Talanta 81 (2010) 1016–1021

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A fast and sensitive enzyme immunoassay for brain natriuretic peptide based on micro-magnetic probes strategy

Ruping Liu [a,b], Juntao Liu [a], Li Xie [c], Mixia Wang [a,b], Jinping Luo [a], Xinxia Cai [a,b,*]

[a] State Key Laboratory of Transducer Technology, Institute of Electronics, Chinese Academy of Sciences, Beijing 100190, China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100190, China
[c] Beijing You'an Hospital, Capital Medical University, Beijing 100054, China

ARTICLE INFO

Article history:
Received 2 November 2009
Received in revised form 21 January 2010
Accepted 24 January 2010
Available online 1 February 2010

Keywords:
Micro-magnetic probe
Immunoassay
Signal amplification
Streptavidin
Brain natriuretic peptide

ABSTRACT

In this paper, a simple, rapid and low-cost method for the high-sensitivity detection of brain natriuretic peptide (BNP) was developed, which adopted three amplification steps: (a) biotin–streptavidin amplification; (b) micro-magnetic probe amplification; (c) HRP (horseradish peroxidase) signal amplification. In the present strategy, the streptavidin-coated micro-magnetic particles (MMPs) were first conjugated with biotin-labeled capture antibody via the biotin–streptavidin interaction, which formed bio-functional micro-magnetic probes. Then, the analyte (antigen) is sandwiched by HRP-labeled antibody and capture antibody bound to MMPs. Finally, the HRP at the surface of sandwich structures catalytically oxidized the substrate and generated optical signals that reflected the amount of the target BNP. The influence of some important parameters such as the size of magnetic particles, the working concentration of HRP-labeled BNP antibody, the stability of magnetic probes, and the assay medium of serum BNP, etc. on the detection ability of present method was investigated in details. It is found that the detection limit of the proposed method could reach 10 pg/mL for BNP, which is much lower than that of sandwich-type ELISA. Furthermore, this detection time for the proposed method just takes about 30 min (two reaction steps and one wash step), which is faster than that of conventional sandwich-type ELISA (taking about 4 h, three reaction steps and three wash steps). Inspired by these advantages, it is expected that this method can probably be applicable to the detection of other hormones and tumor markers that are present in only low concentrations within the human body.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Brain natriuretic peptide (BNP) is a hormone composed of 32 amino acids, which has a pronounced vasodilating effect as well as diuretic and natriuretic activity [1,2]. Generally, the concentration of BNP in serum under normal conditions is less than 20 pg/mL, but it will obviously increase to approximately 1000 pg/mL for the patients diagnosed with acute or chronic heart failure [3,4]. BNP has been one of the most important cardiac markers for the prediction and diagnosis of hear failure. To date, several methods such as the radioimmunoassay (RIA), immunochromatography combined with a fluorescence detection system, and the chemiluminescent enzyme immunoassay, etc. have been employed to detect the concentration of BNP protein in serum [5,6]. Although the above methods offer high sensitivity and specificity, there still exist some drawbacks. The cumbersome radiation of RIA may cause damage to the people and environment. For immunochromatography combined with a fluorescence detection system, its main drawback is high cost and sophisticated instrumentation. The chemiluminescent enzyme immunoassay not only consumes time but also needs complicated experimental procedures.

Immunoassay is currently the predominant technique for the rapid, low-cost and sensitive determination of proteins and biomarkers related to various diseases in clinical fields [7–11]. The immunoassays generally fall into two broad types, competitive immunoassay or sandwich-type immunoassay [12–14]. The sandwich-type immunoassay is known to be superior to competitive ones in terms of sensitivity and working range. Particularly, the sandwich-type ELISA is considered as the most important enzyme immunoassay for some species with the detection limit from 0.1 ng/mL to 1 μg/mL [15–17]. However, its sensitivity for small molecules is insufficient compared with radioimmunoassay or fluorescence-based detection. But the level of disease markers such as BNP protein is usually very low for some diseases, which is beyond the detection limit of sandwich ELISA. And also, the sandwich-type ELISA usually requires long time and multiple steps,

* Corresponding author at: State Key Laboratory of Transducer Technology, Institute of Electronics, Chinese Academy of Sciences, Beijing 100190, China. Tel.: +86 10 58887193; fax: +86 10 58887172.
E-mail address: xxcai@mail.ie.ac.cn (X. Cai).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.01.051

which greatly limit its practical application in clinics [18]. Therefore, searching a novel, rapid, low-cost and sensitive approach for the detection of small-molecule protein in a low concentration is highly desirable and technologically important.

