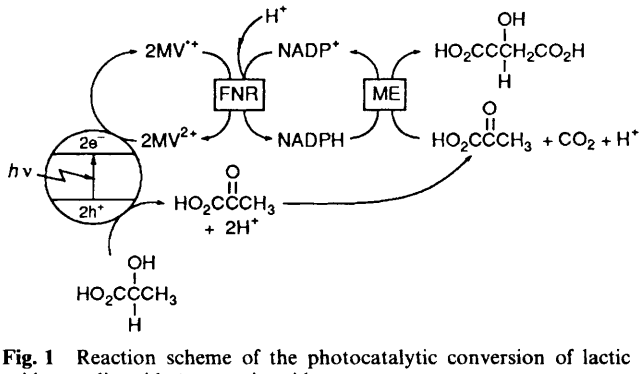

Fig. 1 Reaction scheme of the photocatalytic conversion of lactic acid to malic acid *via* pyruvic acid

Details of the preparation and characterization of the TiO_2/clay were reported in a previous paper.[24] The specific surface area of the TiO_2 microcrystals ranged between 203 and 275 $m^2\ g^{-1}$. The amount of incorporated TiO_2 in the clay was 50 wt.%.

Photocatalytic experiments were carried out using a quartz cell (*ca.* 160 mm height and 18 mm diameter) which contained 10 cm^3 of reaction solution and 1.25 mmol dm^{-3} suspended TiO_2/clay or CdS. After the reaction solution was saturated with CO_2 for 1 h, the cell was closed and illuminated with a 500 W high-pressure Hg arc lamp (Ushio UI-501C) through a Toshiba UV-31 filter for the case of TiO_2/clay, while illumination with a 500 W xenon lamp (Ushio UI-501C) through a Toshiba UV-39 filter was performed in the case of CdS. The cell was set in a water bath at 25 °C. The reaction solution was magnetically stirred during illumination.

Changes in the concentration of NADPH caused by the enzymatic and photocatalytic fixation reactions were followed by absorption spectroscopy at 340 nm (molar absorption coefficient 6200 $dm^3\ mol^{-1}\ cm^{-1}$[25]) using an HP 8452A diode-array spectrophotometer (Hewlett Packard). The production of malic acid and pyruvic acid in the photocatalytic experiments was monitored using a high-pressure liquid chromatograph (Tosoh CCPE) equipped with an organic acid column (Waters N23696) and a UV detector (Tosoh UV-8011). The eluent solution was 0.1% H_3PO_4.

The quantum efficiency for malic acid production at the TiO_2/clay photocatalysts was determined with illumination of monochromatic light of 313 nm obtained by using a monochromater (JASCO, CT-10). Its full width at half maximum (FWHM) intensity was 6 nm. When the CdS photocatalyst was used the quantum efficiency was determined with illumination at 410 nm obtained by using an interference filter (Vacuum Optics Corporation of Japan). Its FWHM was 14 nm. The quantum efficiency for malic acid production is defined by the following equation.

$$\text{quantum efficiency} = \frac{\text{(amount of malic acid production for 1 h)} \times 2}{\text{(number of incident photons for 1 h)}} \times 100$$

The number of incident photons was determined by ferrioxalate actinometry.

Results and Discussion

Photochemical Fixation of CO_2 in Pyruvic Acid using 2-Mercaptoethanol as a Hole Scavenger

Fig. 2 shows the time course of malic acid production obtained in 10 cm^3 of CO_2-saturated TRIS buffer solution (composition is given in the figure caption). Illumination of these suspensions resulted in the selective production of malic acid in proportion to the illumination time for the first few hours. No other reduction products of CO_2 were detected. If the semiconductor photocatalysts were eliminated from the reaction solution containing MV^{2+}, FNR, $NADP^+$, ME and hole scavenger, malic acid was not produced, suggesting that the semiconductor photocatalysts are essential for the production of malic acid. In Fig. 2 the turnover number (TON) of the enzyme is also given. For this estimation, we need the number of moles of active sites per ME molecule, which was estimated to be 5.5×10^{-10} mol with assumption that the molar mass of ME extracted from chicken liver is 2.6×10^5,

