Electronic Supplementary Material (ESI) for RSC Advances

Electronic Supplementary Information

Methane hydroxylation using light energy by the combination of thylakoid and methane monooxygenase

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Materials

All reagents were obtained from commercial suppliers and were of the highest available purity.

Isolation of thylakoid membrane from spinach was performed by reference to the previous methods (detail was showed Supporting Information).[1, 2] Preparation should be conducted at low temperature (approx. 4 °C). Spinach was obtained from a local market. About 90 g of spinach leaves were washed and removed ribs, then packed in wet paper, and left overnight in a cold room. The leaves torn into small pieces were placed in 1 L beaker with 180 mL of Tricine buffer (0.4 M Sucrose, 10 mM NaCl, 2 mM $MgCl_2$, 50 mM Tricine adjusted pH 8.0 with KOH aq.). The leaves were homogenized by hand blender, then the homogenate was filtered twice through two layers of gauze. The filtrate was centrifuged at 5,000 × *g*, 4 °C for 2 min. The supernatant was discarded and the precipitate was resuspended in about 80 mL of Tricine buffer (pH 8.0). The suspension was centrifuged at 5,000 × *g*, 4 °C for 5 min. The pellet was resuspended in a small volume of Tricine buffer (pH 8.0), frozen in liquid nitrogen, and stored at -80 °C. Total chlorophyll concentration of the thylakoid was determined as described previously.[3]

Membrane fraction of *Methylosinus trichosporium* OB3b was isolated according to the method reported procedures.[4, 5] The protein concentration was determined by Lowry method.

NAD^+ photoreduction

The reaction medium (total volume 1.0 mL) containing Tricine buffer (pH 8.0), 2.0 mM NAD^+, and 0.30 mg Chl/mL thylakoid were added to a 5 mL test tube. The sample mixture was stirred in a 30 °C thermostat water bath and irradiated by 10% of output with 250 W metal-halide lamp (MORITEX, MME-250) fitted with yellow filter (Y-47). The reaction mixture was sampled by 30 μL in every fixed time, and then centrifuged at 16,000 × *g* for 5 min. The supernatant was diluted with Tricine buffer (pH 8.0) by a factor of 40, and measured using a UV-vis spectrometer at 340 nm.

NADH production was quantified using the molar extinction coefficient of 6220 M^{-1} cm^{-1}.[6, 7]

NADH dependent MMO assay of membrane fraction from *M. trichosporium* OB3b

The assay protocol was as follows: 500 μL reaction mixtures, consisting of 2.0 mg-protein mL^{-1} of the membrane fraction of *M. trichosporium* OB3b suspended in Tricine buffer (pH 8.0) and 2.0 mM NADH, were placed in a 3 mL vial and sealed with a Teflon-sealed septum. The reaction was initiated by the injection of 300 μL of propylene into the reaction vial using a gas-tight syringe, and the vial was then stirred in a 30 °C thermostat water bath. The amount of produced propylene oxide was determined by FID gas chromatography (HITACHI, 263-30). Quantitation was performed by comparison with pure propylene oxide standards. The specific activity was described as the amount of propylene oxide produced in 1 min by 1 mg of protein (mol propylene oxide min^{-1} mg-$protein^{-1}$). The gas chromatography conditions were as follows: Sorbitol 25%-Gasport B 60/80 glass column (3ϕ × 4 m, GL Science); carrier gas, nitrogen at a flow rate of 20 mL/min; column temperature, 100 °C; injection and detector temperature, 150 °C. In case of methane oxidation, methane (300 μL) was added to the headspace instead of propylene as the substrate. The gas chromatography conditions were as follows: Sorbitol 25%-Gasport B 60/80 glass column (3ϕ × 2 m, GL Science); carrier gas, nitrogen at a flow rate of 20 mL/min; oven temperature, 100 °C; injection and detector temperature, 150 °C.

Propylene oxidation using light energy by the combination of thylakoid and membrane fraction from *M. trichosporium* OB3b

500 μL reaction mixtures, consisting of 0.30 mg Chl mL^{-1} of the thylakoid and 2.0 mg-protein mL^{-1} of the membrane fraction of *M. trichosporium* OB3b suspended in a Tricine buffer (pH 8.0) and 2.0 mM NAD^+, were placed in a 3 mL vial and sealed with a Teflon-sealed septum. The reaction was initiated by the injection of 300 μL propylene into the reaction vial using a gas-tight syringe, and the vial was then stirred in a 30 °C thermostat water bath. The reaction mixtures were illuminated by metal-halide lamp (MORITEX, MME-250). The amount of produced propylene oxide was determined by FID gas chromatography (HITACHI, 263-30).

