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Review article

Molecular mechanisms of heme based sensors from sediment organisms capable of extracellular electron transfer

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ABSTRACT

Bioelectrochemical systems (BES) rely on the metabolism of sediment bacteria capable of forming electrogenic biofilms to generate electrical work. The environment across the thickness of the biofilm is variable and in order for the cells to maintain their viability they require molecular sensors that allow them to adapt their metabolism to their respective environment. The DcrA sensor from *Desulfovibrio vulgaris* and the GSU 0582 and 0935 sensor domains from *Geobacter sulfurreducens* appear to function as redox sensors. The SO2144 sensor domain from *Shewanella oneidensis* MR-1 and the cytochrome c″ from *Methylophilus methylotrophus* appear to function as NO sensors. Although *M. methylotrophus* is not known to colonize electrodes on BES, the characterization of cytochrome c″ serves to illustrate the general mechanism of NO sensing similar to that of the heme based sensors of sediment bacteria used in BES. In all cases, conformational changes initiated by the signal trigger the response. What appears to set these two groups of proteins apart is the poise of the heme in the sensors. In the case of redox sensors the hemes appear to be low spin iron II axially coordinated by two residues of the protein. In the case of NO sensors the heme appears to be high spin iron II with the distal coordination site vacant. Understanding of the molecular bases for signal and ligand discrimination may enable a fine control of biofilm formation in bioelectrochemical systems or the development of novel biosensors.

1. Introduction

Bioelectrochemical systems (BES) are gaining great interest due to their potential for novel industrial processes that combine wastewater treatment with energy generation, water desalination, fine chemical production or environmental bioremediation [1]. These electrochemical devices are often arranged in two compartments separated by a cation-exchange membrane (Fig. 1). Bacteria in the anaerobic compartment donate electrons to the anode upon degradation of organic substrates available in the wastewater. The flow of electrons from the anode to the cathode through an external circuit allows electrical work to be performed. The protons released during the bacterial metabolism diffuse through the cation-exchange membrane into the cathode compartment, where they recombine with the electrons from the circuit. When oxygen from the atmosphere is used, the reaction produces water.

The organisms that can colonize efficiently the anodes of BES must couple their bioenergetic metabolism to the reduction of external electron acceptors [2]. These organisms are typically found in habitats with stratified sediments where the environmental conditions display great variability on daily and seasonal time scales [3]. A member of the genus *Shewanella* was the first organism shown to be capable of powering a microbial fuel cell in the absence of soluble mediators [4,5], and together with the genus *Geobacter* these bacteria attract the bulk of the attention in BES research [6]. Bacteria of the genus *Desulfovibrio* are also competent for powering BES and have attracted great interest in the field of heavy atom and hydrocarbon remediation [7]. In order to interact with the electrodes in the absence of mediators the organisms must contact the electrode and often form biofilms [6,8]. Electron transfer is then performed *via* outer-membrane multiheme cytochromes or electrically conductive bacterial nanowires (direct electron transfer mechanism), or *via* the production of endogenous electron shuttles such as flavins (indirect electron transfer mechanism) [2,9]. Biofilms are environments where conditions change not only with time but also with the biofilm thickness. Gradients of nutrients, redox and pH are known to exist in these structures [10] and therefore it is not surprising that the metabolic status of the cells varies with depth [11].

Coping with the temporal and spatial variability in the natural habitat and within BES requires a considerable metabolic versatility. Efficient signaling cascades enable the organisms to constantly select the fittest metabolic routes in the context of changing conditions. In particular, oxygen is known to regulate the formation and detachment of electrogenic biofilms [12], and therefore the reaction to adventitious oxygen that may diffuse from the cathode compartment is an important factor in BES performance.

Bacteria have evolved sensitive and specific sensors that allow them to monitor redox conditions and the presence of diatomic molecules such as O_2, NO and CO [13]. These sensory systems convert these cues

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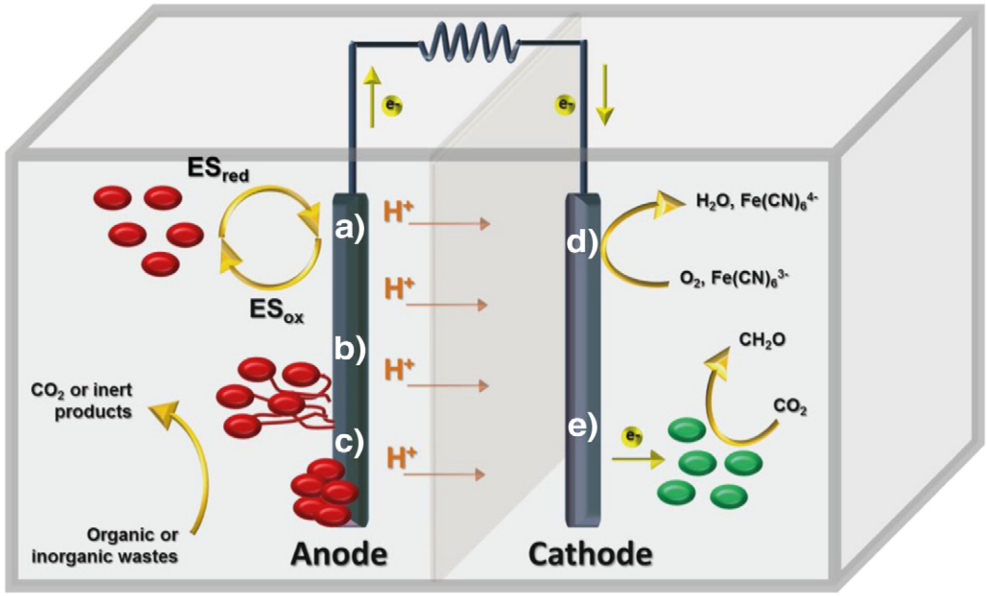