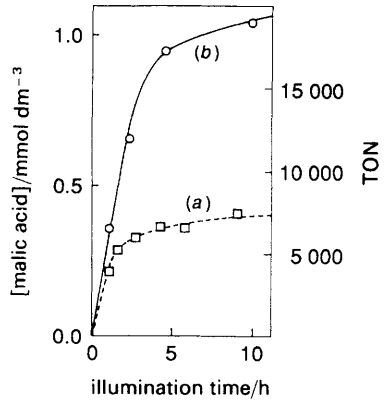

Fig. 2 Time course of malic acid production at the TiO_2/clay (*a*) and CdS (*b*) photocatalysts. The solution was 10 cm^3 of CO_2-saturated TRIS buffer containing 1 mmol dm^{-3} MV^{2+}, 0.1 mmol dm^{-3} $NADP^+$, 0.2 unit of FNR, 1 unit of ME, 2 mmol dm^{-3} pyruvic acid, 27 mmol dm^{-3} 2-mercaptoethanol and 1.25 mmol dm^{-3} TiO_2/clay or 1.25 mmol dm^{-3} CdS. TON of ME in malic acid production is also given

one ME molecule contains four active-centre subunits[26,27] and one unit of ME contains 3.6×10^{-5} g of ME protein.[26,27] The TON obtained was very large.

Malic acid production became stagnated with illumination times >5 h for the CdS suspension and >1.5 h for the TiO_2/clay suspension. It is thought that at these stages, *ca.* 50% of pyruvic acid was converted to malic acid at CdS and *ca.* 15% at TiO_2/clay. It is therefore unlikely that the observed stagnation resulted from a deficiency of the substrate. If 1.25 mmol dm^{-3} TiO_2/clay was suspended in an aqueous solution containing 0.2 mmol dm^{-3} MV^{2+} and 27 mmol dm^{-3} 2-mercaptoethanol and then illuminated by an Hg lamp ($\lambda > 310$ nm), the $MV^{\cdot+}$ production was completed after illumination for *ca.* 10 min. Since 1 mmol dm^{-3} MV^{2+} was used in the above-described photocatalytic fixation of CO_2 in pyruvic acid, the reduction of MV^{2+} must have been completed after illumination for 50 min. However, since the stagnation of malic acid production appeared after illumination for 1.5 h for the case of the TiO_2/clay photocatalyst, as shown in Fig. 2(*a*), it is concluded that this does not result from the stagnation of MV^+ production. Why the malic acid production was stagnated with an increase in the illumination time is a matter for discussion. There may be at least two causes, (i) the photodegradation of ME and (ii) the predominance of the back reaction of the enzymatic CO_2 fixation at ME due to the reaction equilibrium:

$$\text{pyruvic acid} + CO_2 + NADPH + H^+ \rightleftharpoons \text{malic acid} + NADP^+$$

To clarify which is responsible, one unit of fresh ME was added to the reaction solution at the time when a noticeable stagnation was observed, *e.g.* 5 h for TiO_2/clay. The malic acid production then began to occur again, suggesting that the degradation of ME, rather than the equilibrium of the enzymatic CO_2 fixation reaction at ME (see Fig. 1), is responsible for the observed stagnation of the malic acid production. It is more likely that ME was gradually degraded by photoinduced oxidation at the photocatalyst surfaces. In contrast, the addition of FNR or $NADP^+$ or the both did not result in any appreciable improvement in the malic acid production. These results suggest that the incomplete regeneration of $NADP^+$, if any, was not responsible for the stagnation of the malic acid production.

