Aequorin as a reporter system for environmental monitoring in cyanobacteria

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Abstract

Intracellular messengers are basic components of signaling systems; among these, calcium has arisen as probably the most versatile in eukaryotes and also in prokaryotes.
Cyanobacteria are ancient organisms which have evolved signal transduction systems in response to an ever changing environment.

Our group was the first to construct a recombinant strain of the freshwater nitrogen fixing cyanobacterium *Anabaena* sp. PCC7120 constitutively expressing the Ca2+-sensitive photoprotein apoaequorin, *Anabaena* sp.PCC7120 (pBG2001a), which allows continuous and in vivo monitoring of intracellular free Ca2+, [Ca2+]i. Functional recombinant aequorin can be successfully reconstituted on addition of the hydrophobic luminophore coelenterazine; the reconstituted protein has three Ca2+ binding sites and once Ca 2+ ions are bound, aequorin catalyses the oxidation of the substrate coelenterazine by oxygen, resulting in blue light emission.

With this strain we demonstrated that cyanobacteria were able to tightly regulate their basal [Ca2+]i levels ; we have also recorded and analyzed a variety of calcium signatures induced by specific environmental stimuli and by a variety of pollutants: cationic an anionic heavy metals, the metalloid As, naphthalene, organic solvents and several pharmaceuticals including antibiotics; we also recorded Ca2+ signatures induced by some binary mixtures of the pollutants as well as that induced by a real wastewater sample. It was found that, in general, each group of tested pollutants induced a specific Ca2+ signature in a reproducible and dose-dependent way.
We hypothesized that the recorded Ca2+ signatures might be early biomarkers of exposure to pollutants since they seemed to anticipate the toxicological interactions found at larger times of exposure; thus, well before toxicity is evident.

Keywords: Aequorin, bioluminescence, biomarker, pollutants, reporter system

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