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Rapid Communications

Aptamer-Mediated Chemiluminescence Detection of Prion Protein on a Membrane Using Trimethoxyphenylglyoxal

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Effective recognition and quantitative analysis of the prion protein are important in drug discovery and diagnosis for prion diseases, such as bovine spongiform encephalopathy and Creutzfeldt–Jakob diseases. We have developed a high-throughput method for a specific and sensitive determination of prion protein on a solid-phase membrane, based on a chemiluminescence reaction of aptamer with 3,4,5-trimethoxyphenylglyoxal. This method using aptamer is facile, inexpensive and convenient for the detection of the prion protein on a membrane, indicating a lower detection limit of *ca.* 4.2 pmol spot^{-1}.

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Prion diseases are a group of fatal neurodegenerative diseases in animals and humans. They are caused by an unusual transmissible agent that has been termed prion,[1] and may occur as acquired, idiopathic, or hereditary diseases.[2] There is a high demand for convenient methodologies for detecting and measuring the levels of specific proteins in biological samples. A binding study of aptamers with their target is a new strategy in molecular-recognition chemistry.[3] Efforts have been made in the screening of aptamers for prion protein (PrP), and there are a few research groups reporting on the selection of DNA aptamers against PrP.[4] Chemiluminescence (CL) detection of PrP is attractive because general CL detection is highly sensitive with a simple instrumentation that can detect CL-light imaging. CL-imaging detection on a solid-phase membrane or a protein chip has several advantages for selective, sensitive and high-throughput analyses of many samples in a single experiment.

However, a CL-imaging method using aptamer for the quantification of PrP on a solid-phase membrane as well as a protein chip has not been reported. Previously, we developed a chemiluminogenic reagent, 3,4,5-trimethoxyphenylglyoxal (TMPG), for the selective detection of nucleic acids.[5,6] The CL reaction with TMPG is rapid, sensitive and specific, and occurs at room temperature. Recently, we reported on an aptamer-mediated CL-TMPG detection of adenosine using cDNA-modified magnetic beads to capture the aptamer.[7] In the presence of adenosine, the aptamer preferred to form an adenosine-aptamer complex, resulting in an inhibition of forming the aptamer-cDNA complex. Then, the amount of adenosine was measured due to the decrement of the CL intensity on the beads. However, this method was difficult to be applied to high-throughput analyses of a target protein. Therefore, we developed a high-throughput method for the detection of PrP on an inexpensive membrane.

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In this study, mouse recombinant PrP (mrPrP) was expressed in *E. coli*, purified by an amylose resin column, confirmed by SDS-PAGE and immunochemical detection, and three DNA aptamers[4] against PrP were screened for CL detection; then, an aptamer (5′-TTTGGTCCTTGTCTTATGTCCAGAATGCTGG-CGCGCATTTCTCCTACTGGGATAGGTGGATTAT-3′) was finally employed due to its higher specificity to mrPrP. We first investigated the detection conditions, such as solid-phase membranes for adsorbing PrP, binding media for aptamer, aptamer concentrations, temperature and time for incubation, and washing solvents.

Figure 1 shows a comparison of PrP detection between the proposed method with TMPG and an immunochemical method using commercialy available antibodies. The proposed method with TMPG could detect only mrPrP among several other proteins, such as maltose-binding protein (MBP), bovine serum albumin (BSA), casein, catalase, and hemoglobin on a polyvinylidene difluoride (PVDF) membrane. Although the membrane adsorbed various proteins,[8] the aptamer could bind to only the mrPrP on the membrane, and it was directly detected by the TMPG reaction. These results indicate that the proposed method was more facile and specific for PrP than the conventional immunochemical method using two antibodies, although the sensitivity was approximately one fifth lower than that of immunochemical detection. It would be caused by the lower binding force of aptamer.

Figure 2 represents the CL-signal imaging obtained from the aptamer bound to different amounts of mrPrP, and shows a linear relationship between the CL signals and the amounts of mrPrP. We successfully calibrated the mrPrP amount ($y = 1.4607x + 6.161$; y and x indicate the CL intensity and the amount of mrPrP on membrane, respectively) and its detection limit at 3 of the signal per noise ratio (S/N) was 4.2 pmol spot^{-1}, showing a 4.1 – 16.2% relative standard deviation (RSD) for the signals on each spot ($n = 3$). We also found that the dissociation constant (K_d) between mrPrP and the aptamer was about 1.7×10^{-8} M.

