Conjugated oligoelectrolyte represses hydrogen oxidation by Geobacter sulfurreducens in microbial electrolysis cells

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Abstract

A conjugated oligoelectrolyte (COE), which spontaneously aligns within cell membranes, was shown to completely inhibit H2 uptake by Geobacter sulfurreducens in microbial electrolysis cells. Coulombic efficiencies that were 490 ± 95%, due to H2 recycling between the cathode and microorganisms on the anode, were reduced to 86 ± 2% with COE addition. The use of the COE resulted in a 67-fold increase in H2 gas recovery, and a 4.4-fold increase in acetate removal. Current generation, H2 recovery and COD removals by Geobacter metallireducens, which cannot use H2, were unaffected by COE addition. These results show that this COE is an effective H2 uptake inhibitor, and that it can enable improved and sustained H2 gas recovery in this bioelectrochemical system.

1. Introduction

Conjugated oligoelectrolytes (COEs) are oligomers containing semiconducting, π-conjugated backbones with ionic pendant groups. They have long been appreciated as efficient charge transfer components in various optoelectronic applications, such as biosensors, due to their optical and semiconducting properties [1,2]. The hydrophilic charged side groups of COEs result in high solubility in very polar organic solvents and water, and they also have optical fluorescent properties when immersed in bipolar materials. COEs can interact with lipid bilayers in an ordered manner due to hydrophobic interactions between the π-conjugated COE backbones and lipid double layer packing [3,4].

When added to microbial suspensions, COEs spontaneously insert into and span cell membranes and they become fluorescent. For certain microorganisms, COE addition has been shown to enhance extracellular electron transfer to an electrode. For example, the addition of the COE 4,4′-bis[4′-{(N,N-bis(6′-{(N,N,N-trimethylammonium)hexyl)amino)-styryl}stilbene tetrabromide (DSSN+) to cultures of a yeast and Escherichia coli, which normally have limited capabilities for extracellular electron transfer, resulted in dramatic increases in current and power generation in microbial fuel cells (MFCs) [5–7]. COEs have also been shown to support direct transmembrane electron transfer for dissipatory metal reducing bacteria that are already capable of relatively high current production in MFCs. For example, addition of DSSN+ to Shewanella oneidensis MR-1 cultures enabled this microorganism to accept electrons from an electrode and produce succinate, which was otherwise not possible without COE addition [8]. The mechanism that allows COEs to enhance extracellular electron transfer between electrodes and cells is thought to be similar to that of conductive pili used by exoelectrogenic bacteria such as Geobacter sulfurreducens [9]. However, it is also possible that COEs interact with periplasmic and outer membrane complexes in ways that enable electron transfer through direct contact [10], or that they facilitate transfer to electron shuttles that are able to diffuse through the cell membrane and undergo reversible reduction and oxidation cycles with cells and an electrode.

In microbial electrolysis cells (MECs), exoelectrogenic bacteria such as Geobacter sulfurreducens oxidize organic matter (acetate) and release electrons to the anode. By adding a small voltage across the circuit using an external power source, there is sufficient potential to evolve H2 gas from the cathode [11]. In single chamber MECs (no membrane between the electrodes), H2 generated at the cathode electrode is used as an additional electron donor by G. sulfurreducens on the anode. This H2 oxidation results in H2 gas recycling between the anode and cathode, whereby current is maintained by the shuttling of H2 between the two electrodes [12]. In the presence of high concentration of H2, acetate oxidation by G. sulfurreducens (supported by iron reduction) is inhibited [13]. This inhibition of acetate oxidation by H2 has also been shown to occur with other microorganisms, such as Methanosarcina barkeri, Methanosarcina mazei and Methanosaeta thermophile [14,15].

Here we demonstrated that COEs can inhibit microbial uptake of H2 by studying current generation of two exoelectrogenic Geobacter...
species in MECs. About 40% of the Geobacteraceae family is capable of using hydrogen as an electron donor. Two membrane-bonded hydrogenases, Hya and Hyb, and two cytoplasmic hydrogenases, Mvh and Hox are encoded in the G. sulfurreducens genome. Genomic studies indicated that Hya functions as a respiratory-uptake hydrogenase, and that it is required for H₂ to be used as an electron donor [16]. Geobacter metallireducens, which is closely related to G. sulfurreducens, does not contain the Hya hydrogenase. Therefore, while G. sulfurreducens can oxidize H₂ and generate current, G. metallireducens cannot utilize H₂ and thus does not generate current from this electron donor [16]. Microbes most similar to G. sulfurreducens have been found to predominate the anodes of MECs fed with acetate and many other substrates. High H₂ concentrations in MECs could therefore decrease, or even completely inhibit acetate utilization, affecting cell growth and net energy recovery, as electrical power is consumed with no net H₂ gas recovery.

