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Electrochemistry 4 MIPs

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Abstract:

The talk describes the electrosynthesis and the analytical performances of MIPs for the following proteins: Acetylcholine esterase, Cytochrome P450 BM3, Cytochrome P450cam, Laccase, Tyrosinase, Cytochrome c, Ferritin, Transferrin, Concanavalin A and Human Serum Albumin. These MIPs are synthesized with only ONE monomer (and even without a cross-linker) and exhibit measuring ranges in the micromolar up to the sub-nanomolar concentration range.

Key words: Electropolymerization, redox marker, imprinting factor, anchor group, measuring range.

Introduction

Almost 35 years after the pioneering work of Wulff and Mosbach in the synthesis of Molecularly Imprinted Polymers (MIPs) by radicalical polymerization, synthesis of MIPs by the polymerization of electroactive functional monomers gained acceptance.

We describe in the present talk the electrosynthesis of MIPs and present the analytical performance for the following proteins: Acetylcholine esterase (AChE), Cytochrome P450 BM3, Cytochrome P450cam, Laccase, Tyrosinase, Cytochrome c, Ferritin, Transferrin, Concanavalin A (ConA) and Human Serum Albumin (HSA).

Electrochemical MIP Synthesis

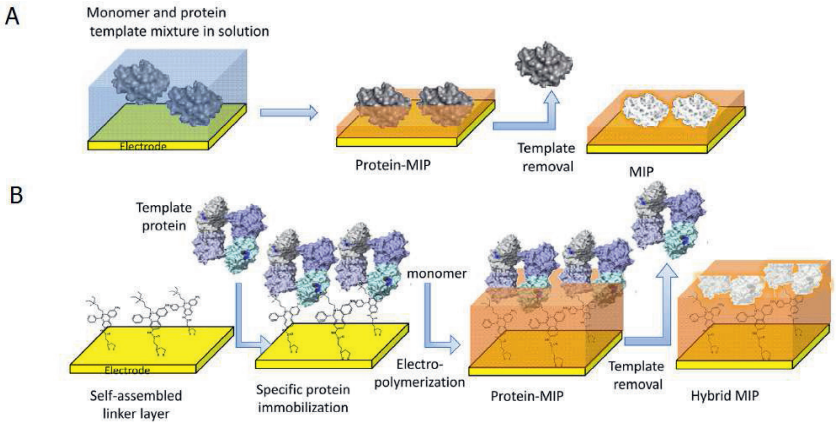

Fig.1 Workflow of electrochemical MIP preparation.

Electropolymerization:

Electrochemical polymerization of monomers in the presence of the target leads to the formation of nano-films on the conducting surface of electrodes, QCM and SPR chips (Fig.1A). Prior to polymerization the protein can be bound to the transducer surface via anchor groups e.g. charged alkane thiol-SAMs, carbohydrates, tags or inhibitors in an oriented fashion in order to generate accessible uniform binding sites (Fig.1B) Most of the electropolymerizable monomers can be deposited from aqueous solutions where protein molecules preserve their natural conformation. Controlling the amount of charge passed during electropolymerization, the layer thickness can be precisely tuned so that the polymer film only partially traps the target molecule.

Template removal

Different methods have been applied to remove proteins from MIPs, which are based on denaturation of the proteins. For oligomeric proteins the dissociation into monomeric units by changing the pH and/or detergents is used. Globular proteins have been extracted by chaotropic reagents, organic solvents or surfactants which induce a collapse of the secondary structure. Degradation of the protein target by proteolytic digestion, e.g. proteinase K, is an effective method which works under mild conditions. Interestingly, removal of cysteine-terminated peptides, but also of proteins can be accomplished by applying anodic pulses of around 1 V.

Electrochemical Readout

A frequently applied method for the characterization of MIPs and NIPs uses redox markers like ferricyanide . It is assumed that the redox peaks in the CVs and the resistance determined by impedance spectroscopy reflect the permeability of the polymer film for the redox marker. It is a simple procedure to follow the work flow of MIP preparation and rebinding. On the other hand, for the determination of quantitative parameters, i.e. binding isotherm , this method is questionable because the change of the current signals is obviously not only caused by the removal or binding of the target but also by the formation of “nonspecific” pores during the extraction of the template. An additional disadvantage of this method is caused by the evaluation: Target binding brings about minute decreases of the big current signal in the absence of the target. Therefore, it is surprising that several papers describing MIPs for both low and high molecular weight targets (using the redox marker ferricyanide) claim measuring ranges over several orders of magnitude with sub-nanomolar lower limits of detection. The evaluation of the enzymatic activity or of direct electron transfer is considerably more specific and gives a direct quantification of the bound target

Conclusion

As compared with antibodies, protein MIPs have been prepared only for a restricted spectrum of proteins. Almost half of the papers use hemoglobin, serum albumin and avidin as model templates.

Point-of-care detection of marker proteins for cardiovascular, tumor diseases, Alzheimer`s disease or virus infections is the prospective aim in the generation of electrochemically addressable MIPs. However, they need still substantial improvement in spite of a few reports claiming routine applicability.

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