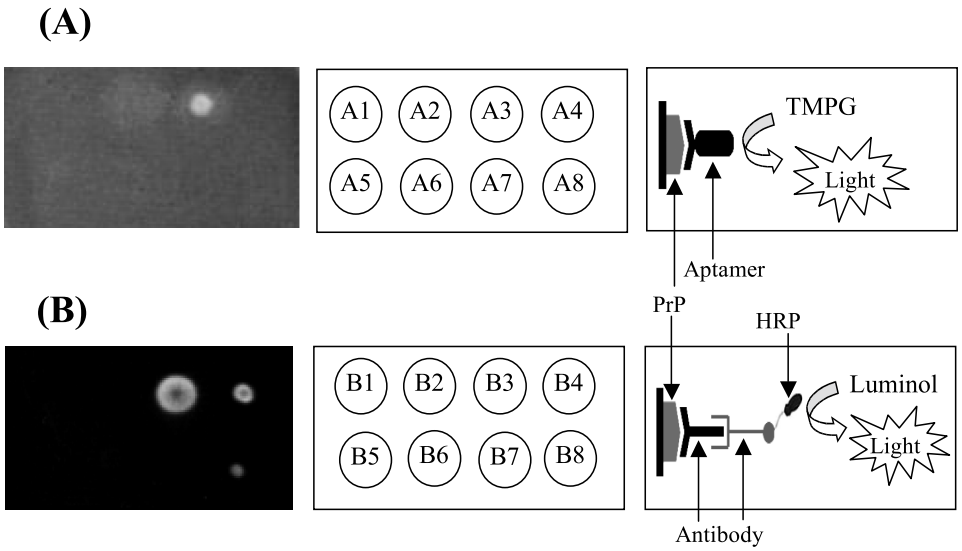

Fig. 1 Specificity of (A) aptamer-based TMPG-CL detection and comparison with (B) immunochemical detection. The right panels indicate schematic principals for detection. The center panels indicate the position of blotted proteins. Dot for (A): A1 = H_2O, A2 = 6 M guanidine hydrochloride as a dissolving solvent, A3 = mrPrP (200 pmol spot^{-1}), A4 = MBP (200 pmol spot^{-1}), A5 = BSA (200 pmol spot^{-1}), A6 = casein (200 pmol spot^{-1}), A7 = catalase (200 pmol spot^{-1}), A8 = hemoglobin (200 pmol spot^{-1}). Dot for (B): B1 = H_2O, B2 = 6 M guanidine hydrochloride, B3 = mrPrP (200 pmol spot^{-1}), B4 = horse radish peroxidase (HRP)-conjugating anti-IgG secondary antibody (1.5 pmol), B5 = BSA (200 pmol spot^{-1}), B6 = casein (200 pmol spot^{-1}), B7 = MBP (200 pmol spot^{-1}), B8 = primary antibody (0.25 pmol). Procedure for (A): proteins were blotted on a PVDF membrane. The membrane was washed with water to remove guanidine hydrochloride and blocked with 5% skimmed milk in a PBS buffer. The membrane was then immersed into 3 mL of 20 mM Tris–HCl buffer (pH 6.5) containing 166 pmol mL^{-1} 5′-FITC-labeled aptamer and kept at 25°C for 1.0 h. After a binding reaction, the membrane was washed each two times with 20 mM Tris–HCl buffer (pH 6.5) and water. The membrane was moistened with 0.1 M tetrapropylammonium hydroxide (pH 7.5) containing 50% methanol for 10 s, and then quickly immersed in a 30 mM TMPG solution dissolved in *N*,*N*-dimethylformamide and tetrahydrofuran (7:3 ratio). Procedure for (B): proteins were blotted onto a nitrocellulose membrane. The membrane was blocked with 50 mg mL^{-1} skimmed milk in a PBS buffer. The membrane was then incubated with a primary anti-mouse PrP monoclonal antibody (5B2), which was diluted to 1/400 with the Tris–HCl buffer for 1.0 h with shaking at 25°C. After three-times washing with the Tris–HCl buffer containing 0.05% Triton X-100, the membrane was incubated with HRP-conjugating anti-IgG secondary antibody (diluted 1/1000) in the Tris–HCl buffer at 25°C for 1.0 h. After incubation, the membrane was washed three times with the Tris–HCl buffer containing 0.05% Triton X-100, and one time with water. Then, LumiGlo reagents containing luminol were added to the membrane. The chemiluminescence image was detected by Lumino CCD AE-6930 densitograph, and processed using Densitometer Analyst Ver. 4.0 Software.

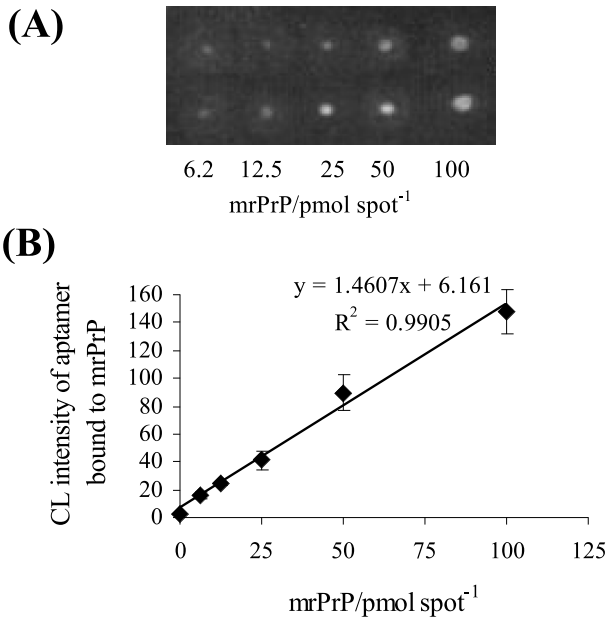

Fig. 2 (A) CL signals of mrPrP (6.2, 12.5, 25, 50, and 100 pmol spot^{-1}) in 6 M guanidine hydrochloride detected by the aptamer-based TMPG-CL method and (B) its calibration curve.

The proposed method was facile, specific and inexpensive, and it has a great possibility to simultaneously determine many samples on a membrane. The reproducible results suggest that this method has a great potential to be applied to the determination of other proteins whose aptamer had been created. The PrP in brain tissue was detected by a dot-blot or western-blot immunoassay after homogenization of the brain tissue in a sucrose solution.[9] Thus, the proposed method using the aptamer instead of an antibody could be applied to the specific detection of PrP in brain tissue. However, the discrimination between normal and abnormal PrP is very important in the diagnosis of prion diseases. Further studies involving the preparation of a specific aptamer for abnormal PrP are now in progress.

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