Recently, the detection of biomarkers has received considerable attention in diseases diagnosis, genetics, pathology, food safety, and many other fields. For example, specific antibody immobilized to solid materials has proven to be a fundamental process in a variety of biotechnological and biomedical applications, including the detection of biomarkers and early diagnosis of diseases. Magnetic micro/nanoparticles have been applied in immunoassays and in various reactions involving enzymes, proteins, and DNA for magnetically controlled transport and target delivery of anticancerous drugs [19–24]. Their reduced size and ability to be transported in biological systems and reacting medium is an advantage over conventional support systems and they can also provide large surface area and unique physicochemical properties [25–28]. Considerable interest over the past two decades has been directed toward the functionalization of micro/nanoparticles because of their excellent biocompatibility, stability, and established synthesis protocols. The micro-magnetic probe strategy developed recently has proven to be a highly sensitive technique for detecting human tumor cells, and is especially well suited to separate and meantime detect the low-concentration proteins [29–32]. However, employing a simple micro-magnetic probe combined with the sandwich ELISA strategy for the detection of low-concentration BNP remains a great challenge and has not reported until now.

In this work, with a view to overcome the challenge for the detection of small-molecule protein at a low concentration, a highly sensitive detection method has been demonstrated by using micro-nanotechnology combined with the sandwich-type ELISA. The streptavidin-coated MMPs were conjugated to biotin-labeled BNP antibody and further formed micro-magnetic probes, which could provide the signal amplification for the detection of low-concentration protein. In this procedure, the magnetic particles acted as the surface for the immobilization of the target BNP and the magnetic separation was further used to increase the concentration of the target protein. The mobility of the MMPs allows a shorter reaction time and a small volume of reagent to be used than that of conventional sandwich-type ELISA, where the antibody is bound to a plate. HRP linked to another BNP antibody, efficiently catalyze substrate, was further used for signal amplification and improved the detection limit [33]. With these three amplification processes, the sensitivity, the detection limits and assay time are greatly improved compared with the sandwich-type ELISA. The optimized detection limit of this proposed method is 10 pg/mL and the assay time is less than 30 min.

2. Experimental

2.1. Apparatus

A Hitachi UV-3010 spectrophotometer has been employed to characterize the optical properties of initial antibody solution and the supernatant. The scanning wavelength was set at 280 nm to measure the rate of anchoring BNP antibody onto MMPs. The optical density of the BNP solution was determined by a Microplate Reader (Bio-Rad Model 680) at 450 nm.

2.2. Reagents and materials

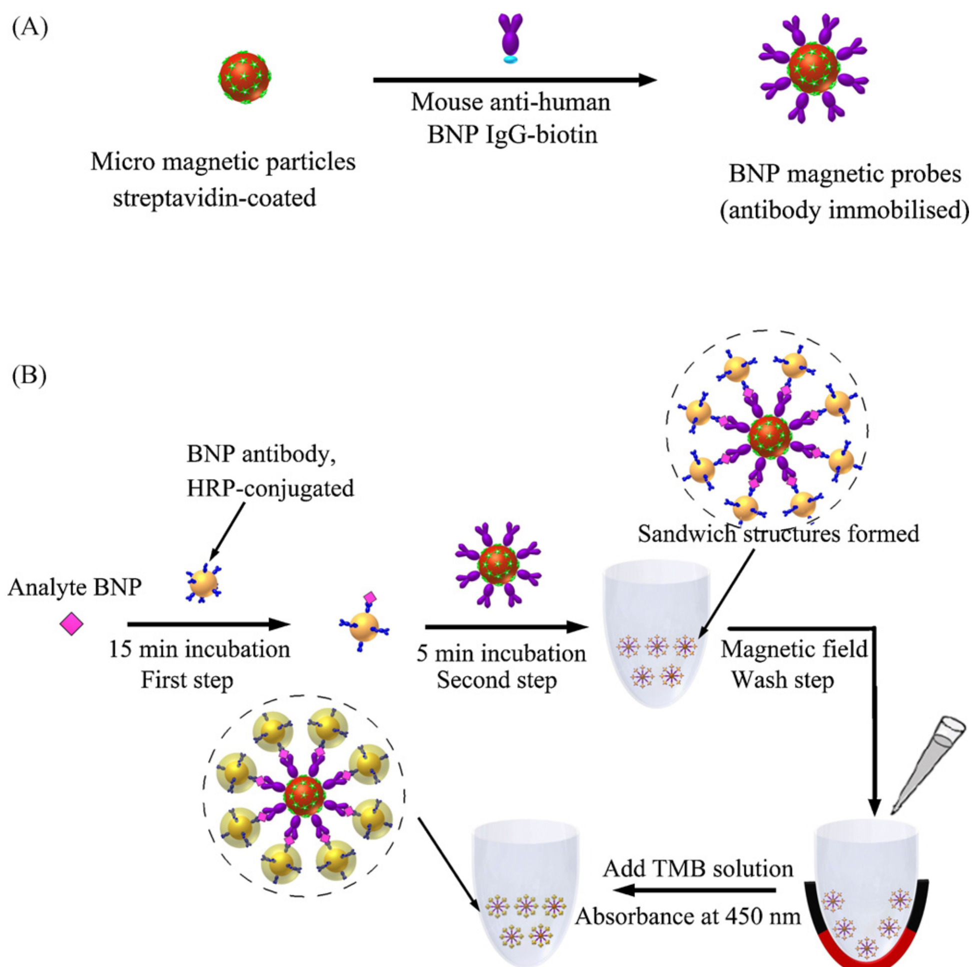
The following chemicals and reagents were used in the experiments: monoclonal mouse anti-human brain natriuretic peptide (BNP), HRP (horseradish peroxidase)-conjugated and monoclonal mouse anti-human brain natriuretic peptide (BNP), biotinylated were purchased from HyTest Ltd. (HyTest, Turku, Finland). Brain natriuretic peptide-32 human was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Streptavidin-coated micro-magnetic particles (MMPs) with the size of about 1 and 2.8 μm were purchased from Dynal Biotech (Dynal, Invitrogen, Spain). The two kinds of micro-magnetic particles were composed of super-paramagnetic beads with a streptavidin monolayer covalently coupled to the surface. 3,3′,5,5′-Tetramethylbenzidine (TMB)–H_2O_2 were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other reagents were in analytical grade. PBS–Tween (10 mM phosphate buffer, 140 mM NaCl, 3 mM KCl, 0.05% Tween 20) was obtained from Calbiochem (Calbiochem, Darmstadt, Germany). Reagent grade water with a specific resistance of 18.2 M was supplied through a Mill-Q Synthesis (Millipore, Bedford, MA, USA). All buffer reagents and other inorganic chemicals were supplied by Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

