Abstract

The recent SARS-CoV-2 pandemic showed how humanity can be threatened by organisms as simple as viruses. This crisis emphasized the need for timely and reliable diagnostics, as well as the fast discovery of effective therapeutics against new diseases. We propose that both challenges can be addressed by deoxyribozymes (catalytically active DNA molecules).

The most common types of signals are light, fluorescence, color and radioactivity. Some of these signals can be generated by deoxyribozymes, but others cannot. A functional DNA molecule that generates a robust fluorogenic or chromogenic signal would have significant advantages for many applications compared to intrinsically fluorescent proteins, which are expensive and labour intensive to synthesize, and fluorescent RNA aptamers, which are more prone to degradation than DNA molecules. To broaden the spectrum of possible outcomes generated by DNA, we have used *in vitro* selection to develop deoxyribozymes, which generate strong fluorogenic and chromogenic signals.

Deoxyribozymes that generate a fluorogenic signal achieve this by dephosphorylating the coumarin substrate 4-methylumbelliferyl phosphate (4-MUP). They transfer the phosphate group from 4-MUP to their own 5' hydroxyl group. This reaction converts the non-fluorogenic 4-MUP into the fluorogenic 4-methylumbelliferone (4-MU). We named the most active deoxyribozyme identified in our studies Aurora. This enhances fluorescence by more than 700-fold and generates a detectable signal in minutes. Deoxyribozymes that produce a yellow color transfer the phosphate group from the colorless substrate 4-nitrophenyl phosphate (4-NPP) to their own 5' hydroxyl group. After dephosphorylation, 4-NPP is converted into yellow 4-nitrophenol (4-NP). We named this deoxyribozyme Apollon. It enhances the chromogenic signal by more than 100-fold.

To improve the catalytic activities of Aurora and Apollon, to identify their minimal catalytic cores, and to solve their secondary structures we used *in vitro* selection in combination with high-throughput sequencing and comparative sequence analysis. Furthermore, to obtain the best possible signal-to-noise ratio, we extensively characterized reaction conditions, including the effects of various mono- and divalent ions, different buffering agents, molecular crowding agents, and pH levels, as well as different substrate and deoxyribozyme concentrations.

Finally, we developed several ways to convert Aurora and Apollon into allosterically regulated sensors. Aurora and Apollon were designed to function as oligonucleotide sensors for specific sequences and also to act as sensors of various types of nucleases in homogeneous assays that did not require washes or purification steps. Notably, an engineered version of Aurora can detect picomolar concentrations of ribonuclease, and was used for rapid identification of novel small-molecule inhibitors of the SARS-CoV-2 ribonuclease Nsp15 in a high-throughput screen. Our results show that deoxyribozymes can generate robust fluorogenic and chromogenic signals and that such deoxyribozymes can be used in applied research to solve real-world challenges.

Key words: deoxyribozyme, *in vitro* selection, sensor, aptazyme, fluorescence, 4-methylumbelliferyl phosphate, colorimetric, chromogenic, 4-nitrophenyl phosphate, homogeneous assay, RNase A, Nsp15, SARS-CoV-2