CHEMCOMM Communication
www.rsc.org/chemcomm

Enzymatic synthesis of pyruvic acid from acetaldehyde and carbon dioxide

Masaya Miyazaki, Mitsukuni Shibue, Kazuya Ogino, Hiroyuki Nakamura and Hideaki Maeda*

Institute for Structual and Engineering Materials, AIST Kyushu, National Institute of Advanced Industrial Science and Technology (AIST), 807-1 Shuku, Tosu Saga 841-0052, Japan.
E-mail: maeda-h@aist.go.jp; Fax: +81(942)81-3695; Tel: +81(942)81-3643

Received (in Cambridge, UK) 4th June 2001, Accepted 3rd August 2001
First published as an Advance Article on the web 22nd August 2001

A new enzymatic synthesis of pyruvic acid from acetaldehyde and carbon dioxide has been developed.

Because global environmental pollution has recently become a serious problem, it is advantageous to develop a clean chemical process, which produces low emissions and organic wastes. Biocatalytic reactions attract attention as environmentally safe catalytic reactions in organic synthesis.[1] Several enzymatic processes have been designed and used as alternative organic processes.

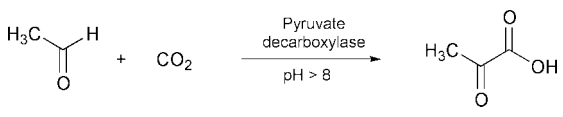
Pyruvate decarboxylase (EC 4.1.1.1) is known as a catalyst of the decarboxylation reaction of pyruvic acid, to produce acetaldehyde. The mechanism of catalytic reaction of pyruvate decarboxylase has been elucidated. This enzyme requires thiamin pyrophosphate as a coenzyme for catalytic activity.[2] As for other thiamin pyrophosphate dependent enzymes, this enzyme has been interesting as a catalyst for carbon–carbon bond formations.[3–5] Pyruvate decarboxylase has been intensively studied as a carboligase for synthesis of chiral α-hydroxy ketones, which are versatile building blocks for organic and pharmaceutical chemistry.

The reverse reaction of this enzyme is also of interest as a catalytic procedure for carboxylation. Several studies have been performed which imitate these enzyme reactions using CO_2 as the reactant.[6–8] A previous study using α-lactoylthiamin showed that production of pyruvate was achieved at higher pH (>10).[8] However, these reactions require organic solvents and severe conditions.

We are interested in the development of novel enzymatic reactions and reactor systems having environmentally safe chemical processes. In the present study, we demonstrate the usefulness of the reverse reaction of pyruvate decarboxylase in the production of pyruvic acid from acetaldehyde and carbon dioxide (Scheme 1).

We chose a sodium bicarbonate buffer system, because not only is this buffer suitable at higher pHs, but it also can be used as the source of carbon dioxide. A typical run was as follows. To a solution of acetaldehyde (100 μM) in sodium bicarbonate buffer (1 ml) in a 1.5 ml microcentrifuge tube, Brewer's yeast pyruvate decarboxylase (1 unit) and thiamin pyrophosphate (the final concentration was 10 μM) were added at 4 °C. The reaction mixture was warmed to 25 °C quickly, and then shaken on a vortex mixer at room temperature. After 1 h, the reaction mixture was chilled on ice, and then subjected to HPLC analysis immediately. The amount of pyruvic acid was calculated from the peak area of HPLC analysis calibrated with commercially available pyruvic acid standards. The yield was estimated based on acetaldehyde.

First, we evaluated the effect of pH on the reaction. The reaction was performed using acetaldehyde (100 μM), thiamin



Scheme 1 Synthesis of pyruvic acid.

(0.1 μM), and pyruvate decarboxylase (1 unit) in 0.1 M $NaHCO_3$–Na_2CO_3 buffer at various pHs (pH 8.5–11.5). The result is shown in Fig. 1. Higher pHs gave a better yield of pyruvic acid. The maximum yield was obtained at pH 11 (61%). The present result agreed well with a previous observation obtained from the hydrolysis of α-lactoylthiamin. In that case, the best yield from hydrolysis was obtained at pH 12. In our case, the best yield was obtained at pH 11, but the yields decreased at much higher pH. Although the hydrolysis proceeds at a maximum rate at pH 12, the enzyme might not be stable over pH 11. Therefore, the maximum yield was obtained at pH 11. Thus, we decided to perform further experiments at pH 11.

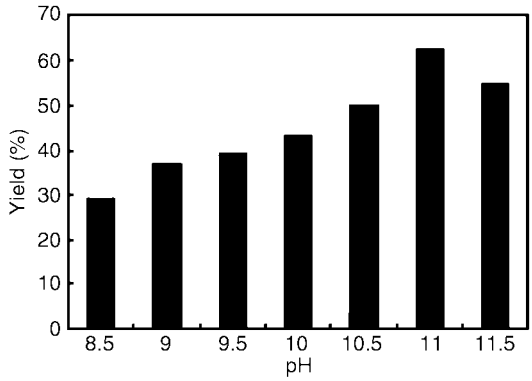


Fig. 1 Effect of pH on pyruvic acid production.