2.3. Anchoring BNP antibody onto micro-magnetic particles

The binding of the biotinylated anti-BNP antibody to streptavidin-coated MMPs was achieved through a streptavidin–biotin bridge. Magnetic probes were prepared according to the following steps. First, 100 μL of streptavidin-coated MMPs (10 mg/mL) were transferred into a 2.0 mL Eppendorf tube. The MMPs were washed three times with 150 μL of wash buffer (10 mM PBS, pH 7.4, 0.05% Tween-20) while physically retaining them on a magnet. Second, the MMPs were resuspended in 100 μL of PBS buffer for the downstream use. 33 and 16 μL biotinylated anti-BNP antibodies (1.2 mg/mL) were added to 100 μL streptavidin-coated MMPs for the preparation of 1 and 2.8 μm magnetic probes, respectively. The mixtures were incubated at room temperature for 30 min with 120 rpm/min, which formed magnetic probes, as illustrated in Scheme 1A. The magnetic probes were separated using a magnet and the supernatant was then carefully removed and set aside for the downstream detection of absorbance. Finally, after the washing steps, the probes were dispersed in 100 μL PBS and were stored in a refrigerator at 4 °C for the downstream use.

2.4. Immunoassay

BNP was detected by a sandwich-type immunoassay using two kinds of monoclonal antibodies: biotinylated anti-BNP antibody and HRP-labeled anti-BNP antibody. Those two antibodies can recognize different epitopes of BNP. The reaction sequence of conventional sandwich-type ELISA was changed. The new method consists of two reaction steps and only one wash step, as illustrated in Scheme 1B. First, 50 μL of HRP-labeled anti-BNP antibody was transferred into an Eppendorf tube and mixed with 50 μL of BNP solution. After incubating for 15 min avoid of light at 37 °C, the antibody–antigen complexes were formed. Second, BNP magnetic probes were added into above complexes and incubated for 5 min avoid of light at 37 °C. Through the interaction between antibody and antigen, the sandwich immunocomplex (magnetic probes–target BNP–HRP) was formed. The Eppendorf tube was placed onto the magnet for 2 min. The formed sandwich complexes were magnetically separated and washed four times with 150 μL of wash buffer (10 mM PBS, 0.05% Tween-20 included). The excess HRP-conjugated antibody was then removed. The immunocomplex was incubated with 50 μL TMB solution and 50 μL H_2O_2 solution for 5 min avoid of light. All the incubation steps were performed at 37 °C. The absorbance at 450 nm was detected after terminating the reaction with 50 μL of 2 mol/L H_2SO_4 solution.



Scheme 1. (A) Preparation procedure of BNP magnetic probes; (B) analytical procedure for the sandwich-type assay based on the magnetic probes.

