LETTER

# Enzymatic Conversion of Atmospheric Aldehydes into Alcohol in a Phospholipid Polymer Film

**Naoki Tanaka,*,† Akihiro Watari,† Tomoko Tada,† Tomoko Asada,† Shigeru Kunugi,† Yin-Fai Lee,‡ Satoshi Yamada,§ Kenshiro Shuto,§ and Shujiro Sakaki||**

Department of Biomolecular Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo, Kyoto 606-8585, Japan, Radiation Oncology and Biology, University of Oxford, Churchill Hospital, Headington, Oxford OX3 7LJ, United Kingdom, Tsukuba Corporate Research Laboratory, NOF Corporation, 5−10 Tokodai, Tsukuba, Ibaraki 300-2635, Japan, and Applied Chemistry Department, Akita National College of Technology, 1-1 Iijimabunkyo, Akita, Akita 011-8511, Japan

**ABSTRACT** We developed a unique method for converting atmospheric aldehyde into alcohol using formaldehyde dehydrogenase from *Pseudomonas putida* (PFDH) doped in a polymer film. A film of poly(2-methacryloyloxyethylphosphorylcholine-*co*-*n*-butyl methacrylate) (PMB), which has a chemical structure similar to that of a biological membrane, was employed for its biocompatibility. A water-incorporated polymer film entrapping PFDH and its cofactor $NAD^+$ was obtained by drying a buffered solution of PMB, PFDH, and $NAD^+$. The aldehydes in the air were absorbed into the polymer film and then enzymatically oxidized by PFDH doped in the PMB film. Interestingly, alcohol and carboxylic acid were produced by the enzymatic reaction, indicating that PFDH catalyzes dismutation of aldehyde in the PMB film. Importantly, a PFDH−PMB film catalyzes aldehyde degradation without consuming the nucleotide cofactor, thereby allowing repeated use of the film. The activity of PFDH in the PMB film was higher than that in other common water-soluble polymers, suggesting that the hydrational state in a phospholipid polymer matrix is suitable for enzymatic activity.

**KEYWORDS:** biofixation • atmospheric aldehyde • formaldehyde dehydrogenase • phospholipid polymer • dismutation • alcohol

## INTRODUCTION

Artificial biofixation is an ideal method for the conversion of atmospheric substances with high yield and selectivity under mild reaction conditions (1−3). We have developed a method for the conversion of atmospheric aldehyde into alcohol using an enzymatic reaction in a polymer film. Formaldehyde and acetaldehyde are possible human carcinogens (4, 5) and cause various health hazards when they are released from plastics and resins. Several photocatalytic methods to degrade atmospheric aldehydes have been studied (6−8), but equivalent enzymatic degradation processes have yet to be exploited. Previously, $NAD^+$-dependent oxidation of aldehydes by dehydrogenases has been used as a sensor to monitor the level of atmospheric aldehydes (9−11). Formaldehyde dehydrogenase isolated from *Pseudomonas putida* (PFDH; EC 1.2.1.46) is a unique enzyme that has yet to be exploited extensively for the treatment of aldehydes in the environment. PFDH, a homotetramer enzyme composed of 42 kDa subunits, can catalyze $NAD^+$-dependent oxidation of aldehyde to carboxylic acid without the external addition of glutathione (12, 13). When PFDH is incubated with $NAD^+$ and either formaldehyde or acetaldehyde in solution, the oxidation of aldehyde with NADH production can be detected as an increase in its light absorbance at 340 nm ($A_{340}$).

