Enhancement of DNA enzyme functions with cationic comb-type copolymers

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（カチオン性くし型共重合体による DNA 酵素機能の強化）

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論　文　内　容　の　要　旨

The DNA enzyme is the DNA sequence that is made up by 4 basic nucleotides. They can catalyze various reactions similar as the natural protein enzymes and natural ribozymes do. The DNA enzyme that is noted as '10-23 DNAzyme' has been studied through theory to application since 1997, it can cleave the ribonucleotide phosphodiester bond in considerable reaction rate. In DNA enzyme dynamics, either the catalyzed phosphodiester bond cleavage or the enzyme turnover determines the overall reaction rate. The DNA enzyme is often questioned to be a true enzyme for a slow turnover rate. The turnover is related to the DNA strand reactions involving Watson-Crick bond formation and dissociation. Changing either binding sequence length of DNAzyme or reaction temperature, the turnover would fail in substrate/enzyme binding or product/enzyme release. To alleviate the limits, I focused on the cationic comb-type copolymer, PLL-*g*-Dex, that produced nucleic acid chaperone-like activity to facilitate the DNA strand reactions. DNA enzyme properties in the absence or presence of PLL-*g*-Dex were studied. The activity of the copolymer was also examined to enhance multi-component nucleic acid enzyme, MNAzyme, a DNAzyme derivative.

The contents of the thesis are as follows:

Chapter 1: The features, species and applications of DNAzymes were described. The biohybrid substances having nucleic acid chaperone activity were summarized. The dextran grafted poly(L-lysine), PLL-*g*-Dex, another cationic compound cetyltrimethylammonium bromide, CTAB, and natural nucleic acid chaperone proteins were introduced and their effects on different nucleic acid strand reactions were reviewed.

Chapter 2: Effects of Cationic comb-type copolymers on RNA cleaving DNAzyme 10-23 was investigated. The copolymer did not influence chemical cleaving activity of the DNAzyme toward ribonucleotide phosphoester bonds as suggested by comparison between the single-turnover and multiple-turnover reactions of the DNA enzyme. PLL-*g*-Dex did not influence DNAzyme reaction under single-turnover reaction condition. Under multiple-turnover reaction condition, the reaction in the presence of PLL-*g*-Dex was faster than that in the absence of PLL-*g*-Dex. These results indicated that the copolymer facilitated

turnover processes of DNAzyme reaction. When reaction temperature was slightly higher than the melting temperature of the substrate binding arm, the DNAzyme showed the highest activity. The copolymer increased the DNAzyme k_{cat}/K_M by fifty fold at 50°C. Electrostatically equivalent amount of PLL-*g*-Dex accelerated the reactions while other cationic nucleic acid chaperone compounds like CTAB caused precipitation of DNA enzyme and substrates.

Chapter 3: MNAzyme, a DNAzyme derivative designed to detect a particular nucleic acid sequence, was investigated in the presence of PLL-*g*-Dex. The PLL-*g*-Dex, that facilitates hybridization, strand exchange reactions and annealing of DNA was utilized to enhance MNAzyme activity. The copolymer promoted the MNAzyme assembling and turnover, and increased the MNAzyme reaction rate about 2 orders of magnitudes, allowing target DNA detection at subpicomolar concentrations. Furthermore, the copolymer enabled us to shorten the substrate-binding arms of the MNAzyme, decreasing the optimum temperature of the MNA assay from 50°C to physiological temperature.

Chapter 4: The research was reviewed and prospective was discussed.