Label-Free and Thermo-Responsive Detection of ATP by Nanoconformational Switching of Hairpin Aptamers

Tatsuro Goda, Yuji Miyahara

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University
2-3-10 Kanda-Surugadai, Chiyoda, Tokyo 101-0062, Japan
goda.bsr@tmd.ac.jp

Increasingly detailed structural designs are highlighting the utility of oligonucleotide aptamers for diagnostic systems. Aptamers are new types of molecular recognition element that can bind to target molecules with high sensitivity and selectivity. Recently, molecularly engineered oligonucleotide aptamers which is designed to induce nanoscaled intra- or inter- molecular displacement upon target binding have been adapted to a wide variety of diagnostic systems. The primary and secondary structures of aptamers are responsible for exhibiting target-induced conformational transitions and signalling -on/-off switching. We report self-complementary hairpin DNA aptamers that can detect target ATP when the hairpin aptamer transformed from the closed-loop into open-loop conformation by temperature-induced denaturation. The nanoscaled conformational switching of the hairpin aptamer on a gold electrode was investigated by simply monitoring the charge transfer resistance of the anionic redox markers by electrochemical impedance spectroscopy (EIS). The EIS can monitor changes in the charge transfer resistance of redox markers at the electrode/solution interface using an electrically equivalent circuit model. We reason that the anti-ATP aptamers, whose sequence is liberated by denaturing the stem duplex during incubation with target ATP above $T_m$, can induce an intramolecular displacement from the close-loop hairpin into ATP/aptamer complex. The consequence of this transition from the compact hairpin to the tertiary ATP/aptamer composite impedes redox reaction of the anionic ferricyanide/ferrocyanide couple at the interface (Figure 1). Whereas, the anti-ATP aptamer cannot interact with non-target GTP above $T_m$ and the open-loop (linear) structure return to the original hairpin duplex after annealing at room temperature so that the impedance does not change. When the hairpin aptamers are incubated below $T_m$, the aptamer region is located in the close-loop hairpin and fails to capture target ATP. Consequently, the aptamer conformation remains unchanged. In conclusion, the hairpin aptamer as a thermo-responsive ligand has much generality for its use as "smart" nano-switches.

Figure 1. Normalized changes in the charge transfer resistance ($\Delta R_{ct}/R_{ct}$) of the redox markers measured by EIS as a function of temperature during incubation with 100 nM ATP or GTP. The gold electrode was functionalized with the hairpin DNA aptamer.