Next, we examined the effects of concentration of bicarbonate buffer on the reaction (Fig. 2). Higher ionic strength of the bicarbonate buffer strongly influenced the yield, as expected. The maximum yield of the reaction was 81% at 500 mM $NaHCO_3$–Na_2CO_3 buffer. This yield was sufficient to use as an organic process, and much higher than that obtained by the reaction in DMF under 20 atm of CO_2.[8] Not only does the latter reaction require multiple steps, but the use of the organic solvent DMF is problematic for environmental safety reasons. The enzymatic reaction does not require any organic solvent and gave a better yield. It has been reported that the thiamin itself

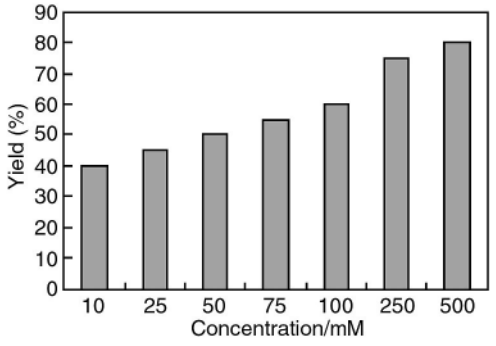


Fig. 2 Effect of ionic strength on enzyme reaction.

 DOI: 10.1039/b104873m

could catalyze a reaction analogous to that of the enzyme, but preparation of the intermediate lactoylthiamin from acetaldehyde was unsuccessful. Thus, it is difficult to reverse the reaction without an enzyme, and pyruvate decarboxylase is the best catalyst for carboxylation of acetaldehyde.

Recently, some enzymatic reactions utilizing supercritical carbon dioxide have been reported.[9,10] In our method, a higher concentration of carbon dioxide is required. The supercritical conditions might be effective at improving the yield of pyruvic acid synthesis, because they should provide a much higher carbon dioxide concentration. The reaction in supercritical carbon dioxide conditions will be our next subject.

Although the effect is much weaker than methane, carbon dioxide is considered to be a greenhouse gas, and therefore its immobilization is desired. The methods currently reported are mainly catalytic or electrochemical reactions, which require much energy.[11–13] One alternative method has been reported, which utilized carbonic anhydrase for the immobilization of carbon dioxide.[14] This biomimetic approach needs almost no energy for the reaction. However, the carbonic anhydrase just improves the solubility of carbon dioxide in aqueous media, and further treatment of dissolved gas is required. Although the reaction requires a large excess of CO_2, our approach can be useful for such a purpose. By our method, the carbon dioxide in the aqueous phase can be condensed with acetaldehyde to produce the pyruvic acid. Because pyruvic acid can easily be converted into lactic acid, which is a constituent of a biodegradable plastic, the carbon dioxide can be immobilized into the biological cycle. An enzymatic reaction by lactic dehydrogenase can be used for hydrogenation of pyruvic acid into lactic acid. Thus, it is possible to design a two-step enzymatic process as a completely environmentally safe method for CO_2 immobilization.

In summary, we have demonstrated the usefulness of the reverse reaction of pyruvate decarboxylase. This reaction might become a recommendable, environmentally safe carboxylation procedure for acetaldehyde. Further studies, such as the reaction utilizing supercritical carbon dioxide, and two-step enzymatic production of lactic acid, are in progress in our laboratories.

We thank Drs Masao Shibata, Tsuyoshi Sakaki, Hiroaki Kodama and Shoji Ando, for their continuous supports.

Notes and references

1 K. M. Koeller and C.-H. Wong, *Nature*, 2001, **409**, 232; A. Schmid, J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts and B. Witholt, *Nature*, 2001, **409**, 258.
2 R. Kluger, *Chem. Rev*., 1987, **87**, 863.
3 W.-D. Fessner, *Curr. Opin. Chem. Biol*., 1998, **2**, 85.
4 G. A. Sprenger and M. Pohl, *J. Mol. Catal. B: Enzym*., 1999, **6**, 145.
5 U. Schorken and G. A. Sprenger, *Biochim. Biophys. Acta*, 1998, **1385**, 229.
6 D. Walther, *Coord. Chem. Rev*., 1987, **79**, 135.
7 P. Braunstein, D. Matt and D. Nobel, *Chem. Rev*., 1988, **88**, 747.
8 M.-A. E. Foppen, Y. M. de Lange, F. van Rantwijk, L. Maat and A. P. G. Kieboom, *Recl. Trav. Chim. Pay-Bas*, 1990, **109**, 359.
9 A. K. Chaudhary, E. J. Bechman and A. J. Russel, *J. Am. Chem. Soc*., 1995, **117**, 3728.
10 Y. Ikushima, N. Saito, M. Arai and H. W. Blanch, *J. Phys. Chem*., 1995, **99**, 8941.
11 Y. Li, G.-H. Xu, C.-J. Liu, B. Eliasson and B.-Z. Xue, *Energy Fuels*, 2001, **15**, 299.
12 M. Aresta, A. Dibenedetto and I. Tommasi, *Energy Fuels*, 2001, **15**, 269.
13 C.-J. Liu, G.-H. Xu and T. Wang, *Fuel Process. Technol*., 1999, **58**, 119.
14 G. M. Bond, J. Stringer, D. K. Brandvold, F. Arzum Simsek, M.-G. Medina and G. Egeland, *Energy Fuels*, 2001, **15**, 309.