Development of rapid aptasensor using graphene oxide and 1,1'-oxalyldiimidazole chemiluminescence detection

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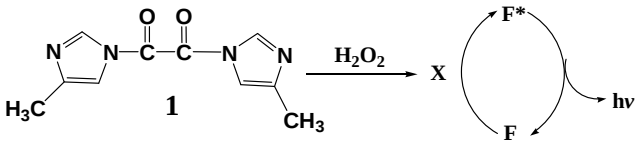
ABSTRACT

Highly selective and sensitive aptasensor capable of rapidly quantifying Vibrio parahaemolyticus in the presence of Vibio cholera and Vibrio vulnificus was developed using an specific DNA aptamer, graphene oxide and 1,1'-oxalyldiimidazole chemiluminescence (ODI CL) detection. A sample containing three different Vibrios was mixed with the aptamer conjugated with TEX615 in TE buffer (pH7.5). Then, complexes of Vibrio parahaemolyticus and aptamer were formed in the mixture incubated for 5 minutes. After the incubation, graphene oxides in PBS (10 mM, pH7.4) were added in the mixture to remove aptamers unbound with Vibrio parahaemolyticus within 1 minute. Trace levels of Vibrio parahaemolyticus in the mixture were rapidly quantified when ODI CL reagents (e.g., ODI, H_2O_2) were inserted. The limit of detection (LOD = average background + 3 × standard deviation) of aptasensor having excellent accuracy, precision, and recovery was 2400 cells/ml. Based on the results, it is expected that highly selective and sensitive aptasensors capable of rapidly quantifying and monitoring food-borne pathogens can be developed using the combination of graphene oxide and ODI CL detection

Keywords: aptasensor, graphene oxide, chemiluminescence, food-borne pathogens

US FDA, USDA, and CDC are looking for appropriate analytical systems capable of rapidly and simply monitoring foodborne pathogens (e.g., salmonella, e-coli, listeria, vibrio) for public health. We developed an accurate, precise and rapid aptasensor for the quantify and monitor vibrio parahaemolyticus using a specific aptamer conjugated with TEX 615, graphene oxide, and 1,1'-oxalyldiimidazole chemiluminescence (ODI-CL) detection as shown in Scheme 1 (1-5).

Fig. 1 shows the principle of aptasensor with ODI-CL detection using graphene oxide and aptamer conjugated with TEX615 (5'-TEX615-ATA GGA GTC ACG ACG ACC AGA ATC TAA AAA TGG GCA AAG AAA CAG TGA CTC GTT GAG ATA CTT ATG TGC GTC TAC CTC TTG ACT AAT-3). Aptamers rapidly bound with vibrios emit stong light when ODI and H_2O_2 are added in the solution. However, aptamer immobilized on the surface



Scheme 1. ODI-CL reaction. **1**: ODI, **X**: high-energy intermediate formed from ODI-CL reaction, **F**: fluorescent dye under the ground state, **F***: fluorescent dye under the excited state.

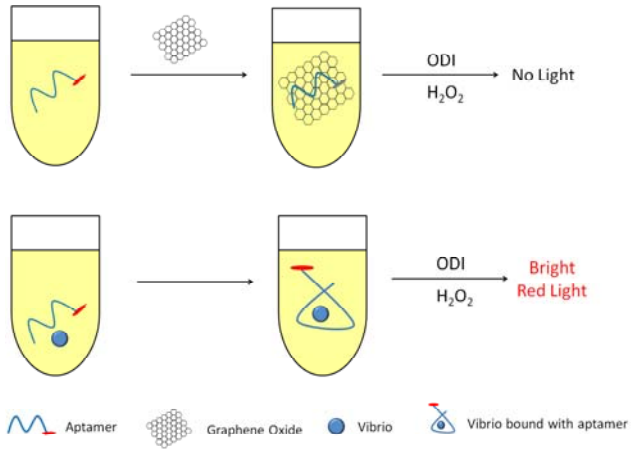


Fig. 1 Principle of aptasensor using graphene oxide and ODI-CL detection.

