Electrochemical biosensing of pesticide residues based on affinity biocomposite platforms

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Abstract

A novel and very sensitive electrochemical immunosensing strategy for the detection of atrazine based on affinity biocomposite transducers is presented. Firstly, the graphite–epoxy composite transducer was bulk-modified with different universal affinity biomolecules, such as avidin and Protein A. Two strategies for the immobilization of the anti-atrazine antibodies on both biocomposite transducers were evaluated: ‘wet-affinity’ and ‘dry-assisted affinity’ immobilization. Finally, the performance of a novel anti-atrazine immunocomposite bulk-modified with anti-atrazine antibodies was also evaluated. The better immobilization performance of the anti-atrazine antibodies was achieved by ‘dry-assisted affinity’ immobilization on Protein A (2%) graphite–epoxy biocomposite (ProtA(2%)-GEB) as a transducer. The immunological reaction for the detection of atrazine performed on the ProtA(2%)-GEB biosensors is based on a direct competitive assay using atrazine-HRP tracer as the enzymatic label. The electrochemical detection is thus achieved through a suitable substrate and a mediator for the enzyme HRP. This novel strategy was successfully evaluated using spiked orange juice samples. The detection limit for atrazine in orange juices using the competitive electrochemical immunosensing assay was found to be $6 \times 10^{-3}$ μg L$^{-1}$ (0.03 nmol L$^{-1}$) thus this biosensing method accomplishes by far the LODs required for the European Community directives for potable water and food samples (0.1 μg L$^{-1}$). This strategy offers great promise for rapid, simple, cost effective, and on-site biosensing of biological, food, and environmental samples.

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1. Introduction

Atrazine has been one of the most widely used herbicides and a persistent environmental contaminant (Papiernik and Spalding, 1998). Because of their polarity, triazine herbicides usually cause contamination of groundwaters and surface waters. Food and environmental regulatory agencies have established control programs in order to avoid those pesticides to enter the food supply. The European Community (EC) has thus established the maximum residue limits (MRL) for pesticide residues. Mass fabrication, low cost, and decentralized in-field analysis are important features of electrochemical sensors to be considered as analytical methodology for the rapid detection of pesticides residues in environmental or food samples (Alegret, 2003). Besides electrochemical biosensing of pesticides, many immunological-based assays have been developed for the sensitive detection of pollutants, including atrazine, in real samples (Schneider and Hammock, 1992). Numerous coupling strategies have been specially developed for the immobilization of antibodies on different surfaces – such as polystyrene microtiter plates for ELISAs or carbon transducer for electrochemical biosensing– through the formation of defined linkages with improved antibody orientation and binding capacity. One of them is based on avidin–biotin interaction (Wilchek and Bayer, 1988, 1990; Wilchek et al., 2006). ‘Chicken egg white’ avidin – a glycosylated and positively charged ($p_I \sim 10.5$) protein – and its bacterial analogue streptavidin, share a similarly high affinity ($K_a \sim 10^{15}$ M$^{-1}$) for the vitamin biotin (Green, 1990).

Another immobilization strategy is based on the antibody bonding through Fc fragment to Protein A or G. The bond strength – from strong to weak – between Protein A (or G) and an antibody is greatly affected by the antibody classes and subclasses (Sjoquist et al., 1972a,b; Akerstrom et al., 1985; Compton et al., 1989; Janis and Regnier, 1989; Larsson, 1990).
immunoassay was used for the determination of atrazine in PBST buffer. Fig. 4 shows the corresponding standard curve for the detection of atrazine on ProtA(2%)-GEB and also shows the main features for this strategy. As can be seen, the atrazine can be easily detected in PBST buffer, showing IC\textsubscript{50} values of 3.6 nmol L\textsuperscript{-1} and the linear range between 0.7 and 13.5 nmol L\textsuperscript{-1} while the LOD was found to be 0.17 nmol L\textsuperscript{-1} (0.037 μg L\textsuperscript{-1}). Similar results were found with the electrochemical magneto immunosensing strategy with Protein A modified magnetic beads (Zacco et al., 2006b and Fig. 4), showing that the immunological reaction is achieved in similar rate over the ProtA-GEB biocomposite as well as in the surface of Protein A magnetic beads which are known to minimize the matrix effect.

4.5. Electrochemical competitive immunosensing of atrazine in real samples

Fig. 5 shows the standard curve obtained when measuring the spiked orange juice with the ‘dry-assisted affinity’ immobilization of the anti-atrazine antibodies on ProtA(2%)-GEB. This preliminary experiments demonstrated that non-specific interferences can be eliminated in part by a very simple pretreatment of the sample, consisting of just adjusting the pH to 7.5 (the original pH of the sample was ∼3.5), filtering the sample through a 0.2-μm filter, and diluting the filtrate five times with PBST.

As shown in Fig. 5, the atrazine can be easily detected in orange juice since the curves obtained with pretreated orange juice samples are similar to those with PBST, showing IC\textsubscript{50} values of 0.5 nmol L\textsuperscript{-1} and the linear range between 0.08 and 6.07 nmol L\textsuperscript{-1} while the LOD was found to be 0.03 nmol L\textsuperscript{-1} (6 × 10\textsuperscript{-3} μg L\textsuperscript{-1}). As the European legislation fixes the maximum level of atrazine at a value of 0.1 μg L\textsuperscript{-1}, the detection limit of the novel electrochemical immunosensing strategy based on ‘dry-assisted affinity’ immobilization of the anti-atrazine antibodies on ProtA(2%)-GEB allows us to measure real samples according to the legislation.

5. Conclusion

A novel electrochemical immunosensing strategy for the detection of atrazine residues in real samples such as orange juice has been developed. Anti-atrazine specific antibodies have been successfully surface-immobilized on both avidin and Protein A modified graphite–epoxy biocomposite (Av-GEB and protA-GEB, respectively). Moreover, the anti-atrazine specific antibodies have been bulk-immobilized on an immunocomposite specific for the detection of atrazine. However, ProtA-GEB has been used in further analysis since better reproducibilities were achieved and it is not necessary to perform the previous biotinylation of the anti-atrazine antibodies for immobilized them. It was found that the binding capacity of the biocomposite showed to be higher when a ‘dry-assisted’ affinity strategy was performed. In this case the hydrophobic bonds – between Protein A and the Fc of the anti-atrazine antibodies – seemed to be favored. Using ProtA-GEB in a competitive immunological assay for the detection of atrazine, excellent detection limits were achieved. The novel competitive electrochemical immunosensing strategy can easily reach the required LOD for potable water and orange juice (MRL 0.1 μg L\textsuperscript{-1}) established by the European Community directives with very simple sample pretreatments. Because of the simplicity of the immunochromical procedure presented here, this strategy can be suitable for fast semiquantitative and quantitative on-site analysis for the presence of atrazine (or atrazine immunoreactive herbicides) in real samples. The fabrication of the biocomposite based biosensor can be easily transferred to industrial scale. The fact that the same biocomposite material could be used for the immobilization of many antibodies is an important practical feature to be considered for the massive fabrications of electrochemical biosensing devices. Moreover, this material can be easily prepared through dry chemistry using procedures that can be transferred to mass fabrication establishing a clear advantage for the development of \textit{biohits}. Additionally, the biosensor design fulfills the requirements desired for these devices: ease of preparation, robustness, sensitivity, low cost of production, ease of miniaturization and simple use and fast response for industrial and environmental applications.

References