Research Article

Rapid and Selective Determination of Folate Receptor α with Sensitive Resonance Rayleigh Scattering Signal

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Received 19 February 2017; Revised 13 April 2017; Accepted 7 May 2017; Published 25 May 2017

A rapid, simple, and novel method for folate receptor α (FRα) determination is reported here. A probe of gold nanoparticles (Au NPs) modified with anti-FRα antibody was synthesized under the optimized conditions first. The antibody-modified Au NPs would aggregate when FRα was added to the probe for the specific interaction between antibody and antigen, resulting in the enhancement of resonance Rayleigh scattering (RRS) intensity. There is a linear relationship between the change of RRS intensity (ΔIRR S) and the concentration of FRα, with the detecting range of 0.50–37.50 ng mL⁻¹ and the limit of determination of 0.05 ng mL⁻¹. The determination of FRα in serum samples was realized with the advantages of high selectivity, high sensitivity, and easy operation.

1. Introduction

Folate receptors (FRs) are a family of glycoproteins on cell membrane [1]. Folate in tissues could be specifically recognized by folate receptors. There are three subtypes of folate receptors expressed on cells, which are FRα (also called FRI), FRβ, and FRγ, respectively. Low level of folate receptors are expressed in normal cells or tissues. However, the expressing level of folate receptors, especially FRα, is greatly increased in most human tumors [2], to fulfill the need of massive folate for the proliferation of cancer cells [3, 4], indicating that the folate receptor could be served as a tumor biomarker for the initiation and progression of cancers and as a therapeutic target for cancer treatments [5]. The expressing level of folate receptor is extremely higher in colon cancers than in normal tissues [5]. Furthermore, the high expression of folate receptor is also associated with other tumors, such as lung cancer [6, 7], breast cancer [8], ovarian cancer [9–11], and brain tumor [5]. So the methodological basis could be provided for early diagnosis and monitoring of cancers by effective and quantitative determination of folate receptor.

Currently, methods for folate receptor determination have been reported, such as fluorescence quenching or imaging [12, 13], electrochemical or electrochemiluminescence biosensors [14–17], and colorimetric detection [18–20]. For these methods, fluorescent dyes, electrochemical luminescent dyes, or expensive instruments are needed, terminal protection of small-molecule-linked DNA should be done first, or the detection is not sensitive enough. So it is still significant to set up simple and rapid ways to determine FRα for the early monitoring of cancers.

In this contribution, FRα is determined using the distinctive resonance Rayleigh scattering (RRS) property of gold nanoparticles (Au NPs). RRS technology is well known for the high sensitivity and the convenience in performance and apparatus (common fluorophotometer). RRS method has been widely used to determine metal ions [21], biomolecules [22], medicines [23], pesticides [24], and so forth. Au NPs could be usually used as RRS probe for the special optical property. In our work, anti-FRα antibody was modified on the surface of Au NPs under optimized conditions, which was accomplished easily. In other words, Au NPs
probe was made first. The antibody-modified Au NPs would aggregate when FRα was added to the probe, resulting in the enhancement of RRS intensity, for the specific interaction between anti-FRα antibody on the surface of Au NPs and FRα antigen. Meanwhile, there is a linear relationship between the change of RRS intensity ($\Delta I_{RRS}$) and the concentration of FRα, with the detecting range of 0.50–37.50 ng·mL$^{-1}$, the limit of determination of 0.05 ng·mL$^{-1}$, and the correlation coefficient of 0.9996. There are other methods reported for the determination of FRα. Compared to these methods, the advantages of our method are listed as follows. Firstly, the sensitivity of determination is guaranteed for RRS intensity is used as the response signal. Secondly, FRα could be determined with high selectivity, since it is based on the specific interaction between anti-FRα antibody and antigen in our method. Finally, the modification of anti-FRα antibody on the surface of Au NPs is simple to be carried out and the RRS performance is convenient to be done with a common and cheap fluorophotometer.

2. Experimental

2.1. Materials and Reagents. Au NPs were synthesized with Na$_3$C$_6$H$_5$O$_7$·2H$_2$O and HAuCl$_4$·4H$_2$O, 1 mg·mL$^{-1}$ Anti-FRα (anti-FOLRα, anti-FOLR1, and FRα) polyclonal antibody was purchased from Sigma-Aldrich Corporation (Missouri, USA). 10 µg·mL$^{-1}$ of Anti-FRα working solution was diluted with sterile PBS buffer (0.01 M, pH 7.4). Lyophilized powder of folate receptor α (FOLR1, FRL, and FRα) was purchased from Sigma-Aldrich Corporation (Missouri, USA). 50 µg·mL$^{-1}$ of FRα stock solution was made by dissolving the lyophilized powder with sterile PBS buffer (0.01 M, pH 7.4). 1 µg·mL$^{-1}$ of working solution was diluted with the sterile PBS buffer. 10.0% BSA, 1.0% NaN$_3$, 10.0% NaCl, and BR buffer were used when anti-FRα is modified on the surface of Au NPs.

2.2. Apparatus. RRS spectra and intensity were measured with a LS55 fluorescence spectrophotometer (Perkin Elmer, USA). Other instruments are the same as that in our previous work [25].

