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Functionalized fluorescent core-shell nanoparticles used as a fluorescent labels in fluoroimmunoassay for IL-6

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Abstract

Nanoparticle labels conjugated with biomolecules are used in a variety of different assay applications. In this paper, a sensitive fluoroimmunoassay for recombinant human interleukin-6 (IL-6) with the functionalized Rubpy-encapsulated fluorescent core-shell silica nanoparticles labeling technique has been proposed. IL-6 was measured based on the specific interaction between captured IL-6 antigen and functionalized fluorescent core-shell nanoparticles-labeled anti-IL-6 monoclonal antibody. The calibration graph for IL-6 was linear over the range 20–1250 pg ml^{-1} with a detection limit of 7 pg ml^{-1} (3σ). The regression equation of the working curve is $I_F = 7.665 + 32.499$ [IL-6] (ng ml^{-1}) ($r = 0.9980$). The relative standard deviation (R.S.D.) for 11 parallel measurements of 78 pg ml^{-1} IL-6 was 3.2%. Furthermore, the application of fluorescence microscopy imaging in the study of the antibody labeling and sandwich fluoroimmunoassay with the functionalized fluorescent core-shell silica nanoparticles was also explored. This proposed method has the advantage of showing the specificity of immunoassay and sensitivity of fluorescent nanoparticle labels technology. The results demonstrate that the method offers potential advantages of sensitivity, simplicity and reproducibility for the determination of IL-6, and is applicable to the determination of IL-6 in serum samples and enabling fluorescence microscopy imaging for the determination of IL-6.

Keywords: IL-6; Functionalized; Fluorescent core-shell nanoparticle; Rubpy; Fluoroimmunoassay; Fluorescence microscopy imaging

1. Introduction

At present, immunoassays using antibody–antigen recognition for the detection and quantification of target analytes are extensively applied in both clinical and environmental monitoring. Each of these methods has its disadvantages. Radioimmunoassays (RIAs) used in the early 1970s were selective and relatively sensitive but required the use of radioactive tracers of the toxins (Lam et al., 1999). The enzyme-linked immunosorbent assay (ELISA) technique has enabled the production of commercial assays for many analytes that provide high selectivity and allow for the use of nonradioactive reagents. However, active enzymes which require special storage and carcinogenic reagents are often required in these colorimetric assays (Giletto and Fyffe, 1998). Chemiluminescence immunoassay has definite advantages such as a high sensitivity, rapidity, simplicity, feasibility, and low cost, both in instrumentation and materials. It is well-applied in bioanalytical and clinical chemistry (Baeyens et al., 1998; Roda et al., 1996; Dodeigne et al., 2000), which can exploit the ultrahigh sensitivity of chemiluminescence and the high selectivity of immunoassay. Although there are many reports of applications of chemiluminescence immunoassay, careful manipulation and ultrapure water are required in the experiment in order to eliminate the interference factors arising from the experimental surroundings and the solution because of the sensitivity of chemiluminescence signal to trace metal ions in the water and samples. Fluoroimmunoassays have been widely used in the clinical monitoring of and scientific research of complex samples (Goldman et al., 2002; Rowe et al., 1999). However, these traditional fluoroimmunoassays suffer from the disadvantages of inefficiency of labeling, poor photostability, and relatively low fluorescence intensities of dyes such as fluorescein isothiocyanate (FITC) (Dyba and Hell, 2003; Fang and Tan, 1999). Other methods are often costly, sophisticated,

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or time consuming (Petrovas et al., 1999). The establishment of both simple and sensitive immunoassay analytical methods is needed to enable earlier detection and quantification of disease markers, thereby improving the likelihood of rapid treatment. This has motivated scientists. Recently fluorescent nanoparticles labels have entered the field as an extremely promising method of replacing established RIAs, ELISA and traditional fluoroimmunoassays (Cummins et al., 2006).

