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What do luminescent bacterial metal-sensors probe? Insights from confrontation between experiments and flux-based theory

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ABSTRACT

Whole-cell bioreporters are routinely operated as sentinels for monitoring the concentration of bioavailable and/or toxic metal ions (M) in aquatic media. Despite the importance of metal bioreporters in environmental risk assessment, their use is often limited to the establishment and exploitation of calibration curves relating bioreporters signal and target metal concentration. In this work, a physicochemical rationale is elaborated for the response of metal-sensitive whole-cell bioreporters beyond the restrictive representation of metal partitioning equilibrium at the microorganism-solution interface. The analysis is conducted under poorly metal complexing conditions for steady-state bioreporter functioning defined by a rate of photons production independent of time. The theoretical framework deciphers how this rate is determined by (i) metal biouptake dynamics with contributions from metal conductive diffusion to the cell surface and metal internalisation kinetics, (ii) formation kinetics and stability of intracellular complexes between M and transcriptional regulators, and (iii) the flux of emitted photons resulting from biochemical reactions initiated after activation of transcriptional regulators. The formalism enables quantitative evaluation of bioreporters performance depending on interfacial cell electrostatics, cell concentration and cell metal-adsorption features. The theory is supported by experimental data on cadmium detection by genetically modified luminescent *Escherichia coli* bioreporters exhibiting various lipopolysaccharidic surface structures.

1. Introduction

Whole-cell bioreporters are genetically modified microorganisms producing a signal −be it chemical [1–4], optical [5–8] or electrochemical [9–11]-following the intracellular accumulation of target analytes dispersed in a given aqueous medium. The dose-dependent signal generated by biosensors may be further used as a proxy to address the toxicity of chemicals of environmental importance [12]. The biologically active sensing element of a biosensor is introduced by proper genetic engineering that often exploits transcriptional regulations involved in homeostasis and/or detoxification strategies operational in unmodified microorganisms. These regulatory elements include a stress-response promoter activated after specific binding of a given target analyte to a regulatory protein, which subsequently leads to the specific expression of a fused reporter gene and to the production of a signaling (or reporter) protein, *e.g.* green fluorescent protein or luciferase [13]. Following this methodology, prokaryotic cells have been routinely employed for the detection of several types of pollutants, including metal ions and organics [5]. The methodology commonly adopted to measure analyte concentration in a given medium with biosensors consists in a two-step procedure. First, the whole-cell bioreporters are incubated in a solution containing the target analyte, and the biosensor signal is recorded as a function of time over a given range of known analyte concentrations. A calibration is then established between the analyte concentration and the output signal selected at a given incubation time [5,6,14,15]. Measurement of the biosensor signal is subsequently performed in a sample of interest and the analyte concentration therein is ‘read’ from pre-established calibration data.

Despite the growing attention drawn by the environmental science community to biosensors, [16] many difficulties remain to interpret quantitatively their response in contact with a solution containing an analyte to be detected. In the case of target metal ions, several reasons may be invoked to explain such difficulties. First, there has been only few attempts to formulate the relationship between biosensors signal

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Nomenclature

List of main symbols

a Radius of the intracellular compartment of the microorganisms (*i.e.* with excluding the peripheral soft surface layer) (m)
Bn^{-1} Inverse of the bioavailability (or Bosma) number (dimensionless) devoid of electrostatic contribution
c_M^a Metal concentration at the outer membrane surface ($mol\,m^{-3}$)
c_M^* Bulk metal concentration at t ($mol\,m^{-3}$)
$c_p = N/V_T$ Cell number density in solution (m^{-3})
$(d\mathcal{L}um(t)/dt)_{max}$ Steady-state flux of photons emitted by the N bioreporters in solution (counts s^{-2})
$\overline{f}_{el}$ Electrostatic Debye correction factor for M diffusion to microorganism membrane surface (dimensionless)
I Solution ionic strength ($mol\,m^{-3}$)
$J_{c,ss}$ Production flux of ZntR-M complexes expressed per unit microorganism surface area under steady-state light emission conditions ($mol\,m^{-2}\,s^{-1}$)
J_u^* Maximum uptake flux of M ($mol\,m^{-2}\,s^{-1}$)
J_u Metal uptake flux ($mol\,m^{-2}\,s^{-1}$)
$J_{\nu,ss}$ Time-derivative of the luminescence emitted per unit microorganism surface area and normalised by the cell concentration c_p (situation where step (ii) in Fig. 1 governs the rate of light production) (in counts m s^{-2})
$k_{a,d}^*$ Effective kinetic constants for the conversion of free M in the intracellular volume V_i of a given cell into M-ZntR complex species ($mol^{-1}\,m^3\,s^{-1}$), and for M-ZntR dissociation (s^{-1}) (subscript 'a' and 'd', respectively)
k_{int} Kinetic constant for metal internalisation (s^{-1})
K_H Henry coefficient for the adsorption of M at the internalisation sites S_u (m)
$\widetilde{K}_H$ Henry coefficient for the adsorption of M at mere adsorption sites on the cell envelope (m)
K_M Reciprocal of the affinity constant of M for the internalisation sites ($mol\,m^{-3}$)
$\overline{K}^* = k_a^* \rho_S^{V_i}/k_d^*$ Stability constant of intracellular M-ZntR complexes (dimensionless)
$\mathcal{L}um(t)$ Luminescence produced at time t by the ensemble of N metal biosensors (counts s^{-1})
M Stands for free metal ions (valence z_M)
N Number of whole-cell bioreporters in the medium
$S_a = 4\pi a^2$ Cell surface area (m^2)
t Delay after introduction of metal ions in the solution containing bioreporters (s)
V_T Volume of the medium where bioreporters are dispersed (m^3)
x^* Dimensionless reciprocal of the affinity of M for the internalisation sites S_u ($x^* = \beta_a c_M^*/K_M$) (equivalently, dimensionless bulk concentration of total metal ions in solution)
x_f^* Dimensionless bulk concentration of bioavailable (free) metal ions in solution ($x_f^* = \beta_a c_{M,f}^*/K_M$)

