Different contribution of carbon nanotubes and graphene oxide in single nucleotide polymorphism for the diagnosis of human mad-cow disease

Wonsoulk Choi[*] and J. H. Lee[**]

[*]Langley High School, McLean, VA 22101
[**]Luminescent MD, LLC, Hagerstown, MD 21742

ABSTRACT

Rapid single nucleotide polymorphism (SNP) with 1,1'-oxalylimidazole chemiluminescence (ODI-CL) detection was developed for the early diagnosis of human mad-cow disease. Mutated single strand DNA (ssDNA) sequence discovered from patients having human mad-cow disease rapidly hybridize with complementary probe conjugated with chemiluminescent dye. The hybridization between target ssDNA and complementary probe was dependent on four different variables such as pH, temperature, incubation time, and the type of nanoparticles (e.g., multi-walled carbon nanotubes, graphene oxide) capable of capturing mismatched ssDNAs and excess complementary probe. However, three different types of mismatched ssDNAs also slowly hybridize with complementary probe with the increase of incubation time. The problem was solved by using ODI-CL detection instead of fluorescence detection of conventional SNP because ODI-CL is about 100 times more sensitive than fluorescence. In other words, it was possible to reduce the interaction between mismatched ssDNAs and complementary probe with short incubation time (15 minutes) using highly sensitive ODI-CL detection. Based on the results, in conclusion, it is expected that SNP with ODI-CL can be applied as a cost-effective, rapid, and simple analytical method to diagnose and prognose various diseases.

Keywords: SNP, graphene oxide, carbon nanotubes, hybridization, chemiluminescence

Mad cow disease commonly known as bovine spongiform encephalopathy is a fatal disease. The symptom of the disease, called variant Creuzfeldt-Jakob Disease (CJD), discovered in people who ate tainted beef containing malfunctioned prion proteins is similar to that of mad cow disease.([1-2]) It is well-known that DNA sequence of prion protein collected from patient having CJD is different from that obtained from normal people because DNAs of prion protein of patient are mutated.([2])

In general, hybridization method using complemantary probe-conjugated fluorescent dye capable of binding mutated single strand DNAs and fluorescence detection is used to sense trace levels of mutated DNAs in normal DNAs.([3-4]) The background of fluorescence generated with light source operated using high voltage power supply is too high to detect low concentration of double strand DNA-conjugated fluorescent dye produced from hybridization between target DNAs (e.g., mutated DNAs) and complementary probe-conjugated fluorescent dye even though fluorescence detection is more sensitive than absorbance detection. In order to enhance the sensitivity of fluorescence detection applied to hybridization method, various nanoparticles such as carbon nanotubes, gold nanoparticles, graphene oxide, silver nanoparticles capable of binding remaining complementary probes after the hybridization of target DNA and complementary probe were used.([5-6])

It is well-known that peroxyoxalate chemiluminescence (PO-CL) detection capable of also quantifying trace levels of fluorescent dyes is more sensitive than fluorescence detection because the background of former, operated without light source and high-voltage power supply, is much lower than that of the latter.([7-9]) In particular, 1,1'-oxalyldiimidazole chemiluminescence (ODI-CL), one of PO-CLs, is at least 10 times more sensitive than conventional PO-CLs such as Bis (2,4-dinitrophenyl) oxalate chemiluminescence (DNPO-CL), Bis (2,4,6-trichlorophenyl) oxalate chemiluminescence (TCPO-CL).([10]) In general, conventional PO-CL is at least 10 times more sensitive than fluorescence detection. Thus, ODI-CL is at least 100 times more sensitive than fluorescence.
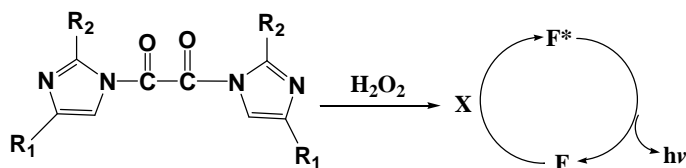
Scheme 1. ODI-CL reaction. R_1 and R_2: H, CH_3, or CH_3CH_2, F: fluorescent dye in ground state, F*: fluorescent dye in excited state.

