Endonuclease Based Hairpin Aptamer Probe for Background Current-eliminated Electrochemical Detection of Thrombin

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In this contribution, *Hpa* II restriction endonuclease is used for the first time to design endonuclease based hairpin aptamer probe and develop background current eliminated electrochemical aptasensor for sensitive signal-on detection of protein biomarker using thrombin as the model target. The hairpin aptamer probe consists of two functional domains: the aptamer sequence for target thrombin and the recognition sequence for *Hpa* II endonuclease. In the absence of target, the ferrocene labeled aptamer probe folds into hairpin structure with a palindromic recognition site which can be digested by *Hpa* II endonuclease, resulting in thorough removal of electro-active ferrocene from the electrode surface and complete elimination of background current for blank sample. While in the presence of target, the aptamer probe changes its conformation when binding with thrombin and dissociates the palindrome structure, resulting in obvious peak current. By eliminating the background current signal instead of amplifying the detection signal, the proposed electrochemical aptasensor reveals an excellent sensitivity towards target thrombin with a low detection limit of 2.67 pM. The sensing system is cost-efficient and easy controllable because only one oligonucleotide probe is involved. Moreover, the design principle of endonuclease based hairpin aptamer probe is easy adapted to other analytes.

Keywords: endonuclease; aptamer; electrochemical; thrombin; *Hpa* II

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