2.5. Assay procedures

The binding efficiency of antibodies to the surface of MMPs was evaluated by measuring the absorbance of the initial antibody solution and the supernatant using a UV–vis spectrophotometer. The scanning wavelength was set at 280 nm. Two kinds of streptavidin-coated MMPs with the size of 1 and 2.8 μm were used for detection of BNP with a series of different concentrations, and the linear correlation coefficient was compared. The optimal dilution for the use of HRP-labeled anti-BNP antibody was determined as the working concentration for HRP. It was established by measuring the absorbance of different dilutions of HRP-labeled anti-BNP reacted with positive sample (100 pg/mL BNP) and negative sample (0 pg/mL BNP) using the magnetic probes by a sandwich ELISA. The absorbance ratio of positive vs. negative reached a maximum value when the HRP-labeled anti-BNP antibody was diluted in the optimal proportions. To determine the stability of the magnetic probes, an experiment was devised to compare the absorbance of positive sample (100 pg/mL BNP) and negative sample (0 pg/mL BNP) by using the probes stored in a refrigerator at 4 °C after various times of storage.

The influence of assay medium on detection of serum BNP level was assayed. For the quantitative determination of BNP in serum samples, BNP standards were diluted with three different assay medium: (1) the assay buffer (10 mM PBS buffer containing 5% BSA); (2) the serum diluent (90% assay buffer + 10% serum); (3) whole serum. Then, the absorbance of the positive sample (1 ng/mL) and negative sample (0 ng/mL) were detected to determine the optimal assay medium of serum samples. To assess the sensitive and the detection limit of the proposed immunoassay, an enzyme immunoassay to detect serum BNP by using the magnetic probes was applied under optimized conditions. In this method, HRP–anti-BNP molecules as tracer and hydrogen peroxide as enzyme substrate. To monitor the feasibility of the newly developed immunoassay, 88 different serum samples, which were provided from Beijing You'an Hospital, China, were examined by the developed immunoassay.

3. Results and discussion

3.1. The ratio of immobilized antibodies on the surface of MMPs

The success of the immobilization that the capture antibody conjugated to MMPs was confirmed by a UV–vis spectrophotometer. The amount of anti-BNP antibody modified on the MMPs could be calculated from the different absorbance at 280 nm between the anti-BNP antibody solution before immobilization and the supernatant after immobilization. Assume the absorbance of initial capture antibody solution to be A_0, while A_t represents the supernatant absorbance, then immobility rate = $[(A_0 - A_t)/A_0] \times 100\%$. It was calculated that the average immobility rate of capture antibody was approximately 81.2% and 93.7% for the preparation of 1 and 2.8 μm magnetic probes, respectively (note that the two values are the mean of three independent detections). This indicates that the BNP capture antibody was effectively immobilized on the surface of MMPs. And the immobilization of antibody on MMPs was

also confirmed by adding TMB solution to detect the absorbance at 450 nm.

3.2. Optimization

3.2.1. Effect of the size of the micro-magnetic particles

The size of micro-magnetic particles (MMPs) is very important for the assay sensitivity [34]. In this study, we compared two kinds of streptavidin-coated MMPs with the size of 1 and 2.8 μm. It is found that the good linear relationship could be obtained between the absorbance and the concentration of BNP in the range of 0–1000 pg/mL by using those two kinds of micro-magnetic probes. The negative signal (no BNP protein) with 1 and 2.8 μm magnetic probes at 450 nm was 0.014 and 0.024, respectively. It is worth noting that in this assay, the negative signal did not increase significantly even when the incubation time of sandwich-type complexes and HRP substrate was greatly prolonged. And also when the experiments were performed with the higher concentrations of HRP-labeled antibody, the development of the color reaction will be accelerated, with no obvious increase in negative signal (date not shown). It is known that prolonging the incubation time of HRP and substrate or raising the concentrations of HRP conjugate will actually enhance the negative signal of analysis in the conventional ELISA. Whereas the micro-magnetic probe strategy can reduce the negative signal and improve the detection sensitivity. Thus, the lower negative signal can make detection sensitivity reaching the picogram level at the present strategy while that of the conventional sandwich-type immunoassay just can reach the nanogram level.

The linear correlation coefficient between the absorbance and a series of different BNP concentrations measured by using 1 and 2.8 μm probes was 0.9933 and 0.9820, respectively. This indicates that the linear correlation coefficient with 1 μm magnetic probes was better than that with 2.8 μm probes. The reason is probably due to the difference of the specific surface area of MMPs. Compared to magnetic probes (2.8 μm in diameter) used in biological separation and detection, the 1 μm probes possess high surface/volume ratio, which can provide more contact surface area for attaching antibody and for capturing target antigen. For instance, the antibody bound to the 1 μm magnetic particles may overcome steric hindrance effect than that of 2.8 μm or larger magnetic particles more easily [35–38]. Therefore, we chose 1 μm magnetic probes for downstream applications.