Using the advantage of ODI-CL detection (see Scheme 1) and nanoparticles, it is possible to sense trace levels of double strand DNAs-conjugated fluorescent dye formed from short hybridization between mutated DNA of prion protein and complementary probe-conjugated fluorescent dye. Thus, I developed, for the first time, a highly sensitive biosensor to accurately and rapidly diagnose and prognose human mad cow diseases such as CJD.

Experimental

Chemicals and materials

Target (mutated) DNA determined based DNA sequence of prion protein for CJD patient shown in Fig. 1(*2*), complementary probe-conjugated TEX 615 of target DNA, four different mismatched DNAs (e.g., MM 0, MM 1, MM 2, MM3) and complementary probes of MM 1, MM2, and MM3 were purchased from Integrated DNA Technologies (see below).

Target DNA: 5'- GTG GTG AAC TGC ATC -3'
Complementary probe of target DNA: 5'- /5TEX615/GAT GCA GTT CAC CAC -3'

Mismatched DNAs
      MM 0: 5'-TTTTTTTTTTTTTTTT-3'
      MM 1: 5'- GTG GTG GAC TGC GTC -3'
      MM 2: 5'- GTG GTG AAC TGC GTC -3'
      MM 3: 5'- GTG GTG GAC TGC ATC -3'

Complementary probe (CP) of mismatched DNAs
      CP 1 of MM 1: 5'- GACGCAGTCCACCAC-3'
      CP 2 of MM 2: 5'- GACGCAGTTCACCAC-3'
      CP 3 of MM 3: 5'- GATGCAGTCCACCAC-3'

Highly concentrated graphene oxide (500 ~ 700 nm, 5.5 mg/ml) dispersed in water was purchased from Graphne Supermarket. Three different sizes of multiwalled carbon-nanotube powders (e.g., < 8 nm, 20 ~ 30 nm, > 50 nm) were purchased from Nanomaterial store. Gold (14 nm, 1000 ppm) and silver (15 nm, 1000 ppm) dispersed in water were purchased from US Research nanomaterial, Inc. Bis-2,4,6-trichloriphenyl oxalate (TCPO) and 4-methylimidazole were purchased from TCI, America. 30 % H_2O_2 was purchased from VWR. DNA free deionized water, HPLC grade ethyl acetate, and isopropyl alcohol were purchased from EMD. PBS and Tris-HCL buffers were purchased from Polysciences, Inc.

Preparations of sample and working solutions

Working solutions of target DNA, complementary probe of target DNA, mismatched DNAs, and complemantary probes of mismatched DNAs were preprared using 10 mM Tris-HCl buffer solution (pH 7.5). ODI was dairly prepared from the reaction between TCPO (5 µM) and 4-methylimidazole (20 µM) in ethylacetate. 0.5 M H_2O_2 was prepared in isopropyl alcohol. 1 ppm gold and silver nanoparticle were prepared in 10 mM Tris-HCl buffer solution (pH 7.5). graphene oxide (55 µg/ml) and carbon nanotubes (40 µg/ml) were prepared in Tris-HCl buffer solution (pH 7.5).

Procedure

The mixure of target DNAs or mismatched DNAs (50 µl) and complementary probe-conjugated TEX 615 of target DNAs with/without complementary probes of mismatched DNAs (50 µl) were incubated for a certain time (15 ~ 90 minutes) in a 1.5-ml centrifuge tube in a shaker (500 rpm) at 37 ℃. After the incubation, one (100 µl) of nanoparticles used in this research was added in the centrifuge tube. Then, the mixture was incubated for a certain time (2 ~ 60 minutes) in a shaker (500 rpm) at 37 ℃. The mixture (10 µl) transferred into a glass text tube (12 ×70 mm) was inserted into a luminometer. H2O2 (25 µl) was injected through a syringe pump. Finally, CL emitted from the test tube was inimmediately measured for 1 second with the addition of ODI (25µl) through another syringe pump.

