Article

Journal of Biomedical Nanotechnology
Vol. 9, 639–643, 2013
www.aspbs.com/jbn

Detection of Tyrosine Hydroxylase in Dopaminergic Neuron Cell Using Gold Nanoparticles-Based Barcode DNA

Jeung Hee An[1], Byung-Keun Oh[2], and Jeong Woo Choi[2,*]

[1] *Division of Food Bioscience, Konkuk University, Chungju 380-701, Korea*
[2] *Department of Chemical and Biomolecular Engineering, Sogang University, Seoul 121-742 Korea*

Tyrosine hydroxylase, the rate-limiting enzyme of catecholamine biosysthesis, is predominantly expressed in several cell groups within the brain, including the dopaminergic neurons of the substantia nigra and ventral tegmental area. We evaluated the efficacy of this protein-detection method in detecting tyrosine hydroxylase in normal and oxidative stress damaged dopaminergic cells. In this study, a coupling of DNA barcode and bead-based immnunoassay for detecting tyrosine hydroxylaser with PCR-like sensitivity is reported. The method relies on magnetic nanoparticles with antibodies and nanoparticles that are encoded with DNA and antibodies that can sandwich the target protein captured by the nanoparticle-bound antibodies. The aggregate sandwich structures are magnetically separated from solution, and treated to remove the conjugated barcode DNA. The DNA barcodes were identified by PCR analysis. The concentration of tyrosine hydroxylase in dopaminergic cell can be easily and rapidly detected using bio-barcode assay. The bio-barcode assay is a rapid and high-throughput screening tool to detect of neurotransmitter such as dopamine.

KEYWORDS: *Bio-Barcode Assay, Gold Nanoparticles, Tyrosine Hydroxylase, Parkinson's Disease.*

INTRODUCTION

Tyrosine hydroxylase (TH) is the first and rate-limiting enzyme in the synthesis pathway of catecholamine in the central and peripheral nervous system.[1] TH is expressed in several cell groups, including the dopaminergic neurons of the substantia nigra, ventral tegmental area, hypothalamus, and olfactory bulb, the noradrenergic neurons of the locus coeruleus and lateral tegmental system, and the adrenergic neuron of the brainstem.[2] TH deficiency was diagnosed after determination of cerebrospinal fluid neurotransmitters and DNA analysis in a child with severe axial hypotonia and hypokinesia associated with dystonic and ballistic movements.[2] The TH concentration in the serum is very low (0.01–1 μM).[3] Therefore, sensitive and selective determination of TH could be employed for molecular diagnostics of Parkinson's disease, design of therapeutics, and evaluation of drug efficacy.[4] Various methods have been developed for detection of TH release by single cells, including fluorometry, chromatography, and RT-PCR technology.[5–7] TH release is rapid; therefore, detection technologies should have high spatial and temporal resolution. Recently, a novel ultrahigh sensitive technique as bio-barcode amplication assay was reported.[8]

However, TH detection from dopaminergic cell was not reported using bio-barcode DNA assay.

Bio-barcode DNA assay have highly sensitive detection of protein and nucleic acid.[8] This methods involves two types of particles, a magnetic nanoparticles functionalized with a group that has an affinity for a target of interest, a nanoparticles functionalized with a second group that has an affinity for the same target along with oligonucleotides barcode DNA that can act as reporter groups for the target of interest.[9] One of the key aspects of the Bio-barcode DNA assay is using gold nanoparticles, which enable simultaneously loading of detection antibodies/probes and a large number of barcode DNA per particle.[10] Because gold nanoparticles are easy to be coupled with antibodies and DNA, it could overcome the complicated preparation of antibody-DNA conjugates required in immuno-PCR.[11]

*Author to whom correspondence should be addressed.
Email: jwchoi@sogang.ac.kr
Received: 9 November 2011
Accepted: 20 April 2012

In this study, we present the first example of TH detection with the bio-barcode assay in the context of the established Parkinson's disease marker. In this study, we show that the modified bio-barcode method can detect TH. In addition, dopamine is suggested as the most effective markers for the diagnosis and monitoring of patient with Parkinson's disease. Also our works show a limit detection of TH from cells.