3.2.2. The working concentration of HRP-labeled BNP antibody

The optimal dilution for the use of the HRP-labeled anti-BNP antibody was determined by measuring the absorbance of different dilutions of HRP-labeled anti-BNP reacted with positive sample (100 pg/mL BNP) and negative sample (0 pg/mL BNP) using the magnetic probes. Thus, the different optical signals were obtained due to the catalytic activity of HRP and the substrates. Fig. 1A showed that both the positive signal and negative signal increased with increasing the concentration of HRP. The higher concentration of HRP led to the higher negative signals, while the lower concentration of HRP led to the lower positive signals. The 1:10,000 diluted HRP-conjugated anti-BNP antibody showed the best absorbance ratio of positive vs. negative (the absorbance of 100 pg/mL BNP antigen vs. that of negative) (Fig. 1B), which meant that almost all of the specific sites of BNP protein were occupied. Therefore, a 1:10,000 dilution of HRP-labeled anti-BNP was chosen as the working solution in this study and thus guaranteed a low detection limit of the immunoassay.

3.2.3. Stability of magnetic probes

To determine the stability of magnetic probes upon long-term storage, an experiment was conducted to compare the performance of the magnetic probes after various times of storage. The magnetic probes were stored in a refrigerator at 4 °C for 30 days. As shown in Fig. 2, it can be found that there was a slow loss in the performance upon long-term storage (30 days) and the detection signal of antigen with 1 μm probes at 30 days was weaker than that at 1, 10 and 20 days. As is known, the positive–negative (P/N) ratio for

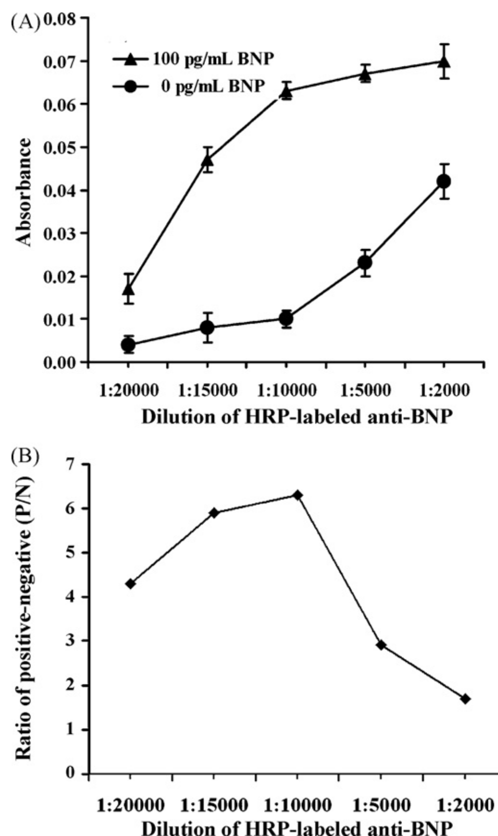


Fig. 1. The absorbance of positive sample (100 pg/mL) and negative sample (0 pg/mL) was monitored at 450 nm by using different dilution rates of HRP-labeled BNP antibody. The best absorbance ratio of positive vs. negative was detected in different dilution rates of HRP-labeled BNP antibody. Each value represents the mean value of three replicates and relative deviation is less than 5%. (A) The absorbance of positive sample (100 pg/mL) and negative sample (0 pg/mL) using different dilutions of HRP-labeled BNP antibody. (B) The absorbance ratio of positive vs. negative in different dilution rates of HRP-labeled BNP antibody.

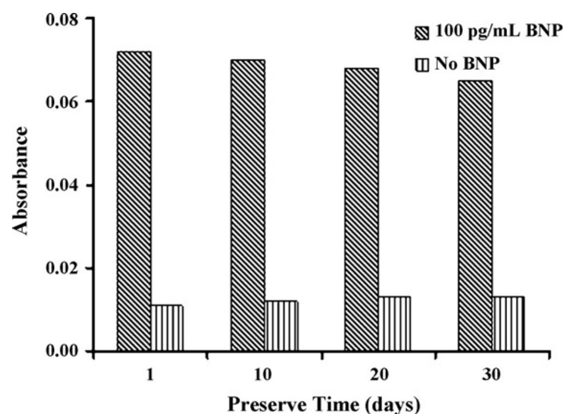


Fig. 2. Detection results of the absorbance of a standard positive sample (100 pg/mL BNP) and a standard negative sample (0 pg/mL BNP) in the assay buffer (10 mM PBS buffer containing 5% BSA) with 1 μm magnetic probes after various times of storage. The volume of magnetic probes was 20 μL (magnetic probes were prepared according to the procedure of Section 2.2, not dilute).

