Selection of Functional Modified DNA Molecules by in vitro Selection Methods

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In vitro selection techniques have been used to select DNA/RNA catalysts and DNA/RNA aptamers that have similar activities as protein-enzymes and antibodies. They could be useful as research tools for molecular biology and as indicators of specific substances for the analysis of clinical and food samples. Recently, to enhance activity or to diversify function, various functionally modified DNAs have been developed. Modified DNAs can be efficiently prepared from modified 2′-deoxynucleoside triphosphates in the absence of the corresponding natural substrates by symmetric PCR (polymerase chain reaction), which is capable of direct exponential amplification of DNA. The production of modified DNAs is limited by the substrate specificity of the DNA polymerases, while there are many examples of the enzymatic preparation of modified DNAs by primer extension or one-primer PCR from modified 2′-deoxynucleoside triphosphates or by symmetric PCR under coexistence of the corresponding natural substrates. Therefore, we synthesized a variety of 2′-deoxynucleoside triphosphates to systematically analyze how the chemical structures of modified bases affect the synthesis of modified DNAs by symmetric PCR. We also examined the influence of the type of DNA polymerase on the production of modified DNAs. Furthermore, to identify the critical step in the synthesis of modified DNA, we measured single nucleotide insertion under single turnover conditions by using primers containing modified dUs (dU = 2′-deoxyuridine) at their 3′-ends and templates containing modified dUs adjacent to the elongation site. Based on the results of these experiments, we prepared modified DNA libraries by the enzymatic method and successfully obtained modified DNA aptamers for analogs of thalidomide and glutamic acid by SELEX.