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Light-mediated regulation of asymmetric reduction of ketones by a cyanobacterium

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Abstract—The stereochemical course of the asymmetric reduction of ketones by a photosynthetic microbe is largely regulated by light. Thus, we find that the enantioselectivity of the reduction of α,α-difluoroacetophenone with *Synechococcus elongatus* PCC 7942 increases as a result of illumination with fluorescent light. Furthermore, DCMU, an inhibitor of photosynthesis, affects the stereoselectivity, and, under illumination decreases the enantioselectivity of the reduction.

1. Introduction

Optically active compounds are useful intermediates for pharmaceuticals, agrochemicals and liquid crystals and hence asymmetric synthesis is one of the most important topics in current organic chemistry. Chiral compounds containing fluorine atoms are of particular interest in the fields of medical and biological chemistry because of their significant biological response properties compared with the parent molecules.[1] Asymmetric reduction of carbonyl compounds with biocatalysts is now recognized as a valuable approach for the synthesis of optically active compounds.[2] To date, two methods have been used for biocatalytic reduction: one involves the use of isolated enzymes and the other involves the use of microbial whole cells. In general, enantiomeric purities are high for isolated enzymatic systems. On the other hand, whole-cell-reactions usually afford unsatisfactory stereoselectivities, because several enzymes in the cell participate in the reaction and the stereochemical preferences are not the same for all of the different enzymes: one enzyme may afford one stereoisomer while another enzyme may produce its antipode. To date, several methods have been developed for improving the selectivity of whole-cell-catalyzed reactions by modifying the reaction conditions: addition of inhibitors of a specific enzyme,[3] reaction in organic solvents,[4] use of hydrophobic polymers such as XAD,[5] and the treatment of microbial cells with acetone.[6] Although these methods are effective for increasing stereoselectivity of microbial reductions and several methods can be applied for a practical synthesis, many reactions are still unsuited to these types of stereochemical control and new strategies to increase the stereoselectivities of whole cell-mediated reactions are therefore desirable.

Herein, we report a novel method for stereochemical control of the asymmetric reduction with a photosynthetic microbe: control by light. Enantioselectivities in asymmetric reduction of ketones are improved by illumination with fluorescent light. This is the first report on light-mediated regulation of an asymmetric reduction.

2. Results and discussion

2.1. Reduction of α,α-difluoroacetophenone by a cyanobacterium

When α,α-difluoroacetophenone **1** was added to a suspension of *Synechococcus elongatus* PCC 7942 and shaken (140 rpm) in the dark, under standard conditions for microbial reduction, the corresponding *R*-alcohol, (*R*)-**2** was obtained with poor enantioselectivities (20–30% ee).

These results suggest that reduction of **1** by *S. elongatus* was conducted by plural enzymes: one transformed the substrates into the corresponding *R*-alcohol, while the other afforded the antipode on reduction. To increase

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the poor enantioselectivity, we tested several previously reported methods for stereochemical control. However, the results of our studies using such methods were unsatisfactory.

Since the microbe we used is a kind of cyanobacteria that grows under illumination, we tested the reaction under illumination with light. We supposed that the relative enzymatic activities were largely influenced by the amount of light in the environment. Phototrophs such as cyanobacteria perform photosynthesis when exposed to light, and all enzymatic activities change depending on whether the environment is dark or light.[7] The relative activities of enzymes that conduct the reduction of **1** may also vary and reactions in the light may afford different stereoselectivities from those in the dark. In fact, when the microbes were incubated with **1** under illumination (13.4 μmol photons m^{-2} s^{-1} (1,000 lux)), (*R*)-**2** was obtained with 68–70% ee. Although the enantioselectivity is still not satisfactory, the selectivity is much higher than that observed for the reaction completed in the dark (20–30% ee) (Table 1).[8]

Thus, we have found that light can regulate the selectivity of asymmetric reduction and induced an increase in the activity of the enzyme that catalyzes the production of (*R*)-**2**. Recently, we reported that chemical yields in the reduction of 2′,3′,4′,5′,6′-pentafluoroacetophenone by *S. elongatus* were improved by light illumination.[9] However, in this case, the enantioselectivity was not affected by light environment. On the contrary, when α,α-difluoroacetophenone is used as a substrate, both the chemical and enantiomeric purities of the corresponding alcohol depend on light. Illumination improved not only the chemical yield, but also the enantiomeric purity. This result shows that illumination induces or activates enzymes that catalyze the conversion of the ketone into (*R*)-**2**.