2. Materials and methods

2.1. Microorganisms and culture media

G. sulfurreducens strain PCA (ATCC 51573) was initially cultured in a medium containing 0.82 g L⁻¹ sodium acetate (electron donor), 0.12 g L⁻¹ sodium fumarate (electron acceptor), 1.5 g L⁻¹ of NH₄Cl, 0.6 g L⁻¹ of NaH₂PO₄, 0.1 g L⁻¹ of KCl, 2.5 g L⁻¹ of NaHCO₃, 10 mL of minerals, and 10 mL of vitamins. G. metallireducens was cultured in a medium containing: 0.82 g L⁻¹ sodium acetate (electron donor), 4.9 g L⁻¹ ferric citrate (electron acceptor), 0.25 g L⁻¹ of NH₄Cl, 0.25 g L⁻¹ of NaH₂PO₄, 0.1 g L⁻¹ of KCl, 2.5 g L⁻¹ of NaHCO₃, 10 mL of minerals, and 10 mL of vitamins. Both media were sparged with anaerobic gas (N₂:CO₂, 80/20%) to adjust the pH to ~7.0, and autoclaved at 121 °C. Same media were used for MEC tests except the soluble electron acceptors were omitted.

2.2. MEC construction, operation and analysis

MECs were made using graphite block anodes (0.6 × 0.45 × 0.15 cm³) and stainless steel mesh cathodes (4.5 × 4 cm²), inserted into 160 mL serum bottles filled with 100 mL of medium. A voltage of 0.9 V was applied across the electrodes, with the current calculated by the voltage drop across a 10 Ω resistor.

Prior to MEC tests, G. metallireducens cells were washed by centrifugation (2422 × g, for 5 min) and resuspended in fresh anaerobic culture medium lacking the original electron acceptor. Washed cell suspensions (10 mL, OD₆₀₀ = 1) of G. metallireducens and G. sulfurreducens (10 mL, OD₆₀₀ = 1) were added to 90 mL of medium in MECs containing a head-space sparged with CO₂/N₂ (20/80%, v/v) unless specified otherwise. DSBN⁺ (5 μM) was injected into half of the MECs for each microorganism once mature biofilms (demonstrated by at least 3 cycles of stable
current production) were formed on the anodes. All the MEC operated using triplicate reactor in feed-batch mode at 30 °C in a temperature-controlled room.

2.3. COE addition

The impact of a COE on current and H₂ gas utilization by *G. sulfurreducens* and *G. metallireducens* was examined using the COE 1,4-bis(4′-(N,N′,N′-bis(6-(N,N,N-trimethylammonium)hexyl)amino)-styril)benzene tetraiodide (DSBN⁺), which had previously been shown to enhance extracellular electron transfer by *E. coli* MFCs [6]. This COE contains a distyrylbenzene (DSB) conjugated region capped with two nitrogen-bound, six-carbon pendant groups containing terminal quaternary ammonium salts (Scheme 1).

2.4. Gas analysis and calculations

Gas in the headspace was sampled using a gas tight syringe (250 μL, Hamilton Syringe). H₂ content was analyzed using gas chromatography (model 2610B, SRI Instruments). Coulombic efficiencies (CE) were defined as the ratio of Coulombs in the consumed substrate based on its COD compared to the current flow through the circuit. MEC performance was evaluated in terms of coulombic efficiency, removal of acetate evaluated in terms of changes in chemical oxygen demand (COD), and energy yield as previously described [11].

3. Results and discussion

DSBN⁺ (5 μM) was injected into half of the MECs (Scheme 1) containing either *G. sulfurreducens* or *G. metallireducens* once mature biofilms had developed on the anodes, as demonstrated by stable cycles of current generation. In the absence of DSBN⁺ addition (controls), there was negligible H₂ gas accumulation in the headspace of the MECs containing *G. sulfurreducens* (0.3 ± 0.04 mL) over 192 h.
also no change in H2 in the headspace for the controls (no DSBN+ addition) (Fig. 2A). As expected, there was a 66% decrease (from 45 ± 0.2 mL to 15.5 ± 0.5 mL after 134 h) for cultures of G. metallireducens (45 ± 1.8 mL of H2 with DSBN+, versus 47 ± 1.3 mL in controls).

