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著者(和文)	WANGJun
Author(English)	Jun Wang
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Cationic Copolymer for Augmentation of Dynamic DNA Circuits

Wang Jun

School of Life Science and Technology, Tokyo Institute of Technology

Academic Supervisor: Professor Atsushi Maruyama

Chapter 1 gives a general review of dynamic DNA nanotechnology and the nucleic acid chaperone-like activity of the cationic copolymer PLL-*g*-Dex. Toehold-mediated strand displacement (TMSD) is the fundamental principle of dynamic DNA nanotechnology, which focuses on the construction of nucleic acid systems whose function is primarily derived from the ability of DNA molecules to undergo dynamically controlled hybridization or reconfiguration.[1] Dynamic DNA circuits based on cascade TMSD reactions, such as hybridization chain reaction (HCR), and catalytic hairpin assembly (CHA), have been extensively applied in the fabrication of DNA nanodevices,[2] biosensing and bioimaging,[3,4] and cancer theranostics[5] due to their programmable self-assembly processes and isothermal amplification properties. However, the further advancement of these DNA circuits is hindered by the limitations such as slow reaction rate and low robustness. Therefore, an approach to augment the performance of DNA circuits is highly desired.

The cationic copolymer, poly(L-lysine)-*graft*-dextran (PLL-*g*-Dex) promotes DNA hybridization and enhances the thermostability of DNA duplex thorough the formation of soluble inter-polyelectrolyte with ionic nucleic acids.[6] The copolymer was utilized to improve the efficiency of functional DNA nanodevices including DNA nanomachines, DNA computer, and DNAzymes.

In Chapter 2, the effects of cationic copolymer and crowding agent on a sodium dependent DNAzyme, EtNa,[7] were investigated. The results showed that a molecular crowded environment containing 10 to 40 wt% PEG enhanced the catalytic activity of EtNa DNAzyme, although dextran did not. The cationic copolymer at 0.03 wt% (0.3 g/L) enhanced the reaction rate of EtNa by 10-fold, which is similar to the acceleration induced by 15 wt% (150 g/L) PEG. A cooperative impact of the copolymer and crowding agent was observed: the combination resulted in an impressive 46-fold acceleration effect. The use of a cationic copolymer and a crowding agent is a promising strategy to improve activity of Na^+-dependent DNAzyme-based nanomachines, DNA circuits, and biosensors.

In Chapter 3, the effect of PLL-*g*-Dex on HCR circuit which generates DNA intermediates and products with high molecular weight was studied. The HCR proceeds through the continuous cross-hybridization of two DNA hairpin reactants which is triggered by a single stranded initiator, resulting in the generation of long DNA polymers.[8] The copolymer significantly enhanced the generation of HCR products with high molecular weight by promoting the initiation reaction and subsequent growth steps of HCR as the copolymer shields the electrostatic repulsion between DNA strands. Therefore, addition of the copolymer accelerated HCR circuit by 42-fold, reducing the reaction time from 4 h to 10 min. The initiator sensitivity of HCR was improved from 3.3 nM in the absence of copolymer to 80 pM in the presence of copolymer. The acceleration effects of PLL-*g*-Dex were commonly observed for both DNA- and RNA-initiated HCR circuits with different hairpin sequences, implying the general function of the copolymer. Moreover, PLL-*g*-Dex protected HCR circuit from nuclease digestion, permitting reliable operation of HCR in the presence of serum components.[9]

In Chapter 4, the effect of PLL-*g*-Dex on CHA circuit, which is another representative DNA circuits, was investigated. In CHA circuit, the cross-opening of two DNA hairpins in the presence of initiator produces numerous DNA duplexes.[10] The fluorescence measurement showed that the copolymer promoted the CHA circuit by 44-fold without causing obvious circuit leakage which is spontaneous reaction between two hairpin reactants in the absence of initiator, indicating the general effectiveness of PLL-*g*-Dex for accelerating DNA circuit. Furthermore, the copolymer greatly reduced the dependence of CHA circuit on toehold length. In the absence of copolymer, the reaction rate was highly dependent on the toehold length of the initiator, with a 35-fold decrease in rate as the toehold length of initiator was truncated from 8 to 4 nucleotides (nt). However, the reaction rate of CHA in the presence of PLL-*g*-Dex was only slightly affected by toehold truncation. Short toeholds can reduce the undesired hybridization between the initiator and bystander NDA strand. Therefore, the combination of short toehold and PLL-*g*-Dex enhanced the robustness of CHA against molecular noise such as the interference from bystander DNA. The function of PLL-*g*-Dex in diminishing the impact of toehold length was further demonstrated by detailed kinetic characterization of a two-input AND logic circuit.[11] Without PLL-*g*-Dex, the truncation of toehold lengths of two inputs or the low salt concentration resulted in the incorrect operation of AND circuit, while the presence of PLL-*g*-Dex ensured the high-speed operation of DNA circuit with various toehold lengths and different salt conditions.[12]

In conclusion, this study demonstrated that use of the copolymer is a simple, efficient, and versatile strategy to augment the performance of dynamic DNA circuits. The addition of copolymer significantly accelerated the operation speed of DNA circuits by more than 40-fold and improved their robustness against nuclease digestion, molecular noise, and reaction

conditions including temperature and salt concentration. This study lays a solid foundation for more flexible design and broader applications of dynamic DNA circuits in biomedical research.

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