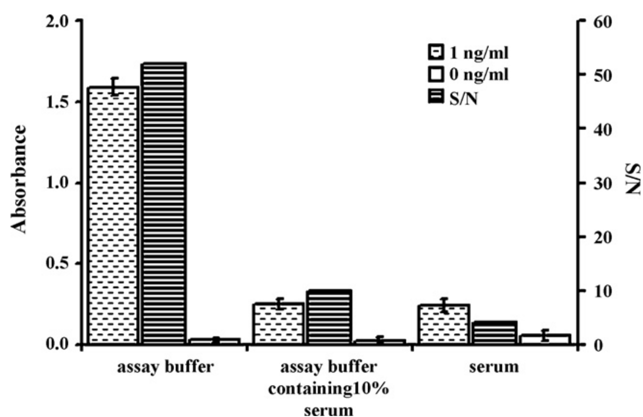


Fig. 3. Three kinds of assay mediums (assay buffer: 10 mM PBS buffer plus 5% BSA; assay buffer containing 10% serum; 100% serum) were investigated. The influence of assay medium on detection of serum BNP level was analyzed by measuring the absorbance of positive sample (1 ng/mL BNP) and negative sample (without analyte). The left and right bars indicate the positive and the negative absorbances, respectively. The center bar represents the S/N value. Each value represents the mean of three replicates and relative deviation is less than 5%. Detection of BNP within the 10% serum environment demonstrates good analytical specificity.

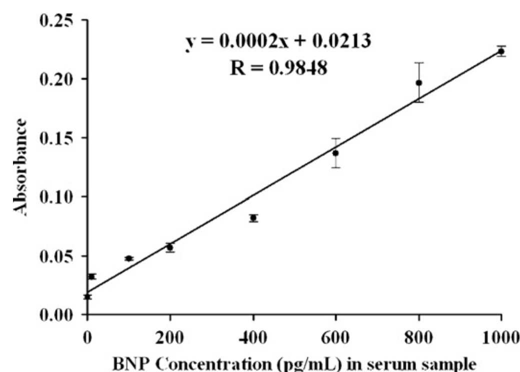


Fig. 4. Detection results of BNP protein in serum sample dilutions. The serum sample (1000 pg/mL of BNP protein) was diluted to 800, 600, 400, 200, 100, 10, and 0 pg/mL in serum diluent (10 mM PBS buffer containing 5% BSA, 10% normal serum) and detected.

the detection of sample would need to be 2.1 or higher to be considered positive [39], the performance of the present probes did not show an obvious decline and positive–negative (P/N) ratio was still high (>5) at 30 days (Fig. 2). The absorbance of a negative sample was 0.013 at 30 days. This demonstrates that, in the absence of analyte, these micro-magnetic probes not only maintain the stability in solution, but also retain their activity for analyte protein in the condition of high saline and protein environments.

3.2.4. The Influence of assay medium on detection of serum BNP level

To determine the immunoassay ability for detecting BNP protein at the picogram level, an immunoassay was performed in three different medium: (1) the assay buffer (10 mM PBS buffer containing 5% BSA); (2) the serum diluent (90% above assay buffer + 10% serum); (3) whole serum. We evaluated the influence of the different assay medium on the test result of BNP serum standards by analyzing the absorbance with a sandwich ELISA. Then, the positive sample (1 ng/mL) and negative sample (0 ng/mL) were assayed to determine the optimal assay medium of serum samples. 50 μL 1:10,000 dilution of HRP-labeled anti-BNP was added into the 50 μL analyte (BNP) solution and incubated for 15 min by gentle shaking, then followed by the addition of 20 μL antibody-coated magnetic probes and additionally incubated for 5 min. The rest of assay procedures were the same as described above. Fig. 3 shows the effects of the assay medium of serum samples on the signal-to-noise (S/N) ratio. The effects of assay medium of serum samples were assayed and the highest signal-to-noise ratio (S/N) was revealed. It is found that the detection of BNP within the 10% serum environment demonstrated good analyte specificity.

3.3. Application

The serum standard sample (1000 pg/mL BNP protein) was diluted to 800, 600, 400, 200, 100, and 10 pg/mL in assay buffer containing 10% serum. Other steps such as incubations, washes and substrate reactions were performed as described above. The assay buffer (10 mM PBS buffer containing 5% BSA) containing 10% serum was also detected as the negative signal. Fig. 4 shows the calibration curve for the detection of BNP in serum dilution using magnetic probe strategy. As shown in Fig. 4, down to 10 pg/mL of BNP protein was detected (S/N $\geq$ 2). The absorbance at 450 nm for 10 pg/mL BNP protein was 0.032. Generally, the BNP concentration in serum of healthy people is less than 20 pg/mL, which goes beyond 100 pg/mL for patients diagnosed with congestive heart failure (CHF), and increases to 1000 pg/mL for patients diagnosed with severe CHF. So, this magnetic probes method can probably be used to detect the serum samples for determining the degree of cardiac failure. As can be seen, the linear range and detection limit of the proposed immunoassay method for BNP are acceptable. Importantly, the consumed time of the developed BNP detection method is shorter than that of the commercial ELISA (>4 h).