Results and Discussion


(a)

(b)
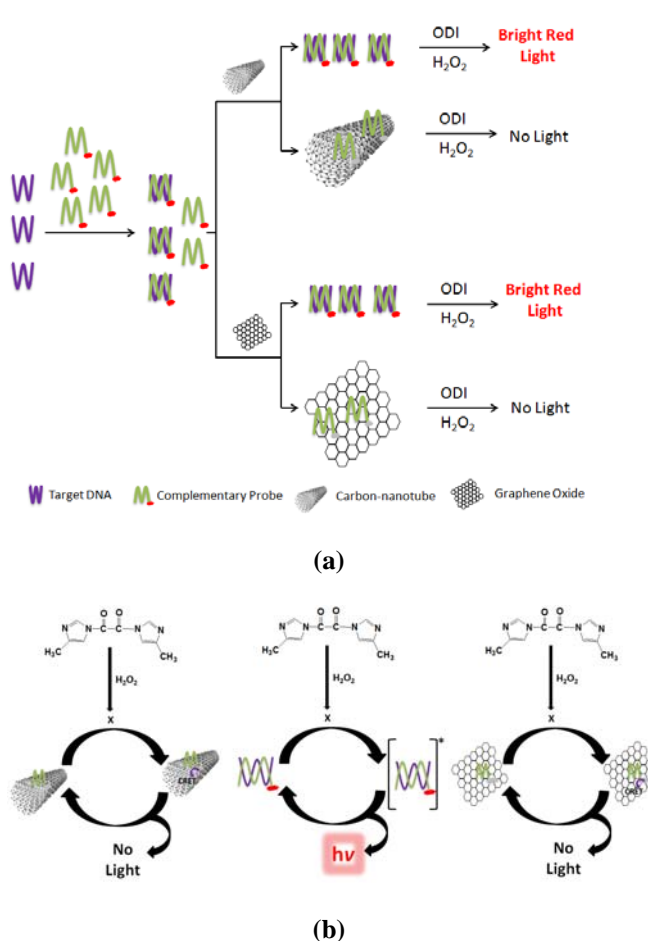
Fig. 1 Principle of hybridization sensor using graphene oxide and ODI-CL detection

Design of biosensor with ODI-CL detection using carbon-nanotube or graphene oxide

Biosensors using the combination of ODI-CL detection and nanoparticles (e.g., carbon-nanotubes, graphene oxide) were designed as shown in Fig. 1 (a). First, target DNA hybridize with complementary probe-conjugated TEX 615. Second, remaining complementary probe after the interaction between target DNAs and complementary probes bind with graphene oxide or carbon-nanotubes. Third, ODI-CL reagents (e.g., ODI, H_2O_2) were mixed with double strand DNA-conjugated TEX 615 and complementary probes immobilized on the surface of graphene oxide or carbon-nanotubes produced from the previous procedures. Finally, double strand DNA-conjugated TEX 615 emit bright light, whereas complementary probe bound with graphene oxide or carbon-nanotubes can't emit light due to the rapid chemiluminescent resonance energy transfer between excited TEX 615 conjugated with complementary probe and the surface of graphene oxide or carbon-nanotubes as shown in Fig. 1(b).

Based on the principle of biosensor shown in Fig. 1, additional experiments were conducted to develop highly selective and rapid biosensor capable of diagnosing and monitoring mad cow disease of human.
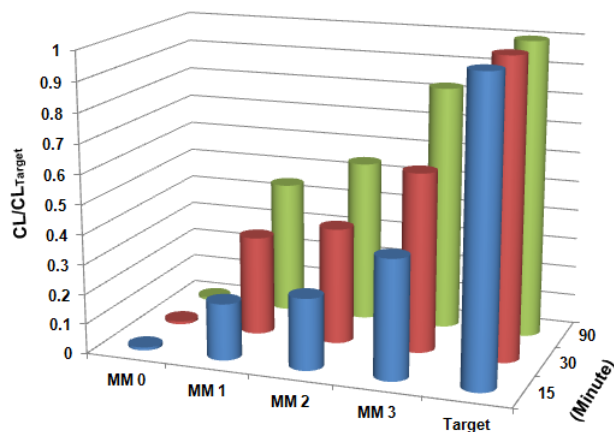
Effects of incubation time and pH for DNA hybridization in the presence of graphene oxide
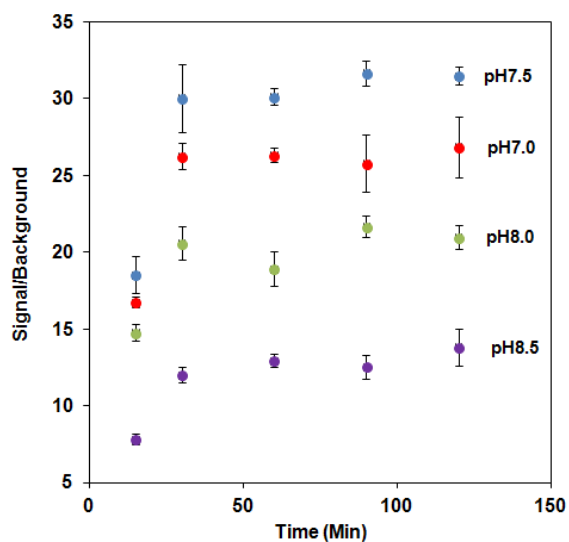
Fig. 2 pH effect of DNA hybridization sensor with ODI-CL detection.

