氏　　名	CARLOS RAÚL ARIAS BARREIRO
授与した学位	博　士
専攻分野の名称	学　術
学位授与番号	博甲第４２３５号
学位授与の日付	平成２２年　９月３０日
学位授与の要件	自然科学研究科　バイオサイエンス専攻 （学位規則第５条第１項該当）
学位論文の題目	Ecotoxicological implications of biosensing oxidative stress with the *E.coli*-roGFP2 （大腸菌 roGFP2 を用いた酸化ストレスバイオセンシングの生態毒理学的意義）
論文審査委員	准教授　且原　真木　　教授　鈴木　信弘　　教授　金原　和秀（静岡大学）

学位論文内容の要旨

Homeostasis of oxidative (redox) processes is quintessential to the maintenance of cellular physiological activities. In the environment, a myriad of anthropogenically produced chemical toxicants, contributes to factors like UV radiation, O_2, O_3 among others, to the alteration of redox homeostasis. There is plenty of evidence linking abnormal changes of redox potential to events that lead to the damage of cellular structures and ultimately to cell death. Redox-sensing, which is carried out with specifically designed GFP proteins is currently an expanding field with applications in research areas like plant and medical sciences. However, the concept of using redox-sensing expressing organisms in mainstream regulatory applications, like environmental monitoring, has not yet been explored.

Constitutive expression of roGFP2 in bacteria clashes with the widely accepted concept of whole-cell biosensor, which generally exemplifies bacteria with a promoter/reporter (bioreporter) system and implies that a measurable signal should be attained after expression protein in question. The redox-sensing-based system provides a measurable signal straightforwardly and also, because the nature of the response is based on conformational changes of the protein, the analysis time is significantly reduced when compared to promoter/reporter biosensor systems.

Using a common mechanism in an attempt to universalize the detection of toxic effects of chemicals is commonplace in environmental science, with the Microtox®, as the bacterial paradigm. Likewise, our initial hypothesis approached the concept of universalizing oxidative stress to evaluate in a fast, high-throughput and precise fashion, the smallest alterations in the redox potential. Initially, statistical analysis demonstrated that EC (Effective concentration) would not be usable, as a "maximum" concentration would also elicit a reduction of the net fluorescence by means of cytotoxic effects.

Lowest observable effect concentrations (LOECs) seem more appropriate as endpoint values as they would express the minimal shifts from the cellular homeostatic conditions induced in an oxidizing environment. This provides a reasoning basis of why correlations were performed based on LOEC values. Moreover, despite the still lower R^2 (~0.5) for organic chemicals, the results point the way to a preference for compounds/mixtures with no thiophilic activity, where interference activity of heavy metals towards roGFP2 was pointed out. It is highly possible that addition of more organic-compound endpoints would increase linear correlation between toxicity and oxidation.

The results obtained for structurally similar groups such as calcogens and PAHs further indicate that the path to establish the *E.coli*-roGFP2 biosensor into a standardized bioassay would require more chemical-based structural differentiations based on oxidative patterns and/or toxicity endpoints.

As a final prospect, future research should focus on the increasing the viability of cells in order to provide longer intervals where readings will not be affected by auto-oxidation. Initial results have provided insights on immobilization of cells onto an agarose-based matrix, with successful evaluation of oxidation by standards like H_2O_2 with slightly increased detection times, but with unchanged auto-oxidation rates.

In conclusion, the *E.coli*-roGFP2 biosensor holds the potential to become a standardized system for the evaluation of oxidative stress. Access to a variety to environmental samples should also provide enough standardization data to evaluate its use in a regulatory framework.

論文審査結果の要旨

環境中にはさまざまな有毒有害物質が存在する。その定量には機器分析による方法とバイオアッセイによる方法とがある。本研究では蛍光タンパク質roGFPを定常発現している大腸菌を用いてハイスループットの新しいバイオアッサエイ技術の開発に取り組んだ。多くの毒物は細胞内で酸化ストレスを介して細胞に傷害を与えると考えられている。roGFPは酸化還元状態に応じて蛍光スペクトルが変化し、その変化は400 nm と490 nmの蛍光強度比の値で定量化できる。本研究ではroGFPを定常発現している生きた大腸菌を用いて、さまざま有毒有害物質が数十秒から数分の間で蛍光変化させること、すなわちきわめて迅速に細胞内の還元状態の変化をモニターできることを始めて明らかにした。また多くの有毒有害物質に対して検出感度も十分に高いことを示し、またモバイルセンサー化へ向けて細胞をゲルで固定化した基礎実験もおこなった。このような博士論文内容について議論した結果、本件は審査基準を満たしており、その内容は博士に値するものであるとの結論を得た。また論文発表と発表後の質疑応答についても問題は見いだされなかった。したがって最終試験の結果として、博士号に相当すると結論した。