2.3. Synthesis of Anti-FRα-Antibody-Modified Au NPs Probe. The synthesis procedure of original Au NPs was mentioned in [25]. The concentration of Au NPs was calculated based on Lambert-Beer law; $A = ebc$. The extinction coefficient $e$ for 13-nm Au NPs is $2.7 \times 10^8$ M$^{-1}$·cm$^{-1}$ [26]. The average size of Au NPs we synthesized is about 13 nm according to TEM images in Figure 2. So we use this extinction coefficient to calculate the concentration of Au NPs. The absorption intensity of Au NPs solution was 0.487 after fivefold dilution, which was detected in a 1-cm absorption cell. According to the equation $A = ebc$, the original concentration of Au NPs solution was calculated to be 9.0 nM. Afterward, Au NPs need to be modified with anti-FRα antibody, which can be served as a probe for FRα determination. The principle and the detailed method of how we optimize the condition of pH and antibody concentration were described in [25], but the concentrations of certain substances are different. For pH optimization in this contribution, 200 µL of Au NPs solution with onefold dilution, 35 µL of doubly distilled water, 30 µL of BR buffer with certain pH, and 15 µL of 10 µg·mL$^{-1}$ anti-FRα antibody were added. Then 20 µL of 10.0% NaCl was added to test the stability of the antibody-modified Au NPs on certain pH conditions. The optimizing process of anti-FRα antibody was carried out in the same way.

2.4. Procedure in Detail for FRα Determination. First, the interaction between antibody-modified Au NPs probe and FRα was carried out in a 1.5 mL EP tube. 50 µL of well-modified Au NPs probe solution and various concentrations of FRα working solution were added to EP tubes. Second, different volume of doubly distilled water was added to keep the total volume of 400 µL, with mixing thoroughly and keeping the tubes at room temperature for 10 min. The color of the mixture changed from light red to light blue. Third, RRS spectra and intensity were measured with the detecting wavelength range of 500 nm to 700 nm, for there is a characteristic RRS peak within this range. RRS signal was obtained using a LS55 fluorescence spectrophotometer, by means of synchronous scanning at $\Delta \lambda = 0$ ($\lambda_{ex} = \lambda_{em}$) with slit width of 10 nm.

2.5. Pretreatment and Determination for Real Samples. The serum sample was obtained from the Southwest Hospital in Chongqing and stored at 4°C. The serum was diluted 10 times with sterile PBS (0.01 M, pH 7.4) before use. Two different concentrations of standard FRα were added to the serum samples, respectively. And then the determination was realized by calculating the recovery of standard addition.

3. Results and Discussion

3.1. Characteristics of RRS Spectra and TEM Images. RRS spectra of the interaction between antibody-modified Au NPs and FRα are shown in Figure 1. Curves 1 and 2 represent the original Au NPs and antibody-modified Au NPs, respectively. What we can see from Curves 1 and 2 is that RRS spectra of the original Au NPs and antibody-modified Au NPs are slightly distinct, which is because the surface of Au NPs had been changed after Au NPs were modified with anti-FRα antibody. A characteristic RRS peak is located at 590 nm when the interaction occurs between FRα and antibody-modified Au NPs probe (from Curve 3 to Curve 6). And the characteristic RRS intensity is enhanced gradually with the increasing concentration of FRα. So the quantitative determination of FRα is set up, based on the linear relationship between the change of RRS intensity and FRα concentration.

The distance among antibody-modified Au NPs was shortened and antibody-modified Au NPs aggregated when
FRα was added, for the specific interaction between antibody and antigen. The more FRα was added, the stronger RRS intensity would be, within the certain concentration range of FRα. The enhancement of RRS intensity when FRα is added to the antibody-modified Au NPs probe is essentially caused by the aggregation of Au NPs, which could be proved by TEM images (Figure 2). It is shown that the antibody-modified Au NPs are dispersed well (Figure 2(a)) but aggregate dramatically when FRα is added (Figure 2(b)).

3.2. Optimization of pH and Antibody Concentration When Au NPs Were Modified with Anti-FRα Antibody. The process of Au NPs modification with anti-FRα antibody could be affected by pH of the buffer. So pH condition was optimized first. The well-modified Au NPs would not aggregate and RRS intensity would not increase with relatively high concentration of NaCl and the appropriate pH condition was got under this principle. Anti-FRα antibody was added to Au NPs solutions at different pH conditions. After mixing and incubating, NaCl solution was added to the mixture. It is shown in Figure 3 that Au NPs aggregate and RRS intensity is increased with the addition of NaCl when pH is less than 6.09 and RRS intensity remains stable when pH is higher than that value, which demonstrates that Au NPs could be well modified under neutral and alkaline conditions. Finally, a neutral pH of 7.00 is chosen based on the result.

