Kinetic Control of DNA Oligomerization

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Introduction

DNA is widely used for programmed molecular construction at the nanometer scale.[1] One, two and three-dimensional structures have been built using protocols that assume that the most stable structure will be formed at each assembly step. In some cases assembly was assisted by a single-stranded DNA template. DNA has also been used to make active machines, including autonomously moving devices powered by DNA hydrolysis, ATP hydrolysis or the energy released when unpaired nucleotides hybridize. Non-equilibrium DNA hybridization can be used to create artificial biochemical reaction circuits. Catalysis of DNA hybridization has also been used to control the assembly of DNA oligonucleotides into larger structures. A first example was the use of DNA hybridization energy to drive the oligomerization of metastable single-stranded hairpin-loop monomers. This principle has recently also been used to fabricate branched structures. Here we present a more complex system for the assembly of oligomeric DNA which has the advantage that the resulting assemblies do not incorporate self-complementary sequence motifs. This greatly increases the flexibility of this assembly system and the stability of its products.

Experimental

Reaction Scheme

The assembly chain reaction has the components: two two-strand monomers (M1, M2), two auxiliary rubbish collectors (R1, R2) and a seed (S). Each monomer consists of a closing strand (C1 or C2) hybridized to complementary domains of a loop strand (L1 or L2) to create two duplex necks, isolating the central domain of the loop strand as a single-stranded (ss) loop and leaving an exposed toehold at one end. The loop domain of L1 is complementary to the toehold of L2 and vice versa, and the neck domains of L1 and L2 are complementary. The designed product of the kinetically controlled assembly process is a linear chain formed by staggered hybridization of many copies of L1 and L2. Waste products W1, W2 are formed by hybridization of a closing strand to a rubbish collector strand.

Assembly is initiated by seed S which interacts with M1 by hybridizing to the external toehold on L1. S displaces half of C1 from L1, opening the loop. The last six bases of C1 to be revealed form a toehold to which R1 can hybridize. This allows R1 to displace all but three bases of L1 from C1 by branch migration. Displacement is completed by spontaneous dissociation of W1 (R1+C1) from L1. The opened loop of L1 can now bind the toehold of M2, allowing the newly uncovered neck of L1 to displace the first half of C2 from L2 and allowing rubbish collector R2 to remove C2 completely, creating a ss overhang that has the same sequence as S. This overhang can now bind the toehold of a new M1, initiating its incorporation in the growing chain - etc.

Assembly is kinetically controlled. The secondary loop structures of the monomers are kinetically stable. Only the monomer currently attached to the end of the growing chain is reactive because its loop domain is opened, revealing the toehold required to initiate the next strand displacement reaction. A closing strand is almost completely unreactive while hybridized to a loop strand: reaction with a rubbish collector is enabled only when part of the closing strand is displaced from a monomer by reaction with the growing chain.

Assembly is driven by the energy released when unpaired bases in opened loops hybridize to complementary toeholds. In the metastable initial state each monomer contains 32 bp and 14 unpaired nucleotides, of which 7 are constrained in a bulge loop.[10] Rubbish collectors are ss. When the loop strand of a monomer is incorporated into the growing chain its loop is opened and it forms 23 bp with the free end of the chain. The waste complex contains 19 bp. Overall, incorporation of a monomer leads to the formation of 10 bp with a free energy change $\Delta G° \approx 20$ kcal Mol^{-1}.

The kinetically controlled reaction initiated by addition of the seed generates linear oligomers: only one end of the growing chain is reactive, the other end is a non-reactive blunt duplex formed by hybridization of the first monomer with the seed.

Methods and Materials

Oligonucleotide concentrations were deduced from uv absorbance measurements (260 nm). Hybridization reactions were carried out in 100 mM NaCl, 5 mM $MgCl_2$, 20 mM Tris•HCl and 1 mM EDTA at pH 8.0. The two monomers were prepared separately by heating and rapid cooling (quenching): mixtures of loop strands (1 µM) and closing strands (1.25 µM) were heated to 95°C for five minutes and then placed on ice. (Slow annealing also yielded sharp monomer bands.) An excess of closing strands was used to ensure that all loop strands were properly closed. Assembled monomers were mixed stoichiometrically to give final concentrations of loop strands of 0.5 µM. Rubbish collectors were added to final concentrations of 0.75 µM. Different amounts of seed were then added to start the oligomerization reactions. Reactions were allowed to proceed for one hour at room temperature (18°C). Samples were then loaded onto a

non-denaturing polyacrylamide gel (15% 29:1 acrylamide:bis-acrylamide, TAE).

