A One-Pot, Two-Step Enzymatic Synthesis of L-Lactic Acid from Acetaldehyde

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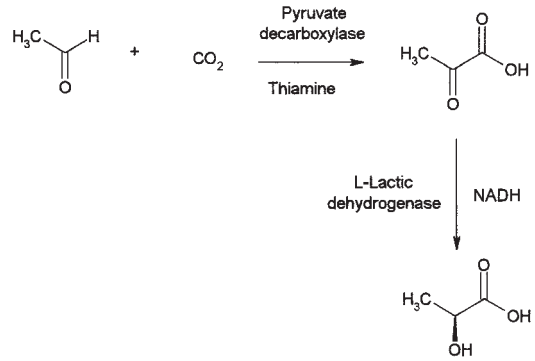
A one-pot, two-step enzymatic synthesis of L-lactic acid from acetaldehyde and carbon dioxide has developed. The reaction was performed using a combination of reverse reaction of pyruvate decarboxylase and hydrogenation of pyruvate by lactic dehydrogenase. The maximum yield was obtained at pH 9.5.

Recent interest of the problems of environmental pollution forces to develop a green chemistry process for the chemical industry.[1] Several environmental friendly processes have been developed. The biocatalytic processes such as fermentation and enzyme reactions have attracted attentions as environmentally safe chemical process.[2,3]

The biodegradable polymers have also been interested for environmental safety.[4,5] Among the biodegradable plastics, the poly(L-lactic acid) has been widely studied. Several methods have been developed to produce the lactic acid from many sources.[6–9] However, most of them produce racemic form and require relatively longer time and multiple steps to obtain pure L-lactic acid. These disadvantages are problematic not only for large-scale production but also for the environmental safety. Therefore, a simple method, which can produce optically pure L-lactic acid, is desired.

We are interested in the development of novel enzymatic reactions and reactor systems having environmentally safe chemical processes. Pyruvate decarboxylase (EC 4.1.1.1) is known as a catalyst of the decarboxylation reaction of pyruvic acid to produce acetaldehyde, and has been utilized as a catalyst for C–C bond formation such as carboligase for synthesis of chiral α-hydroxy ketones, which are versatile building blocks for organic and pharmaceutical chemistry.[10,11] We investigated the reverse reaction of pyruvate decarboxylase and found that the reverse reaction can be achieved in the basic condition.[12] Because pyruvic acid can be easily hydrogenated asymmetrically by L-lactic dehydrogenase, it is possible to produce L-lactic acid from acetaldehyde and carbon dioxide by combining these enzyme reactions (Scheme 1). Here we report a one-pot, two-step enzymatic synthetic procedure for L-lactic acid from acetaldehyde.

The experimental was performed as follows. To a solution of acetaldehyde (0.1 μM), thiamine pyrophosphate (0.1 μM), NADH (0.2 μM) in various concentration and pH of $NaHCO_3$-Na_2CO_3 buffer. The reaction was initiated by adding pyruvate decarboxylase (1 unit) and L-lactic dehydrogenase (1 unit) at room temperature. The reaction was performed 1 h, then the mixture was analyzed by RP-HPLC. The amount of each compound was estimated by peak area calibrated by commercially available standards. The absolute configuration of lactic acid was confirmed by the optical rotation, and the yield was calculated based on acetaldehyde. For confirmation, the yielded lactic acid was subjected to the analysis. The ^{1}H-NMR spectra



Scheme 1. Synthetic process of L-lactic acid.

and optical rotation value were identical to the commercially available L-lactic acid.[13]

Because the reverse reaction of pyruvate decarboxylase proceeds at higher pH, the yield should become better than that of neutral pH. Therefore, the effect of pH (7 to 10.5) on the reaction was examined, first. The results were summarized on Table 1 (entries 1 to 8). In the present study, the yield of lactic acid was maximized at pH 9.5. Much higher pH gave lower yield of L-lactic acid and gave relatively better recovery of pyruvic acid. This result can be explained as follows. The first step proceeds at higher pH, whereas the second step, hydrogenation by L-lactic dehydrogenase, gave lower yield at much higher pH, yielded decrease of production of L-lactic acid and therefore the recovery of pyruvic acid was increased (Table 1).

