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Conversion of pyrrole to pyrrole-2-carboxylate by cells of *Bacillus megaterium* in supercritical CO_2

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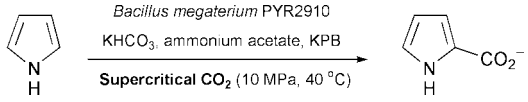
Pyrrole was converted to pyrrole-2-carboxylate in supercritical CO_2 using cells of *Bacillus megaterium* PYR 2910, and the yield of the carboxylation reaction in supercritical CO_2 was 12 times higher than that under atmospheric pressure.

The development of CO_2 fixation reactions on organic molecules is one of the challenges in synthetic chemistry. An increasing number of chemical CO_2 fixation reactions[1] have been reported, especially using supercritical CO_2 (CO_2 above its critical temperature (31.0 °C) and pressure (7.38 MPa, 72.8 atm)), such as the synthesis of urethane,[1b] dimethyl carbonate,[1c] styrene carbonate[1d] methyl ethanoate.[1e] On the other hand, biocatalysis is now one of the most powerful and indispensable tools for organic synthesis due to its environmentally friendliness and excellent enantio-, regio- and chemo-selectivities.[2] Some enzymatic CO_2 fixation reactions have also been reported including the central CO_2 fixation reaction in photosynthetic organisms catalyzed by ribulose-1,5-diphosphate carboxylases,[3] the reduction of CO_2 to formic acid or methanol by dehydrogenases,[4] the reductive CO_2 fixation on 2-oxoglutarate and pyruvate by isocitrate[5] or malate[6] dehydrogenases, and the CO_2 fixation on pyrrole and phenolic compounds (phenol and catechol) by decarboxylases from *B. megaterium*[7] or *Clostridium hydroxybenzoicum*,[8] respectively.

Although enzymes in nature catalyze reactions in aqueous media, their use in supercritical CO_2 has been attracting increasing attention due to its gas-like low viscosities and high diffusivities and its liquid-like solubilizing power,[9] and mostly hydrolytic enzymes, with the exception of the cholesterol oxidases[10] and the alcohol dehydrogenase,[11] have been used in supercritical fluids to improve their functions, *i.e.*, faster reaction rates.[12] Here we report that cells of *Bacillus megaterium* PYR2910 catalyzes the reverse reaction, CO_2 fixation, in supercritical CO_2 for the first time. As shown in Scheme 1, CO_2 was fixed on pyrrole to produce pyrrole-2-carboxylate at 10 MPa and 40 °C. The yield of the reaction in supercritical CO_2 was much higher than that at atmospheric pressure.

The cells of *Bacillus megaterium* PYR2910[7] were employed for the CO_2 fixation reaction. The reaction was conducted by adding CO_2 to 10 MPa to the mixture of pyrrole, the cells, $KHCO_3$, and NH_4OAc in potassium phosphate buffer. For the reaction at atmospheric pressure (0.1 MPa), the evolved CO_2 was released to keep the pressure atmospheric. The yields of the reaction at 40 °C are listed in Table 1. The yield is much higher for the reaction in supercritical CO_2 than at atmospheric pressure (Table 1, Entries 1–4). It was also confirmed by the control experiment without the cells that the non-biocatalytic carboxylation of pyrrole did not proceed (Table 1, Entry 5).[13] Therefore, the cell is surely catalyzing the CO_2 fixation reaction more effectively in supercritical CO_2 than at atmospheric pressure.



Scheme 1

The time courses of the reaction at 10 MPa and at atmospheric pressure in Fig. 1 also have a higher yield for the supercritical reaction than that of the atmospheric reaction. The reaction reached an equilibrium position within a few hours and did not proceed further. As listed in Table 1, the doubling of the quantity of cells (Entry 6) as well as the change in the initial pH value from 5.5 to 7.0 to prevent a pH decrease caused by CO_2 (Entry 7) did not have any significant effect on the equilibrium position.

