IMMOBILIZATION OF ANTI-GLYPHOSATE ANTIBODIES ONTO GLASS

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Glyphosate (N-(phosphonomethyl)glycine; trade name Roundup^{TM}) is currently the most widespread agrochemical in the whole world. Although it is officially announced that "glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet", glyphosate has been classified as probably carcinogenic based on the evidence of its carcinogenicity in humans and animals since 2015 [1]. At present, HPLC methods are commonly used for the detection of glyphosate. For the conduction of rapid glyphosate analyses we propose to use an optical immunobiosensing system. The aim of the present study was to find optimal conditions for immobilization of anti-glyphosate antibodies onto glass surfaces applicable for the selective detection of glyphosate.

Commercial glass slides (Paul Marienfeld GmbH & Co, Germany) were pre-cleaned and activated in plasma chamber before silanization with N-[3-Trimethoxysilyl)propyl]ethylenediamine. Silanized slides were functionalized with the mixture of biotin-polyethylene glycol (PEG) and methoxy-PEG (mass ratio 1:50) which generates specific binding points and avoids nonspecific binding onto the surface. Silanized slides were also functionalized only with methoxy-PEG. This revealed that less than 1 % of phycoerythrin conjugated streptavidin detected on the surface with total internal reflection fluorescence microscope (TIRFM) was bound nonspecifically.

The biotin-labelled capture antibodies were immobilized onto the surface via NeutrAvidin bridge. IgY-type anti-glyphosate antibodies were biotinylated with biotin-PEG-hydrazide after oxidation with periodate solution to assure the directed attachment of antibodies. NeutrAvidin was added before attachment of capture antibodies. Anti IgY antibodies, marked with fluorescein isothiocyanate were used to determine the number of bound antibodies. We found that over 250 000 IgY per 1 mm^2 were available for the detection of glyphosate. The developed bioselective surface can be further integrated with immunobiosensing system, enabling to achieve high selectivity and low detection limit.

References

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