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Effect of carbon dioxide concentrations on asymmetric reduction of ketones with plant-cultured cells

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ABSTRACT

Enantioselectivities in asymmetric reduction of ketones were controlled by atmospheric carbon dioxide concentrations: the reaction in high carbon dioxide concentrations under illumination of fluorescent light afforded the corresponding L-alcohol while that in low carbon dioxide concentrations in the presence of glucose under dark conditions gave the antipode, D-alcohol.

Recently, carbon dioxide (CO_2) concentrations have been increasing steadily in the global atmosphere and this phenomenon is thought to affect largely the life of animals and plants on earth. Then reducing the concentration of global atmospheric CO_2 is one of the most important targets for the world community as represented by 'Kyoto Protocol in 1997'. Since a large amount of CO_2 emitted into the atmosphere should be collected, chemical industries are forced to use CO_2 as a starting material or as a medium (i.e., supercritical CO_2) for various chemical and biological reactions. Thus the novel use of CO_2 is worth investigating. Here, we would like to demonstrate that a CO_2 concentration is a useful factor for controlling stereoselectivity in biotransformation.

In recent years, asymmetric reductions of artificial ketones with microbes have been widely used for the synthesis of optically active alcohols,[1] however, plant cells have hardly been used for this purpose[2] because of lack of useful knowledge for controlling stereoselectivity in reactions. On the contrary, until now, stereochemical controls of microbial reductions have been well known: the use of selective inhibitors and organic-aqueous biphasic system.[1] However, these methods could be difficult to apply in biotransformation using plant cells. Then a novel method is required if we intend to use plant cells as biocatalysts.

Now we would like to report that the use of high atmospheric CO_2 concentrations in the reaction system using plant-cultured cells effectively increases the enantioselectivity of asymmetric reduction of ketones. Thus we carried out the asymmetric reduction of ketones **1a–e** using cultured cells of *Nicotiana tabacum*[3] as biocatalysts. Reaction conditions were optimized by switching the concentrations of CO_2 ('air' vs 'high CO_2' conditions[4]) or the conditions of light ('dark' vs 'light' conditions[5]). The results are summarized in Table 1. In the case of air conditions, the reduction of *t*-butyl acetoacetate (**1a**) gave low enantiomeric excess (ee) of the corresponding L(*S*)-alcohol **2a**[6] (46% chemical yield and 13% ee after 24 h[7,8]) under dark conditions, however, both the chemical yield and the ee of L-**2a** were enhanced under illumination of fluorescent light[5] (83% chemical yield with 75% ee) (Table 1, entries 1 and 2). Similar phenomenon has been observed in the reduction with cyanobacteria.[9] Since the enantioselectivity of the reduction in the present system was not satisfactory even under light conditions, further efforts were required to increase the enantioselectivity. We kept our eyes on CO_2 concentrations because it is well known that photosynthesis including sugar production is strongly affected by atmospheric CO_2 concentrations.[10] Thus the effect of CO_2 concentrations on the ee of L-**2a** under light conditions was examined. As expected, high atmospheric CO_2 concentrations in the cultivation box increased the enantioselectivity of the asymmetyric reduction (0.03% CO_2, 75% ee; 0.3% CO_2, 88% ee; 0.47% CO_2, 97% ee; 1.54% CO_2, 98% ee) as shown in Figure 1. Especially, 98% ee with 98% chemical yield of L-**2a** was obtained under the high CO_2/light conditions (Table 1, entry 4). Other ketones were also used as the substrates. Thus, the reduction of isopropyl acetoacetate (**1b**) under high CO_2 conditions gave high ee of L-**2b** under light conditions (Table 1, entry 8) while low ee of D-**2b** was obtained under dark conditions (Table 1, entry 7). When acetophenone (**1c**) was used as the substrate, the tendency of stereochemical course was similar to that of **1b** although chemical yields were very low (Table 1, entries 9–12). In all cases, it was found that the enantioselectivity of the asymmetric reduction was shifted toward L-direction by using the high CO_2/light conditions.

