

Enzyme Biosensor Based on *Tobacco Mosaic Virus*-Modified Field-Effect Structures

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Abstract

A novel approach for the development of enzyme biosensors based on field-effect structures modified with *tobacco mosaic virus* (TMV) as enzyme nanocarrier is presented. TMV nanoparticles serve as scaffolds for precisely positioned high-density enzyme immobilization with retained enzyme activity. To prove this new concept, a capacitive penicillin biosensor based on an electrolyte-insulator-semiconductor structure modified with penicillinase-functionalized TMV nanotubes was realized as model system. The fabricated sensor has been electrochemically characterized by capacitance-voltage and constant-capacitance measurements. The sensor surface morphology with TMV nanotubes was physically investigated by scanning electron microscopy and atomic force microscopy. The results of this study demonstrate the promising potential of virus particles as universal scaffolds for the immobilization of biomolecules on sensor surfaces and for the development of highly sensitive enzyme biosensors.

Key words: penicillin biosensor, *tobacco mosaic virus*, enzyme nanocarrier, field-effect structure, enzyme penicillinase

Introduction

Currently, viruses are not only considered as infectious agents, they are also recognized as promising functional materials for application in nano- and biotechnologies [1]. *Tobacco mosaic virus* (TMV) is one of the most studied plant viruses and is, due to its high chemical and physical robustness, attractive for the integration with different transducers for applications in biosensing [2]. TMV is non-pathogenic for mammals and consists of 2130 identical coat proteins which enable the selective binding of a high amount of biomolecules, in particular, enzymes [3]. Recently, we presented an amperometric sensor modified with glucose oxidase functionalized TMV nanotubes for the detection of glucose [4]. In this study, we introduce a capacitive penicillin biosensor based on an electrolyte-insulator-semiconductor (EIS) structure modified with enzyme penicillinase functionalized TMV nanotubes.

Results and Discussion

For the fabrication of the penicillin sensors, Al-p-Si-SiO₂-Ta₂O₅ EIS structures have been modified with biotinylated TMV nanotubes. A penicillinase (from *Bacillus cereus*) was conjugated with streptavidin ([SA]-penicillinase) and immobilized on the TMV nanotubes via biotin-streptavidin bioaffinity binding (see Fig. 1). The fabricated penicillin sensors were electrochemically characterized in buffer solution as well as in bovine milk containing different amounts of penicillin G using capacitance-voltage and constant-capacitance modes. To investigate the sensor surface morphology with TMV nanoparticles, atomic force microscopy (AFM) and scanning electron microscopy (SEM) (see Fig. 2) was used. Details of the experiments and the obtained results will be presented. The functioning mechanism of the TMV-modified field-effect enzyme biosensor will be discussed.

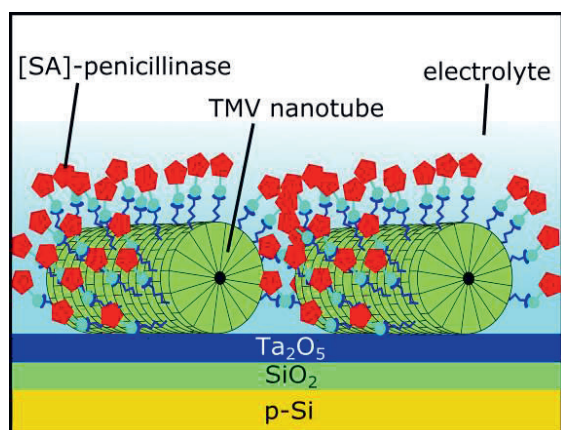


Fig. 1. Schematic layer structure of the capacitive EIS sensor modified with TMV nanotubes as enzyme nanocarriers with a typical length of 300 nm, an outer diameter of 18 nm, and an inner diameter of 4 nm.

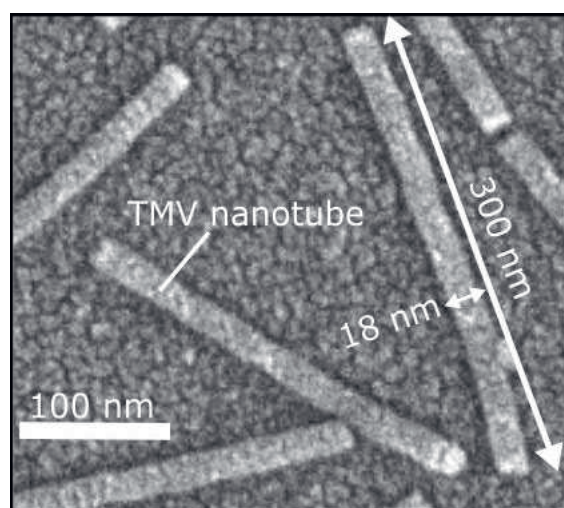


Fig. 2. SEM image of the TMV-modified Ta_2O_5 sensor surface.

Conclusion

The results achieved in the present work represent the enormous potential of TMV nanotubes as scaffolds for biomolecule binding and their integration with electronic transducers offers a new universal platform for the creation of various enzyme biosensors.

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