EXPERIMENTAL DETAILS

DNA Oligonucleotides, Biobarcode DNA, and PCR Primers

The sequence of the thio-capped oligo nucleotide and the bio-barcode DNA were 5'-TTT GGG GAA CAC CGT GGA GGG GCA TAG CAT CTC-$(A)_{10}$-$(CH_2)_3$-SH-3' and 5'-GAG ATG CTA TGC CCC TCC ACG GTG TTC CCC-3, respectively. The forward (F5-GAT GGA TGC CTC ATT GGG G-3) and reverse (R5-GCA GCT CCA GGC CCC CCA GC-3') primers for amplification of the bio-barcode DNA were synthesized by Bioneer (Seoul, Korea).

Magnetic Probe Preparation

Tosyl-activated magnetic microparticles (MyOne™ Dynabeads®; Invitrogen, Inc., Carlsbad, CA, USA) were heavily functionalized with a mixture of rabbit polyclonal antibodies (abcam). Briefly, 10 μl TH polyclonal antibody (1 mg/ml), 20 μl magnetic microparticles, 84 μl 3 M $(NH_4)_2SO_4$ and 66 μl borate buffer were combined in a 0.2 ml PCR tube and incubated at 37 °C at 1400 oscillations/min for 24 h. The microparticles were separated magnetically after incubation. To block any remaining active sites on the microparticles, 250 μl of blocking buffer, which was consisted of phosphate-buffered saline (PBS) pH 7.4 with 0.5% bovine serum albumin (BSA) and 0.05% Tween 20, was added and the microparticles were then incubated at 37 °C for 24 h. Following incubation, the microparticles were once again separated magnetically and were washed twice with 1 ml of magnetic-probe solution that contained PBS pH 7.4, 0.1% BSA and 0.05% Tween 20.

Preparation of Functionalized Gold Probes

The anti-TH and thiol-capped oligonucleotides were conjugated onto gold nanoparticles by a previously described one-step method.[11] The antibody- and thiol-capped oligonucleotides-conjugated gold nanoparticles were resuspended in 4 ml PBS. 0.8 OD of bio-barcode DNAs were included and hybridized for 4 h at room temperature. The solution was centrifuged (13,000 × g, 30 min) at 4 °C, and the resultant anti-TH–nanoparticles-dsDNA complexes were resuspended in NaCl (0.15 M)/PBS (0.01 M) and stored at 4 °C. The anti-TH- and DNA-conjugated gold nanoparticles were detected by using a UV-visible spectrophotometer, the antibody activity on the gold nanoparticles surface was evaluated by ELISA, and the oligonucleotides on the gold nanoparticles were detected by RT-PCR.

Capture of Tyrosine Hydroxylase in SH-SY5Y Cells by the Bio-Barcode DNA Assay

In a typical experiment, a sandwiched immunoassay of magnetic immunoprobe/TH/gold immunoprobe was carried out, and then the DNA bio-barcodes were collected by a magnetic separation and thermal dehybridization. Detection of tyrosine hydroxylase by RT-PCR for matrix gene was performed according to a procedure published elsewhere with some modifications.[11] The obtained RT-PCR products were analysed using 2% agarose gel electrophoresis.

Cell Culture

SH-SY5Y cells have been cultured in the presence of CO_2 (5%) at 37 °C in Dulbecco's Modified Eagle's Medium supplemented with fetal bovine serum (10%) and 0.5% antibiotics.

