ABSTRACT

In vitro selection was used to isolate a series of deoxyribozymes from a pool of random-sequence DNAs that catalyze an ATP-dependent self-capping reaction. Each deoxyribozyme catalyzes the transfer of the nucleoside and α-phosphate moieties of ATP to the phosphate group located at its 5′ terminus, thereby creating a 5′,5′-pyrophosphate cap. This same pyrophosphate cap structure is formed by T4 DNA ligase during the classical process of DNA ligation. These DNA capping enzymes represent a collection of self-processing deoxyribozymes that can be used for the directed modification of DNA.

INTRODUCTION

DNA in its single-stranded form is known to be capable of adopting intricate tertiary structures that can bind various ligands and that can promote chemical transformations (1,2). The catalytic repertoire of DNA includes many of the same reactions that are catalyzed by ribozymes (3), suggesting that engineered deoxyribozymes may make versatile catalysts that could be used for a variety of applications (4-6). Our studies have primarily focused on the ability of DNA to promote self-processing reactions and the ability of DNA to catalyze various reactions on separate DNA substrates. If catalysis by DNA proves to be broadly applicable, then self-processing DNA constructs might be of significant utility.

Several classes of self-processing deoxyribozymes already have been isolated from random-sequence pools of DNA using in vitro selection (3). For example, deoxyribozymes have been isolated that catalyze chemically activated DNA ligation (7), oxidative DNA cleavage (8), and DNA phosphorylation (9). In this study, we set out to isolate deoxyribozymes that catalyze the formation of a 5′,5′-pyrophosphate cap structure like that formed by T4 DNA ligase during the cascade of reactions involved in the enzymatic ligation of DNA. Similar cap structures can be formed and utilized by ribozymes that have been created by in vitro selection (10,11), which provide a precedence for such nucleic acid catalyzed reactions. Presumably, the cap intermediate of the T4 DNA ligase-mediated reaction could also be formed by a deoxyribozyme, and subsequently used by a ligase deoxyribozyme, thereby completing the reaction cascade for DNA ligation without requiring protein enzymes.

MATERIALS AND METHODS

The procedure used for the in vitro selection of self-capping deoxyribozymes outlined in Fig. 1A was adapted from that described by Li and Breaker (9). Optimized deoxyribozyme reaction buffer: 50 mM HEPES (pH 7 at 23°C), 400 mM NaCl, 10 mM MgCl₂, and 10 μM CuCl₂.

RESULTS AND DISCUSSION

Using the selection procedure outlined in Fig. 1A, we isolated a population of self-capping DNAs after 21 rounds (G21) that exhibits significant catalytic activity (Fig. 1C). Cloning and sequencing of G21 DNA revealed the presence of at least 12 distinct classes of deoxyribozymes as determined by sequence comparison (data not shown). "Class I" deoxyribozymes, which display ATP- and Cu²⁺-dependent catalytic activity, were arbitrarily chosen to be further optimized by reselection.

Although the original DNA recovered by selection is 97 nt (including the 5′-donor, randomized domain, and 3′-primer domains), only the 5′-donor domain and the first 28 nt of the original random-sequence domain remain highly conserved (Fig. 2A). This suggests that the nucleotides that are critical for catalytic activity are located within this smaller region.

Therefore, we synthesized and tested the activity of several truncated DNAs to define the 3′ terminus of the deoxyribozyme. The 41-nt DNA terminating in G27 of the original random-sequence domain is the shortest DNA that retains activity (Fig. 2B). Under optimized reaction conditions, this minimized deoxyribozyme exhibits a K_M for ATP of 800 nM and a k_tera of 5 x 10³ min⁻¹. These results again demonstrate that surprisingly small DNAs can catalyze DNA self-processing reactions.
Figure 1. *In vitro* selection of self-capping DNA. (A) The selective-amplification process begins with a pool of ~10\(^{14}\) distinct DNA molecules (70 random-sequence nucleotides) that are (I) incubated (20 or 1 hr) in the presence of ATP (1 or 0.1 mM) at 23°C as indicated for each round. Excess ATP is removed from the DNA by denaturing 10% polyacrylamide gel electrophoresis (PAGE), then (II) ligated to an acceptor DNA using T4 DNA ligase in the absence of added ATP. Ligated DNAs are (III) isolated by PAGE, amplified by PCR using the appropriate primers, and (IV) single stranded DNAs are recovered as described previously (9). The resulting single-stranded DNAs are (V) phosphorylated by polynucleotide kinase and the purified molecules are (VI) subjected to the next selection reaction. Individuals within a DNA population analyzed by (VII) PCR amplification followed by cloning and sequencing. (B) Sequences of the acceptor, donor and template DNAs. (C) Self-capping activity of DNA populations reflected by ligation with T4 DNA ligase. Subsequent analyses have confirmed the formation of a 5',5'-pyrophosphate cap. Asterisks indicate the introduction of mutations by PCR (12).

Figure 2. A small self-capping DNA. (A) Sequence of the highly-conserved portion of a prototypic class I deoxyribozyme. (B) Catalytic activity of 5' \(^{32}\)P-labeled variants of the class I self-capping deoxyribozyme. Open and filled arrowheads depict the capped and precursor DNAs in lane 5, with preceding lanes (right to left) containing progressively shorter DNAs.

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**REFERENCES**


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