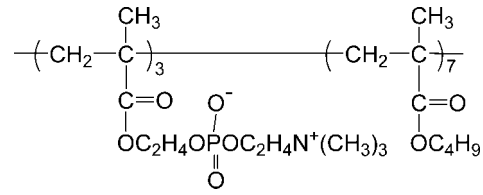



**FIGURE 1. Chemical structure of PMB.**

In this study, we developed a method using PFDH immobilized in a polymer film for enzymatic treatment of aldehydes in the air. A film of the phospholipid polymer, a water-soluble polymer containing 2-methacryloyloxyethylphosphorylcholine, was employed to entrap PFDH in the film. The polymer was specifically designed to adopt a structure similar to that of a biological membrane (14−17) and extensively used biomaterials for its biocompatiblity. We used a derivative of the polymer, poly(2-methacryloyloxyethylphosphorylcholine-*co*-*n*-butyl methacrylate) (PMB; Figure 1) because of its ability to stabilize the native conformation of the protein. The enzymatic reaction of PFDH

* Corresponding author. E-mail: tanaka@kit.ac.jp. Tel. and Fax:+81-75-724-7861.
Received for review September 21, 2008 and accepted October 29, 2008
† Kyoto Institute of Technology.
‡ University of Oxford.
§ NOF Corporation.
|| Akita National College of Technology.
DOI: 10.1021/am800068a



Published on Web 12/01/2008

doped in the PMB film against aldehydes in air was examined in this study.

## EXPERIMENTAL SECTION

**Materials.** Formaldehyde dehydrogenase from *Pseudomonas putida* (PFDH) was obtained from Toyobo Co. (Osaka, Japan). We confirmed that the purity of the enzyme was >90% by SDS-PAGE (except for the content of bovine serum albumin (BSA) used as the stabilizer). Nicotinamide adenine dinucleotide ($NAD^+$, oxidized form) was purchased from Oriental Yeast (Tokyo, Japan). Formaldehyde (37% in aqueous solution with 10% methanol) was obtained from Wako Pure Chemicals (Osaka, Japan). Acetaldehyde (>99.5%) and BSA (further purified fraction V, >98%) were purchased from Sigma-Aldrich (Piscataway, NJ). Poly(2-methacryloyloxyethylphosphorylcholine-*co*-*n*-butyl methacrylate) (PMB) was prepared by the radical copolymerization of 2-methacryloyloxyethylphosphorylcholine and *n*-butyl methacrylate in ethanol with *tert*-butyl peroxyneodecanoate as the initiator at 60 °C for 6 h and purified by precipitation from *n*-hexane and ether. Poly(sodium *p*-styrenesulfonate) (PSS; $M_w$ = 70K) and poly(*N*-vinylpyrrolidone) (PVP; $M_w$ = 55K) were obtained from Sigma-Aldrich. A PFDH-doped polymer film was prepared by drying 4.1 mL of a 3.3% PMB aqueous solution (buffered in 50 mM phosphate at pH 7.5) with 6.3 $\mu$M PFDH and 2.2 mM $NAD^+$ in a glass Petri dish (92 mm diameter) at 25 °C and 45% relative humidity.

**Enzymatic Assay.** The enzymatic activity of PFDH doped in a PMB film against aldehyde vapor was assayed by measuring the concentration change of aldehyde in a glass desiccator from Schott Duran with an approximate volume of 5.8 L in the presence or absence of a PFDH−PMB film. The aldehyde vapors were generated in the desiccator by dropping the aqueous aldehyde solution from the top outlet on a preheated beaker placed on a porcelain desiccator plate. The concentration of aldehyde in the desiccator was measured using a gas detector tube (Gastec Co., Kanagawa, Japan). The gas detector tube was connected to the side outlet of the desiccator, and sample air was collected by drawing into the tube using a manual sampling pump. All measurements were performed at 25 °C and 45% relative humidity, in which the concentration of environmental aldehydes in air is low and not detectable using a gas detector tube (<0.1 ppm).

A GL Science (Tokyo, Japan) GC353B gas chromatograph with a flame ionization detector was used to analyze the enzymatic products. A Varian GC-PLOT CP-PolaPLOT U capillary column was used for chromatographic separation, and helium, with a flow rate of 90 mL/min, was used as the carrier gas. The 500 $\mu$L sample vapor from the glass desiccator was injected using a Hamilton gastight syringe into the injection port.