To clarify the mechanism for the observed stereochemical control in the microbial reduction, the effect of a photosynthetic inhibitor was investigated.

2.2. Effect of addition of DCMU on reduction of α,α-difluoroacetophenone by a cyanobacterium

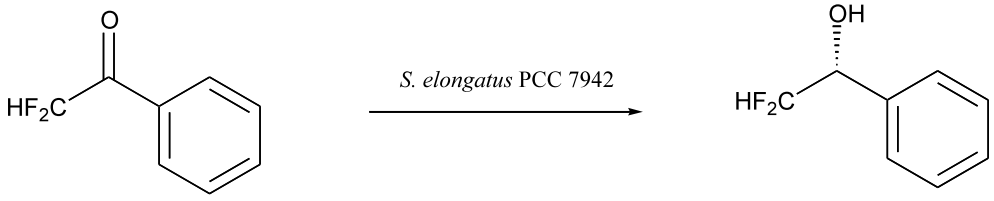
DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is known to inhibit photosynthesis.[10] This inhibitor is widely used because of its exceptionally potent and specific inhibitory effect on the Hill reaction (light-induced transport of electrons from water to acceptors). Therefore the use of DCMU as an additive under illumination with light can change reaction conditions so that they are similar to darkness with respect to the electron transport system. Thus, under these conditions, although the microbe is exposed to light, reducing power resulting from photosynthesis (i.e. NADPH) is not generated, just as in the dark.

Actually, as shown in Fig. 1, enantioselectivity of the reduction of **1** in the light decreased almost to the level of that in the dark conditions when 10 μM of DCMU was added to the system. DCMU also decreases the chemical yield of the reduction down to similar levels as those observed for the reaction completed in the dark.

The effect of DCMU on enantioselectivity was investigated by changing its concentrations, with the results shown in Fig. 2. Thus, increasing the DCMU concentration led to decreases in the chemical and enantiomeric purities. This result undoubtedly indicates that inhibition of photosynthesis causes a decrease in enzymatic activities that selectively produce the *R*-alcohol. This is a strong evidence for the association of photosynthesis with enzymatic activities in reduction of the ketone by *S. elongatus* PCC 7942 (Fig. 3).

The mechanism of stereochemical control is proposed as follows: Under illumination, chlorophyll in cyanobacteria captures light energy and generates the reducing agent NADPH. The *R*-enzymes (the enzymes that selectively produce *R*-alcohol) use thus generated NADPH[11] selectively while the *S*-enzymes (the enzymes that produce *S*-alcohol) would not use the same coenzyme. Light also changes the physiology of cyanobacte-

Table 1. Reduction of difluoroacetophenone by *S. elongatus* PCC 7942



1 (*R*)-**2**

Light	Days	Yield (%)	Ee (%)	Config.
On	1	88	68	*R*
On	4	>99	70	*R*
Off	1	61	27	*R*
Off	4	96	31	*R*

Substrate; 10 μmol, biocatalyst as dry weight: 1 g/L, medium; 20 mL.

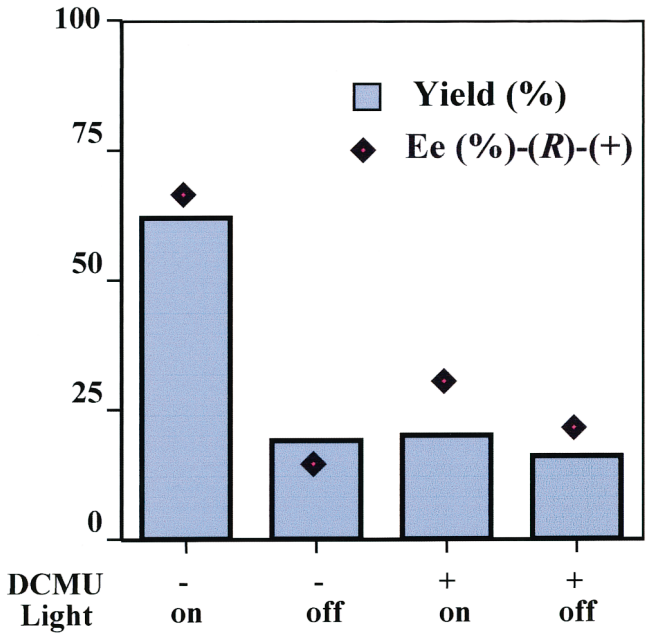


Figure 1. The effects of DCMU on reduction of α,α-difluoroacetophenone by *S. elongatus* PCC 7942.

