2011 International Conference on Environmental Science and Engineering
(ICESE 2011)

Aptamer-based assay for prion diseases diagnostic

Saijin Xiao, Yongzhong Ou-Yang, Xinglei Zhang, Xue Li, Shuiping Yang and Huanwen Chen

Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, College of Chemistry Biology and Materials Science
East China Institute of Technology, Nanchang, Jiangxi Province, China
Xiaosj66@126.com

Abstract

Prion diseases is a group of fatal neurodegenerative diseases which cause serious damages in human and animals. Prion diseases-associated isoform (PrPSc), the only marker of these diseases, is abundant in brain only at the late stage which makes the disease diagnostic at the presymptomatic stage difficult. Aptamers owing to their intrinsic advantages such as excellent stability, easy manipulation and reproducible, nontoxic and diagnostic potential, have been applied in protein detection, molecular recognition, cancer diagnostic and cell imaging. Recent years, various aptamers that bind to prion protein (PrP) with high affinity and selectivity were selected by different groups and the application of aptamers to prion diseases diagnostic is arousing more and more attentions as the intrinsic advantages of aptamers. In this context, we will introduce prion diseases, the challenges of prion diseases diagnostic at the presymptomatic stage and aptamers against PrP, and then summarize the latest progress of aptamers-based assay for prion diseases diagnostic.

Keywords: prion diseases; prion protein; prion diseases diagnostic; aptamer

1. Introduction

1.1. Prion diseases

Prion diseases, a group of fatal neurodegenerative diseases, were firstly found in sheep and gradually found in other species. Prion diseases include Kuru, Gerstmann-straussier-scheinker disease (GSSD), Creutzfeldt-Jakob disease (CJD), Fatal familiar insomnia (FFI) and viriant Creutzfeldtl-Jakob disease

(vCJD) in human, Scrapie, Transmissible mink encephalopathy (TME), Bovine spongiform encephalopathy (BSE), Feline spongiform encephalopathy (FSE), Chronic wasting disease (CWD) in animal [1]. The hallmark of these diseases is the accumulation of abnormal prion protein (PrP^{Sc}) which is converted from the cellular prion protein (PrP^{C}) [2]. The emergency of vCJD in 1996, which are found to be transmitted from BSE to human, trigger urgent development of methods for prion disease diagnositc at the presymptomatic stage so as to prevent the potential epidemic of vCJD in human [3].

1.2. The major challenge for prion diseases diagnostic

The main problem is that PrP^{Sc} is abundant in brain only at the late stage of the diseases, thus how to sensitively and selectively detect the minute quantity of PrP^{Sc} is the main challenge for prion diseases diagnostic at the presymptomatic stage. Nowadays, two strategies are adopted to improve the sensitivity of prion diseases detection. The first one depends on the specific interaction of PrP^{Sc} with its antibodies in virtue of different spectroscopic technique such as raman spectroscopy, fluorescence correlation spectroscopic and Fourier-transformed infrared spectroscopy (FT-IR) [4-9]. However, the widespread of these methods cumbered by the specificity and useless of antibodies, expensive equipments and the need of high level skill in interpretation of results. The other interesting alteration to improve the sensitivity of prion diagnostic is to increase the minute quantity of PrP^{Sc} in samples. For example, sodium phosphotungstic acid and plasminogen are used to concentrate PrP^{Sc} [10, 11] while protein misfolding cyclic amplification (PMCA) and cell infection assay are applied to amplify the minute quantity of PrP^{Sc} [12-13]. Among the methods mentioned above, PMCA is considered to be the most promising one for prion diagnostic at the pre-symptomatic stage, however the time required to achieve optimal sensitivity is 3 weeks at least.