The effect of pressure on the carboxylation of pyrrole was also investigated, and the result is shown in Fig. 2. The maximum yield was between 4 and 7 MPa; the yield at just above its critical pressure (7.6 MPa) is about 12 times that at atmospheric pressure (0.1 MPa). Similar pressure dependencies of the yield on pressure were also observed using an increased quantity of the cells, shorter reaction times, and different temperatures (data not shown). At present, it is not clear why the increased concentration of CO_2 in the range greater than the

Table 1 Conversion of pyrrole to pyrrole-2-carboxylate in supercritical CO_2 at 40 °C

Entry	Pressure/MPa	Cells/mL[a]	pH	Time/h	Yield (%)[b]
1	0.1 (Atmospheric)	0.5	5.5	1	7
2	0.1 (Atmospheric)	0.5	5.5	3	6
3	10 (Supercritical)	0.5	5.5	1	54
4	10 (Supercritical)	0.5	5.5	3	55
5	10 (Supercritical)	0.0	5.5	3	0
6	10 (Supercritical)	1.0	5.5	3	59
7	10 (Supercritical)	0.5	7.0	1	59

[a] OD_{610} = 32; decarboxylation activity for pyrrole-2-carboxylate = 0.024 mmol min $^{-1}$ml^{-1}. [b] Based on the starting amount of pyrrole determined by HPLC analysis.

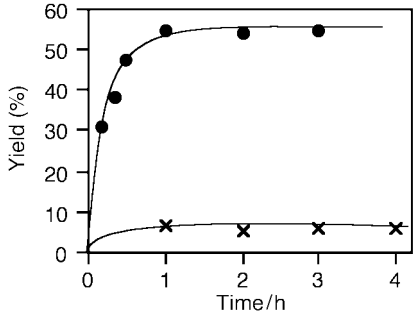


Fig. 1 Time course of conversion of pyrrole to pyrrole-2-carboxylate [●: 10 MPa (supercritical), × 0.1 MPa (atmospheric)].

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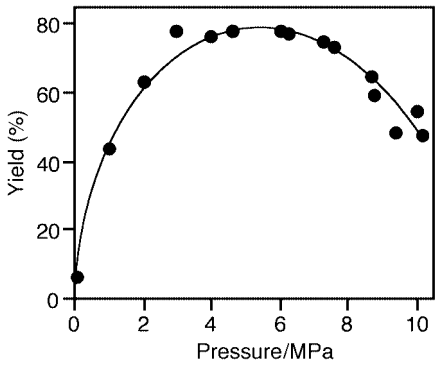

Fig. 2 Effect of pressure on conversion of pyrrole to pyrrole-2-carboxylate by the cells.

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critical pressure did not favorably shift the carboxylation equilibrium.

In a typical experiment, *B. megaterium* PYR2910 was grown as previously described.[7b] To a stainless steel pressure-resistant vessel containing a magnetic stirrer bar as previously described,[11] pyrrole (0.40 M, 0.50 mL), potassium phosphate buffer (pH 5.5, 0.40 M, 0.50 mL), ammonium acetate (0.56 M, 0.50 mL), the cells (OD_{610} = 32, 0.50 mL) and $KHCO_3$ (0.60 g) were added. The vessel was then warmed to 40 °C, and CO_2 preheated to 40 °C was introduced to a final pressure of 10 MPa. The mixture was stirred at 40 °C for 3 h, and the CO_2 was liquefied at −10 °C and then the gas pressure was released. The chemical yield was measured by HPLC analysis as previously described.[7b]

In conclusion, cells of *B. megaterium* catalyzed the conversion of pyrrole to pyrrole-2-carboxylate in supercritical CO_2 at 40 °C with pressures up to 10 MPa, and a higher yield was obtained under supercritical conditions than at atmospheric pressure. This finding will play a significant role both in investigating enzyme species suitable for the biocatalysis in supercritical CO_2 and in developing synthetic methods utilizing CO_2.

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13 Control reaction using inactivated cells of *B. Megaterium* [inactivation conditions: 90 °C, 2 h, reaction conditions: 10 MPa, 40 °C, 3 h, cells: 1.0 mL (OD_{610} = 32)] afforded no carboxylation product, which indicates that a biocatalyst is at work and that the carboxylation is not an unexpected process promoted by non-enzymic constituents of the cell.