EFFECTS OF BIOLOGICAL MATRICES ON THE PP2A ENZYMATIC REACTION: SIGNIFICANCE FOR THE DEVELOPMENT OF PP2A-BASED BIOSENSORS

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Problem and solution

Okadaic acid (OA) and derivatives are marine toxins produced by some microalgae, which are ultimately incorporated by shellfish. Their fast and reliable detection is of utmost importance in order to avoid food intoxications. Biochemical assays and biosensors can overcome the limitations of the mouse bioassay (reference analysis method for these toxins) and provide faster, more specific and more sensitive screening tools.

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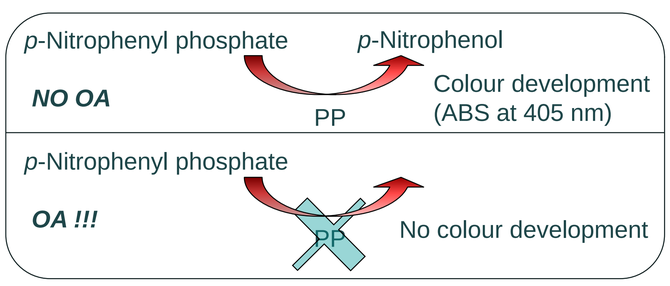
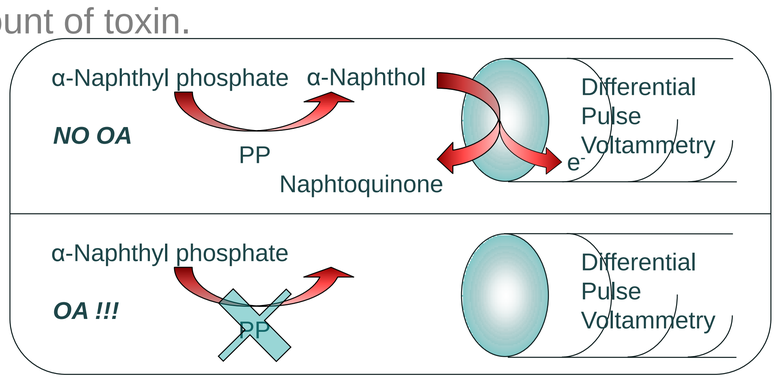
Colorimetric and electrochemical strategies

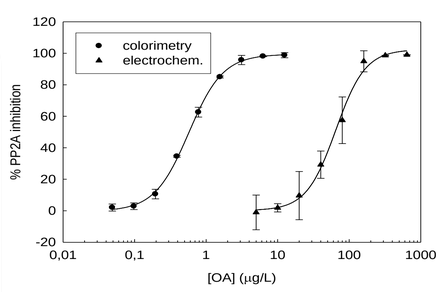
OA and some derivatives bind to the receptorial site of **protein phosphatases (PPs)** in a reversible mechanism, inhibiting their enzymatic activity. The colorimetric assay and the electrochemical assay (enzyme in solution) and biosensor (immobilised enzyme) are based on the extent of inhibition, which is proportional to the amount of toxin.



