1-21-2011

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Abstract. The potential for inferring the presence of cancer by the detection of miRNA in human blood has motivated research into the design and operation of DNA-based chemical amplifiers that can operate in bodily fluids. As a first step toward this goal, we have tested the operation of a DNA-based autocatalytic network in human serum and mouse serum. With the addition of sodium dodecyl sulfate to prevent degradation by nuclease activity, the network was found to operate successfully with both DNA and RNA catalysts.

1 Introduction

Worldwide, approximately 1.3 million deaths per year are caused by lung cancer [1]. Early detection and diagnosis of cancer can lead to decreased mortality rates, yet current screening methods require significant resources [2]. Recently, microribonucleic acids (miRNAs) have been detected in human blood serum [3]. MicroRNAs are small, single-stranded, non-coding RNAs that are 21-23 nucleotides in length and regulate genes by suppression of messenger RNAs [4, 5]. Several miRNAs are amplified in various cancers [6], and expression profiling reveals that miRNA signatures can be used for cancer classification and prognosis.

Current diagnosis technology requires reverse-transcription polymerase chain reaction (RT-PCR) to detect miRNAs in serum [7]. Developments in DNA computing have shown that it is possible to construct metastable DNA-based chemical networks that accept DNA as catalytic inputs and generate output DNA strands whose concentration increases exponentially to produce an easily detectable signal [8]. As miRNAs occur in blood in low abundance, such amplification networks would allow for detection without using PCR. In this study, we report the operation of the DNA-based autocatalytic network reported by Zhang *et al.* in human blood serum with sodium dodecyl sulfate (SDS). The autocatalytic DNA system accepts the input of either DNA or RNA catalysts and produces output signal strands that generate an easily detectable fluorescence signal.

2 Autocatalytic Network

To assess the feasibility of detecting miRNA in human serum using a DNA-based catalytic network, the entropy-driven autocatalytic system developed by Zhang *et al.* was selected as a test network. Figure 1(a) reproduces the autocatalytic network with the same domain naming convention [8]. In this network, strand **4 2bc** is the autocatalyst that initiates signal strand production by toehold-mediated strand invasion of the substrate complex via the **2b** domain. With an exposed $\overline{\mathbf{3}}$ domain, the fuel strand displaces two autocatalyst strands by two strand invasion processes and forms the waste complex. In each cycle, the amount of autocatalyst strand is doubled, leading to exponential growth of the signal strand. In Fig. 1(b), the released signal strand reacts with a reporter complex to displace a tetrachlorofluorescein (TET) labeled strand. An increase in signal strand concentration is detected by an increase in the TET fluorescence intensity. Zhang *et al.* were able to demonstrate exponential behavior of this autocatalytic network in buffer and in a solution with total mouse liver RNA and rabbit reticulocyte lysate [8], demonstrating successful operation in a complex biological environment.

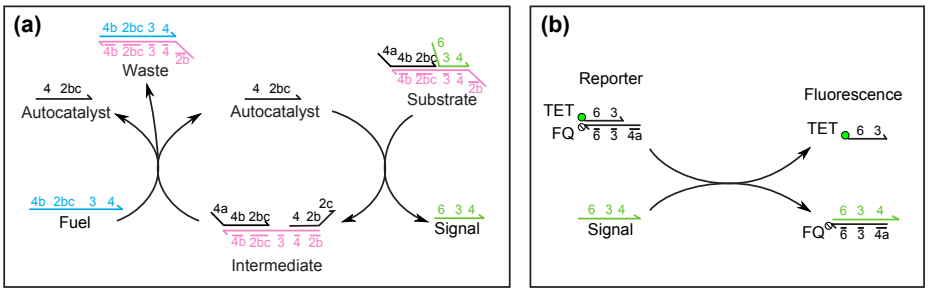

Fig. 1. The DNA-based autocatalytic network reported by Zhang *et al.* [8]. In (a) the autocatalyst initiates the release of the signal strand. The fuel strand displaces both autocatalysts, producing the waste complex. Both released autocatalysts can then initiate new cycles. In (b) the reporter complex consists of a dye-quencher pair in the quenched state. The signal strand from (a) reacts with the reporter complex, displacing the TET dye labeled strand and producing an increase in fluorescence intensity.