RESULTS AND DISCUSSION

Synthesis and Characterization of Anti-Tyrosine Hydroxylase Conjugate

The barcode assay utilized 60 nm Au nanoparticles probes, each confunctionalized with an antibody (polyclonal) that recognizes for the target antigen (Fig. 1). Note that half of the barcode for each target is the target-reporting oligonucleotide probe, while the other half is identified for all the barcodes and is in effect a universal sequence. The target antigens are captured insolution by magnetic nanoparticles probe, each conjugated with a monoclonal specific for an epitope of the target antigen different from the one recognized by the gold probe.[12] The target-magnetic nanoparticles probe complexes are the sandwiched by the Au nanoparticles probe with an antibody that can bind to the target in a different region than the magnetic nanoparticles probe (Fig. 1). These complexes are isolated with a magnetic field and washed, and the barcode strands are release by a ligand exchange process induced by the addition of dithiothreitol. The bio-barcode strands are then identified by the RT-PCR method. Au nanoparticles probe is functionalized with a detection of antibody and barcode DNA strands (43 bp/particle). The Au nanoparticles probe are stored at a concentration of 0.8 O.D., with the excess barcode DNA removed in 0.15 M NaCl, 0.025% Tween 20, 0.1% BSA, and 10 mM phosphate buffer, pH 7.4 solution.

The SEM image of the immobilized gold colloidal nanoparticles is shown in Figure 2(a). The average particle

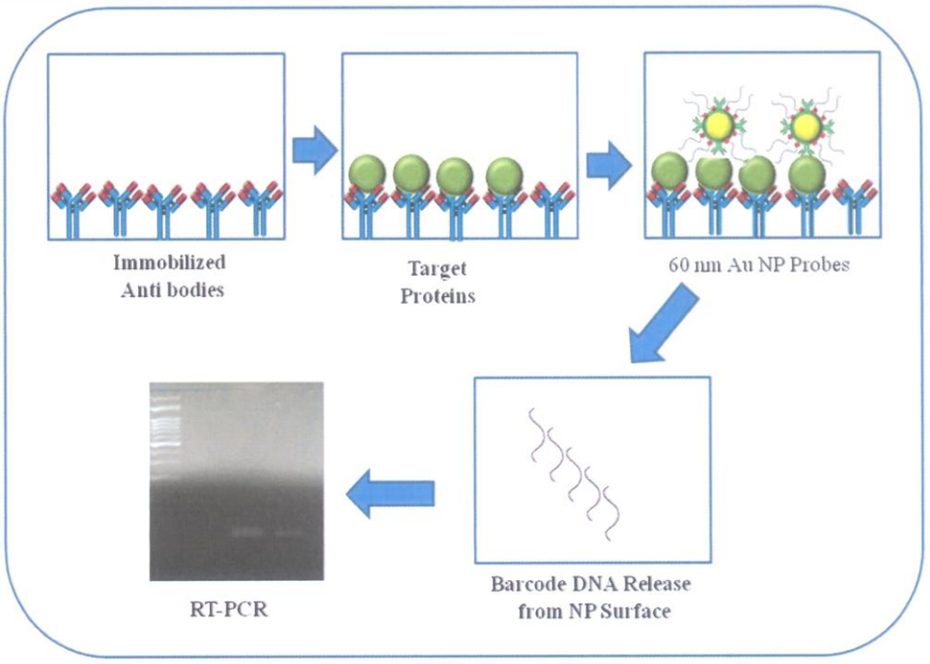

Figure 1. Schematic diagram of the surface immobilized biobarcode assay protocol.

diameter was about 60 nm. Obviously, most gold nanoparticles self-assembled in close proximity to each other by forming randomly distributed one-dimensional patterns.[13]

The UV-VIS absorption spectrum of the immobilized gold colloidal nanoparticles after TH addition is illustrated in Figure 2(b) according to gold size. The gold ranging from 5 to 100 nm in diameter exhibited increasing binding affinity following subsequent immunizations, with the highest affinity associated with 60 nm gold nanoparticles. We observed the binding activity for the conjugate prepared with 50 and 60 nm diameter gold nanoparticles. The antibody response to carries plays an important role in determining the immunogenicity of the conjugated nanoparticles.