## RESULTS AND DISCUSSION

In order to obtain a stable polymer film, we prepared PMB using different copolymerization ratios and examined their properties. The homopolymer of poly(2-methacryloyloxyethylphosphorylcholine) (MPC; $M_w$ = 100K) and the copolymer consisting of 80 mol % MPC residues ($M_w$ = 600K) formed a film by drying of their aqueous solution, but these films deliquesced after 1 month of storage at 25 °C and 45% relative humidity. However, a film from the copolymer consisting of 30 mol % MPC residues ($M_w$ = 100K; Figure 1) maintained its stable form after more than 1 month of storage at 25 °C within a humidity range of 45−60%. This copolymer was selected for the enzyme-entrapping film. Next, the effect of PMB on the enzymatic activity of PFDH was examined by measuring the kinetics

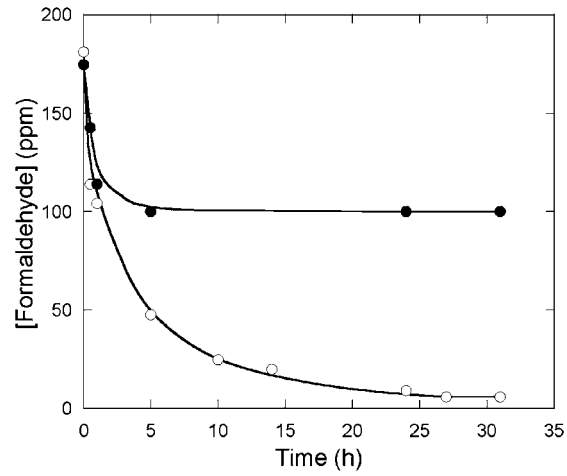



**FIGURE 2. Concentration (ppm) of formaldehyde in the desiccator monitored by a gas detector tube: ●, in the presence of a PMB film; ○, in the presence of a PFDH−PMB film.**

of the oxidation of formaldehyde monitored through an increase in $A_{340}$ in solution. The enzyme kinetics of PFDH was unaffected by 1% PMB in solution (data not shown), indicating that PMB does not inhibit the activity of PFDH.

A PFDH-doped polymer film was prepared by drying of a PMB aqueous solution with 6.3 $\mu$M PFDH and 2.2 mM $NAD^+$ in a glass Petri dish. We estimated that the obtained film contained ca. 10% (w/w) water by measurement of its weight. To assay the activity of PFDH in the PMB film against atmospheric substrates, the aldehyde vapor concentration in the desiccator was measured using a gas detector tube. The 180 ppm aldehyde vapor was generated by dropping of the aqueous aldehyde solution on a preheated beaker in a desiccator. In the presence of a PMB film without PFDH, the formaldehyde concentration decreased from 180 to 100 ppm in the first 6 h to reach an equilibrium (● plots in Figure 2), indicating that 80 ppm formaldehyde has been absorbed into the film. However, the concentration of formaldehyde was decreased from 180 to 5 ppm after 30 h (○ plots in Figure 2) in the presence of a PFDH−PMB film. This result can be explained either by enzymatic reaction of PFDH or by the increased surface area with protein doping in the PMB film. To confirm that this result is due to the enzymatic activity of PFDH, the properties of a PMB film entrapping BSA were examined as a control. The change of the aldehyde vapor concentration in the presence of a BSA−PMB film was almost the same as the PMB film without PFDH (i.e., ● plots in Figure 2; data not shown). These results indicate that the drastic decrease of the aldehyde vapor in the presence of a PFDH−PMB film is due to the enzymatic reaction.

The concentrations of formaldehyde and $NAD^+$ in the PMB film in the first 6 h are estimated to be much higher than 10 mM from the approximate film volume of ca. 150 $\mu$L. These concentrations are much higher than the respective $K_m$ values for formaldehyde and $NAD^+$ of 67 and 56 $\mu$M (12). Thus, PFDH active sites must be saturated with substrate and cofactor in the PMB film. However, the apparent reaction rate of PFDH in the PMB film is estimated as ca. 24 nmol/mg/min from the concentration decrease of formal-

dehyde in the initial stage, which is much slower than the $V_{max}$ value of 10 $\mu$mol/mg/min in aqueous solution (12). This may be because the polymer matrix affected the enzymatic activity of PFDH by changing the hydration state of the enzyme or the diffusion rate of the molecules. We have confirmed that atmospheric acetaldehyde was also degraded by a PFDH–PMB film in a similar way.