3 Network Operation in Serum

To test the autocatalytic system in human serum, the reported DNA sequences of the network were purchased without modification from Integrated DNA Technologies with the same purification processes. Substrate and Reporter complexes were prepared in 1× phosphate buffered saline (PBS) and filtered by polyacrylamide gel electrophoresis to remove excess single-stranded components. Both

DNA and RNA versions of the Autocatalyst strand were used to initiate the network.

Whole blood was collected from volunteers and allowed to clot for 30 minutes at room temperature. The clotted solutions were centrifuged at room temperature for 10 minutes leaving the serum as the supernatant, which was extracted to separate vials for storage at -80 °C. In order to ensure successful operation of the autocatalytic network in serum, the use of the ionic detergent sodium dodecyl sulfate (SDS) added to serum was used as a means to suppress nuclease activity without disrupting DNA hybridization. SDS is commonly used to denature proteins, and with 10% SDS, DNA lifetime and hybridization rates in serum are increased [9]. Figure 2 shows the results for operating the autocatalytic network in a solution of 50% human serum, 10% SDS and 0.5×PBS with both DNA, Fig. 2(a), and RNA, Fig. 2(b), catalysts. The data represent the normalized fluorescence intensity of TET dye integrated over one minute intervals with every 75^{th} data point marked with a symbol. For the data shown in Fig. 2(a), the Substrate and Fuel components of the network were present in solution at concentrations of 100 nM, while the Reporter was present at 200 nM. The network was operated with zero added catalyst and with the DNA catalyst added at 10 and 100 nM. The times to half completion for the DNA catalyst were 5.5 min. at 100 nM, 15.6 min. at 10 nM, and 21.7 min. with no catalyst added. For the data shown in Fig. 2(b), the Substrate and Fuel components of the network were present in solution at concentrations of 25 nM, while the Reporter was present at 50 nM. The network was operated with zero added catalyst and with the RNA catalyst added at 2.5 and 25 nM. For the RNA catalyst, the times to half completion were 54.7, 82.1, and 101.3 min. for 25, 2.5, and 0 nM, respectively. The resulting fluorescence versus time data are in qualitative agreement with the results reported previously [8], exhibiting a similar concentration dependence and initial exponential intensity increase. Although the times to half completion for the DNA catalyst are similar to previous results, it should be noted that here the concentrations of all components is 10 times greater. The longer times to half completion for the RNA catalyst are expected for the factor of four reduction in the component concentrations. It should be noted that operation of the autocatalytic network without added catalyst indicates a non-zero leak rate of the system, as observed previously [8]. Methods to reduce this leak rate are currently being studied.

Initial experiments in detecting cancer-related miRNA will be performed in mouse models of lung cancer. To verify that the autocatalytic network can serve as a test system for the detection of miRNA in mouse models, the network was operated in mouse serum. Figure 3 shows the results for autocatalytic network operation in a solution of 50% mouse serum, 10% SDS, and 0.5×PBS with RNA catalyst added at concentrations of 10 and 100 nM, as well as operation with no added catalyst. The measured times to half completion were 13.6, 37.8, and 47.4 min. for 100, 10, and 0 nM, respectively. These half completion times are only slightly longer than those for DNA catalysts in human serum at the same

