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Cathodic supply of electrons to living microbial cells via cytocompatible redox-active polymers

Masahiro Kaneko [a], Masahito Ishikawa [b,c], Jieun Song [a], Souichiro Kato [c,d], Kazuhito Hashimoto [e,*], Shuji Nakanishi [c,*]

[a] Department of Applied Chemistry, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
[b] Department of Biotechnology, Graduate School of Engineering, Nagoya University, Furocho, Chikusa-ku, Nagoya 464-8603, Japan
[c] Research Center for Solar Energy Chemistry, Osaka University, 1-3, Machikaneyama, Toyonaka, Osaka 560-8531, Japan
[d] Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology, 2-17-2-1 Tsukisamu-Higashi, Toyohira-ku, Sapporo, Hokkaido 062-8517, Japan
[e] National Institute for Materials Science, 1-2-1 Sengen, Tsukuba-city, Ibaraki 305-0047, Japan

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ABSTRACT

Redox-active polymers composed of 2-methacryloyloxyethyl phosphorylcholine (MPC) and redox-active units are a new category of cytocompatible electron mediators which possess permeability of cell membranes. However, supply of electrons to living cells through the cytocompatible redox polymers has not been achieved so far due to the high redox potential of the redox polymers. Here we report that electrons were successfully supplied from a cathode into *Escherichia coli* cells, generating the current density of 7.8 μA cm^{-2} at −0.40 V vs. SHE. It was also revealed that the cytocompatibility of viologen was improved simply by co-polymerization with MPC.

1. Introduction

Microbial electrosynthesis involves supplying electrons to living microbes via a cathode to improve the efficiency of target chemical production [1–4]. Cathodic electrons can be transferred to microbes via two main mechanisms. The first is extracellular electron transfer (EET) via redox-active proteins in the outer cellular membrane [5–8]. Although the molecular mechanism of the EET process has not been clarified, several studies have demonstrated that certain microbial species are able to directly utilize electrons supplied from cathodes as reducing equivalents for converting CO_2 to valuable chemicals [8–10]. For example, Nevin et al. recently reported that *Sporomusa ovata* produces acetic acid and oxo-butyrate from CO_2 when utilizing a cathode as the sole electron donor [6].

The second mechanism for cathodic electron transfer is via small lipophilic molecules that serve as electron mediators with redox activity [11–13]. Viologen [14,15] and quinone [16] derivatives are representative electron mediators capable of supplying electrons to living microbes. For example, the production of butanol in fermentative *Clostridium acetobutylicum* cells is enhanced by supplying reducing equivalents via methyl viologen [15]. The utilization of artificially synthesized electron mediators for microbial electrosynthesis is advantageous, as it is applicable to microbes lacking specific redox-active proteins in the outer membrane. However, many electron mediators are cytotoxic [11,17,18], and therefore cannot be used in long-term cultivation.

To overcome the cytotoxicity of common electron mediators, we synthesized copolymers composed of redox-active units and 2-methacryloyloxyethyl phosphorylcholine (MPC) [19,20]. MPC-based polymers exhibit high cytocompatibility due to their protein adsorption-resistance [21,22], and we previously reported that a copolymer of MPC and vinyl ferrocene is capable of capturing electrons from living microbes with low cytotoxicity [19,23,24]. However, due to the high redox potential of ferrocene (+0.50 V vs. SHE), the MPC-vinyl ferrocene copolymer is not able to supply electrons to living cells. Herein, we synthesized an MPC-based copolymer composed of a viologen derivative as the redox active unit and demonstrated that the copolymer transferred electrons directly into living *Escherichia coli* cells. In addition, we investigated whether copolymerization of the redox-active units with MPC improved the cytocompatibility of the novel electron mediator.

2. Experimental

2.1. Synthesis of Vi monomer and pMBVi

pMBVi was synthesized by the free radical polymerization of MPC (NOF Co., Ltd., Tokyo, Japan), BMA and Vi monomer with α,α′-

* Corresponding authors.
E-mail address: nakanishi@chem.es.osaka-u.ac.jp (S. Nakanishi).

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azobisisobutyronitrile (AIBN; Tokyo Chemical Industry Co., Inc., Tokyo, Japan) as an initiator. The other organic reagents and solvents used in this study were commercially available reagents of extra-pure grade and were used without further purification. The Vi monomer, *N*-methyl-*N*′-(4-vinylbenzyl)-4.4′-bipyridinium chloride iodide, was synthesized from 4.4-bipyridyl by the two-step synthesis according to previously described method [25]. MPC (0.443 g, 1.50 mmol), BMA (120 μL, 0.750 mmol), Vi monomer (0.407 g, 0.900 mmol) and AIBN (20.5 mg, 0.150 mmol) were dissolved in EtOH/H_2O (5 mL, 1/1 [v/v]). The solution was sealed in a test tube, purged with argon gas for 15 min, and polymerization was then conducted at 60 °C for 48 h. After the reaction, the pMBVi polymer solution was precipitated in a mixed solvent composed of diethyl ether/chloroform (90/10 [v/v]). The obtained precipitate was collected by filtration, dried in vacuum for 10 min, and then dissolved in distilled water for dialysis treatment through a regenerated cellulose membrane (MWCO: 1000) for 4 days. The polymers were then freeze-dried and the obtained dark yellow powder was used for experiments. The molecular weight of the polymers was measured by gel permeation chromatography in a mixed solution of water and methanol (30/70 [v/v]) containing 10 mM lithium bromide. The composition of pMBVi was determined by ^{1}H NMR.

2.2. Cell culture

Escherichia coli K12 was aerobically pre-cultured in Luria–Bertani (LB) medium with shaking at 37 °C. The defined medium used for the electrochemical measurements with *E. coli* was composed of 7 g K_2HPO_4, 3 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 10 g NaCl, and 0.1 g Bacto yeast extract (per liter).

