REAL-TIME AND LABEL-FREE EVALUATION OF T7 DNA POLYMERASE IMMOBILIZATION

Julija Dronina[1,2], Deivis Plaušinaitis[2], Urte Samukaite Bubniene[1,2], Arunas Ramanavicius[1,2]

[1] Center for Physical Sciences and Technology, Laboratory of Nanotechnology, Department of Functional Materials and Electronics, Sauletekio av. 3, LT-10257 Vilnius, Lithuania; email: julija.dronina@ftmc.lt

[2] Vilnius University, Department of Physical Chemistry, Faculty of Chemistry and Geoscience, Naugarduko str. 24, LT-03225 Vilnius, Lithuania

During the COVID-19 pandemic, the development of RNA or DNA-sensors and accurate analytical methods based on DNA-modifying enzymes for nucleic acid detection is especially relevant. Recently, DNA-modifying enzymes have been widely used for the diagnosis of viral or pathogenic disease by “gold” (PCR, qPCR, RT-qPCR) methods. The immobilization of biologically active proteins is the primary key in reusing expensive enzymes for many analytical cycles. However, the immobilization of DNA-modifying enzymes on any surface technique with the ability to detect viral or bacterial nucleic acid is individual for each DNA-modifying enzyme group. Generally, DNA-modifying enzymes facilitate the transformation of DNA-based substrate. These enzymes can accurately recognize, amplify, and/or cleave target and non-target DNA fragments [1]. Therefore, immobilization of the DNA-modifying enzymes is still a complex and challenging task in biotechnology and biosensing.

T7 DNA polymerase is a promising DNA-modifying enzyme that catalyzes DNA replication in vitro. The enzyme has an extraordinarily strong 3′→5′ exonuclease (nucleotide cleavage) activity at a single site. However, immobilization of a fully functional T7 DNA polymerase is a challenging task due to the fundamental properties of the enzymes. All enzymes are proteins and are amphiphilic, which causes them to have an affinity with different interfaces and they have multiple strong more than one active centers. Effectively used inherent T7 DNA polymerase properties can be successfully employed for developing DNA-sensors.

This study was successful in investigating the kinetics of immobilization of T7 DNA polymerase with masked active site [2] on different mixed thiol surfaces by QCM method. Study have shown that increasing concentrations of carboxyl groups in the thiol mixture increases the yield (ng) of T7 DNA polymerase immobilization. Commonly, we have demonstrated by this study/research that immobilization is best described according to the first-order kinetic equation. Moreover, our results revealed that immobilized 10 ng of T7 DNA polymerase corresponds to 1.20 Hz frequency change. To evaluate the performance of the engineered T7 DNA polymerase-sensor, we measured the specificity of the immobilized enzyme. In our study, DNA-sensor with immobilized 427.95 ± 0.60 ng of T7 DNA polymerase could accurately cleave 15 ng of double-stranded DNA fragments during overnight incubation at room temperature. In particular, the results explained that immobilized T7 DNA polymerase is entirely functional on all thiol mixture surfaces, and the enzyme's active site was well protected from inactivation. Therefore, the T7 DNA polymerase-sensor can be used more widely in DNA detection research.

References

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