2. BIOSENSOR SYMPOSIUM
TÜBINGEN 2001 http://barolo.ipc.uni-tuebingen.de/biosensor2001

Neurotoxin detection in food using disposable AChE-biosensors

Holger Schulze, Francois Villatte, Rolf D. Schmid, Till T. Bachmann
Institut für Technische Biochemie, Allmandring 31, D - 70569 Stuttgart
Tel. 0711-685-3197
itbtba@po.uni-stuttgart.de www.itb.uni-stuttgart.de
Registriernummer der Online-Anmeldung: 292

Poster

The extensive use of pesticides to protect agricultural crops necessitates reliable tools for the detection of residues in food and water, thus ensuring environmental protection and consumer safety. Neuroinhibitors such as organophosphates and carbamates are widely used in agriculture because of their high insecticidal activity and their rapid mineralisation in the environment. Since they do not only inhibit insect acetylcholinesterase (AChE) but also interfere with neural transmission in other organism, including humans, they represent a potential hazard for human health and environmental food chains, and thus require continuous assessment. Recent reports on organophosphate contamination in baby food have initiated the reassessment of permitted residual concentrations of these compounds in view of the US Food Quality Protection Act. Here we present a rapid detection method for neurotoxins in food, which can increase the number of tested foodstuff and thus improve the consumer safety.

We developed a highly sensitive and rapid food analysis biosensor based on disposable screen-printed thickfilm electrodes [1, 2]. Matrix problems were solved by using isooctane as extraction solvent. The performance of this amperometric test was checked by three different food matrixes, namely orange juice, apple and peach baby food. The detection limit was comparable to chemical standard methods and met the requirements for monitoring the maximum residue levels in baby food set by the EU.

As such screening tests use a large amount of enzyme a production of recombinant acetylcholinesterase is strongly promoted. A promising expression system is the yeast *Pichia pastoris* [3, 4]. Expression and enzyme optimisation studies require a rapid and efficient screening test to detect positive yeast colonies after transformation. Using indoxylacetate as a substrate, we designed a chromogenic test that is not interfered by the culture media, and thus is suitable for microtiter plate screening. Moreover, it was possible to adapt the test for direct on-plate detection of acetylcholinesterase-expressing colonies.

Literatur

1. Bachmann, T.T. and Schmid, R.D. (1999) A disposable multielectrode biosensor for rapid simultaneous detection of the insecticides paraoxon and carbofuran at high resolution, *Anal. Chim. Acta*, **401**, 95-103.
2. Bachmann, T.T., Schmid, R. D., Léca, B., Marty, J. L., Villatte, F., and Fournier, D., (2000) Improved discrimination of organophosphate and carbamate insecticides using recombinant mutants of *Drosophila* acetylcholinesterase and a disposable multielectrode biosensor, *Biosens. Bioel.,* **15**(1-4), 193-201.
3. Heim, J., Schmidt-Dannert, C., Atomi, H., and Schmid, R. D., (1997) Functional expression of a mammalian acetylcholinesterase in *Pichia pastoris*: comparison to acetylcholinesterase, expressed and reconstituted from *Escherichia coli*, *Biochim. Biophys. Acta,* **1396**, 306-319.
4. Morel, N. and Massoulié, J. (1997) Expression and processing of vertebrate acetylcholinesterase in the yeast *Pichia pastoris*, *Biochem. J.,* **328**, 121-129.