The concentration of antibody must be taken into account as well when Au NPs were modified with anti-FRα antibody. The modification efficiency would be affected by the antibody concentration, which was described in this part. It is shown in Figure 4 that RRS intensity is increased with the addition of NaCl when the antibody concentration is less than 0.40 μg·mL⁻¹, which is because the whole surface of Au NPs could not be adsorbed thoroughly with fewer antibodies. However, RRS intensity stays stable when the antibody concentration is over the range of 0.40–0.60 μg·mL⁻¹, indicating that Au NPs could be well modified under this condition. Considering the losses in the real process of operation, we choose 0.50 μg·mL⁻¹ as the optimal concentration of anti-FRα antibody.

3.3. Selectivity for FRα Determination. We analyze the impact of the coexisting substances listed in Table 1 to investigate the selectivity for the determination of FRα. FRα with the final concentration of 25.00 ng·mL⁻¹ and a certain concentration of a coexisting substance were added to the determination system. The change in RRS intensity was compared with the situation without the coexisting substance. What is shown in Table 1 is that the foreign substances, such as saccharides, proteins, amino acids, and metal ions, would not affect the detection of FRα, for the change in RRS intensity at 590 nm is within the permissible range. Theoretically, the determination for FRα is realized through the interaction between antibody and antigen. Anti-FRα antibody modified on the surface of Au NPs could specifically recognize FRα, which guarantees the high selectivity of FRα determination.

| Coexisting substances | Conc. (μg mL⁻¹) | Change in RRS intensity (%) | Coexisting substances | Conc. (μg mL⁻¹) | Change in RRS intensity (%) |
|----------------------|-----------------|-----------------------------|----------------------|-----------------|-----------------------------|
| Sucrose              | 85.58           | +1.02                       | Vc                   | 0.25            | −2.63                       |
| Glucose              | 45.04           | +0.93                       | Histone              | 2.50            | +3.21                       |
| Lactose              | 85.58           | +0.85                       | Myoglobin             | 5.00            | +1.95                       |
| Starch               | 25.00           | +1.08                       | K⁺                   | 4.88            | −2.62                       |
| His                  | 0.39            | +3.18                       | Ca²⁺                 | 0.79            | −1.14                       |
| Pro                  | 2.87            | −1.39                       | Al³⁺                 | 0.67            | +2.99                       |
| Thr                  | 2.98            | +2.19                       | Zn²⁺                 | 1.63            | +1.08                       |
| Phe                  | 4.13            | +0.98                       | Mg²⁺                 | 0.60            | +1.52                       |
| Cys                  | 3.03            | +1.85                       | NH₄⁺                 | 45.00           | +4.23                       |
| BSA                  | 12.50           | +1.28                       |

Figure 1: RRS spectra for the interaction between antibody-modified Au NPs probe and FRα. Curve 1, original Au NPs; Curve 2, antibody-modified Au NPs probe; Curves 3–6, interaction between the probe and FRα. Concentrations: FRα (Curves 3–6, ng·mL⁻¹), 2.50, 12.50, 25.00, and 37.50.
Figure 2: TEM images for antibody-modified Au NPs probe (a) and the interaction between the probe and FRα ((b) concentration of FRα, 12.50 ng·mL⁻¹).

Table 2: Determination of FRα in real samples (n = 10).

| Sample number | FRα added (ng·mL⁻¹) | FRα detected (ng·mL⁻¹) | Recovery (%) | RSD (%) |
|---------------|----------------------|------------------------|--------------|---------|
| 1             | 12.50                | 12.69                  | 93.77–105.59 | 4.12    |
| 2             | 25.00                | 23.64                  | 90.88–98.64  | 2.91    |

Figure 3: Effect of pH condition on the modification of Au NPs with anti-FRα antibody. Concentrations: Au NPs, 3.0 nM; anti-FRα, 0.50 μg·mL⁻¹; NaCl, 0.67%; pH, 1.98, 2.87, 4.10, 5.02, 6.09, 7.00, 7.96, 8.95, 9.91, 10.88, and 11.92.

The relationship between ΔI_RRS and FRα concentration over the range of 0.50–37.50 ng·mL⁻¹, with the linear regression equation, ΔI_RRS = 200.72 + 11.93c. The limit of determination (LOD) is 0.05 ng·mL⁻¹, and the correlation coefficient is 0.9996.

To further validate the accuracy and feasibility of the method presented here, we determined the concentration of FRα in two real samples with ten parallel repeats for each one. The determination was realized by calculating the recovery of standard addition in diluted human serums, which is shown in Table 2. The detection recovery is between 90.88 and 105.59% and RSD is 4.12% and 2.91%, respectively. What we can see from the result is that the method is accurate and applicable to quantitatively determine FRα in serums.

4. Conclusion

In this contribution, anti-FRα antibody was stably modified on the surface of Au NPs under optimal conditions, so that effective Au NPs probe was synthesized for the determination
of the tumor biomarker FRα. The quantitative determination of FRα is realized with resonance Rayleigh scattering signals, according to the linear relationship between $\Delta I_{RBS}$ and FRα concentration, with the advantages of easy operation, high sensitivity, and excellent selectivity.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

**Acknowledgments**

All authors herein are grateful for the support from the Key Program of National Natural Science Foundation of China (81230064) and Third Military Medical University Young Creative Talent Fund (2011XQN10).

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