Methane hydroxylation using light energy by the combination of thylakoid and membrane fraction from *M. trichosporium* OB3b

The procedure for propylene oxidation using light energy by the combination of thylakoid and membrane fraction of *M. trichosporium* OB3b was used with the following modifications. Methane

(300 μL) was added to the headspace instead of propylene as the substrate. The gas chromatography conditions were as follows: Sorbitol 25%-Gasport B 60/80 glass column (3ϕ × 2 m, GL Science); carrier gas, nitrogen at a flow rate of 20 mL/min; oven temperature, 100 °C; injection and detector temperature, 150 °C.

Methanol consumption by the combination of thylakoid and membrane fraction of *M. trichosporium* OB3b

500 μL reaction mixtures, consisting of 0.30 mg Chl mL^{-1} of the thylakoid, 2.0 mg-protein mL^{-1} of the membrane fraction of *M. trichosporium* OB3b, 2.0 mM NAD^+ and 110 μM methanol, suspended in a Tricine buffer (pH 8.0) were placed in a 3 mL vial and sealed with a Teflon-sealed septum. The vial was then stirred in a 30 °C thermostat water bath. The amount of produced methanol was determined by FID gas chromatography (HITACHI, 263-30) in every fixed time.

In a control experiment carried out in the dark conditions, we found small amount of oxidation products. This is probably due to the existence of some metabolites originally found in chlorophyll or in a membrane fraction and these organic compound(s) served as electron source for oxidation of substrate by pMMO. In the case of methane oxidation, methanol was found at the initial of reaction. Methanol is used for crystallization of tricine by chemical supplier and small amount of methanol was observed in tricine.

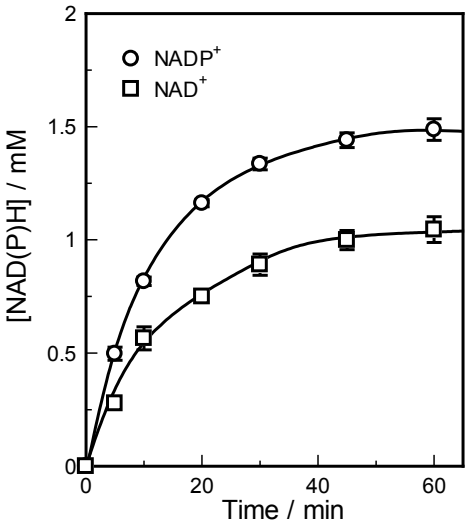

Fig. S1 Time dependence of NAD(P)H production with thylakoid from spinach irradiated visible light.

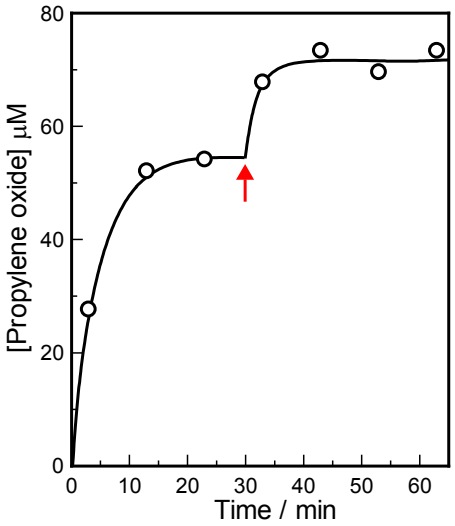

Fig. S2 Effect of the addition of membrane fraction from *M. trichosporium* OB3b on pMMO assay using propylene oxidation. At 30 minute, membrane fraction from *M. trichosporium* OB3b (1.0 mg-protein) was added.

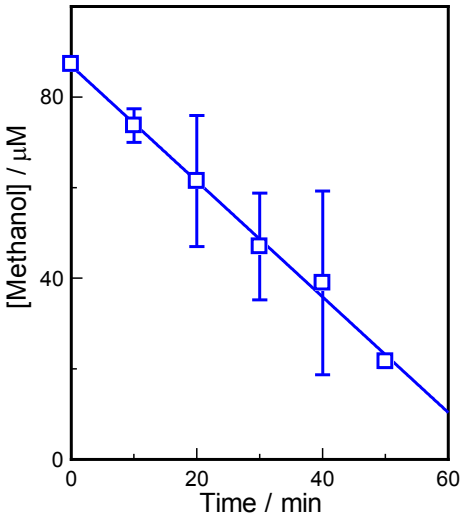

Fig. S3 Time dependence of methanol consuming process containing the thylakoid, membrane fraction of *M. trichosporium* OB3b and NAD^+ in Tricine buffer (pH 8.0).

Notes and references

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