The activity of the PFDH–PMB film was compared with that of PFDH entrapped within the films of other common water-soluble polymers, PVP ($M_w$ = 55K) and PSS ($M_w$ = 70K). PFDH in these polymer films exhibited enzymatic activity for the degradation of atmospheric aldehyde, but the reaction rate was less than half that of PFDH–PMB. In addition, the enzymatic activity of PFDH–PMB was unaffected after 1 month of storage at 25 °C and 45% relative humidity, suggesting that the enzyme is stabilized in the phospholipid polymer matrix. These results could be related to the way water molecules are incorporated into the polymer film. The structure and hydrogen-bonding network of water molecules in a phospholipid polymer matrix have been shown to be distinct from those found in other polymer films and closely resemble conditions in the bulk aqueous state (18, 19). The activity of PFDH may be less affected by the hydration state in the PMB matrix compared with those in the PVP or PSS matrix.

To assay the enzymatic activity in the polymer film, we measured the absorbance spectrum of the PFDH–PMB film (coated on the quartz plate) before and after reaction with aldehydes in the desiccator to monitor the increase in $A_{340}$ of NADH. Interestingly, the $A_{340}$ value of NADH in the absorbance spectrum of the PFDH–PMB film did not increase. In addition, the amount of formaldehyde catalyzed by the PFDH–PMB film (ca. 20 $\mu$mol) was much higher than that of $NAD^+$ present in the film (ca. 9 $\mu$mol). These results suggested that the oxidization of aldehydes in PFDH–PMB was not accompanied by a stoichiometric conversion of cofactor from $NAD^+$ to NADH. A similar phenomenon has been reported for the catalysis of propanal and other longer-chain aldehydes by PFDH in aqueous solution, where two molecules are converted to one molecule each of the corresponding carboxylic acid and alcohol, i.e., a dismutation. The dismutation of aldehydes by PFDH in solution has been suggested to consist of two coupled half-reactions (12, 13).

$$RCHO + H_2O + PFDH{-}NAD^+ \rightarrow RCOOH + PFDH{-}NADH + H^+ \quad (1)$$

$$RCHO + PFDH{-}NADH + H^+ \leftrightarrow RCH_2OH + PFDH{-}NAD^+ \quad (2)$$

In the first half-reaction (1), one aldehyde molecule is oxidized by the enzyme–$NAD^+$ complex to form a corresponding carboxylate and enzyme–NADH complex. In the second half-reaction (2), another aldehyde molecule binds to this enzyme–NADH complex and is reduced to an alcohol. Either the PFDH–NADH complex formed by the oxidation of aldehyde in (1) can dissociate to yield free NADH and enzyme, or it can combine with another molecule of aldehyde to form the productive PFDH–NADH–RCHO complex. When the dissociation rate of NADH is slower than the association rate with aldehyde, PFDH catalyzes the dismutation of aldehyde, where $NAD^+$ is not converted to NADH. The molar ratio of the substrate (aldehyde) concentration to the product (carboxylic acid and alcohol) concentration is expected to be 2:1 in a dismutation of PFDH.