Detection of the Tyrosine Hydroxylase Using Barcode Probes

There are many factors that may that may limit performance of the DNA barcode-based immunoassay. These factors include the type of particles or antibodies, attachment chemistry, effectiveness of removal and washing steps, etc. Among them, immunological affinity of the immunoprobes towards their target analytical plays the most important roles. All target concentrations over the 0.156 ng/ml to 10 ng/ml concentration range could be easily differentiated from negative control (0 ng/ml). The image of band in RT-PCR was showed a linear relationship between target concentration and intensity over a concentration range.

The magnetic nanoparticles immunoprobe were successively incubated with 0.156 ng/ml of the TH, the capture the TH validate magnetic separation. After the incubation period, the samples were treated protease K, and then barcode DNA was eluted by the DNA elution kit. Finally, 50 ul DNA-free water was added to dissolve the DNA for RT-PCR analysis. Figure 4 shows the gels electrophoresis image of the RT-PCR products. False negative results could be obtained if the washing step is not performed adequately in any immunoassays. Moreover, both washing solutions were dopamine free after 3 times of washing as shown by PCR negative signal in 0 ng/ml.

Detection of the Tyrosine Hydroxylase in SH-SY5Y Cells by the Barcode Analysis

To study release of neurotransmitters from a neuronal cell model, we chose to exploit the fact that TH can be derivatized to be fluorescent dye. The TH phenotypes were confirmed by immunofluorescene staining (Fig. 4(A)). The TH expression showed cytosolic fraction in SH-SY5Y cells.

In Figure 4(B), the SH-SY5Y cells extract was treated with Ripa protein lysis buffer. The result shows that one can still detect as low as 10 nM dopamine concentration in SH-SY5Y cells by using bio-barcode analysis. It is necessary to ensure that the immnunoprobes are able to capture the TH secretion from dopaminergic cells. To determine this, conventional RT-PCR was exploiting as a straight forward approach to validate the usefulness of the immunoprobe. This could be because of blocking effect of the microbeads or non-specific interactions of the beads and the TH. Non-specific interation of the immunoprobe with other catecholamine is also important information that should be elucidated to evaluate the specificity of the

