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Elucidating the Action Mechanism of Herbicides using Chemical Force Microscopy and Molecular Modeling

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Abstract – This work has been instituted to produce nanobiosensors to detect herbicides and other environmental pollutants, which are expected to provide new opportunities for the rapid screening of environmental samples and to investigate action mechanism of neurodegenerative diseases e.g., Parkinson's disease. An atomic force microscopy (AFM) was used to directly evaluate specific interactions enzymatic-inhibitor herbicides using tips functionalized with enzymes. The oriented immobilization of enzymes against molecules of the herbicide diclofop-p on gold was carried out to create active nanobiosensor surfaces. The specific interaction between the enzyme acetyl-coenzyme A carboxylase – ACCase, immobilized on the AFM tip was measured by force spectroscopy and corroborated with molecular modeling techniques.

The aim of this work was to generate a nanobiosensor to detect herbicides and other environmental pollutants. These biosensors [1] are expected to provide new opportunities for the rapid screening of environmental samples. We use enzyme-inhibitor herbicides, particularly acetyl-Coa carboxylase (ACCase), which is necessary for the synthesis of fatty acid in plants. We use cantilever biosensors to transduce the recognition event from its receptor-coated surface into a mechanical response. The receptors (enzymes) were covalently anchored to the cantilever (tip surface functionalization) and adsorbed on interdigitated electrodes. Enzyme inhibitors bind to enzymes and decrease their activity. Since blocking an enzyme activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors. They are also used as herbicides and pesticides. Specific interactions between surfaces can be studied at the molecular scale using AFM. Adhesion, in particular, is governed by short-range intermolecular forces that may be controlled by appropriate surface modification, thus leading to the so-called Chemical Force Microscopy (CFM) [2]. One way to functionalize the AFM tip is to cover it with an ordered monolayer of organic molecules (a self-assembled monolayer), in special, the enzyme ACCase. The force of interaction can be estimated from the excess force required to pull the tip free from the surface. For the case of an electronic tongue used here, we adorb an enzyme monolayer on interdigitated electrodes using a self-assembly method. With CFM we could distinguish between nonspecific adhesion and specific interactions – brought about by the herbicide. This force curve was acquired with a thin film of diclofop-methyl in contact with ACCase for 5 hours. These results will now be compared with those obtained with an electronic tongue as the sensing device and molecular modeling results. The adhesion force revealed a distribution with average rupture forces of 103±12 nN and 36±7 pN for diclofop-p and atrazine, respectively. As expected, a higher force – above 100 nN – was measured between the enzyme and its enzymatic inhibitor (diclofop-p), while for atrazine the lower force should be non-specific, van der Waals or electrostatic forces. Furthermore, the adhesion force increased drastically with contact time for diclofop-p, but there were negligible changes for the non specific atrazine. Our study indicates that AFM can be utilized as a convenient nanobiosensing tool for confirming the presence and assessing the strength of herbicide-enzyme interactions on biosensor surfaces. Preliminary results of molecular modeling showed that the enzyme ACCase has positive and negative charged groups located at different and specific regions on the protein surface, suggesting that functionalized tips surfaces can affect strongly this enzyme orientation. Thus, based on the electrostatic potential, AFM tips functionalized with silanes, thiois, or any other positively charged groups are more suitable to immobilize the ACCase enzyme and preserve it biological activity. Such results have been applied in the study of Parkinson's disease. Scientists have recently found a link between environmental factors and the development of Parkinson's disease, some evidence implicates herbicides as important factors in many cases this disease. A higher incidence of parkinsonism has long been noted in people who live in rural areas, particularly those who drink private well water or are agricultural workers [3].

References

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