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Triggered amplification by hybridization chain reaction

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Abstract

We introduce the concept of hybridization chain reaction (HCR), in which stable DNA monomers assemble only upon exposure to a target DNA fragment. In the simplest version of this process, two stable species of DNA hairpins coexist in solution until the introduction of initiator strands triggers a cascade of hybridization events that yields nicked double helices analogous to alternating copolymers. The average molecular weight of the HCR products varies inversely with initiator concentration. Amplification of more diverse recognition events can be achieved by coupling HCR to aptamer triggers. This functionality allows DNA to act as an amplifying transducer for biosensing applications.

Biosensors require both a recognition component for detection and a transduction component for readout. In gene chips, recognition is performed by single-stranded DNAs that screen for complementary nucleic acid fragments, and transduction is typically performed by optical or electrochemical means (1, 2). Nucleic acid aptamers obtained by in vitro selection methods (3, 4) generalize this recognition capability to a wide range of target analytes (5, 6) and are amenable to optical transduction approaches (7, 8). Here, we demonstrate that DNA can also play the transduction role via an amplification approach termed hybridization chain reaction (HCR). This class of mechanisms suggests the possibility of constructing biosensors solely from unmodified single-stranded DNA.

Single-stranded DNA is a versatile construction material (9) that can be programmed (10–15) to self-assemble into complex structures (10, 16–23) driven by the free energy of base pair formation. Synthetic DNA machines can be powered by strand displacement interactions initiated by the sequential introduction of auxiliary DNA fuel strands (24–31). Typically, various DNA strands begin to associate as soon as they are mixed together. Catalytic fuel delivery provides a conceptual approach to powering autonomous DNA machines by storing potential energy in loops that are difficult to access kinetically except in the presence of a catalyst strand (32). In the system described here, monomer DNA building blocks are mixed together but do not hybridize on an experimental time scale. Exposure of an initiator strand triggers a chain reaction of hybridization events similar to living chain polymerization but without covalent bond formation. This system introduces the concept of triggered self-assembly of DNA nanostructures.

Methods

System Specifications. DNA sequences were designed by using a combination of criteria (15): sequence symmetry minimization (10), the probability of adopting the target secondary structure at equilibrium (12), the average number of incorrect nucleotides at equilibrium relative to the target structure (15), and hybridization kinetics (33). For the kinetic studies, H1(2AP) was used in place of H1 with the third base (A) replaced with 2-aminopurine (34). DNA was synthesized and purified by Integrated DNA Technologies (Coralville, IA). For the basic HCR system, concentrated DNA stock solutions were prepared in 50 mM Na2HPO4/0.5 M NaCl (pH 6.8). For the ATP aptamer HCR system, stocks were diluted to 5 mM MgCl2/0.3 M NaCl/20 mM Tris (pH 7.6).

Native Gel Electrophoresis. Samples were heated to 95° C for 2 min and cooled to room temperature for 1 h before use. Agarose gels (1%) contained 0.5 g of ethidium bromide per ml and were run at 150 V for 60 min. Reactions were incubated at room temperature for 24 h before loading.

Fluorescence Kinetics. Fluorescence data were obtained using a fluorometer at 22° C. Excitation and emission wavelengths were 303 nm and 365 nm, respectively. Stock solutions of 0.40 M H1(2AP) and 0.48 M H2 were heated to 90° C for 90 sec and cooled for 1 h. Fluorescence was monitored before and after addition of initiator.

Results

The simplest HCR mechanism employs two hairpin species (H1 and H2). The key to this system is the storage of potential energy in short loops protected by long stems. This situation contrasts with that for molecular beacons (36), where short stems protect long loops. In the present HCR system, each hairpin is caught in a kinetic trap, preventing the system from rapidly equilibrating. Introduction of an initiator strand (I) triggers a chain reaction of alternating kinetic escapes by the two hairpin species corresponding to "polymerization" into a nicked double helix. Amplification of the initiator recognition event continues until the supply of H1 or H2 is exhausted. The average molecular weight of the resulting polymers is inversely related to the initiator concentration, suggesting the potential for quantitative sensing.

The kinetics of HCR can be explored by using fluorescence quenching. The adenine analog 2-aminopurine (2AP) is fluorescent when single-stranded but is significantly quenched when in a stacked double-helical conformation (34). Monomer usage can be monitored as polymerization occurs by replacing H1 with the labeled hairpin H1(2AP). Monitoring 2AP fluorescence is preferable to using standard end-labeled strands because the local environment of quenched 2AP will be the same regardless of whether I or H2 performs the quenching. The hairpin monomers H1(2AP) and H2 do not hybridize in the absence of initiator. Addition of I at a lower concentration leads to fluorescence quenching via HCR. With H2 present, HCR performs fluorescent amplification, allowing each I molecule to alter the fluorescence of multiple hairpins. Addition of insufficient initiator provides only partial quenching, demonstrating that HCR, and not I alone, is responsible for exhausting the supply of H1(2AP) monomer.

More diverse biosensors can be created by triggering HCR with molecular recognition events based on DNA or RNA aptamers (3, 4). The aptamer HCR system demonstrates successful detection of ATP, as well as specificity in differentiating ATP from GTP.

Discussion

These data suggest that HCR can be described by a coarse-grained, structure-function relationship in which secondary structure (as opposed to tertiary atomic coordinates) is sufficient to determine the qualitative interactions of components in the system. The basic HCR system and the aptamer HCR system have identical hairpin secondary structures, with stems of length 18 and loops of length 6; both sets of hairpins are stable as monomers when mixed but undergo HCR in response to triggering. The sequence identity between analogous hairpins in these systems is only 30%.

A series of experiments on hairpins with different combinations of loop and stem lengths gave widely different results. For example, systems with stems of length 18 and loops of length 8 or with stems of length 10 and loops of length 6 were not stable at room temperature. If the loops were too small (length 4 with stems of length 10), polymerization in the presence of initiator occurred very slowly. While sequence and tertiary structure are central to aptamer function (37), secondary structure appears to be the appropriate level of description to represent and design the core functionality of HCR.

The concept of HCR is not limited to polymerization of monomer hairpins. The HCR mechanism represents linear growth in response to initiator. It is possible to envision more complicated sets of monomers that would undergo triggered self-assembly to create branched structures corresponding to quadratic growth or even dendritic systems exhibiting exponential growth. Nonlinear amplifiers of this type are currently under investigation.

Here, HCR detection was performed by gel electrophoresis or fluorescence quenching. By using inexpensive synthesized DNA components and standard gel electrophoresis equipment, HCR schemes could potentially serve as economical and easily adopted amplifiers for molecular detection. Alternatively, it may be possible to develop a nanogold-based colorimetric assay (38, 39) for HCR that could be used for readout in

nonlaboratory settings.

HCR amplification could be applied to a wide range of sensing applications by developing a general approach to aptamer triggering. Furthermore, the trigger need not be based on molecular recognition because any physical process that exposes an initiator strand will suffice. For some applications in which detection is the primary objective of amplification, HCR may have the potential to become an attractive protein-free, room-temperature alternative to PCR (40).

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