Result and discussion

The DNA sequences, color-coded, are shown in Figure 1.

S	ACTGGAACTAGTTGATGAAGCTG
L1	**GTGTGCGTATTATGTC**TCCTCCTC
	AGCTTCATCAACTAGTTCCAGT
C1	CTAGTTGATGAAGCTGGACATAAT
	ACGCACAC
L2	AGGAGGAGACATAATACGCACACA
	CTGGAACTAGTTGATGAAGCTG
C2	**CAGCTTCATCAACTAGGTGTGCGT**
	ATTATGTC
R1	**TGCGTATTATGTCCAGCTT**
R2	GCACACCTAGTTGATGAAG

Figure 1. DNA sequences. The sequences are color-coded, DNA sequences of the same color but with different font (normal or bold) are complementary.

Analysis of the reaction was done by polyacrylamide gel electrophoresis (PAGE) [2]. Lanes 1 and 2 contain both monomers (lane 2 also contains seed) but no rubbish collectors: products of high molecular weight are almost completely absent, indicating a very low background rate of polymerization. Lane 3 contains monomers and rubbish collectors but no seed: little evidence of assembly is visible, even after 24 hours (data not shown), indicating that the monomers are very stable. (Waste complexes in lane 3 result from hybridization between rubbish collectors and excess closing strands.) Upon adding seed (lanes 4 to 6) monomers disappear and a ladder of slower bands appears, showing that oligomers are forming, as designed. There is a broad distribution of product sizes, with an inverse dependence of the average length on seed concentration. At the highest seed concentration (lane 6) the reaction is expected to take on average five steps producing an oligomer containing 115 bp and a 23-nucleotide ss overhang. At 2.5% seed concentration (lane 4) it is expected that the average oligomer contains forty monomers or around 900 bp. Oligomer mobilities are reduced by nicks in the DNA backbone at 23 bp intervals, preventing direct comparison with the DNA marker ladder. Residual monomers in lanes 4-6 indicate a mismatch (<20%) between monomer concentrations. Faint bands in all lanes, running to around 50 bp and 150 bp, are attributed to dimerization of closing and loop strands. Also visible in lanes 4 to 6 are waste complexes.

This assembly system may be of practical use in the synthesis of repetitive linear DNA. Use of two-strand monomers removes the requirement for substantial self-complementarity in the strands making up the oligomer (self-complementarity is characteristic of a hybridization chain reaction based on hairpin loops): this allows flexible sequence design and increases the stability of the product. If all loop strands were 5´ phosphorylated then the components of the oligomer could be joined by ligation to create a continuous duplex. Phosphorylation of only one loop strand to allow ligation and subsequent separation of one strand of the duplex offers advantages over other techniques for creating repetitive single-stranded DNA: with assembly PCR isolation of one strand is difficult unless its sequence is restricted to contain only three out of four possible nucleotides; with rolling circle replication the position of the 3´ end of the daughter strand is undetermined. The seed could be extended to attach a unique sequence motif or modification at one end of the chain. The single-stranded overhang on the growing end of the chain could be used to attach a unique oligonucleotide at that end.

The presence of the seed at one end of the chain prevents the formation of rings. DNA rings, which form sharp bands with low mobilities, are absent in the kinetically controlled assembly reaction. They are, however, the dominant products when the reactants are annealed to overcome the designed kinetic barriers to reaction.

Conclusion

In conclusion, we have demonstrated the synthesis of linear DNA oligomers by non-equilibrium assembly. The triggered assembly reaction avoids ring structures that dominate when the reactants are annealed. The use of two-strand monomers avoids any requirement for substantial self-complementarity in the oligomeric reaction products. Oligomer lengths can be controlled by adjusting the seed concentration. This assembly system may be of practical use in the synthesis of repetitive linear DNA.

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References

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