1.3. Aptamers against prion protein

Taking the advantage of the known capacity of PrP^{Sc} to bind nucleic acids such as RNA and DNA, several groups are attempting to identify artificial ligand (aptamer) by in vitro selection in large randomized library (see in Table 1). The first aptamer that specifically interact with PrP^{C} but not prion diseases-associated isoform in brain homogenate, were selected by Weiss and other RNA aptamers such as SAF-93, DP7 and 60-3 were also selected later [14-18]. It is known that DNA aptamer is more suitable for diagnostic purpose than RNA aptamer since DNA is more resistant against nucleases, which is important in cases of prion diagnostic with brain and tissue homogenate [19]. Two DNA aptamers were selected by SELEX now, the first one recognizing human recombinant PrP can also bind to cell cultured- and brain-derived PrP^{C} of various species such as cattle, sheep and deer [20]. The binding site of this DNA aptamer which locates within 23-90 epitope of PrP is similar to that of many RNA aptamers. To avoid the interaction with the N-terminal of PrP, Bibby *et al* selected the second DNA aptamer [21], which appear to be more promising in prion diagnostic since a trivalent pool of aptamers was proved to reactive against guanidinium-denatured PrP^{Sc}. However, it is pity that the two DNA aptamers only recognize PrP^{C} and denatured PrP^{Sc}.
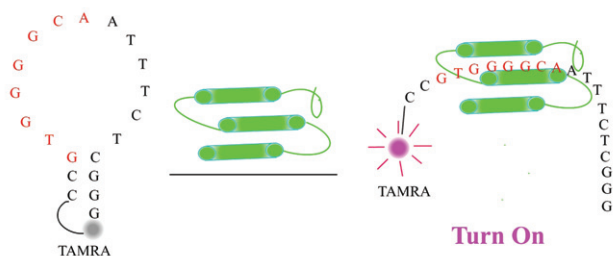
Fig. 1 Schematic presentation of PrP^C detection with aptamer-mediated fluorescence assay developed by Xiao *et al* [2].

1.4. Aptamer-based assays for prion protein detection

Aptamers owing to the intrinsic advantages of excellent stability, readily manipulation and reproducibility, non toxic and non-immunogenic have been applied in protein detection, molecular recognition, cancer diagnostic and cell imaging [22-25]. However, the application of aptamers, especially DNA aptamers, in prion diseases diagnostic is just underway. Xiao *et al* designed the first aptamer-based assay for PrP^C detection. In their assay, the core sequence of DNA aptamer was located in the loop, three guanine bases were added at 3'-terminal and tetramethyl-6-carboxyrhodamine (TAMRA) was modified at 5'-terminal, see in figure 1 [2]. In the native state, TAMRA was proximity to guanine bases through the base-pair of G-C bases, and thus the fluorescence of TAMRA was quenched. However, when target PrP^C was coexistence with aptamer assay, the specific interaction of aptamer with PrP forced TAMRA away from guanine with the results of fluorescence restored. When the concentration of PrP^C ranges from 1.1 to 44.7 μg/mL, a linear relationship was constructed between the fluorescence intensity and the concentration. The selectivity of the aptamer-based fluorescence assay was also examined using other proteins.

