Development of biomimetic D1 peptides as novel photosynthetic based-biosensors for environmental monitoring

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Abstract

Synthetic biomimicry approaches can help to build more robust biorecognition elements for biosensors, overcoming the problem of instability of current biomediators that limits their market applicability. Synthetic peptides that mimic parts of the photosynthetic apparatus as biorecognition elements lead to an increased performance for the detection of environmental contaminants, including pesticides able to bind to D1 protein in the photosystem II and inhibit the photosynthetic process. Thus, the combination of computational analysis, molecular biology and biomimicking tools makes possible the use of more stable, sensitive, selective and specific biomediators for the development of effective biosensors. Based on this approach, this work describes the use of biomimetic peptides of the photosynthetic plastoquinone binding niche of the green alga *Chlamydomonas reinhardtii* for pesticide measurement in environmental or food samples. In previous studies, we identified point mutations in D1 protein of photosystem II of the alga that increase atrazine binding affinity, particularly the mutant S268C which presents a sensitivity of 1 nM, a 10-fold increase over wild type[1,2]. After identification of key binding residues, biomimetic peptides containing the plastoquinone-binding site in a loop shaped by two alpha-helices were designed and characterized, showing high stability and affinity for the pesticide atrazine (affinity constant 3.52×10^5 M^{-1} for the selected peptide D1pepmut)[3]. In further steps, this 70 aminoacid-peptide was modified to increase the solubility in aqueous solvents by adding two histidines in the N- and C-terminus, and three new peptides were selected. A cysteine was included in two of the modified peptides (S264C or S268C), peptides were labeled with different types of commercial carboxylated quantum dots (peak emissions from 510 to 710 nm) by carbodiimide reaction coupling and fluorometric detection was performed. The results confirmed that mutation S264C conferred resistance to atrazine and diuron, while the change S268C increased the sensitivity. The application of new technologies based on quantum dots, nanoparticles and magnetic particles that could lead to improved response of the photosynthetic material and, therefore, to increased biosensor sensitivity -up to the picomolar range- was further explored.

References

1. G. Rea, *Protein Sci.* 18, 2139–51, **2009**.
2. M. T. Giardi, *Biosens. Bioelectron.* 25(2), 294-300, **2009**.
3. V. Scognamiglio, *Phys. Chem. Chem. Phys.* 15, 13108, **2013**.

Figures

A- Schematic representation of the 3D structure of *Chlamydomonas reinhardtii* photosynthetic reaction centre (D1-D2 heterodimer). In green, the region corresponding to synthetic D1 peptide
B- Structural model of one of the mutated D1 synthetic peptides
C- Structural simulation of the putative D1 peptide-pesticide complex
D- Quantum dot-labeled D1 peptides for direct fluorescent detection of pesticides using Biosensor's prototype instrument