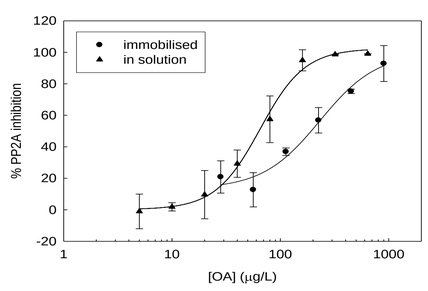
PP inhibition-based colorimetric assay



PP inhibition-based electrochemical biosensor



Calibration curves for OA with the colorimetric and electrochemical assays

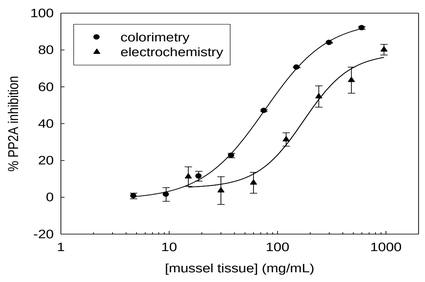


Calibration curves for OA with the electrochemical assay and biosensor

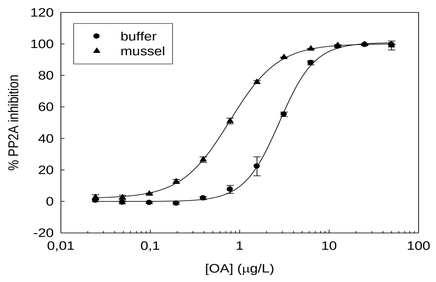
The electrochemical assay provides with higher limits of detection (LODs) than the colorimetric one, due to the higher amount of enzyme required. In the biosensor, the diffusion barrier created by the polymer used in the immobilisation of PP increases the LOD. A new immobilisation protocol based on the conjugation of the enzyme to magnetic particles is in progress in order to improve the performance of the biosensor.

Shellfish matrix effects

At increasing mussel tissue equivalents, matrix inhibits PP activity even in the absence of toxin. At a mussel flesh concentration that should not inhibit PP, OA-spiked mussel inhibits more than OA-spiked buffer (**synergistic effect**).

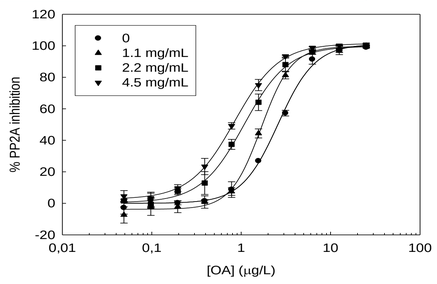


Effect of non-toxic mussels on the assay and the biosensor



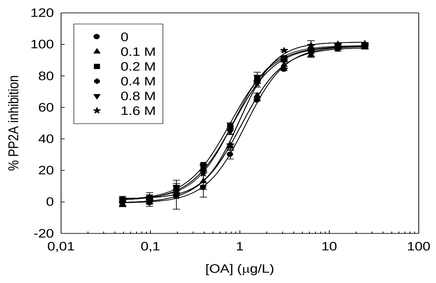
Synergistic effect of spiked OA in the presence of mussel flesh at 4.5 mg/mL

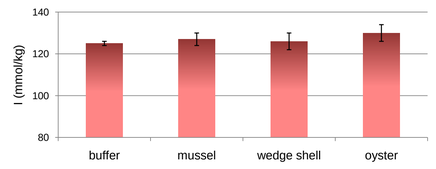
Matrix dilution



The synergistic effect decreases with mussel flesh dilution, but it never disappears. To work at extremely diluted solutions, much more sensitive assays/biosensors would be required.

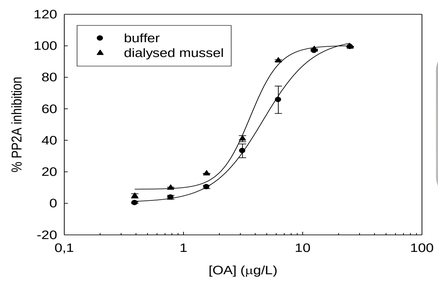
Ionic strength





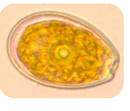
The ionic strength is not responsible for the synergistic effect. Moreover, there are no significant differences in ionic strength between buffer and dissolved shellfish matrices.

Dialysis



Dialysis of mussel flesh was performed to investigate the MW of the synergistic compound. That effect decreases at fraction MW<3,5 kDa but not completely and, moreover, OA (MW = 805) would also be in that fraction, which makes dialysis non-suitable, apart from being time-consuming.

In progress



- Correction factors for OA quantification in different shellfish matrices are being established.
- Preliminary experiments indicate that matrix effects of toxic microalgae (*e.g. Prorocentrum lima*) are lower than matrix effects of shellfish.

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