With the purpose of improving the sensitivity of aptamer-based assays, nanomaterials with exceptional electrical, optical or magnetical capability were introduced. For example, Hu *et al* developed an ultrasensitive method for PrP detection by making use of the high fluorescence quality of quantum dots and the excellent plasmon resonance properties of gold nanoparticles [26]. They also proved that the aptamer-based assay was able to detect PrP^C in serum without isolation prior to detection. Multi-walled nanotubes with high conductivity were used by Hianik and coworkers to develop a high sensitive and selective aptamer-based method for PrP^C detection [27]. The basic sequence of DNA aptamer reported in Ref. [20] was extended by 16-mer thymidine tail modified at the 3'terminal. The designed DNA aptamer is part of loop-like structure, as seen in Fig. 2. When the designed aptamer-based assay was coexistence with target PrP^C, the changes of oscillation frequency could be observed, and at relatively low PrP^C concentrations frequency decreased rather faster and then saturated for DNA aptamer. The limit of detection is 20-50 pM for the aptamer assay developed by Hianik and coworkers.
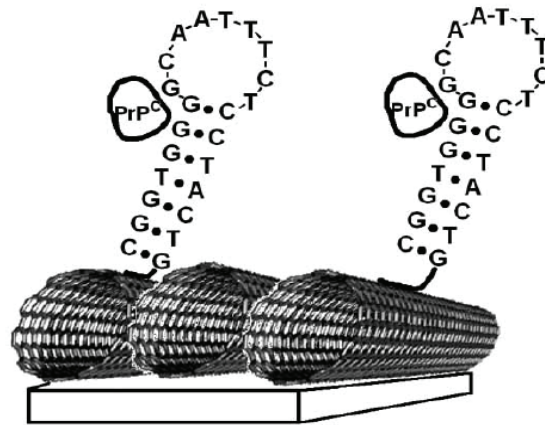
Fig. 2 Schematic representation of the biosensor for PrP^C detection which is based on DNA aptamer and MWNTs and developed by Hianik *et al* [27].
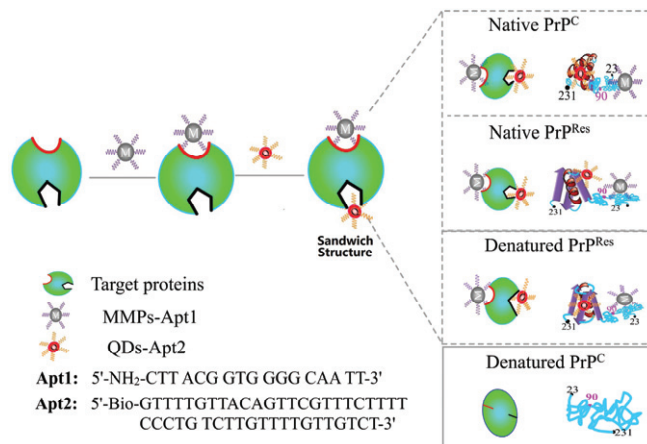

Fig. 3 Schematic presentation of our dual-aptamer strategy for sensitive discrimination and detection of prion disease-associated isoform developed by Xiao *et al* [29].

Compared with single-aptamer configuration that has been adopted in most of protein detection assays, the sensitivity and selectivity of protein detection assay could be improved dramatically with the employment of dual-aptamer configuration [28]. Taking the advantages of dual-aptamer configuration, Xiao *et al* developed an intelligent dual-aptamer strategy for prion diseases-associated isoform discrimination and detection both in buffer solution and serum, see in Fig. 3 [29]. The two aptamers, which have distinct binding epitopes on PrP, were firstly modified with magnetic microparticles (MMPs) and quantum dots (QDs), forming the probes of MMPs-Apt1 and QDs-Apt2. In the presence of target PrP, the two probes coassociated with PrP simultaneously and formed MMPs-Apt1-PrP-Apt2-QDs sandwich structure through the specific interaction of aptamers and PrP. Owing to the excellent separation ability of MMPs and high fluorescence quality of QDs, the sandwich structures could be separated easily from the medium and represented high fluorescence. The discrimination of PrP^C and PrP^{Res} (prion diseases-

associated isoform) was based on the distinct binding affinities of aptamers to denatured-PrP^C and -PrP^{Res}, which was induced by denaturing detergent. For PrP^C, it is sensitive to denaturing detergent and the binding epitopes of the two aptamers destroyed, thus the fluorescence of MMPs-Apt1-PrP^C-Apt2-QDs sandwich structure disappeared after treated with denaturing detergent. On the contrary, PrP^{Res} is resistant to denaturing detergent and the epitope of Apt2 become more accessible after treated with denaturing detergent, thus the fluorescence of MMPs-Apt1-PrP^{Res}-Apt2-QDs sandwich structure enhanced dramatically compared with that of native state PrP^{Res}. The further identifications showed that the present dual-aptamer assay developed by Xiao *et al* could be successfully applied to the detection of PrP in 0.01% brain homogenate, about 1000-fold lower than that of commonly applied antibody-mediated assays, which can detect PrP just in 10% brain homogenate. This is the first addressing that dual-aptamer configuration was applied in prion diseases detection and the selectivity is high enough for direct PrP detection in serum and brain homogenate without isolation and purification pior to assay.