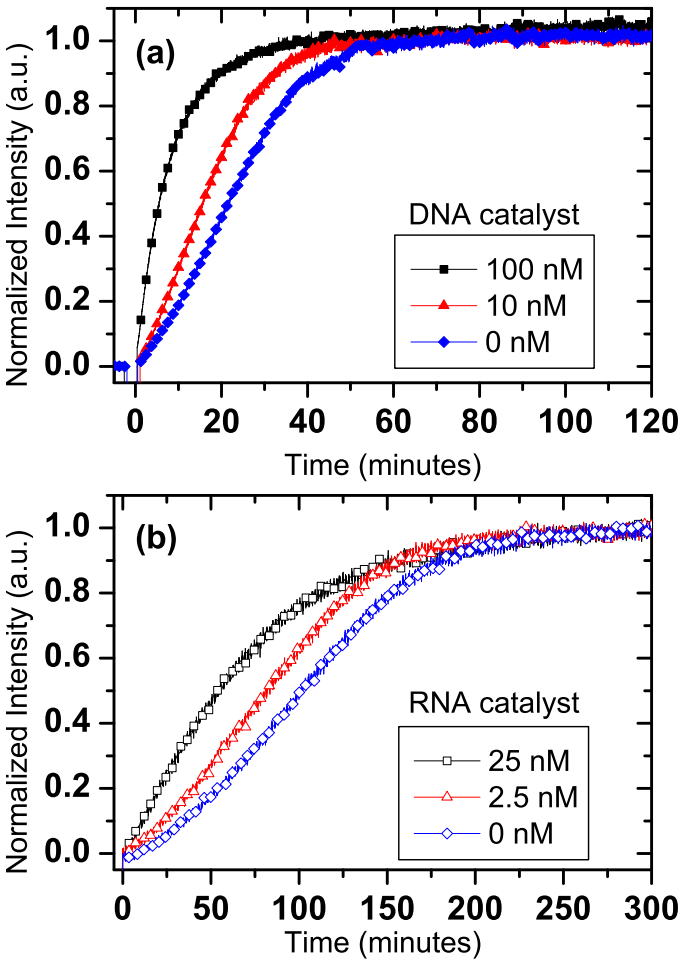

Fig. 2. Autocatalytic network operation in 50% human serum, 10% SDS and 0.5×PBS with (a) DNA and (b) RNA catalysts. Both the DNA and RNA catalysts successfully initiated the autocatalytic network. The observed fluorescence increase and catalyst concentration dependence are in qualitative agreement with the results for network operation in buffer as reported previously [8].

component concentrations, Fig. 2(a), which suggests that test results from mouse models should be readily applicable to human systems.

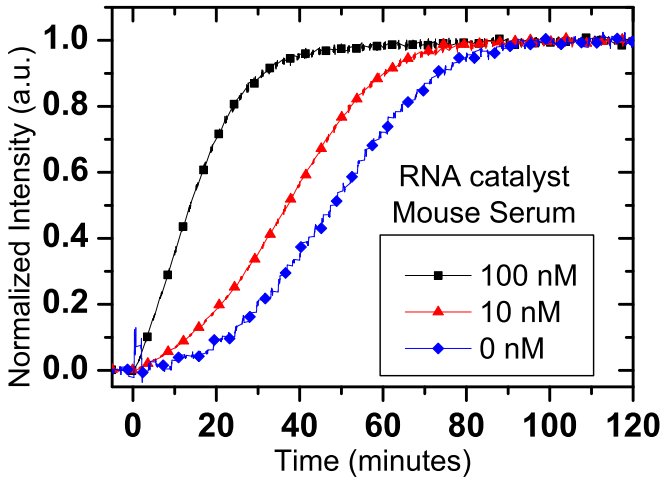

Fig. 3. Autocatalytic network operation in 50% mouse serum, 10% SDS and 0.5×PBS with 100, 10, and 0 nM of RNA catalysts. Network operation in mouse serum was successful with times to half completion comparable to operation in human serum with DNA catalysts.

4 Conclusion

A DNA-based autocatalytic network was successfully operated in 50% human serum, 10% SDS, 0.5×PBS using both DNA and RNA catalysts. Network operation was also confirmed in mouse serum using an RNA catalyst. Operation in serum with 10% SDS was shown to be sufficient to prevent rapid degradation of the network. Times to half completion were similar to those for operation in buffer solution, although the strand concentrations were an order of magnitude higher in the experiments reported here. In all cases, the network exhibited an apparent exponential increase in fluorescence intensity and clear dependence on the catalyst concentration. These results clearly support the feasibility of detecting miRNA in human serum and mouse serum using a DNA-based catalytic network.

Acknowledgments. The authors thank David Y. Zhang, Erik Winfree, Lulu Qian, and William B. Knowlton for valuable assistance with this work. This work was supported by NIH Grant No. P20 RR016454 from the INBRE Program of the National Center for Research Resources, DARPA Contract No. N66001-01-C-80345, and NSF CCF 0855212.

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