To monitor the feasibility of the newly developed immunoassay, a random sample of 88 patients (age range, 4–79 years), were examined by the developed immunoassay. Among these patients, male: 49, female: 39. Patients were divided into 4 age groups: 45 years of age or younger, older than 45–54 years of age, older than 55–64 years of age, and older than 65 years of age. Blood samples were obtained under sterile conditions by venipuncture and collected into EDTA-containing plastic tubes. After blood withdrawal, the samples were stored at 4 °C (up to 1 h) before the measurement of BNP. The experimental results are listed in Table 1. The serum BNP concentration under normal conditions is less than 20 pg/mL, and it increases to more than 100 pg/mL for patients diagnosed with congestive heart failure (CHF) and exceeds 1000 pg/mL for patients diagnosed with severe CHF. By analysis, it is found that the number of patients with CHF increased progressively with age. The patients from 45 years of age or younger could yield an absolute reduction

Table 1
The results of 88 BNP serum samples in clinical trials were classified according to statistics.

	Men and women				
	Total	<45 years	45–54 years	55–64 years	≥65 years
Median (pg/mL)	38	19	59	93	156
≥100 pg/mL (%)	11.4%	3.2%	23.5%	33.3%	60.0%
Minimum (pg/mL)	<10	<10	10	35	55
Maximum (pg/mL)	674	131	142	103	674
Samples/N	88	63	17	3	5

of 8.2% (3.2% vs. 11.4%) compared with the total patients. The risk of CHF was lower for 45 years of age or younger patients (3.2%). However, among patients older than 65 years of age, the risk of CHF could reach to 60% and a relative reduction of 33.3% for older than 55–64 years of age. But the risk of CHF was higher in patients older than 45 years of age (23.5%). Despite an increased risk for CHF in patients older than 45 years of age, a routine detection of BNP strategy could significantly provide a basis for the accurate diagnosis and timely treatment in elderly patients with CHF. This is a preliminary study with relatively small numbers of patients; however, the statistical findings showed the good agreement between the proposed method and clinic results.

4. Conclusions

In this work, a highly sensitive and fast immunoassay method for the measurement of BNP with picogram level was carried out by using a novel magnetic probe strategy. Here, the magnetic probes play a double role. First, the particles are solid support for biomolecules. Second, they are carrier for the antibody, which could be easily manipulated for improving the kinetic of the antibody–antigen interaction and increasing the immunoassay sensitivity. The small size of magnetic particles allowed the affinity of multiple target antigens onto the magnetic probes, which facilitated magnet-mediated separation and fast detection. Furthermore, avidin, which has four capturing sites for biotin, provided both sufficient binding opportunity and binding strength for biotin-labeled antibody. The simple biotin–streptavidin interaction forming the bio-functional magnetic probe, could probably be extended to other detection of protein immunoassay systems, creating a robust immunoassay capable of detecting a variety of clinically relevant serum analyte. The simplicity of this assay required less technical proficiency than conventional sandwich-type ELISA and reduced the risk of experimentation error. The detection limit of this assay for BNP determined is 10 pg/mL, which is about 100-fold improvement compared to the classic ELISA system. Furthermore, the overall analysis time was shortened form 4 h to 30 min. And, these assays also displayed good stability after storage for 30 days.

Acknowledgments

This project is sponsored by the Hi-Tech R. & D. Program of China (No. 2007AA03Z428), the NSFC (No. 60801032), and IECAS Program (07QNCX-9240).