Nanoparticle labels contribute to the design of next generation immunoassays with many advantages, such as lower detection limits, low consumption of reagents, and excellent specificity and sensitivity (Harma et al., 2003; Seydack, 2005; Valanne et al., 2005). In the past few years, one of the most widely studied nanoparticles for labeling was the fluorescent core-shell nanoparticles (Karst, 2006; Yang et al., 2004; Santra et al., 2001a,b; Bagwe et al., 2004). In comparison to conventional organic dye labels, the fluorescent core-shell nanoparticle labels offer the advantages of both higher photostability to the excitation of light and stronger luminescence resulting in an amplification factor similar to that of enzyme labels because thousands of fluorescent dye molecules are encapsulated in the matrix that also serves as a shield to protect dye from photobleaching (Santra et al., 2001a,b; Lian et al., 2004; Yang et al., 2004). Fluorescent core-shell nanoparticles also allow for faster analysis because one fewer reaction step is required than when enzyme labels are used. Moreover, they can be prepared easily under mild conditions with inexpensive reagents and simple procedures. With their uniform sizes and high functionality, they can be modified easily. These advantages make them the ideal dye for various science research and clinical applications (Bagwe et al., 2004). For example, Valanne and Lian et al. (Lian et al., 2004; Valanne et al., 2005; Tan et al., 2004, 2006; Ye et al., 2004, 2005; Huhtinen et al., 2004, 2005; Soukka et al., 2001, 2003) have developed several kinds of novel rare earth-doped nanoparticles for immunoassay. Their experimental results suggest that immunoassay based on rare earth-doped nanoparticle labels is sensitive and significantly more rapid and convenient compared to free dye labels immunoassay. Yang has reported a new fluoroimmunoassay for α-fetoprotein and Hepatitis B Surface Antigen based on fluorescent hybrid silica nanoparticle labels demonstrating easy preparation, good photostability and low toxicity of nanoparticles and high sensitivity in the biochemical assays (Yang et al., 2003, 2004). A solid-substrate room-temperature phosphorescence immunoassay based on an antibody labeled with nanoparticles containing dibromofluorescein and Rhodamine 6G luminescent molecules was also developed by Liu for the determination of human IgG (Liu et al., 2005a,b). Other researchers have reported successful results with microarray immunoassay, room temperature phosphorescence immunoassay, and two-photon excitation assay technology based on the nanoparticles labels (Feng et al., 2003; Nichkova et al., 2005; Koskinen et al., 2004).

In this work, a novel sandwich fluoroimmunoassay for IL-6 with functionalized Rubpy-encapsulated fluorescent core-shell silica nanoparticles labels has been developed. IL-6 was measured based on the specific interaction between captured IL-6 antigen and functionalized fluorescent core-shell nanoparticles-labeled anti-IL-6 monoclonal antibody. The method offers potential advantages of sensitivity, simplicity and reproducibility for the determination of IL-6, and is applicable to the determination of IL-6 in serum samples. In addition, we explore the application of fluorescence microscopy imaging in the determination of the IL-6 with the functionalized fluorescent core-shell nanoparticles.

2. Experimental

2.1. Reagents and solutions

IL-6, two monoclonal antibodies against rh IL-6 were kindly donated by Prof B.Q. Jin (Department of Immuno, The Fourth Military Medical University, Xi'an, China). Bovine serum albumin (BSA) and $Ru(bpy)_3Cl_2$ (Rubpy) were obtained from Sigma (USA). (3-Aminopropy1)triethoxysilane (APS) was obtained from Acros (Belgium). Na_2CO_3, $NaHCO_3$, KH_2PO_4, Na_2HPO_4, NaCl, KCl, Tween-20, NaH_2PO_4 and $NH_3{\cdot}H_2O$ (28–30 wt.%) were purchased from Xi'an Chemical Reagent Company (Xi'an, China). TEOS, *n*-hexanol, Triton X-100 (TX-100) and cyclohexane were obtained from Shanghai Chemical Plant (Shanghai, China). Distilled, deionized water was used for the preparation of all aqueous solutions. Unless otherwise stated, all chemicals and reagents used in this study were of analytical grade quality. The 96-well transparent microtiter plates used for the assay were obtained from Corning Incorporated (USA).

All solutions were prepared in deionized, distilled water. The coating solution was 0.05 mol l^{-1} carbonate buffer, pH 9.6, containing 1.59 g Na_2CO_3 and 2.93 g $NaHCO_3$/l. 0.15 mol l^{-1} PBS buffer, pH 7.4 was prepared by dissolving 0.2 g KH_2PO_4, 2.9 g Na_2HPO_4, 8.0 g NaCl and 0.2 g KCl in 1 l water. Ninety-six-well plates were rinsed with PBST solution (PBST: PBS solution containing 0.05% (v/v) Tween-20). IL-6 antigen and antibody were diluted with PBSTB solution (PBSTB: PBST containing 0.1% (w/v) BSA).

2.2. Synthesis of functionalized fluorescent core-shell nanoparticles

Synthesis of functionalized fluorescent core-shell nanoparticles was carried out according to methods described by a previous paper (Santra et al., 2001a,b; Bagwe et al., 2004) with little change. The details of the procedure are described in the following. The W/O microemulsion was prepared at room temperature first by mixing 1.77 ml surfactant TX-100, 7.5 ml oil phase cyclohexane and 1.8 ml cosurfactant *n*-hexanol. 0.48 ml Rubpy dye solution was then added. Then the resulting mixtures were homogenized with magnetic force stirring to form a water-in-oil microemulsion. In the presence of 100 μl of TEOS, a hydrolyzation reaction was initiated by adding 60 μl of $NH_3{\cdot}H_2O$ under stirring. The reaction was allowed to stir for 12 h. Then, 100 μl of APS and 60 μl of $NH_3{\cdot}H_2O$ were added to the mixture under stirring. The reaction was allowed to stir for another 12 h. After the reaction was completed, acetone was added to break the microemulsion and recover the particles. The contents were then centrifuged and