Although large progresses have been made in the development of aptamer-based assays for prion protein detection, there is still a long way for the application of aptamer-based assay in direct clinical prion diseases diagnostic at the presymptomatic stage.

Acknowledgment

References

[1] K. Schneider, H. Fangerau, B. Michaelsen, W. H. M. Raab, The early history of the transmissible spongiform encephalopathies exemplified by scrapie. *Brain Research Bulletin* 2008, vol. 77, no. 6, pp. 343-355.

[2] S. J. Xiao; P. P. Hu; Y. F. Li; C. Z. Huang; T. Huang; G. F. Xiao. Aptamer-mediated turn-on fluorescence assay for prion protein based on guanine quenched fluophor. *Talanta*, 2009, vol.79, pp. 1283-1286.

[3] V. Zomosa-Signoret, J. D. Arnaud, P. Fontes, M. T. Alvarez-Martinez, J. P. Liautard, Physiological role of the cellular prion protein. *Vet. Res.* 2008, vol. 39, no. 4, pp. 9.

[4] Bellon, W. Seyfert-Brandt, W. Lang, H. Baron, A. G. Ner, M. Vey, Improved conformation-dependent immunoassay: suitability for human prion detection with enhanced sensitivity. *J. Gen. Virol.* 2003, vol. 84, pp. 1921–1925.

[5] H. Englund, D. Sehlin, A.-S. Johansson, L. N. G. Nilsson, P. R. Gellerfors, S. Paulie, L. Lannfelt, F. E. Pettersson, Sensitive ELISA detection of amyloid-β protofibrils in biological samples. *J. Neurochem.* 2007, vol. 103, pp. 334-345.

[6] M. Coleman, R. M. Nisbet, S. Han, R. Cappai, D. M. Hatters, A. F. Hill, Conformational detection of prion protein with biarsenical labeling and FlAsH fluorescence. *Biochem. Biophys. Res. Commun.* 2009, vol. 380, pp.564-568.

[7] F. Fujii, M. Horiuchi, M. Ueno, H. Sakata, I. Nagao, M. Tamura, M. Kinjo, Detection of prion protein immune complex for bovine spongiform encephalopathy diagnosis using fluorescence correlation spectroscopy and fluorescence cross-correlation spectroscopy *Anal. Biochem.* 2007, vol. 370, pp. 131-141.

[8] Krafft, G. Steiner, C. Beleites, R. Salzer, Disease recognition by infrared and Raman spectroscopy, *J. Biophotonics* 2009, vol. 2, pp. 13-28.

[9] Soto, Diagnosing prion diseases: needs, challenges and hopes. *Nature. Rev.* 2004, vol. 2, pp.809-819.

[10] M. B. Fischer, C. Roeckl, P. Parizek, H. P. Schwarz, A. Aguzzi, Binding of disease-associated prion protein to plasminogen. *Nature*, 2000, vol. 408, pp.479-483.

[11] M. Maissen, C. Roeckl, M. Glatzel, W. Goldmann, A. Aguzzi, Plasminogen binds to disease-associated prion protein of multiple species. *Lancet*, 2001, vol. 357, pp. 2026-2028.

[12] G. P. Saborio, B. Permanne, C. Soto, Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* 2001, vol. 411, pp. 810–813.

I.   Vorberg, A. Raines, B. Story, S. A. Priola, Susceptibility of common fibroblast cell Lines to transmissible spongiform encephalopathy agents. *J. Infect. Dis.* 2004, vol. 189, pp. 431-439.

[13] S. Weiss, D. Proske, M. Neumann, M. H. Groschup, H. A. Kretzschmar, M. Famulok, E.-L.Winnacker, RNA aptamers specifically interact with the prion protein PrP. *J. Virol.* 1997, vol. 71, pp. 8790-8797.

[14] S. Sekiya, F. Nishikawa, K. Noda, P. K. R. Kumar, T. Yokoyama, S. Nishikawa, In vitro selection of RNA aptamers against cellular and abnormal isoform of mouse prion protein. *Nucleic Acids Symp. Ser.* 2005, vol. 49, pp. 361-362.