Thus a novel factor, a high atmospheric CO_2 concentration, for stereochemical control was observed. Until now, the use of plant cells has not been recognized as one of the general methods for biotransformations, especially in asymmetric reductions, because

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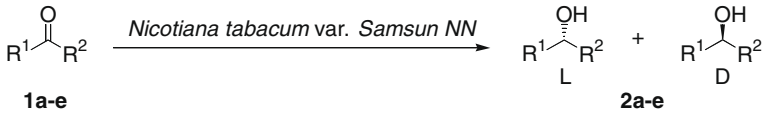
Table 1
Asymmetric reduction of ketones **1a–e** using Nicotiana tabacum var. Samsun *NN*



a, R^1 = Me, R^2 = $CH_2CO_2Bu^t$ b, R^1 = Me, R^2 = $CH_2CO_2Pr^i$
c, R^1 = Me, R^2 = Ph d, R^1 = CF_3, R^2 = Ph e, R^1 = CF_3, R^2 = 2-thienyl

Entry	Ketone **1**	Conditions[a]	Product **2**	Yield[b] (%)	ee[b] (%)	Conf.[b]
1	**1a**	Air/dark	**2a**	46	13	L(*S*)
2	**1a**	Air/light	**2a**	83	75	L(*S*)
3	**1a**	High CO_2/dark	**2a**	45	19	L(*S*)
4	**1a**	High CO_2/light	**2a**	98	98	L(*S*)
5	**1b**	Air/dark	**2b**	49	25	D(*R*)
6	**1b**	Air/light	**2b**	61	46	L(*S*)
7	**1b**	High CO_2/dark	**2b**	46	8	D(*R*)
8	**1b**	High CO_2/light	**2b**	94	83	L(*S*)
9	**1c**	Air/dark	**2c**	1	38	D(*R*)
10	**1c**	Air/light	**2c**	6	65	L(*S*)
11	**1c**	High CO_2/dark	**2c**	2	20	D(*R*)
12	**1c**	High CO_2/light	**2c**	7	74	L(*S*)
13	**1d**	Air/dark	**2d**	66	50	D(*S*)
14	**1d**	Air/light	**2d**	80	15	D(*S*)
15	**1d**	High CO_2/dark	**2d**	68	43	D(*S*)
16	**1d**	High CO_2/light	**2d**	96	5	L(*R*)
17	**1e**	Air/dark	**2e**	100	81	D(*R*)
18	**1e**	Air/light	**2e**	100	63	D(*R*)
19	**1e**	High CO_2/dark	**2e**	97	70	D(*R*)
20	**1e**	High CO_2/light	**2e**	100	48	D(*R*)

[a] The reaction was conducted for 24 h at 25 °C.
[b] Determined by chiral GC analysis using DEX-CB.

of lack of knowledge for controlling stereochemistry in reactions as mentioned above. The present finding has opened new possibility to the use of plant cells as biocatalysts.

Table 1 also shows that the product alcohols **2a–e** under the high CO_2/light conditions were obtained in high chemical yields. These results indicate that more reducing agents may be produced under photosynthetic conditions. Then we examined the effect of sugar such as glucose on the reaction system. However, contrary to our expectation, the addition of glucose (1%) to the medium in the reduction of **1a** under dark conditions gave the D-**2a** (not L-**2a**) in 83% chemical yield with 82% ee (Table 2, entry 1). Similar results were obtained in the reduction of **1b–e** with glucose (Table 2, entries 2–5). Consequently, we have found that addition of glucose under dark conditions changed the enantioselectivity of the asymmetric reduction from L-configuration to D-configuration. Surely, we obtained the antipode, the D-alcohol, by the same biocatalyst.

Now we have succeeded in stereochemical control of plant cell-mediated reduction of ketones (e.g., **2a** shown in Scheme 1).

The reason why a high atmospheric CO_2 concentration affected the enantioselectivity of the reduction of artificial ketones was uncertain. One possibility is that some reducing agents that accelerate the reduction toward the L-direction would be stored under photosynthetic conditions.