Because the reverse reaction of pyruvate decarboxylase prefers higher concentration of bicarbonate buffer, we also examined the effect of concentration of bicarbonate buffer at pH 9.5. As shown on Table 1 (entries 6, 9–13), higher concentration of bicarbonate gave better yield, as expected. The maximum yield was obtained at 500 mM bicarbonate buffer (51%). Our previous study of the reverse reaction of pyruvate decarboxylase yielded about 43% of pyruvic acid production in 500 mM bicarbonate buffer at pH 9.5. In the present study, the combined yield of lactic acid and pyruvic acid exceeds 65%, meaning the yield of the reverse reaction of pyruvate decarboxylase was improved than that by alone. This might result from change of equilibrium condition of the reaction, namely pyruvic acid consumption by the second step might promote better yield of the first step. Further studies are required to solve the detail of this mechanism.

Recent interest of poly(L-lactic acid) demands the effective method to produce the material, L-lactic acid. The fermentation method has been focused as effective solution, because it can produce relatively pure L-isomer.[6–9] Our method utilizes enzymatic reaction, which proceeds in shorter times and gives the product in higher purity than that by fermentation. Although further studies are required to establish efficient pilot-scale

Table 1. Reaction yield of L-lactic acid and pyruvic acid in various conditions

Entry	pH	Concentration of buffer/M	Pyruvic acid (A)%[a]	L-Lactic acid (B)%[a]	(A) + (B)%[a]
1	7.0	0.1	1	11	12
2	7.5	0.1	3	15	18
3	8.0	0.1	9	18	28
4	8.5	0.1	11	21	32
5	9.0	0.1	13	26	39
6	9.5	0.1	17	31	48
7	10.0	0.1	21	29	50
8	10.5	0.1	28	27	55
9	9.5	0.01	5	21	26
10	9.5	0.02	9	24	33
11	9.5	0.05	10	26	36
12	9.5	0.2	12	43	55
13	9.5	0.5	14	51	65

[a]The yield was calculated based on acetaldehyde.

production, these features are advantageous than the classical fermentation method for lactic acid production.

In conclusion, we have developed a one-pot, two-step enzymatic synthesis method of L-lactic acid from acetaldehyde and carbon dioxide. This method might become a recommendable, environmentally safe procedure for L-lactic acid production. Further studies are in progress in our laboratory.

References and Notes

1 P. T. Anastas and J. C. Warner, ''Green Chemistry: Theory and Practice,'' Oxford University Press, New York (1998).
2 K. M. Koeller and C.-H. Wong, *Nature*, **409**, 232 (2001).
3 A. Schmid, J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, and B. Witholt, *Nature*, **409**, 258 (2001).
4 W. Amass, A. Amass, and B. Tighe, *Polym. Int.*, **47**, 89 (1998).
5 S. Jacobsen, P. H. Degree, H. G. Fritz, P. H. Dubois, and R. Jerome, *Polym. Eng. Sci.*, **39**, 1311 (1999).
6 J. Lunt, *Polym. Degrad. Stab.*, **59**, 145 (1998).
7 H. Ohara, U. Doi, M. Otsuka, H. Okuyama, and S. Okada, *Seibutsu Kagaku*, **79**, 142 (2001).
8 A. Ishizaki, G. Kobayashi, P. Vonktaveesuk, T. Tsuge, and K. Tanaka, *Kagaku Kogaku Ronbunshu*, **25**, 136 (1999).
9 K. Hoshino, M. Taniguchi, H. Marumoto, K. Shimizu, and M. Fujii, *Agric. Biol. Chem.*, **55**, 479 (1991).
10 G. A. Sprenger and M. Pohl, *J. Mol. Catal. B: Enzym.*, **6**, 145 (1999).
11 U. Schorken and G. A. Sprenger, *Biochim. Biophys. Acta*, **1385**, 229 (1998).
12 M. Miyazaki, M. Shibue, K. Ogino, H. Nakamura, and H. Maeda, *Chem. Commun.*, **2001**, 1800.
13 The reaction was terminated by addition of 1 mol dm^{-3} HCl (100 μl). Then L-lactic acid was immediately isolated from the reaction mixture and purified by RP-HPLC and crystallization. $[\alpha]_D^{20}$ +3.9 (c = 1.0, H_2O), −13.7° (c = 2.0, 1.5 mol dm^{-3} NaOH); ^{1}H-NMR (300 MHz, D_2O) δ 1.32 (3H, d, CH_3) and 4.13 (1H, s, C*H*OH). Authentic sample: $[\alpha]_D^{20}$ + 3.9 (c = 2.0, H_2O), −13.5° (c = 2.5, 1.5 mol dm^{-3} NaOH); ^{1}H-NMR (300 MHz, D_2O) δ 1.33 (3H, d, CH_3) and 4.12 (1H, s, C*H*OH).