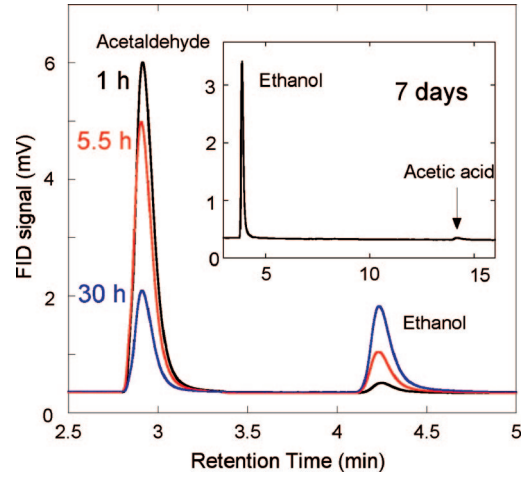
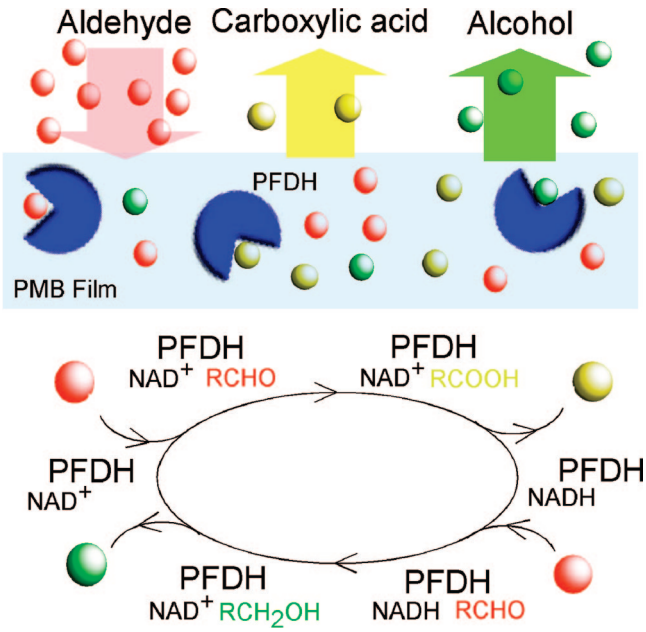



**FIGURE 3. Gas chromatograms of the air in the desiccator containing 540 ppm acetaldehyde and a PFDH–PMB film. Inset: expanded chromatogram.**

To gain insight into the reaction mechanism of PFDH in the PMB film, we analyzed the vapor in the desiccator by gas chromatography. Because an aqueous formaldehyde solution contains methanol as the stabilizer, which may interfere with the analysis, acetaldehyde was used as the substrate. The gas chromatograms in Figure 3 show that ethanol and acetic acid were produced with a corresponding decrease in acetaldehyde. These peaks did not appear when the enzyme is absent from the film, supporting the notion of a dismutation reaction for acetaldehyde in the film. After 7 days, acetaldehyde was not detectable. The concentrations of ethanol and acetic acid produced from 540 ppm acetaldehyde in the desiccator were calculated to be 250 and 100 ppm, respectively, based on the chromatogram peak intensity. The molar ratio of the initial acetaldehyde concentration to the final ethanol concentration was 2:0.93, which is close to the expected molar ratio of 2:1 for a dismutation. Some acetic acid may have been retained in the film, leading to a lower level of acetic acid as detected by gas chromatography. In fact, when the pH of the solution in which the polymer film was dissolved was measured, the $H^+$ concentration in the polymer film was found to be higher after the enzymatic reaction.

The reaction mechanism of PFDH in the films of PVP and PSS was also studied by gas chromatography. We found that PFDH also catalyzed acetaldehyde dismutation in these polymer films. Such changes in the reaction mechanism in the presence of polymer may be related to changes in the diffusion of molecules in the polymer matrix. The diffusion rate of NADH, being a bulky molecule, would be significantly decreased in a polymer matrix compared with the smaller acetaldehyde molecule. Thus, under these conditions, PFDH–NADH may have a greater chance of combining with

**Scheme 1. Dismutation of PFDH Converting Atmospheric Aldehyde into Carboxylic Acid and Alcohol in the PMB Film**




aldehyde to form the PFDH−NADH−RCHO complex before the dissociation of NADH from PFDH (Scheme 1).