Fig. 1. Schematic representation of a MFC. Bacteria at the anode chamber (left half, red (dark) ellipsoids) feed on organic or inorganic wastes and transfer electrons to the anode: a) Indirectly *via* electron shuttles (ES) or b)/c) directly *via* nanowires and outer membrane cytochromes. The protons produced in the metabolism flow through the selective permeable membrane to the cathode chamber and the electrons flow through an electrical circuit to the cathode. The electrons are then transferred to the final electron acceptor. This can be d) Abiotic such as oxygen or e) Biotic such as phototrophic anoxygenic bacteria (right half, green (light) ellipsoids).

into regulatory outputs, by modulating expression of genes, which allows the cells to adapt to the altered environment [14,15].

The genomes of bacteria capable of creating electrogenic biofilms reveal the presence of a great diversity of sensor proteins that contain redox-active cofactors. These cofactors can be pyridine nucleotides, redox-sensitive amino-acid side chains such as cysteine thiols, quinones, iron–sulfur clusters, flavins, or hemes [16]. Their diversity is correlated with the genome size and the metabolic versatility of the bacteria [17]. Of the various sensor systems we will focus on some well-characterized bacterial heme redox sensors and what is known about the heme mediated molecular basis for sensing external stimuli such as environmental redox levels or small molecule ligands.

2. Heme based sensors

Hemes are some of the most versatile cofactors found in biological molecules [18]. Hemes of types *b* and *c*, both of which are derived from protoporphyrin IX [19], represent the most common hemes found to be associated with proteins. The reduction potentials of heme proteins range from −550 mV to +450 mV (*versus* standard hydrogen electrode (SHE)) depending on the type of porphyrin, the axial ligands and the global protein fold [20]. In particular, the heme propionates provide an efficient and strong thermodynamic coupling of redox state with protonation state which provides a concerted modulation of reduction potentials and pKas known as the redox-Bohr effect [21,22]. In the heme system, four- or five-coordinated iron is typically high spin whereas six-coordinated iron can be high or low spin depending on the nature of the axial ligands. Strong field ligands such as histidines or methionines promote the low spin state of the iron, which can be diamagnetic for the case of Fe(II). Therefore, the reactive properties of a heme can be modulated by modification of the redox-, spin-, and coordination-state of the iron and protonation of the propionates. All of these factors contribute to define the functional properties of each heme in a protein.

Sensor proteins containing hemes in their active site play important roles in various biological processes such as chemotaxis, energy taxis and regulation of gene expression [23,24]. Typically, the heme is bound at the N-terminal region, while the C-terminal region contains the functional domain. In these sensors the heme must elicit the conformational changes upon signal detection that activate the functional domain [23,24]. In the case of sensors that bind small diatomic molecules such as CO, NO or O_2, fulfillment of their role requires the discrimination between these diatomic ligands of similar electronic properties in order to achieve a selective response [25]. In this mini-review four proteins are used to illustrate general aspects of the functional properties of heme based sensors.

2.1. DcrA

In 1992, a putative sensor protein, DcrA, with a *c*-type heme was reported by Dolla et al. in the sulfate reducing bacterium *Desulfovibrio vulgaris* Hildenborough [26]. DcrA was proposed to be responsible for O_2 and/or redox sensing [27]. Various studies show that the transcription level of this gene is unresponsive to the presence of oxygen which may indicate that this sensor is constitutively expressed [28–31].

DcrA possesses the typical architecture of a methyl-accepting chemotaxis protein (MCP), with a periplasmic and a cytoplasmic domain connected by two membrane spanning regions [32]. *In vitro*, the methylation levels of the methyl-accepting domain decrease upon addition of O_2 and increase upon subsequent addition of sodium dithionite, as expected for a redox-dependent or oxygen sensor of the MCP family [27]. Resonance Raman (RR) spectroscopic studies showed that the *c*-type heme attached to the periplasmic domain of DcrA displays redox-linked axial ligand exchange. In the oxidized state the heme is six-coordinate high-spin with spectral features typical of a water molecule in the distal position. Upon reduction this water molecule is displaced by a strong field endogenous ligand giving origin to a diamagnetic hexa-coordinated heme [32]. A structure for this protein does not exist but the low reduction potential of DcrA (−250 mV) suggests that the endogenous distal ligand may be a histidine, several of which exist in the polypeptide. Reduced DcrA reverts to the ferric high spin state upon exposure to O_2, probably due to the low reduction potential of the heme and the absence of a distal amino acid capable of stabilizing a bound O_2 [32]. The very negative reduction potential of the heme in