Main greek symbols

α Luminescence produced per unit concentration of ZntR-M complexes over the whole sample volume (in counts $s^{-1}\,mol^{-1}\,m^3$)
β_a Boltzmann factor applying at the membrane surface (dimensionless)
Γ_{ads} Surface concentration of adsorbed metal ions ($mol\,m^{-2}$)
κ^{-1} Electric double layer thickness (m)
$\rho_S^{V_i}$ Molar concentration of binding sites S carried by ZntR and smeared-out over the intracellular compartment of an individual cell ($mol\,m^{-3}$)

intensity and concentration of internalized metal that triggers the chain of biochemical reactions leading to signal emission [17]. Second, the analysis of speciation and distribution of metal species from bulk solution to biosensors surface is often restricted to the use of thermodynamic models, *e.g.* the Biotic Ligand Model (BLM) and the Free-Ion Activity Model (FIAM) [18–24]. By definition, the latter ignore the possible limitation of metal biouptake by diffusion (supported or not by complexants) [19,22,25–30] and the physicochemical features of the soft interphase formed between biosensors and aqueous medium [31–36]. Finally, the performance of metal-responsive whole-cell bioreporters is tied to intricate energetic requirements that are more or less satisfied depending on bioavailability and quality of nutrients and other essential elements [5,16,37].

In order to broaden the range of conditions in line with a predictable functioning of metal-sensing whole-cell bioreporters, there is therefore an urgent need to elaborate on a mechanistic *and* quantitative levels how biosensors response mirrors the cascade of extracellular and intracellular biophysicochemical processes involving the target analyte. As a first step toward achievement of this objective, a theoretical rationale is here formulated for the time-dependent luminescence produced by genetically modified metal-sensing bacterial reporters under poor metal-complexing conditions where metal speciation in the extracellular solution is kept simple, *i.e.* metal is predominantly present in the form of free metal ion species. The formalism integrates the interplay between chemodynamics of the intracellular complexes formed between metal ions and transcriptional regulatory promoters, and the dynamics of the extracellular metal transfer processes leading to metal biouptake and bioaccumulation. It further establishes the connection between flux of photons emitted by the bioreporters, flux of metal biouptake and concentration of bioavailable metal fraction in solution. Highlights of this work include a comprehensive understanding of the impacts of solution ionic strength, cell surface electrostatics and cell concentration on biosensors response. The work further details a successful comparison between theory and experiments on genetically modified *Escherichia coli* strains producing constitutively luminescence (bioreporter strains) or in the presence of cadmium ions, and differing in terms of lipopolysaccharide (LPS) surface layer structure. The selected cadmium-based bioreporters are commonly adopted in studies dealing with evaluation of metal bioavailability in soils and freshwater samples. In addition, the metal adopted, Cd, is suitable to test the fundaments of the here-reported theory as, under the medium conditions examined, Cd is predominantly present in the form of free ionic species with no significant formation of complexes, in line with the aforementioned objective of the work. Finally, the modulation of the surface phenotypes of the bacterial reporters by varying the extent of LPS truncation enabled to tune the electrostatic properties and the metal biosorption capacities of the bacteria, both shown here to significantly determine the biosensors response. Considering LPS layers with distinct thicknesses further rendered possible the analysis of the LPS-mediated effects on metal diffusion transfer to the cell membrane and therewith on the magnitude of the biouptake flux and the rate of biosensor light production.

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