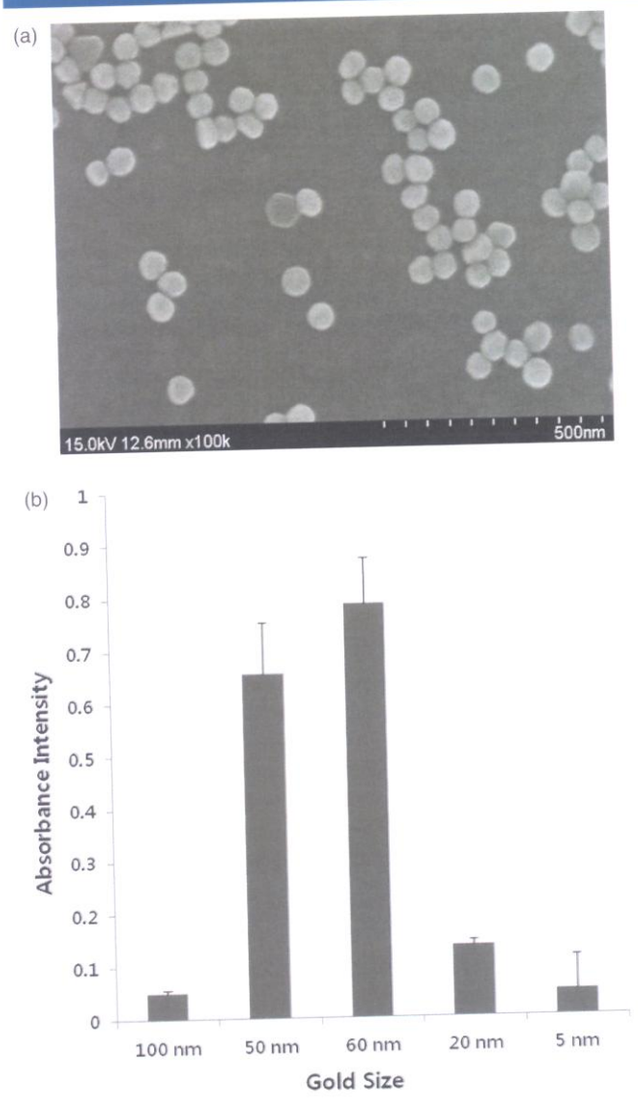

Figure 2. Scanning electrode microscopic image of the anti–tyrosine hydroxylase conjugated gold nanoparticles (A) and the affinity activity showed in anti-tyrosine hydroxylase and gold nanoparticles (B).

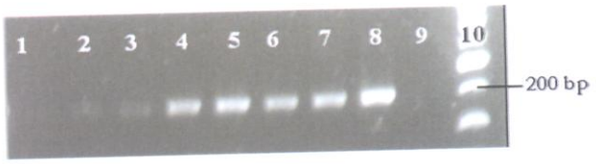

Figure 3. Agarose gel electrophoresis image illustrating validation of the immunoprove by RT-PCR for tyrosine hydroxylase detection. On gel 1 lane: negative control, 2 lane: 0.156 ng/ml, 3 lane: 0.312 ng/ml, 4 lane: 0.625 ng/ml, 5 lane: 1.25 ng/ml, 6 lane: 2.5 ng/ml, 7 lane: 5 ng/ml, 8 lane: 10 ng/ml, 9 lane: albumin (non specify protein) 10 lane: DNA ladder. All PCR procedures were conducted for 35 cycles. 20 ul PCR products were electrophoresed on a 2% agarose gel was visualized with ethidium bromide.

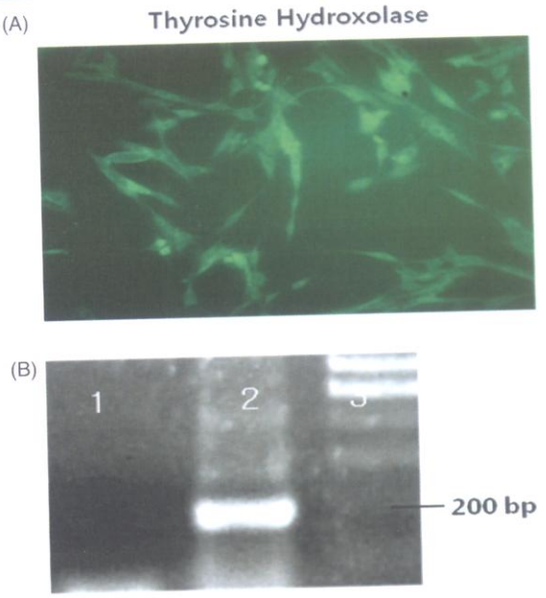

Figure 4. Tyrosin hydroxylase flurescence image in SH-SY5Y cell (A) and Agarose gel electrophoresis image illustrating validation of the immunoprove by RT-PCR for tyrosine hydroxylase detection in dopaminergic cells (B).

immunoprobes. To evaluate the clinical application potential of the proposed method, the recovery experiments were performed. The dopamine extracts originated from 1×10^6 cell/ml SH-SY5Y cells. The recovery of TH detection was from 94.6% to 102%.

CONCLUSION

In this study, gold nanoparticles dual-modified with antibody and dsDNA were employed in an immunosorbent bio-barcode assay for detection of trace amounts of TH. The dual-modified gold nanoparticles were characterized by UV–visible spectrophotometer, and the conjugated anti-dopamine and oligonucleotides were examined by RT-PCR. These experiments provide concrete evidence that modified gold nanoparticles exhibit robust stability and reactivity, and retain their natural bioactivities. The immunosorbent bio-barcode assay using modified gold nanoparticles is sensitive enough to detect TH at a low 0.156 ng/ml level. In addition, the sensitivity may be further enhanced by optimizing the molecular ratio of antibody and oligonucleotide, hybridization temperature, or using RT-PCR. Thus, the immunosorbent bio-barcode assay is a rapid and high-throughput screening tool to detect tyrosine hydroxylase in dopaminergic cells.