If the amount of malic acid is obtained as a function of illumination time for several concentrations of pyruvic acid

and the rate of malic acid production is estimated at an initial stage of the illumination, then the rate of malic acid production is related to the concentration of pyruvic acid, as shown in Fig. 3. Similar relations were obtained at the CdS photocatalyst. In the case of the TiO_2/clay photocatalyst, the rate of the malic acid production was proportional to the concentration of pyruvic acid up to 1 mmol dm^{-3}, beyond which the rate became saturated. It is then thought that below 1 mmol dm^{-3} of pyruvic acid, enzymatic fixation of CO_2 at ME (see Fig. 1) must be the rate-determining step. For concentrations >1 mmol dm^{-3}, one of the other four electron-transfer steps constituting the photochemical enzymatic fixation process of CO_2 (see Fig. 1) should be rate determining.

In order to clarify the rate-determining step, the influence of the amounts of ME, FNR and $NADP^+$ on the rate of malic acid production was investigated at fixed concentrations of pyruvic acid and MV^{2+} of 2 mmol dm^{-3} and 1 mmol dm^{-3}, respectively. An increase of ME from 1 unit to 5 units or $NADP^+$ from 0.1 to 0.2 mmol dm^{-3} did not increase the rate of malic acid production, while an increase of FNR from 0.2 to 1 unit resulted in a 2.2 times increase in the rate of malic acid production. Therefore it is concluded that the electron transfer between MV^+ and FNR is the rate-determining step. The apparent quantum efficiencies for malic acid production determined for 1.25 mmol dm^{-3} photocatalysts in the presence of 1 mmol dm^{-3} MV^{2+}, 0.08 unit of FNR, 0.1 mmol dm^{-3} $NADP^+$ 0.4 unit of ME and 2 mmol dm^{-3} pyruvic acid were 9.7% for TiO_2/clay and 0.9% for CdS. In the case of the former photocatalyst, NADPH also absorbs the monochromatic light. Since the absorbance at 313 nm of 0.1 mmol dm^{-3} NADPH was one tenth that of the 1.25 mmol dm^{-3} TiO_2/clay photocatalyst, it is thought that 90% of the irradiated photons must have been used in the photo-excitation of the TiO_2/clay. The net quantum efficiency in the use of the TiO_2/clay would then be 10.7%. One may think from the results shown in Fig. 2 that the CdS photocatalyst should give a higher quantum efficiency than the TiO_2/clay. However, the light source used in the experiments was different, and the absorption threshold of the semiconductor photocatalysts was different. The low quantum efficiency obtained at the CdS photocatalyst was improved slightly to 2.5% if the amount of suspended photocatalyst was increased to 6.25 mmol dm^{-3}.

Judging from results reported for TiO_2 photocatalysts,[28] pyruvic acid used as a substrate may be reduced to lactic acid. This was found to be true at the TiO_2/clay photocatalyst if the concentration of the photocatalyst was increased from 1.25 to 6.25 mmol dm^{-3} without changing the concentration of MV^{2+} (1 mmol dm^{-3}). The reduction of pyruvic acid must take place if the concentration of MV^{2+} becomes, in a relative sense, too small to be reduced selectively at the photocatalyst surface. As long as the concentration of the photocatalyst was kept at 1.25 mmol dm^{-3} in the presence of 1 mmol dm^{-3} MV^{2+}, MV^{2+} alone was photoreduced, allowing the selective fixation of CO_2 in pyruvic acid.

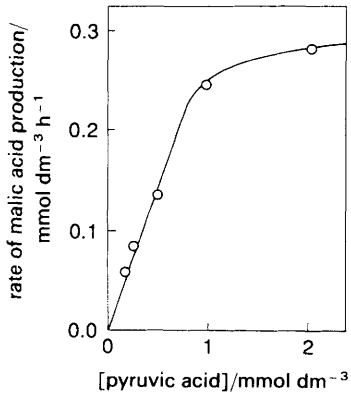

Fig. 3 Rate of the malic acid production at the TiO_2/clay photocatalyst as a function of the concentration of pyruvic acid