of graphene oxide, based on the π-π stacking interaction between two materials, don't emit light because of chemiluminescent resonance energy transfer fromTEX615 conjugated with aptamer to graphene oxide. The stacking interaction between graphene oxide and aptamer was dependent on the concentation of NaCl. As shown in Fig. 2, aptamer strongly bind with graphene oxide and size of graphene oxide was bigger in the presence of high concentration of NaCl. Thus, we have used TE buffer (pH7.5) containing 0.01 M NaCl.

We studied the effect of dye conjugated with aptamer. The CL emission of TEX615 was much brighter than other dyes (HEX, 6-FAM) that we used in this study. Thus, we selected TEX615 as a dye conjugated with aptamer for the quantification of Vibrio.

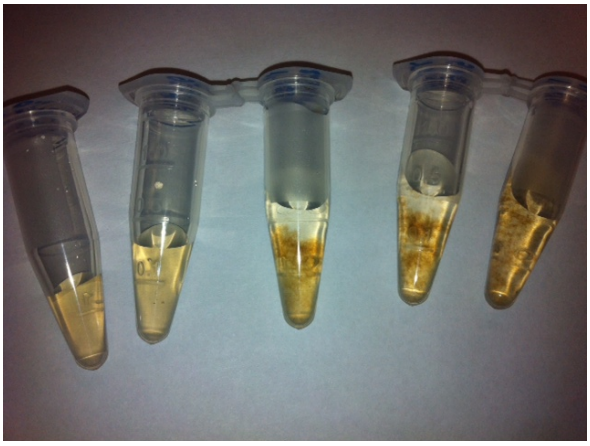


Fig. 2 Effect of NaCl concentraion to immobilized aptamer on the surface of graphene oxide.

Vibrio rapidly binds with aptamer within 3 minutes in TE (pH 7.5). In addition, aptamer bound with vibrio emit strong light in TE (ph 7.5). With the increase of pH of TE, relative CL intensity of aptamer bound with vibrio was decreased.

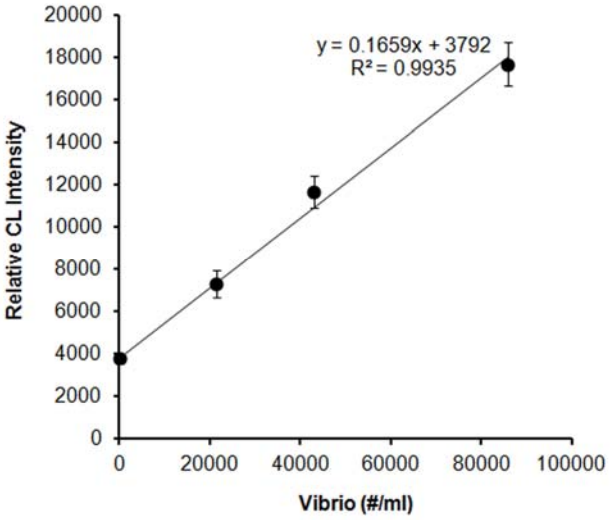


Fig. 3 Calibration curve for the rapid quantification of vibrio.

As shown in Fig. 3, aptasensor we developed in this research was able to quantify low number of vibrios within 3 minutes. The limit of detection (LOD = backgropund + 2 standard deviation, n =10) and the limit of quntifcation of vibrio using the aptasensor were 1,818 and 6,060 vibrios in 1 ml sample. The reproduciblity (recovery: 91 ~ 108 %) of aptasensor was good within the acceptable error range.

We confirmed in this research that aptasensor with ODI-CL detection can quantify trace levels of vibrio in a sample. The result indicates that it is possible to develop rapid and simple aptasensors capable of accuratly and precisely quantifying and monitoring various foodborne pathogens in food.

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