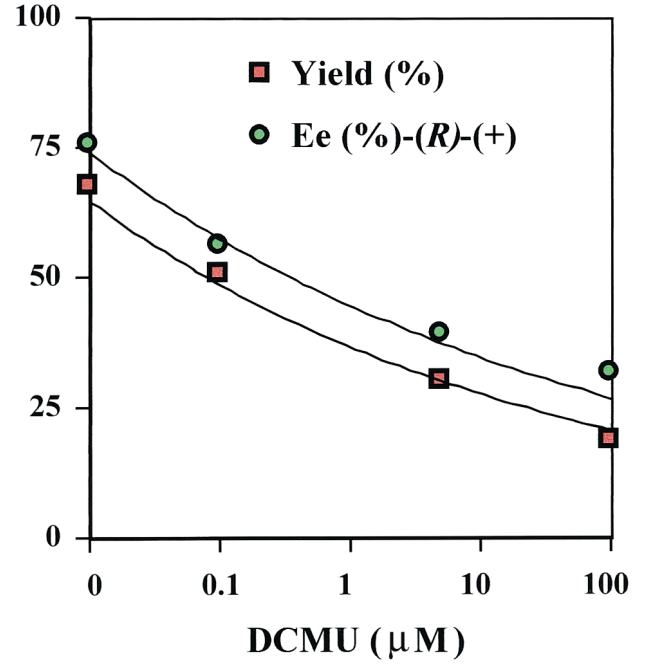


Figure 2. The effects of concentration of DCMU on reduction of α,α-difluoroacetophenone by *S. elongatus* PCC 7942.

ria through the photosynthetic process. These physiological changes induce expression and/or activation of enzymes. Thus, enzymes that produce the *R*-alcohol become active. We are not able to determine which mechanism is plausible at the present time. Under darkness, the reducing ability may stem from the oxidation of alcohols such as saccharides; the *S*-enzymes preferentially use the thus generated reduced form of the coenzyme. The *R*-enzymes can also participate in the reduction under dark and consequently, both *R*- and *S*-alcohols are produced. Under illumination, only the *R*-enzymes are activated and, as stated above, these produce the *R*-alcohol selectively.

2.3. Reduction of acetophenone derivatives by the cyanobacterium

Acetophenone derivatives such as acetophenone, α-monofluoroacetophenone, α,α-difluoroacetophenone, and α,α,α-trifluoroacetophenone were reduced by *S. elongatus* PCC 7942, and the effect of illumination was observed (Table 2).

Although acetophenone was reduced to the *S*-alcohol with an excellent ee (>99% ee *S*) in the light,[12] the chemical yield was poor and the reduction could not proceed under darkness. When α-monofluoroacetophenone was used as the substrate, the reduction under illumination produced the *R*-alcohol with higher enantioselectivity than that in the dark, just as in the reduction of **1**. However, in the reduction of α,α,α-trifluoroacetophenone, no effect of light on enantioselectivity was observed. Although illumination affects chemical yield (63% under illumination and 19% in the dark), enantioselectivities were not influenced by illumination conditions.

The difference in sensitivity to brightness between trifluoroacetophenone and mono- and difluoroacetophenone can be explained by the idea that the quantity of enzymes that can reduce trifluoroacetophenone is less than that for mono- and difluoroacetophenone. In the case of using *Geotrichum candidum* as the biocatalyst, α,α,α-trifluoroacetophenone was reduced only by *S*-enzyme and gave *S*-alcohol with high enantioselectivity.[13] On the contrary, reductions of α-monofluoroacetophenone and α,α-difluoroacetophenone were conducted by both *R*- and *S*-enzymes, resulting in the production of low enantioselectivities.[13] The bulkiness of the trifluoromethyl group in α,α,α-trifluoroacetophenone may decrease the number of enzymes that can participate in the reaction.