The results shown in Fig. 2 indicate that the hybridization of target DNA and complementary probe is dependent on incubation time and pH of tris-HCl buffer solution. Fig. 6 shows that the appropriate incubation time necessary for the hybridization of target DNA and complementary probe is

30 minutes because the signal/background ratio determined after a 30-minute incubation was the same as those calculated after more than 30 minutes of incubation within the acceptable error range ($\pm$ 5 %). The signal/background ratio at pH 7.5 is higher than those at the rest pHs (e.g., 7, 8, 8.5). Also, the high signal/background ratio determined after a 15-minute incubation at pH 7.5 indicates that the hybridization of target DNA and complementary probe can be rapidly quantified.


Fig. 3 Effect of incubation time for the quantification of target using DNA hybridization sensor with ODI-CL detection

The results shown in Fig. 3 indicate that the hybridization of target DNA and complementary probe is faster than those of mismatched DNAs and complementary probe. Thus, CL/CL_{target} for the hybridization of target DNA and complementary probe with a 15-minute incubation is much higher than those for the hybridizations of mismatched DNAs and complementary probe after the 15-minute incubation. As shown in Fig. 2, the hybridization of target DNA and complementary probe was completed with 30 minutes of incubation. Thus, the hybridization of target DNA and complementary probe with a 30-minute incubation was the same as that with a 90-minute incubation, while CL/CL_{target} ratios for the hybridization of mismatched DNAs and complementary probe enhanced continually with the increase of incubation time. As shown in Fig. 3, the hybridization between completed mismatched DNA (MM 0) and complementary probe did not occur.

Development of rapid biosensor capable of selectively sensing target DNAs

Based on the results, rapid biosensor was developed to selectively sense and quantify target DNAs using the complementary probe-conjugated TEX 615 for target DNA and the complementary probes, which is not conjugated TEX 615, for mismatched DNAs. As shown in Fig. 4, complementary probe-conjugated TEX615 capable of
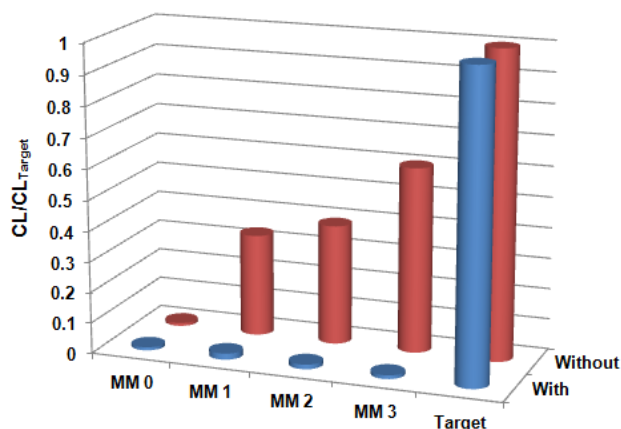
Fig. 4 Accuracies of DNA hybridization sensor with ODI-CL detection in the absence and presence of complementary probe of mismatched ssDNA.

completely hybridizing with target DNA hybridize with mismatched DNAs (e.g., MM 1, MM 2, MM 3) in the absence of complementary probes of mismatched DNAs. When mismatched DNAs were mixed with complementary probes of mismatched DNAs and complementary probe-conjugated TEX 615 of target DNA, however, negligible CL emission like background was observed. This is because the mismatched DNA rapidly interacts with complementary probe, which is not conjugated with fluorescent dye, of the mismatched DNA instead of the complementary probe-conjugated TEX 615 for target DNA.