Acknowledgment: This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2011-0000384) and by the Original Technology Research Program for brain Science through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science

and Technology (2009-0003456) and by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0011876).

REFERENCES

1. J. Mallet, Tyrosine hydroxylase from cloning to neuropsychiatric disorderstes. *Brain. Res. Bull.* 50, 381 (**1999**).
2. S. C. Daubner, T. Le, and S. Wang, Tyrosine hydroxylase and regulation of dopamine synthesis. *Arch. Biochem. Biophys.* 508, 1 (**2011**).
3. E. Oliverira, C. R. Pinheiro, A. P. Santos-Silva, I. H. Trevensoli, Y. Areu-Villaca, J. F. Nogueira Neta, A. M. Reis, M. C. F. Passos, E. G. Moura, and P. C. Lisbora, Nicotine exposure affects mothers and pops nutritional, biochemical and hormonal profiles during lactation in rats. *J. Endocrinol.* 205, 159 (**2010**).
4. W. Yeung, V. C. N. Wong, K. Chan, J. Hui, C. Hung, E. Yau, C. Ko, C. Lam, C. M. Mak, S. Siu, and L. Low, Expanding phenotype and clinical analysis of trosine hydroxylase defiency. *J. Child. Neurol.* 26, 179 (**2011**).
5. T. Nagatsu and T. Yamamoto, Flurescence assay of tyrosine hydroxylase activity in tissues homegrnate. *Cellular Molec. Life Sci.* 24, 1183 (**1968**).
6. T. Lloyd and M. A. Walega, Purification of tyrosine hydroxylase by high pressure liquid chromatography. *Analyl. Biochem.* 116, 559 (**1981**).
7. S. T Lee, Y. L. Suh, Y. Ko, C. Ki, K. W. Sung, H. Kim, J. Kim, S. Kim, H. Chueh, S. H. Lee, K. H. Yoo, and H. H. Koo, Measure of tyrosine hydroxylase transcripts in bone marrow using biopsied tissue instread of aspirates for neuroblastoma. *Pediatr Blood Cancer* 55, 273 (**2010**).
8. C. Cao, R. Dhumpa, D. D. Bang, Z. Ghavifekr, J. Høgberg, and A. Wolff, Detection of avian influenza virus by fluorescent DNA barcode-based immunoassay with sensitivity comparable to PCR. *Analyst* 135, 337 (**2010**).
9. B. K. Oh, J. M. Nam, S. W. Lee, and C. A. Mirkin, A fluorophore-based bio-barcode amplification assay for proteins. *Small* 2, 103 (**2006**).
10. R. Duan, X. Zhou, and D. Xing, Electrochemiluminescence bio-barcode method based on cysteamine–gold nanoparticle conjugates. *Anal. Chem.* 82, 3099 (**2010**).
11. L. Chen, H. Wei, Y. Guo, Z. Cui, Z. Zhang, and X. E. Zhang, Gold nanoparticle enhanced immuno-PCR for ultrasensitive detection of Hantaan virus nucleocapsid protein. *J. Immunol. Methods* 346, 64 (**2009**).
12. Y. Tang, H. Wang, J. Xiang, Y. Chen, W. He, N. Deng, and H. Yang, A sensitive immunosorbent bio-barcode assay combining PCR with icELISA for detection of gonyautoxin 2/3, *Anal. Chim. Acta* 657, 210 (**2010**).
13. S. U. Kim, Y. J. Kim, J. W. Choi, and B. K. Oh Thin film fabrication of electroactive protein withhem group. *Biochip J.* 1, 188 (**2007**).