3. Conclusion

We have developed a novel method to improve enantioselectivities in the reduction of ketones by a cyanobacterium (*S. elongatus* PCC 7942). The enantiomeric purities of the products increased when using light, and we found that light is a new regulator of enantioselectivities in asymmetric reduction of ketones. Adding DCMU, an inhibitor of photosynthesis, decreased the chemical and enantiomeric purities of reduction products even under illumination.

These results suggest that physiological changes through photosynthesis have effects on the enzymatic activities in the reduction of ketones by cyanobacteria.

Cyanobacteria are photosynthetic algae that perform photosynthesis with the aid of light energy. Light con-

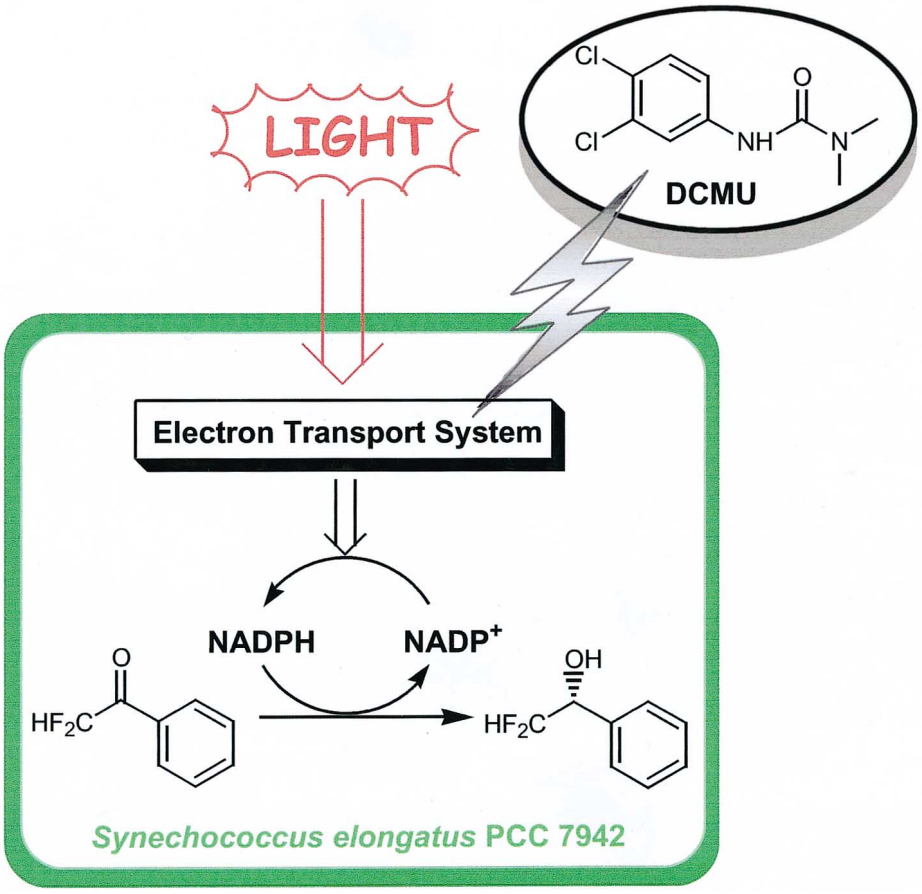
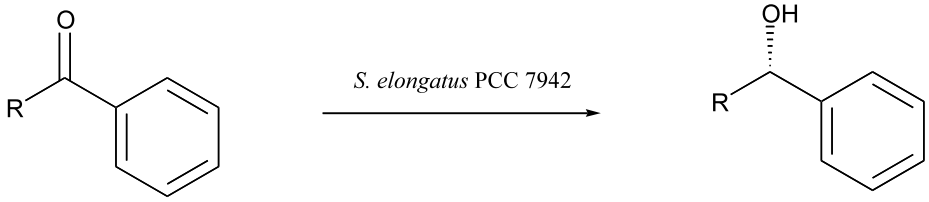


Figure 3. Inhibition mechanism of DCMU in reduction of ketones by *S. elongatus* PCC 7942.