In conclusion, we have demonstrated that atmospheric aldehyde can be efficiently converted to alcohol by PFDH dismutation in a phospholipid polymer film. The observed dismutation in the polymer film may involve the slow diffusion of $NAD^+$ within the polymer matrix. We are currently investigating the potential mechanism of the reaction. Importantly, the PFDH−PMB film catalyzes the conversion of aldehyde without converting $NAD^+$ to NADH, and thus the film can be used repeatedly without consuming the nucleotide cofactor. The PMB film entrapping various enzymes may be widely applicable for the conversion of atmospheric substances such as $CO_2$, $NO_x$, and $SO_x$ into useful chemicals such as alcohol.

**Acknowledgment.** We thank Prof. K. Ishihara (The University of Tokyo) and Prof. Y. Iwasaki (Kansai University) for their helpful comments and encouragement during the course of this research.

## REFERENCES AND NOTES

(1) Willner, I.; Mandler, D.; Riklin, A. *J. Chem. Soc., Chem. Commun.* **1986**, 1022–1024.
(2) Itoh, T.; Asada, H.; Tobioka, K.; Kodera, Y.; Matsushima, A.; Hiroto, M.; Nishimura, H.; Kamachi, T.; Okura, I.; Inada, Y. *Bioconjugate Chem.* **2000**, *11*, 8–13.
(3) Obert, R.; Dave, B. C. *J. Am. Chem. Soc.* **1999**, *121*, 12192–12193.
(4) Feron, V. J.; Til, H. P.; Woutersen, R. A. *Toxicol. Ind. Health* **1990**, *6*, 637–639.
(5) Chiarpotto, E.; Biasi, F.; Scavazza, A.; Camandola, S.; Aragno, M.; Tamagno, E.; Danni, O.; Dianzani, M. U.; Poli, G. *Alcohol Alcohol.* **1995**, *30*, 721–728.
(6) Fujishima, A.; Rao, T. N.; Tryk, D. A. *J. Photochem. Photobiol. C* **2000**, *1*, 1–21.
(7) Nishijima, K.; Ohtani, B.; Yan, X.; Kamai, T.-a.; Chiyoya, T.; Tsubota, T.; Murakami, N.; Ohno, T. *Chem. Phys.* **2007**, *339*, 64–72.
(8) Sopyan, I. *Sci. Technol. Adv. Mater.* **2007**, *8*, 33–39.
(9) Williams, A. K.; Hupp, J. T. *J. Am. Chem. Soc.* **1998**, *120*, 4366–4371.
(10) Yao, T.; Handa, S. *Anal. Sci.* **2003**, *19*, 767–770.
(11) Nikitina, O.; Shleev, S.; Gayda, G.; Demkiv, O.; Gonchar, M.; Gorton, L.; Csoregi, E.; Nistor, M. *Sens. Actuators B* **2007**, *Chemical 125*, 1–9.
(12) Ogushi, S.; Ando, M.; Tsuru, D. *J. Biochem.* **1984**, *96*, 1587–1591.
(13) Tanaka, N.; Kusakabe, Y.; Ito, K.; Yoshimoto, T.; Nakamura, K. T. *J. Mol. Biol.* **2002**, *324*, 519–533.
(14) Ishihara, K.; Nomura, H.; Mihara, T.; Kurita, K.; Iwasaki, Y.; Nakabayashi, N. *J. Biomed. Mater. Res.* **1998**, *39*, 323–330.
(15) Sakaki, S.; Nakabayashi, N.; Ishihara, K. *J. Biomed. Mater. Res.* **1999**, *47*, 523–528.
(16) Iwasaki, Y.; Tabata, E.; Kurita, K.; Akiyoshi, K. *Bioconjugate Chem.* **2005**, *16*, 567–575.
(17) Watanabe, J.; Ishihara, K. *Bioconjugate Chem.* **2007**, *18*, 1811–1817.
(18) Kitano, H.; Sudo, K.; Ichikawa, K.; Ide, M.; Ishihara, K. *J. Phys. Chem.* **2000**, *B 104*, 11425–11429.
(19) Kitano, H.; Imai, M.; Mori, T.; Gemmei-Ide, M.; Yokoyama, Y.; Ishihara, K. *Langmuir* **2003**, *19*, 10260–10266.

AM800068A