It is sure that the enantioselectivity of the reduction was strongly affected by photosynthetic activities of the cells: low ee

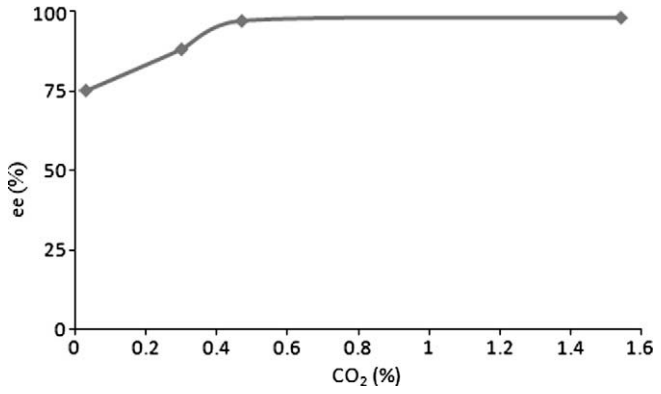
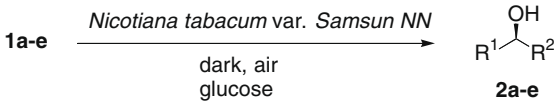


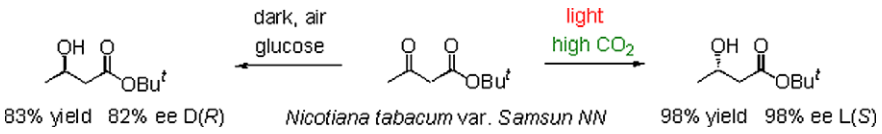
Figure 1. The effect of CO_2 concentrations on the ee of L-**2a** under light conditions.

Table 2
Reduction in the presence of glucose



Entry	Ketone **1**[a]	Product **2**	Yield[b] (%)	ee[b] (%)	Conf.[b]
1	**1a**	**2a**	83	82	D(*R*)
2	**1b**	**2b**	64	43	D(*R*)
3	**1c**	**2c**	5	49	D(*R*)
4	**1d**	**2d**	62	59	D(*S*)
5	**1e**	**2e**	100	92	D(*R*)

[a] The reaction was conducted in the presence of glucose under dark conditions for 24 h at 25 °C.
[b] Determined by chiral GC analysis using DEX-CB.



Scheme 1. Synthesis of both enantiomers with *Nicotiana tabacum* var. *Samsun NN*.

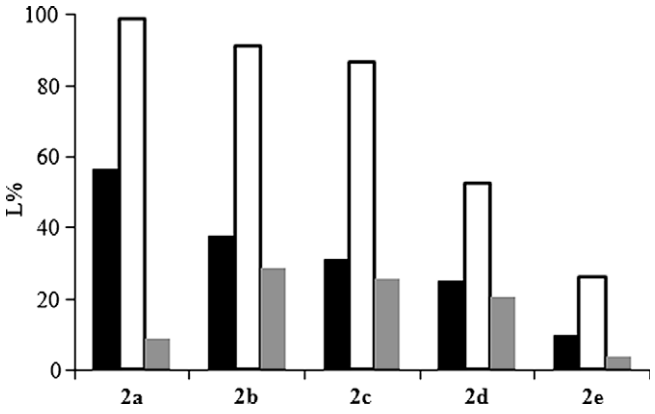


Figure 2. The L% of products **2a–e**. Conditions: air/dark (■); high CO_2/light (□); air/glucose/dark (■).

of L-alcohols was obtained under dark conditions and high L-selectivity was found under light conditions. Photosynthesis will be largely participating in the present stereochemical control since addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (1×10^{-6} M), a photosynthetic electron transport inhibitor, lowered the enantioselectivity of the reduction of **1a** even under light conditions: 33% ee of L-**2a** was observed. However, sugars, which are well-known products of photosynthesis, are not the candidates because the reaction with glucose shifted the reduction course to the D-selectivity, not to the L-selectivity, and addition of starch gave the similar effect to that of glucose on the enantioselectivity of the reduction of **1a** (1% starch: **2a**, 71% ee (D)).