Table 2. Reduction of acetophenone derivatives by *S. elongatus* PCC 7942



R	Light	Yield (%)	Ee (%)	Config.
CH_3	On	3	>99	*R*
CH_3	Off	n.r.	–	*R*
CH_2F	On	35	71	*R*
CH_2F	Off	8.5	27	*R*
CHF_2	On	77	66	*R*
CHF_2	Off	21	14	*R*
CF_3	On	62	63	*R*
CF_3	Off	19	61	*R*

Substrate; 10 μmol, biocatalyst as dry weight: 1 g/L, medium; 20 mL.

trols the life of algae. Besides driving photosynthesis—the process supporting algal autotrophic life, light controls the signals for circadian phenomena and photomorphogenesis. These physiological phenomena are usually induced by changes in the activities of enzymes.[14] Therefore, light is the most important regulator for selectivities in photosynthetic whole-cell-catalyzed reactions.

4. Experimental

4.1. General

Synechococcus elongatus PCC 7942 was obtained from the Institut Pasteur. Gas chromatographic analyses were performed using chiral GC-column (CP-cyclodextrin-B-2,3,6-M-19 [CPCD]; 25 m; He 2 mL/min)

equipped on Shimadzu GC-14B with C-R6A. Yields were determined by GC analyses using naphthalene or chlorobenzene as internal standards. Enantiomeric purities of the products were determined by GC analyses. The absolute configurations of the products, alcohol were determined by comparing the GC retention times with those of authentic samples.

4.2. Cultivation

Synechococcus elongatus PCC 7942 was grown in BG-11 medium[15] (pH 8.0) under continuous illumination provided by fluorescent lamps (40 μmol photons s^{-1} m^{-2}) with air-bubbling at 25°C. Cell density was measured by A_{720} with a spectrophotometer (Hitachi U-3210).

4.3. General procedure for the reduction of ketones with *S. elongatus* PCC 7942

The ketone (10 μmol) was added to a suspended culture of *S. elongatus* PCC 7942 (1 g/L as dry weight) in BG-11 medium (20 mL). The mixture was shaken at 140 rpm and 25°C, and the resulting mixture was extracted with ether and eluted by Extrelut. The chemical and enantiomeric purities were determined by GC analyses. The GC conditions and the retention times of substrates, products, and internal standards are as follows: acetophenone; CPCD 110°C, ketone 9.8 min, *S*-alcohol 14.4 min, *R*-alcohol 13.9 min, chlorobenzene 3.6 min: α-monofluoroacetophenone; CPCD 120°C, ketone 5.6 min, *S*-alcohol 12.6 min, *R*-alcohol 13.7 min, naphthalene 10.5 min: α,α-difluoroacetophenone; CPCD 120°C, ketone 4.1 min, *S*-alcohol 15.8 min, *R*-alcohol 17.0 min naphthalene 10.5 min: α,α,α-trifluoroacetophenone; CPCD 120°C, ketone 3.6 min, *S*-alcohol 17.5 min, *R*-alcohol 18.1 min, naphthalene 10.5 min.

4.4. Method used to investigate the effect of addition of DCMU on the reduction of α,α-difluoroacetophenone by *S. elongatus* PCC 7942

α,α-Difluoroacetophenone (10 μmol) was added to a suspended culture of *S. elongatus* PCC 7942 (1 g/L as dry weight) in BG-11 medium (20 mL) with or without DCMU (10 μM) in DMSO (100 μL). The mixture was shaken at 140 rpm and 25°C for 24 h under illumination (fluorescent light; 13.4 photons μmol s^{-1} m^{-2}) or darkness and the resulting mixture was extracted with ether and eluted by Extrelut. The chemical and enantiomeric purities were determined by GC analyses.

4.5. Method used to investigate the effect of DCMU concentration on the reduction of α,α-difluoroacetophenone by *S. elongatus* PCC 7942

α,α-Difluoroacetophenone (10 μmol) was added to a suspended culture of *S. elongatus* PCC 7942 (1 g/L as dry weight) in BG-11 medium (20 mL) with DCMU (0, 0.1, 5, 100 μM) in DMSO (100 μL). The mixture was shaken at 140 rpm and 25°C for 30 h under illumination (fluorescent light; 13.4 photons μmol s^{-1} m^{-2}) and the resulting mixture was extracted with ether and eluted by Extrelut. The chemical and enantiomeric purities were determined by GC analyses.

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