[15] S. Sekiya, K. Noda, F. Nishikawa, T. Yokoyama, P. K. R. Kumar, S.Nishikawa, Characterization and application of a novel RNA aptamer against the mouse prion protein. *J. Biochem.* 2006, vol. 139, pp.383-390.

[16] S. Gilch, C. Kehler, H. M. Schätzl, Peptide aptamers expressed in the secretory pathway interfere with cellular PrP[Sc] formation. *J. Mol. Biol.* 2007, vol. 371, pp. 362-365.

[17] J. G. Safar, K. Kellings, A. Serban, D. Groth, J. E. Cleaver, S. B. Prusiner, D. Riesner, Search for a prion-specific nucleic acid. *J. Virol.* 2005, vol. 79, no. 16, pp. 10796-10806.

[18] S. Gilch, H. M. Scha̋tzl, Aptamers against prion proteins and prions. *Cell. Mol. Life Sci.* 2009, vol. 66, pp. 2445-2455.

[19] K. Takemura, P. Wang, I. Vorberg, W. Surewicz, S. A. Priola, A. Kanthasamy, R. Pottathil, S. G. Chen, S. Sreevatsan. DNA aptamers that bind to PrPC and Not PrPScshow sequence and structure specificity. *Exp Biol Med* 2006, vol. 231, pp. 204-214.

[20] F. Bibby, A. C. Gill, L. Kirby, C. F. Farquhar, M. E. Bruce, J. A. Garson, Application of a novel in vitro selection technique to isolate and characterise high affinity DNA aptamers binding mammalian prion proteins. *J. Virol. Meth.* 2008, vol. 151, pp.107-115.

[21] J. Hu, P. C. Zheng, J. H. Jiang, G. L. Shen, R. Q. Yu, G. K. Liu, Electrostatic interaction based approach to thrombin detection by surface-enhanced raman spectroscopy. *Anal. Chem.* 2009, vol. 81, no. 1, pp. 87-93.

[22] W. Li, X. Yang, K. Wang, W. Tan, Y. He, Q. Guo, H. Tang, J. Liu, Real-time imaging of protein internalization using aptamer conjugates. *Anal. Chem.* 2008, vol. 80, pp. 5002-5008.

[23] C. Walther, K. Meyer, R. Rennert, I. Neundorf, Quantum dot#carrier peptide conjugates suitable for imaging and delivery applications. *Bioconjugate Chem.* 2008.

[24] L. Q. Chen, S. J. Xiao, L. Peng, T. Wu, J. Ling, Y. F. Li, C. Z. Huang, Aptamer-based silver nanoparticles used for intracellular protein imaging and single nanoparticle spectral analysis. *J. Phys. Chem. B* 2010, vol. 114, pp. 3655-3659.

[25] P. P. Hu, L. Q. Chen, C. Liu, S. J. Zhen, S. J. Xiao, L. Peng, Y. F. Li, C. Z. Huang, Ultra-sensitive detection of prion protein with a long range resonance energy transfer strategy. *Chem. Commun.* 2010, DOI: 10.1039/c0cc02600j.

[26] T. Hianik, A. Porfireva, I. Grman, G. Evtugyn, EQCM biosensors based on DNA aptamers and antibodies for rapid detection of prions. *Prot. Pept. Lett.* 2009, vol. 16, no. 4, pp. 363-367.

[27] Y.-H. Lao, K. Peck, L.-C. Chen, Enhancement of Aptamer Microarray Sensitivity through Spacer Optimization and Avidity Effect, *Anal. Chem.*, 2009, vol. 81, no. 5, pp. 1747-1754.

[28] S. J. Xiao, P. P. Hu, X. D. Wu, Y. L. Zou, L. Q. Chen, L. P., J. Ling, S. J. Zhen, L. Zhan, Y. F. Li, C. Z. Huang, Sensitive Discrimination and Detection of Prion Disease-Associated Isoform with a Dual-Aptamer Strategy by Developing a Sandwich Structure of Magnetic Microparticles and Quantum Dots, *Anal. Chem.* 2010, DOI: 10.1021/ac101865s