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Light mediated cofactor recycling system in biocatalytic asymmetric reduction of ketone

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Reduction of an artificial ketone by *Synechococcus elongatus* PCC 7942 proceeds smoothly by the aid of light. The efficiency of the reaction is very high since the coenzyme NADPH is regenerated by using light energy.

Biocatalytic reduction is a useful tool for obtaining optically active alcohols, and many scientists have studied reactions using isolated enzymes, microbes and plant cell cultures as biocatalysts.[1] Reduction of substrates usually requires a large input of energy, and in microbial reductions, carbohydrates such as sugars have been used to recycle the coenzyme. These carbohydrates are generated through photosynthesis with sunlight energy. In other words, we have been *indirectly* using light energy for asymmetric reduction.

Now we propose the direct use of light energy for such reactions by using a biocatalyst that falls into a new category, the phototroph, because it can *directly* use light energy.

For the majority of redox enzymes, nicotinamide adenine dinucleotide [NAD(H)] and its respective phosphate [NADP(H)] are required. These cofactors are prohibitively expensive if used in stoichiometric amounts. Since it is only the oxidation state of the cofactor that changes during the reaction, it may be regenerated *in situ* by using a second redox-reaction to allow it to re-enter the reaction cycle. Usually, formate,[2] glucose,[3] and simple alcohols such as ethanol[4] and 2-propanol[5] are used to generate the oxidized form of the coenzyme to the reduced form. These reductants originally stem from bioproducts of CO_2 by the aid of sunlight with phototroph.

Phototrophs such as algae and plants capture light energy to generate NADPH from $NADP^+$through photosynthetic electron-transfer reactions. Subsequently, CO_2 is converted into sugar, generally using NADPH[6].

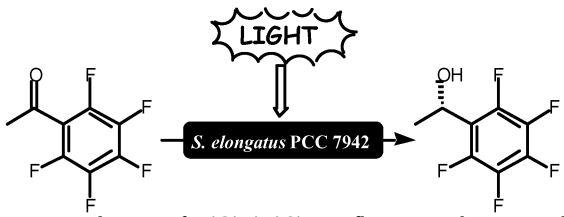
We propose that the reducing power of NADPH generated through photosynthesis also can be used in the reduction of exogenous substrates such as unnatural ketones to yield useful optically active alcohols. Thus, cofactor-recycling is no problem when photosynthetic living cells are used as biocatalysts for reduction. Accordingly, we can use solar energy directly for bioconversion of artificial substrates.

We focus on cyanobacteria (microalgae) since they belong to both phototroph and microbe categories, in other words, they are plant-like photosynthetic bacteria. Therefore, the growth rate of cyanobacteria is as high as that of typical microbes. The problem of using cultured plant cells is that they usually grow very slowly.

Now we propose a new system for cofactor-recycling, in which cyanobacteria converts light energy to reducing power. The advantage of this reaction is that light energy, which is a cheap resource, can be used since the microalgae possesses a system that is required for production of reduced coenzymes, NADPH.

We tried to reduce ketones by using a cyanobacterium under illumination (see Experimental†).

We already reported that aryl methyl ketones are reduced to the corresponding *S*-alcohols by *Synechococcus elongatus* PCC 7942, which is one of the cyanobacteria, that is a photosynthetic prokaryote, with high enantioselectivity (> 96% ee).[7] When 2′,3′,4′,5′,6′-pentafluoroacetophenone is used as a substrate, the reaction proceeds in an excellent chemical yield (> 90%) with high enantioselectivity (> 99% ee) (Scheme 1).



Scheme 1 Reduction of 2′,3′,4′,5′,6′-pentafluoroacetophenone with *S. elongatus* PCC 7942.

Since the perfluorinated phenyl group has a striking stacking ability with electron-rich arenes,[8] optically active alcohols having pentafluorophenyl moiety are potentially useful chiral building blocks for fine chemicals.[9]

An advantage of the proposed biotransformation system is its high substrate/biocatalyst (s/b) ratio (= 2.0), which is comparable to a low s/b ratio of the other biocatalysts (baker′s yeast: 0.003–0.02 and plant cell cultures of *Marchantia polymorpha*: 0.001), therefore, a cyanobacterium-catalyzed reaction is very effective. This supports the idea that the reaction proceeds by a cofactor-recycling system that uses light energy.

To clarify our proposal, we investigated the effect of light and the cofactor-dependency on the reduction of ketones. We examined the effect of light on the reduction of a ketone by a microbe (Fig. 1). This figure illustrates the time-course of the yields of the alcohol. The line with the circles shows the yields of the reduction under illumination, and the line with the triangles shows the yields of the reduction under darkness. The reaction rate under illumination is higher than that under darkness. Actually, the initial rate under illumination is about four times higher than that in the dark.