H2 gas utilization by G. sulfurreducens reduced the rate of acetate consumption, evaluated on the basis of changes in COD concentrations over time (Fig. 1B). There was little COD removal by G. sulfurreducens after 72 h (12 ± 5% COD removed) in the absence of DSBN+ addition. When DSBN+ was added, COD was continuously removed in MECs containing G. sulfurreducens, with 53 ± 1% removed after 192 h. DSBN+ addition to G. metallireducens MECs did not affect the COD removal (89 ± 1% with DSBN+, versus 87 ± 0.4% for controls after 192 h) (Fig. 1B).

Current generation was unaffected by DSBN+ addition to the MECs for either G. sulfurreducens or G. metallireducens (Fig. 1C). DSBN+ addition therefore did not improve current production by Geobacter biofilms, as it did for microorganisms such as E. coli that otherwise produce very little current in bioelectrochemical systems. However, a lack of a decrease in current production also indicated that DSBN+ addition, at the dose used here, was not toxic to these microorganisms.

Coulombic efficiencies were decreased with DSBN+ addition, which further supported the inhibition of H2 gas recycling in the presence of DSBN+. G. sulfurreducens had a coulombic efficiency of 490 ± 95% without DSBN+ addition, indicating substantial hydrogen gas recycling (Fig. 1D). When DSBN+ was added, the coulombic efficiency was reduced to 86 ± 2%, which was only slightly higher than that obtained with G. metallireducens in the presence (81 ± 1%) or absence (80 ± 6%) of DSBN+.

To further examine the effect of DSBN+ on H2 uptake, MECs were prepared with H2 added to the headspace and no acetate in the medium. There was no change in the H2 headspace concentration using cultures of G. sulfurreducens with DSBN+ addition, compared to a 66% decrease (from 45 ± 0.2 mL to 15.5 ± 0.5 mL after 134 h) for the controls (no DSBN+ addition) (Fig. 2A). As expected, there was also no change in H2 in the headspace for G. metallireducens with or without DSBN+ addition (Fig. 2B). These tests with H2 addition in the absence of acetate demonstrated that DSBN+ addition completely and specifically inhibited H2 uptake by G. sulfurreducens at a concentration as low as 5 μM in the solution. From these results, we concluded that this COE can completely inhibit H2 oxidation by exoelectrogenic biofilms of Geobacter in MECs.

The impact of DSBN+ and other COEs on hydrogen utilization by these two exoelectrogenic species likely has implications for other microorganisms as well. For example, we previously observed that addition of DSBN+ to mixed cultures in MECs repressed hydrogenotrophic methanogenesis [17]. While the H2 uptake mechanisms by methanogenic Archaea [18,19] are different from Geobacter, this observation suggests that COEs might repress hydrogen oxidation pathway of G. sulfurreducens in a similar way important for hydrogenotrophic methanogenesis [17]. Whether COEs impact hydrogen uptake through direct interactions with these enzymes, or indirectly affects hydrogen uptake through its interactions with the inner or outer membranes, however, is not presently known. Previous studies have shown that DSBN+ interacts intercalates in cell membranes in an ordered manner [5,6], but its possible presence on the inner membranes, or within the cell periplasm, has not been examined. Thus, the specific mechanism of COE inhibition of hydrogen uptake will require further investigation.

4. Conclusions

The addition of the conjugated oligoelectrolyte, DSBN+, into MECs inoculated with G. sulfurreducens, completely inhibited H2 uptake by microorganisms on the anode, preventing H2 recycling. H2 gas recovery from acetate was increased 67-fold, and COD removal was enhanced 4.4-fold through DSBN+ addition. MECs inoculated with G. metallireducens, which is unable to oxidize H2, were not affected by DSBN+ addition. Further studies on DSBN+ impacts on hydrogen uptake by microorganisms, especially on whether they directly interfere with hydrogenases, are needed to better understand the mechanism by which this COE inhibits hydrogen gas utilization by bacteria.

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References