Background free biosensor using the combination of graphene oxide and Fe_3O_4 nanoparticles

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ABSTRACT

Cost-effective and rapid biosensor with background free chemiluminescence (CL) detection was developed using ssDNA aptamer-conjugated 6-FAM fluorescein (DAF), graphene oxide bound with magnetic nanoparticle (GMN), and 3,4,5-trimethoxyphenylglyoxal (TMPG). When thrombin was incubated in TE buffer (pH 7.5) containing 200 nM DAF for 10 minutes, thrombin binds strongly to DAF. Free DMF, which does not bind with thrombin in the solution, binds with GMN through the π-π interaction between the DAF and GMN. Thus, we were able to remove DMF-bound GMN in the solution using a magnetic bar. When thrombin-bound DMF was mixed with TMPG (0.1 M) in the presence of tetrapropylammonium hydroxide (0.01 M), strong CL was observed based on the chemiluminescence resonance energy transfer from high-energy intermediate formed from the reaction of TMPG and guanine, the main base of DAF, to 6-FAM. The background-free biosensor having excellent accuracy and precision was more sensitive than conventional and time-consuming analytical methods.

Keywords: 3,4,5-trimethoxyphenylglyoxal (TMPG), chemiluminescence, Aptamer, Magnetic, Graphene oxide

1 PAPER LAYOUT

Background free aptasensor was developed for the rapid quantification of thrombin applied to diagnose and monitor various diseases using aptamer (5'- GGG GGGGTTGGTGTGGTTGG-3'), capable of specifically binding thrombin, and magnetic graphene oxide we developed.

0.01 M $FeCl_2$ and 0.01 M $FeCl_3$ (1:4 volume ratio, 5 ml) were mixed in a 20-ml glass vial. Then, 1mg/ ml graphene oxide (4 ml) was added in the vial. The vial was in a preheated (85 °C). Then, ammonium hydroxide (200 µl) was added in the vial. Finally, chemicals in the vial were reacted to produce magnetic graphene oxide, as shown in Fig. 1, for 50 minutes at 85 °C. The stock was stored in a refrigerator.

Fig. 2 shows that the principle of aptasensor capable of sensing trhombin using 3,4,5-trimethoxyphenylglyoxal

Fig. 1. Magnetic graphene oxide

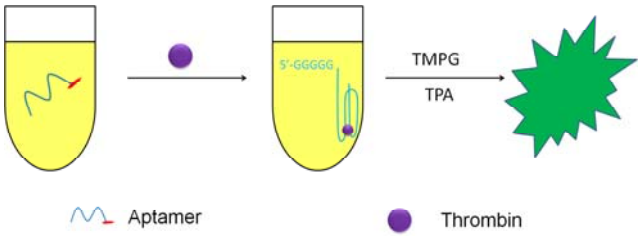

Fig. 2. Aptasensor with TMPG chemiluminescence detection capable of sensing thrombin.

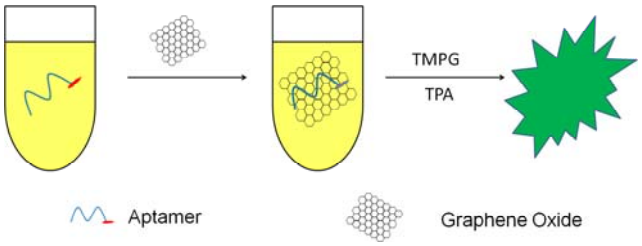

Fig. 3. CL emission of aptamer immobilized on the surface of graphene oxide in TMPG chemiluminescence system.

Tris-HCl, (TMPG) chemiluminescence(*1-3*) detection. Aptamer bound with trombin emitted light when TMPG

(0.02 M in DMF) and tetrapropylammonium hydroxide (0.02 M in TPA) were added in the tube. Unfortunately, aptamer immobilized on the surface of graphene oxide also emitted light when TMPG and TPA were added as shown in Fig. 3. This result indicates that it is difficult to develop rapid and simple aptasensor with TMPG chemiluminescence detection using conventional graphene oxide

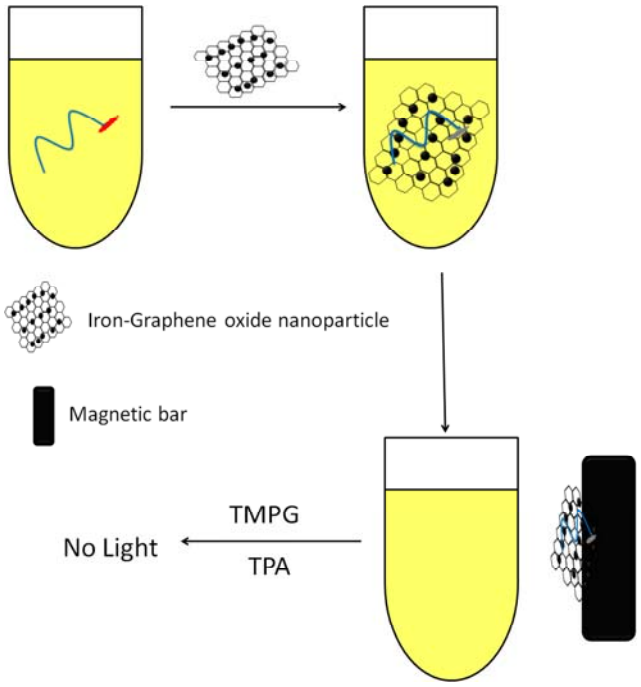

Fig. 4. Removal of aptamer immobilized on the surface of magnetic graphene oxide using a magnetic bar.

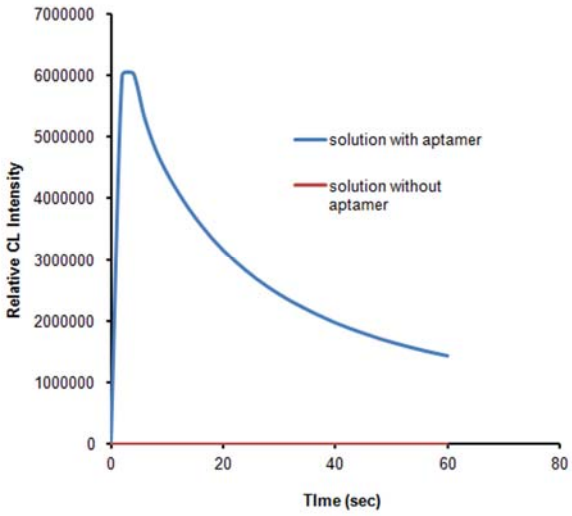

Fig. 5. CL emissions in the absence and presence of aptamer conjugated with 6-FAM in TMPG chemiluminescence reaction.

In order to solve the problem, we have used magnetic grphene oxide, instead of conventional graphene oxide, for removing aptamer not binding with thrombin. As shown in Fig. 4, aptamer bound with magnetic graphene oxide was removed using the magnetic bar. Also, no CL emission was measured when TMPG and TPA were added in the tube.

As shown in Fig. 5, aptamer conjugated with 6-FAM emits strong light when TMPG and TPA were added. However, no CL emission was measured with the addition of TMPG and TPA after removing aptamers using magnetic graphene oxide. Based on the results shown in Fig. 5, it is possible to develop background free aptasensor capable of trace levels of thrombin in human serum. We will show the results in the TechConnect World 2013 - Nanotech, Microtech, Biotech, Cleantech Joint 2013 Conferences in Washington DC, USA.

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