Furthermore, the line with the squares shows the yields in the reduction under illumination after an initial two days under darkness. Apparently, the reaction increased rapidly under illumination. This graph supports the assumption of a direct effect on the reaction using light.

Next, we investigated cofactor-dependency in reducing ketones by using the cyanobacterium. A cell free extract (acetone-dried powder) was prepared from the cyanobacterium,

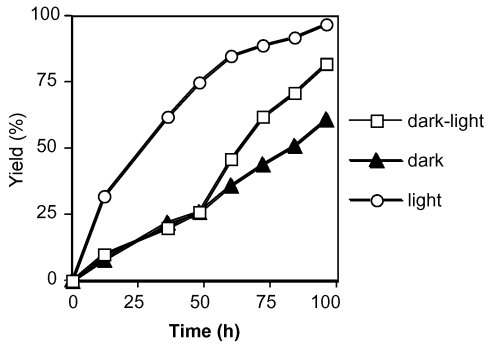


Fig. 1 Effect of light on reduction of 2′,3′,4′,5′,6′-pentafluoroacetophenone with *Synechococcus elongatus* PCC 7942.

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and the reaction was conducted by using the extract in the presence of the coenzymes (Table 1). As a result, NADPH was effective for the reaction but NADH was not. This result demonstrates that cyanobacterial reactions are NADPH-dependent.

Table 1 Cofactor-dependency in reduction of 2′,3′,4′,5′,6′-pentafluoroacetophenone with *Synechococcus elongatus* PCC 7942

Coenzyme	Activity (%)	Ee (%)-(*S*+)
None	0	—
NADH	6.7	> 99
NADPH	100	> 99

Substrate: 0.2 mmol, acetone-dried cyanobacterium: 5 mg, phosphate buffer (pH 7.0): 2 mL, coenzyme: 5 mg, 40 h.

Thus, the reaction mechanism of the cyanobacterial reduction is thought to be part of the following process (Fig. 2).

As shown in Fig. 2, chlorophyll in cyanobacteria captures light energy to generate NADPH, and the substrate, a ketone, is converted into the corresponding optically active alcohol by the aid of NADPH. We propose a new strategy for production of useful compounds by using microalgae *directly* with the aid of light energy instead of *indirectly via* fossil fuels.

Although plant cells[10] and algae[11] have been used as biocatalysts for reduction, the effect of light on the reduction has never been investigated. In these studies, resting cells of a phototroph are used. We investigated a novel system using living cells. In the proposed novel system for asymmetric reduction, light energy is effectively used for biotransformation.

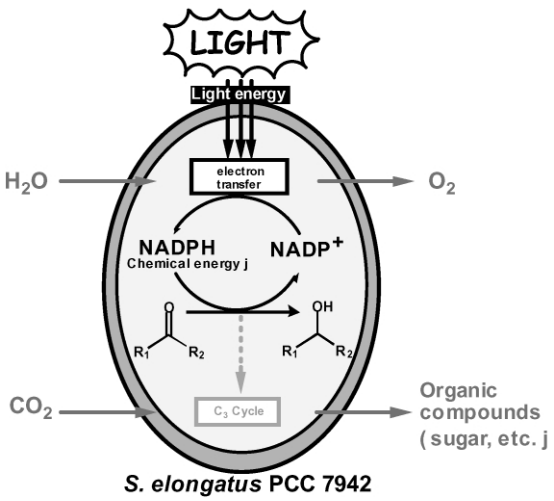

Fig. 2 Reaction mechanism of reduction of ketones using *Synechococcus elongatus* PCC 7942.

We used the living cells of algae, a natural cellular environment that absorbs carbon dioxide, discharges oxygen, and also synthesizes a useful optically active alcohol at the same time. Consequently, we have developed a new method using algae that could benefit the world without destroying natural ecological systems.

Notes and references

† *Experimental.*

Culture conditions. Cyanobacterium (*Synechococcus elongatus* PCC 7942) was grown in 300 mL conical glass culture flasks containing 100 mL BG-11 medium photoautotrophically at 25 °C under continuous fluorescent light (13.4 μmol m^{-2} s^{-1}) with shaking at 110 rpm.

Reduction of 2′,3′,4′,5′,6′-pentafluoroacetophenone by Synechococcus elongatus PCC 7942. Substrate (0.04 mmol) was added to a 300 mL conical glass culture flask containing a suspension of cyanobacterium (60 mg as a dry weight)[12] in 100 mL BG-11 medium and reacted at 25 °C under continuous fluorescent light (13.4 μmol m^{-2} s^{-1}) with shaking at 110 rpm. The chemical and optical yields of the reaction were determined by chiral GC (CP-cyclodextrin β-2,4,6-M-19) using naphthalene as an internal standard.

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12 The reaction rate varies in proportion to mass of the biocatalysts.