2.3. Electrochemical experiments

A single-chamber, three-electrode system was used for all electrochemical measurements. The system was equipped with a glassy carbon (GC) electrode (3 cm^2) at the bottom of the reactor as the working electrode, and Ag/AgCl (saturated KCl) and platinum wire were used as the reference and counter electrodes, respectively. To conduct electrochemical measurements, 4 mL of the defined medium for *E. coli* was supplemented with glucose (20 mM), pMBVi (1.0 g/L ≈ 1.1 mM Vi monomer) and potassium nitrate (10 mmol). Oxygen was removed from the system by nitrogen purging for 15 min, as oxygen immediately oxidizes the reduced form of Vi. After a pre-reduction of the solution at −0.40 V for approximately 24 h, *E. coli* was introduced into the system to give an optical density at 600 nm (OD_{600}) of 1.0 and current was measured without agitation at 30 °C in an anaerobic chamber. The turnover number of pMBVi was calculated using the following equation:

$$\text{turnover number} = \frac{S/F}{CV} = \frac{S}{CFV}$$

S (=3.74C): Integrated area of the *I*-t curve in the presence both of pMBVi and nitrate for 60 h after the introduction of *E. coli* into the system, F (=9.65 × 10^4 C/mol): Faraday constant, C (=1.11 × 10^{-4} mol/L): molarity of the Vi component in 1.0 g/L pMBVi solution, V (4.00 × 10^{-3} L): electrolyte volume.

3. Results and discussion

A novel electron mediator composed of MPC, butyl methacrylate (BMA), and redox-active viologen (Vi) was synthesized by a free radical polymerization method. The synthesized copolymer, poly(MPC-*co*-BMA-*co*-Vi), is hereafter referred to as pMBVi (Fig. 1a). As amphiphilicity is required for the cell-membrane permeability of the polymer [26,27], the hydrophobic BMA unit was included, because both MPC and the oxidized form of Vi are hydrophilic. The composition of pMBVi was determined by ^{1}H NMR to be MPC:BMA: Vi = 46:18:36 mol%, and the weight-average molecular weight was estimated to be 4.9 kDa (M_w/M_n = 1.3). From the characterizations, the average number of Vi, MPC, BMA in a molecule is estimated to be 6.3, 4.9, and 2.8, respectively. Cyclic voltammetry (CV) of pMBVi in 0.1 M phosphate buffer solution (pH 7) showed two reversible redox waves with mid-point potentials of −0.66 and −0.28 V (Fig. 1b), similar to those observed for the viologen monomer (Fig. 1c). The −0.66 and −0.28 V redox peaks can be assigned to the redox couples of Vi^+/Vi and Vi^{2+}/Vi^+, respectively. The estimated mid-point potential of pMBVi would allow this compound to supply electrons to various intracellular redox species, such as ubiquinone (+0.10 V) and flavin adenine dinucleotide (−0.22 V).

Using *E. coli* as a model microbial species, we attempted to verify whether pMBVi could serve as an electron mediator and supply electrons to living cells. As pMBVi is spontaneously oxidized by oxygen, the following experiments were conducted under anaerobic conditions. We first conducted non-electrochemical batch-culture experiments using nitrate as the sole electron acceptor of *E. coli*. After 3 h of

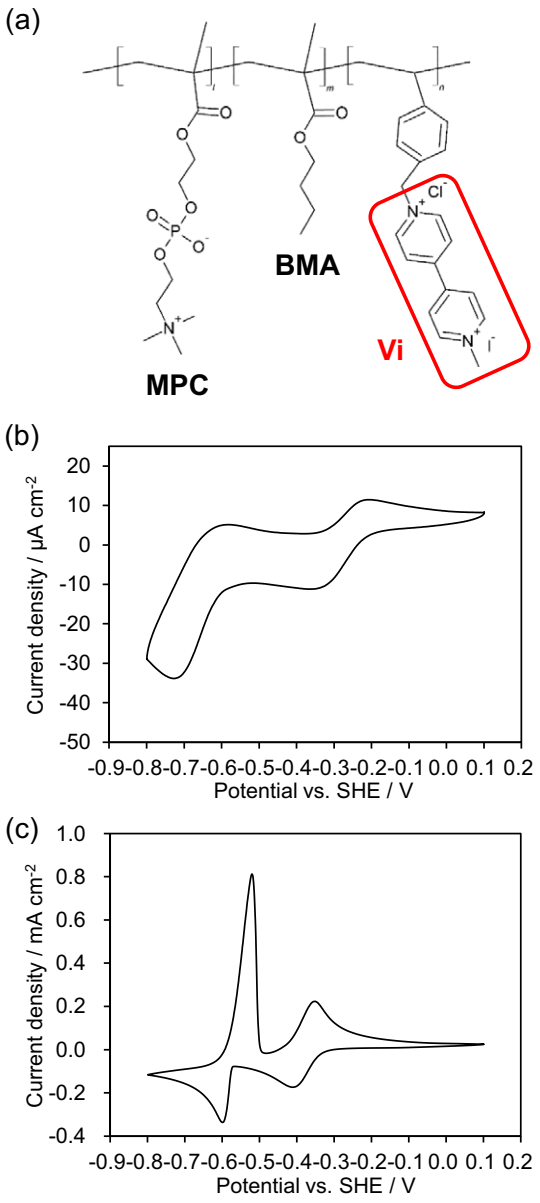

Fig. 1. (a) Chemical structure of pMBVi. (b) Cyclic voltammograms obtained in 0.10 M phosphate buffer solution (pH 7) containing 1.0 g L^{-1} of pMBVi or (c)Vi monomer. The scan rate was 10 mV s^{-1}.