As described above, the photochemical fixation of CO_2 in pyruvic acid at TiO_2/clay and CdS photocatalysts occurred with quantum efficiencies of 10.7 and 0.9%, respectively. On the other hand, the quantum efficiencies for photoreduction of MV^{2+} to $MV^{\bullet+}$ obtained under the same experimental conditions, but in the absence of $NADP^+$, FNR and ME, were 16.4% for TiO_2/clay and 8.3% for CdS. The obtained quantum efficiencies for $MV^{\bullet+}$ production were much higher than that for malic acid production, giving support to the above-described view that the rate-determining step is not the reduction of MV^{2+} to $MV^{\bullet+}$ but the electron transfer between $MV^{\bullet+}$ and FNR. It was found that the illumination intensity greatly influences the quantum efficiency for malic acid production, and the smaller the illumination intensity, the larger the quantum efficiency. For example, the quantum efficiencies obtained under the same solution conditions as described above, except for the concentration of the CdS photocatalyst which was 6.25 mmol dm^{-3}, were 2.5, 4.8 and 5.6% for illumination intensities of 1.73×10^{-3}, 1.61×10^{-4} and 2.59×10^{-5} W cm^{-2}, respectively.

Photocatalytic Conversion of Lactic Acid to Malic Acid through Pyruvic Acid

As shown above, the quantum efficiency for malic acid production at the CdS photocatalyst was smaller than that at the TiO_2/clay photocatalyst. However, the use of the CdS photocatalyst is preferred from the viewpoint that lactic acid is photo-oxidized to pyruvic acid. This photo-oxidation reaction did not occur at TiO_2/clay.

Fig. 4 shows the time course of the photoreduction of MV^{2+} and photo-oxidation of lactic acid to pyruvic acid on the CdS photocatalyst, which was obtained in CO_2-saturated TRIS buffer containing MV^{2+}, lactic acid and CdS. No other products were obtained by the photo-oxidation of lactic acid. Photogenerated electrons must be used in reducing MV^{2+} to $MV^{\bullet+}$, while photogenerated holes must be used in oxidizing lactic acid to pyruvic acid.

$$MV^{2+} + e^- \rightarrow MV^{\bullet+}$$

$$CH_3\underset{\underset{OH}{|}}{C}HCO_2H + 2h^+ \rightarrow CH_3\overset{\overset{O}{\|}}{C}CO_2H + 2H^+$$

According to the results given in Fig. 4, the rate of MV^+ production was twice that of pyruvic acid, satisfying the chemical stoichiometry for the light-induced reaction.

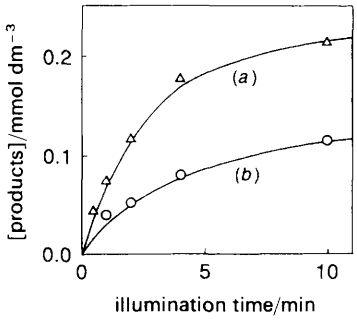

Fig. 4 Time course of the production of $MV^{\bullet+}$ (*a*) and pyruvic acid (*b*) under illumination by a Xe lamp. The solution was 4 cm^3 of CO_2-saturated TRIS buffer solution containing 0.25 mmol dm^{-3} MV^{2+}, 100 mmol dm^{-3} sodium lactate and 1.25 mmol dm^{-3} CdS

When FNR, $NADP^+$ and ME were added to the above solution, both pyruvic and malic acid were produced, as shown in Fig. 5, suggesting that a fraction of the photo-produced pyruvic acid was converted to malic acid by enzymatic CO_2 fixation. However, the amount of malic acid produced was *ca.* 1 μmol at most even after 10 h of illumination, which is much smaller than that achieved when using 2-mercaptoethanol as the hole scavenger. This result suggests that lactic acid exerts a negative effect on the enzymatic CO_2 fixation reaction. To confirm this view the effect of lactic acid on the catalytic activities of ME and FNR was investigated.