Addition of fatty acids to the system was examined but stereochemical course of the reductions was not affected by low fatty acid concentrations and the cells were damaged at high fatty acid concentrations. Thus the mechanism of the effect of CO_2 on enantioselective reduction which shifted the reaction course toward the L-selectivity is obscure in the present time.

As described above, the addition of glucose changed the stereoselectivity toward giving the D-alcohol. To clarify the effect of glucose, photosynthetic activities were measured using PAM.[11] As the result, maximum photochemical efficiencies were decreased in the presence of glucose. Thus, addition of glucose resulted in lowering photosynthetic activities. While conditions at high photosynthetic activities gave the L-alcohol in high ee, addition of glucose inhibited photosynthesis and decreased the L-selectivity. Enzymes or products in metabolic pathway of glucose will participate in the stereochemical control directly or indirectly.

Trifluoromethyl ketones **1d** and **1e** were also used as the substrates (Table 1, entries 13–20). The stereochemical courses of the reductions of these substrates also shifted toward the L-selectivity by the high CO_2/light conditions. Figure 2 shows the L% of the products **2a–e** in the air/dark, the high CO_2/light, and the air/glucose/dark conditions. Thus, in every case, the stereochemical course of the reduction shifted to give the L-alcohols by the high CO_2/light conditions and the course was changed to give the D-alcohols by the air/glucose/dark conditions.

The present method indicates that CO_2 is a useful compound for controlling stereoselectivity in biotransformation. Further studies for clarifying the mechanism of the present system are in progress in our laboratories. The efficiency enhancement in the current systems and the use of other plant-cultured cells are challenges for the future.

References and notes

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4. Conditions at a high atmospheric CO_2 concentration were attained by placing the buffer containing 2 M K_2CO_3/2 M $KHCO_3$ (1:4, v/v)[12] in other wells. A part of gas in the culture cluster was taken up by a syringe and the CO_2 concentration was determined by measuring its IR spectrum (2361 cm^{-1}) and by comparing the spectrum with that of the standard sample: the concentration of atmospheric CO_2 in the box was 1.54%. The CO_2 concentration of global atmosphere is 0.03%.
5. Fluorescent light (HITACHI FL40SW, 400–700 nm, max 580 nm, 30–35 μmol photons m^{-2} s^{-1}).
6. D-L notation was adopted here since methyl and trifluoromethyl ketones were used in this Letter and (*R*)-configuration of (*R*)-1-phenyl-2,2,2-trifluoroethanol is the same configuration as (*S*)-1-phenylethanol by definition.
7. Pre-sterilized 12-well cell culture clusters (Corning Incorporated, NY) was used for pre-cultivation and also for reaction of the ketone. In clean bench, culture cells (240 mg in 2 mL LS medium) were put in the wells and pre-cultivated for 5 days. The substrate (1 mg in 10 μL DMSO) was added and the reaction was carried out for 24 h. Conditions for the high CO_2/light were as follows: the CO_2 concentration was 1.54% (v/v) in the absence of glucose; 30–35 μmol photons m^{-2} s^{-1}. Conditions for the air/glucose/dark were as follows: CO_2 (0.03%, v/v); glucose (1%, v/v); under dark conditions. The reaction mixture was extracted with ether. The chemical yields and ee were obtained from GC-analysis using a Varian Chirasil-DEX-CB (25 m × 0.32 mm) column (100 °C isotherm). The retention times of ketone **2a**, the L-alcohol, and the D-alcohol were 1.64 min, 7.89 min, and 8.28 min, respectively.
8. Average data for more than 3–5 times.
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11. PAM: pulse amplitude modulation fluorometer (PAM200, Walz Corporation, Effeltrich, Germany). Minimum chlorophyll fluorescence (F_o) and maximum chlorophyll fluorescence (F_m) were measured after 30 min dark adaptation. The variable chlorophyll fluorescence (F_v) was calculated from (F_m–F_o) and F_v/F_m gives an estimate for the maximum photochemical efficiency of PS II. cf. Schreiber, U.; Hormann, H.; Neubauer, C.; Klughammer, C. *Aust. J. Plant Physiol.* **1995**, *22*, 209.
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