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Abstract

Amplified Detection of the Aptamer—Vanillin Complex with the Use of Bsm DNA Polymerase †

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The decreased sensitivity for electrochemical detection of low-molecular-weight targets with the use of aptamers is a great problem of scientific importance. The amplification of the signal is a promising approach for analyzing the formation of the aptamer-analyte complex. For this purpose, a biocatalytic amplification technique using Bsm DNA polymerase (EP0691, Thermo Fisher) was chosen. At first, we showed that Bsm reaction can be detected by the ion-sensitive field effect transistor (ISFET) with Ta2O5 gate at 22oC. Main components of this reaction are a hairpin fluorescence probe (FP), a short primer (PR) and target probe (TP), which can hybridize with FP and the stem of the hairpin is opened. After that, the hairpin anneals with the primer and triggers the polymerization reaction (https://doi.org/10.1093/nar/gkn1024). Polymerase has strong strand displacement activity and lacks exonuclease activities, so TP is displaced and hybridized to another FP. Thus, this is the amplification of the signal at a low concentration of TP. We showed the detection of 1 fmol of TP with the ISFET. Secondly, these results allowed the detection of the dehybridization probe (DP), which is released from the aptamer during addition of the target molecule. As a proof-of-concept, we used immobilized aptamer for vanillin, which was obtained by us during Capture-SELEX (http://dx.doi.org/10.1155/2012/415697), and designed DP that can act as TP in the Bsm reaction. This approach allowed us to decrease the limit-of-detection (LoD) of vanillin by the ISFET compared to simple dehybridization and, to date, the LoD of vanillin is 1.0×10^{-8} M.