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HIGHLY SENSITIVE BIOSENSOR FOR PROTEIN ANALYSIS BASED ON AMPLIFICATION STRATEGY

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Abstract - Calmodulin (CaM) is an essential protein that participates in many intracellular activities, and it has been found to intimately associate with cancer diagnosis and treatment. In this paper, we report a highly sensitive electrochemical immunosensor for the detection of CaM with based multifunctional biolabel. Reduced grapheme oxide (rGO)/gold popcorn nanoparticles (GPCs) with high conductivity and large surface area were employed as the sensing matrix of the immunosensor to immobilize CaM. Multifunctional biolabel (HRP-GMC-Ab1) was introduced not only to specifically recognize the captured CaM, but also to provide a large amount of HRP for the catalytic current output. Attributed to this strategy, enhanced attached amount of HRP could be obtained and resulted in amplified electrochemical signals. Analytical results show that this immune sensor exhibited good performance with high sensitivity and stability, and could be extend to other important protein analysis schemes.

Index Terms - Biosensor, Cancer, electrochemical, Protein Biomarker.

I. INTRODUCTION

Calmodulin (CaM) is a widely distributed protein that mediates many crucial processes such as inflammation, metabolism, apoptosis, and the immune response. It is notable that CaM also has played an important role in the regulation of cancer progression, and it is an crucial factor in various cancer cellular activities.1 The CaM level in cancer cells is closely related to their proliferation, and may function as a significant regulator to predict the progress. CaM is now widely confirmed to be a potential target for cancer therapy, and many chemotherapeutants have been shown to inhibit CaM expression in cancer cells. For example, Tamoxifen is now frequently used in lung cancer therapy, which is also a well-known CaM-inhibitor in reducing the CaM expression in lung cancer cells. The change of CaM level could directly reveals cancer cellular progression related information; therefore, development of CaM assay is of great importance in cancer study and has shown high potency for clinical diagnostics and therapy.

II. RESULTS AND DISCUSSION

The detection mechanism of the electrochemical immunosensor for CaM based on layer by layer assembling of Au nanomaterials.3 First, the immunosensor was prepared by casting rGO chitosan solution on the GCE surface as the immobilization matrix. After it was incubated with CaM at different concentrations via the glutaraldehyde linking, the synthesized HRP-pGNR-Ab1 was introduced on the CaM/IL-rGO/GCE via the immunocomplex specific recognition.4 EIS and CV were further used to confirm the preparation of immunosensor. Curve a in Figure 6A presents the nyquist plots of EIS at the bare GCE. When IL-rGO chitosan solution was modified on the bare GCE, the electron-transfer resistance (Ret) was greatly decreased because of the combined conductivity of ILs and rGO (curve b). Upon the conjugation of CAM on the electrode, Ret was increased due to the electron-transfer inhibition by the inert biomolecules (curve c). HRP-pGNR-Ab1/CaM/IL-rGO/GCE displays an increased Ret, implying that HRP-pGNR-Ab1 recognizes the surface CaM and blocked the interfacial electron transfer (curve d). The further increase of Ret, owing to the immobilization of HRP-pGNP-HRP-Ab2, indicates the effective recognition to the HRP-pGNR-Ab1 (curve e). After the biocatalyzed precipitation, it increased obviously owing to the generated insoluble precipitates that retarded the electron exchange on the electrode surface (curve f). Figure 6 B shows the CV curves for each fabrication steps, the results are completely in accord with that from EIS, which further confirmed the successful designing of the proposed immunosensor. These results proved the successful construction of the immunosensor, and therefore it can be applied in CaM determination.

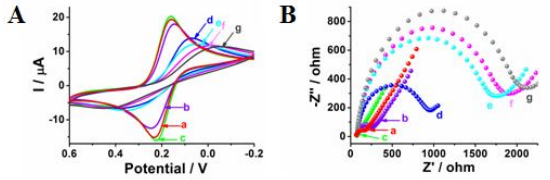

Fig 1. (A) EIS with amplitude of 5 mV and frequency range of 0.01 Hz-100 kHz and (B) CVs on (a) bare GCE, (b) rGO/GCE, (c) Aupcs/rGO/GCE, (d) CaM/Aupcs/rGO/GCE, (e) BSA/CaM/Aupcs/rGO/GCE, (f) HRP-GMC-Ab1/BSA/CaM/Aupcs/rGO/GCE, (g) HRP-PAu-Ab2/HRP-GMC-Ab1/BSA/CaM/Aupcs/ rGO/GCE, which were measured in pH 7.4 PBS containing 5.0 mM Fe(CN)63-/4-.

CONCLUSION

In summary, we have proposed a highly sensitive sensor for calmodulin analysis. The amplification

strategy could provide much more amount of HRP, and the proposed immunosensor therefore exhibited good analytical performance for CaM analysis. Furthermore, this assembling approach is of great potential for the preparation of various biocomposites and the established strategy is robust for the design of immunosensors to detect many other important proteins.

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