Conclusions

The results observed in this research indicate that biosensor developed with the combination of graphene oxide and ODI-CL detection can rapidly diagnose and prognose human mad cow disease with high selectivity and sensitivity. This is because (1) graphene oxides can rapidly bind with remaining complementary probe-conjugated TEX 615 after the interaction between target DNAs and complementary probe-conjugated TEX 615, (2) complementary probe-conjugated TEX 615 bound with graphene oxide can't emit light due to the CRET principle when ODI CL reagent are added in the solution, (3) target DNA can selectively bind with complementary probe-conjugated TEX 615 while mismatched DNAs interact with complementary probes of mismatched DNAs in the solution, and (4) ODI-CL detection, which is at least 100 times more sensitive than fluorescence detection, can quantify trace levels of fluorescent dye like TEX 615.

In conclusion, research results reported in this paper indicate that the biosensor capable of selectively and rapidly sensing mutated (target) DNAs without additional procedures (e.g., time-consuming sample separation, washing) can be applied to various research fields such as biochemistry, clinical chemistry, food safety, homeland security, molecular biology, and toxicology.

REFERENCES

[1] Mastrianni, J. A. "The genetics of prion diseases", *Genet. Med. 12*, 187-195, 2010.

[2] Jeong, B. H., Ju, W. K., Lee, E. A., Choi, I. S., Im, J. H., Choi, E. K., and Kim, Y. S. Molecular analysis of prion protein gene (PRNP) in Korean patients with Creutzfeldt-Jakob disease., *J Korean Med Sci. 13*, 234-240, 1989.

[3] Schutz, E., Scharfenstein, M., and Brenig, B. Genotyping of ovine prion protein gene (PRNP) variants by PCR with melting curve analysis, *Clin. Chem. 52*, 1426-1429, 2006.

[4] Gurjar, A. A., Hegde, N. V., Love, B. C., and Jayarao, B. M. Real-time multiplex PCR assay for rapid detection and toxintyping of Clostridium perfringens toxin producing strains in feces of dairy cattle, *Mol. Cell. Probe. 22*, 90-95, 2008.

[5] Sheng, L. F., T.Ren, J., Miao, Y. Q., Wang, J. H., and Wang, E. K. PVP-coated graphene oxide for selective determination of ochratoxin A via quenching fluorescence of free aptamer, *Biosens. Bioelectron. 26*, 3494-3499, 2011.

[6] Guo, Z. J., Ren, J. T., Wang, J. H., and Wang, E. K. Single-walled carbon nanotubes based quenching of free FAM-aptamer for selective determination of ochratoxin A, *Talanta 85*, 2517-2521, 2011.

[7] Motoyoshiya, J., and Maruyama, T. A CIEEL switching controlled by metal ion-ligand interaction in peroxyoxalate chemiluminescence, *Luminescence 25*, 181-182, 2010.

[8] Cistato, L. F. M., Bartoloni, F. H., Bastos, E. L., and Baader, W. J. Direct kinetic observation of the chemiexcitation step in peroxyoxalate chemiluminescence, *J. Org. Chem. 74*, 8974-8979, 2009.

[9] Park, P. S., Rho, T. H. D., Kim, Y. T., Ko, S. O., Schlautman, M. A., Carraway, E. R., and Lee, J. H. Evidence of multiple pathways capable of emitting peroxyoxalate chemiluminescence using a charge coupled device spectrometer, *Chem. Commun. 47*, 5542–5544, 2011.

[10] Lee, J. H., Je, J. T., Schlautman, M. A., and Carraway, E. R. Fast peroxyoxalate chemiluminescence for minimized analytical separation systems, *Chem. Commun.*, 270-271, 2003.