It is expected that in the enzymatic fixation of CO_2 in pyruvic acid NADPH is oxidized to $NADP^+$ at ME with the consumption of two electrons and the release of one proton (see Fig. 1). The decrease in NADPH concentration was experimentally confirmed; see the time course given in Fig. 6(*b*). NADPH concentration steadily decreased if the solution did not contain lactic acid. However, in the presence of 100 mmol dm^{-3} lactic acid the rate of decrease of the NADPH concentration was very low [see Fig. 6(*a*)].

According to the reaction scheme given by Fig. 1, $NADP^+$ is reduced to NADPH with the consumption of two electrons from two MV^+ and one proton. Fig. 7 confirms this result, where the time course of the production of NADPH is given. The results were obtained for 4 cm^3 of CO_2-saturated TRIS buffer solution containing MV^{2+}, FNR, CdS and 2-mercaptoethanol as the hole scavenger in the presence and absence of lactic acid. It is seen in Fig. 7 that lactic acid retards a little the reduction of $NADP^+$ to NADPH. By comparing the results shown in Fig. 6 with those in Fig. 7 it is concluded that lactic acid has a more serious effect on ME than on FNR.

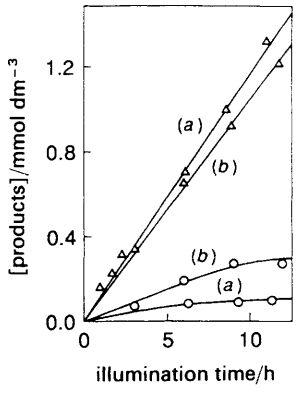

Fig. 5 Time course of the photoproduction of pyruvic acid (—△—) and malic acid (—○—) in the presence of 1 unit (*a*) and 5 units (*b*) of ME in 10 cm^3 of CO_2-saturated TRIS buffer solution containing 1 mmol dm^{-3} MV^{2+}, 0.2 unit of FNR, 0.1 mmol dm^{-3} $NADP^+$, 100 mmol dm^{-3} sodium lactate and 1.25 mmol dm^{-3} CdS

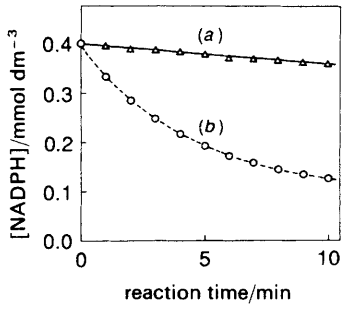

Fig. 6 Time course of the consumption of NADPH in the presence (a) and absence (b) of 100 mmol dm^{-3} lactic acid, obtained after 1 unit of ME was added to 4 cm^3 of a CO_2-saturated TRIS buffer solution containing 2 mmol dm^{-3} sodium pyruvate and 0.4 mmol dm^{-3} NADPH

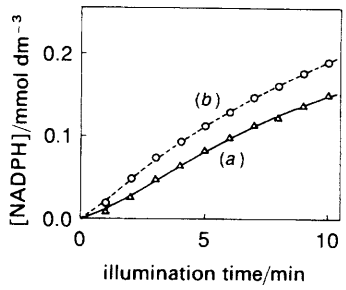

Fig. 7 Time course of the production of NADPH in the presence (*a*) and absence (*b*) of 100 mmol dm^{-3} lactic acid in 4 cm^3 of CO_2-saturated TRIS buffer solution containing 0.3 mmol dm^{-3} MV^{2+}, 0.4 mmol dm^{-3} $NADP^+$, 0.1 unit of FNR, 1.25 mmol dm^{-3} CdS and 27 mmol dm^{-3} 2-mercaptoethanol

As mentioned above, lactic acid has unfavourable effects on the activities of ME and FNR, especially on the former. This imposes a serious limitation in the light-induced enzymatic production of malic acid from lactic acid. However, the unfavourable effect of lactic acid on ME may be weakened by appropriately choosing the relative concentrations of these substances, as the results shown in Fig. 5 suggest. In that case, the use of 5 units of ME resulted in a greater amount of malic acid being produced with less suppression of the pyruvic acid production than in the case of 1 unit of ME.

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