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A universal approach for electrochemically DNA detection based on mediator displacement LAMP

Zahra Bagheryan[1], **Martin Trotter**[2], **Lisa Becherer**[1], **Nadine Borst**[2] and **Felix von Stetten**[2]
[1]IMTEK-University of Freiburg, Germany
[2]Hahn-Schickard, Germany

Recently, a wide variety of isothermal amplification methods, like loop mediated isothermal amplification (LAMP), has been developed as a powerful alternative to PCR to respond the demand of a simplified method without the requirement of thermocycling process. Furthermore, it was considerably explored that amplified products can be detected electrochemically to reduce some limitations of optical methods. However, these methods are still suffering from some shortcomings, as the requirements of chips tailored for each panel of targets. Here we have demonstrated a universal approach for DNA amplification detection using the mediator displacement technology. The mediator displacement approach in general relies on the release of a mediator (here containing an electroactive label) during DNA amplification and its subsequent interaction with a universal reporter (UR) immobilized on the electrode. The mediator displacement probe consists of a loop primer with a target-specific region and a generic mediator hybridization site. The actual mediator is hybridized to this site and is only available for hybridization, if the target DNA is present. In this regard, URs allow the generation of the detection signal independently of the analyte and can be used for specific signal generation in various applications. The ability of the developed method to detect mediator probes was assessed by performing LAMP in a reaction mixture. After 25 min of amplification, the product (target HIV-1) was added to the UR-functionalized electrode surface and electrochemical signal was detected using square wave voltammetry (SWV) without further treatment. The results showed that signal appeared in the presence of target and no unspecific signal is detected for NTC as the mediator remains bound to its site. The key feature in electrochemical mediator displacement LAMP is the possibility of simultaneous target amplification and detection in multiplex assays, which presents an especially attractive option for real-time applications.

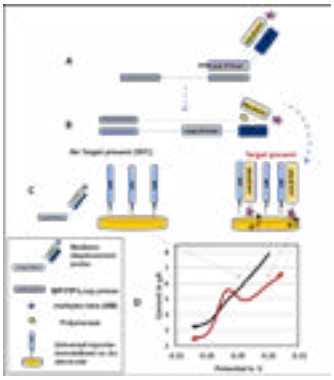

Figure: 1. Schematic illustration of the mediator displacement LAMP. The modified primer contains a target specific primer sequence and a mediator hybridization site, which is complementary to the mediator (A). The signal generation of this approach relies on release of mediator, containing methylene blue (MB) as electroactive label, based on the displacement activity of polymerase in the presence of the target (B). The released mediator then anneals to the universal reporter (UR) immobilized on the electrode surface, which leads to signal generation due to the electron transfer between MB and working electrode. No displacement and no hybridization taking place in the absence of the target (NTC) (C). Square wave voltammetry (SWV) signals for two LAMP reactions that contained no (NTC) or 10^3 initial copies of HIV DNA per reaction are displayed (D). Hybridization time was 30 min at room-temperature.

Recent Publications

1. Hsieh K (2012) Rapid, sensitive and quantitative detection of pathogenic DNA at the point of care through microfluidic electrochemical quantitative loop mediated isothermal amplification. Angewandte Chemie. 51(20):4896-900.
2. Zhang X (2014) Brief review of monitoring methods for loop-mediated isothermal amplification (LAMP). Biosensors and Bioelectronics 61:491-499.
3. Patterson A S (2013) Electrochemical real-time nucleic acid amplification: towards point-of-care quantification of pathogens. Trends in biotechnology 31(12):704-712.

4. Goda, T (2015) Electrical and electrochemical monitoring of nucleic acid amplification. Frontiers in bioengineering and biotechnology 3:29.
5. Becherer L (2018) Simplified real-time multiplex detection of loop-mediated isothermal amplification (LAMP) using novel mediator displacement probes with universal reporters. Analytical Chemistry 90(7):4741-4748.

Biography

Zahra Bagheryan has completed her BSc in Pure Chemistry; MSc in Analytical Chemistry and PhD in Electrochemistry. Her research interests cover electrochemistry nanobiotechnology, biosensors, DNA and aptamer arrays. She is currently focusing on electrochemical sensors for nucleic acid detection which includes some related topics such as amplification procedures (PCR and isothermal approaches), electrode miniaturization and microfluidic.

Zahra.Bagherian@imtek.uni-freiburg.de

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