References

[1] T. Sudoh, K. Kangawa, N. Minamino, H. Matsuo, Nature 332 (1988) 78–81.
[2] M. Yoshimura, H. Yasue, E. Morita, N. Sakaino, M. Jougasaki, Circulation 84 (1991) 581–1518.
[3] K. Ryoji, K. Yoshimi, S. Yukari, M. Fumio, N. Osamu, Anal. Chem. 78 (2006) 5525–5531.
[4] Y.J. Teramura, A. Yusuke, I. Hiroo, Anal. Biochem. 357 (2006) 208–215.
[5] C.W. Knudsen, T. Omland, P. Clopton, A. Westheim, W.T. Abraham, A.B. Storrow, J. Mccord, R.M. Nowak, M.C. Aumont, P. Duc, J.E. Hollander, A.H.B. Wu, P.A. Mccullough, A.S. Maisel, Am. J. Med. 116 (2004) 363–368.
[6] H.H. Chen, J.G. Lainchbury, G.J. Harty, J.C. Burnett, Circulation 105 (2002) 999–1003.
[7] X.M. Li, X.Y. Yang, S.S. Zhang, Trends Anal. Chem. 27 (2008) 543–553.
[8] J. Wang, G. Liu, B. Munge, L. Lin, Q. Zhu, Angew. Chem. Int. Ed. 43 (2004) 2159.
[9] N. Rosi, C.A. Mirkin, Chem. Rev. 105 (2005) 1547–1562.
[10] E. Engvall, P. Perlman, Immunochemistry 8 (1971) 871–874.
[11] R. Lequin, Clin. Chem. 51 (2005) 2417.
[12] M. Mukoyama, K. Nakao, K. Hosoda, S. Suga, Y. Saito, Y. Ogawa, G. Shirakami, M. Jougasaki, J. Clin. Invest. 87 (1991) 1402–1412.
[13] H. Shimizu, K. Masuta, K. Aono, H. Asada, K. Sasakura, M. Tamaki, K. Sugita, K. Yamada, Clin. Chim. Acta 316 (2002) 129–135.
[14] M. Mukoyama, K. Nakao, Y. Saito, Y. Ogawa, K. Hosoda, S. Suga, G. Shirakami, M. Jougasaki, H. Imura, Lancet 335 (1990) 801.
[15] A. Frey, B. Meckelein, J. Immunol. Methods 233 (2000) 47–56.
[16] R.S. Yalow, S.A. Berson, Nature 184 (1959) 1648–1649.
[17] C.P. Jia, X.Q. Zhong, B. Hua, M.Y. Liu, F.X. Jing, X.H. Lou, S.H. Yao, J.Q. Xiang, Q.H. Jing, J.L. Zhao, Biosens. Bioelectron. 24 (2009) 2475–2482.
[18] M. Kono, A. Yamauchi, T. Tsuji, A. Misaki, K. Igano, K. Ueki, Nucl. Med. Technol. Soc. 13 (1993) 2–7.
[19] V. Kourilov, M. Steinitz, Anal. Biochem. 311 (2002) 166–170.
[20] J.M. Nam, C.S. Thaxton, C.A. Mirkin, Science 301 (2003) 1885.
[21] E. Zacco, M.I. Pividori, S. Alegret, M.P. Marco, Anal. Chem. 78 (2006) 1780–1799.
[22] M. Tudorache, M. Co, H. Lifgren, J. Emneus, Anal. Chem. 77 (2005) 7156–7162.
[23] M. Tudorache, A. Tencaliec, C. Bala, Talanta 77 (2008) 839–843.
[24] D.Q. Tang, D.J. Zhang, D.Y. Tang, H. Ai, J. Immunol. Methods 316 (2006) 144–152.
[25] R.S. Molday, D. Mackenzie, J. Immunol. Methods 52 (1982) 67–353.
[26] B.Y. Kularatne, P. Lorigan, S. Browne, S.K. Suvarna, M.O. Smith, J. Lawry, Cytometry 50 (2002) 7–160.
[27] S. Morisada, N. Miyata, K. Iwahori, J. Microbiol. Methods 51 (2002) 8–141.
[28] R.E. Zigeuner, R. Riesenberg, H. Pohla, A. Hofstetter, R. Oberneder, J. Urol. 169 (2003) 5–701.
[29] P.A. Liberti, C.G. Rao, L.W.M.M. Terstappen, J. Magn. Magn. Mater. 225 (2001) 7–301.
[30] F. Paul, D. Melville, S. Roath, D. Warhurst, IEEE Trans. Magn. 17 (1981) 4–2822.
[31] N. Seesod, P. Nopparat, A. Hedrum, A. Holder, S. Thaithong, M. Uhlen, J. Lundeberg, Am. J. Trop. Med. Hyg. 56 (1997) 8–322.
[32] J. Zhang, S. Song, L. Zhang, L. Wang, H. Wu, D. Pan, C. Fan, J. Am. Chem. Soc. 128 (2006) 8575–8580.
[33] N.M. Green, Methods Enzymol. 184 (1990) 51–67.
[34] Y. Zhuo, P.X. Yuan, R. Yuan, Y.Q. Chai, C.L. Hong, Biomaterials 30 (2009) 2284–2290.
[35] A. Ambrosi, M.T. Castaneda, A.J. Killard, M.R. Smyth, S. Alegret, A. Merkoci, Anal. Chem. 79 (2007) 5232–5240.
[36] S.P. Kent, K.H. Ryan, A.L. Siegel, J. Histochem. Cytochem. 26 (1978) 618–621.
[37] G. Jie, H. Huang, X. Sun, J. Zhu, Biosens. Bioelectron. 23 (2008) 1896–1899.
[38] O.A. Saleh, L.L. Sohn, Proc. Natl. Acad. Sci. 100 (2003) 820–824.
[39] E. John, M. Roger, F. Marilyn, J. Clin. Microbiol. 10 (1979) 210–217.