Chip-Based Energy Transfer System between Quantum dots and Gold Nanoparticles for Analysis of Protein Glycosylation

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ABSTRACT

We demonstrate a chip-based detection of protein glycosylation by using the energy transfer between quantum dot (QDs) and gold nanoparticles (AuNPs). Our system relies on modulations in the energy transfer between the nanoparticles on a surface; that is, the photoluminescence (PL) of lectin-coated QD (energy donor) immobilized on a glass slide is quenched by carbohydrate-coated AuNPs (energy acceptor), and the presence of the glycoprotein causes the increase of the PL of QD. As a model system, Concanavalin A-coated QD (ConA-QD) and dextran-coated AuNP (Dex-AuNP) were employed to detect the mannosylated proteins, showing that the linear signals were observed for the concentration as well as the number of glycan moiety of the glycoprotein. Our system will be promising for analysis of glycoproteins with high selectivity and sensitivity in a multiplexed and high-throughput manner.

Keywords: glycosylation, glycan, energy transfer, quantum dot, gold nanoparticle

1 INTRODUCTION

Protein glycosylation is one of the most essential processes in the living systems, and often is a major target for developing therapeutics and diagnosis [1, 2]. To this end, many methods such as affinity chromatography [3], capillary electrophoresis-mass spectrometry [4], and surface plasmon resonance (SPR) [5] have been developed to analyze glycoproteins. However, most of them are restricted in a rapid read-out. Alternatively, carbohydrate- or lectin-based microarrays have been employed for high-throughput analysis of glycoproteins by using fluorescently labeled probes [6, 7]. Despite to many advantages of such array-based methods, a major hurdle is that several cycles of affinity binding and washing steps are necessary together with labeling molecules such as lectins, antibodies or glycoproteins. In addition, the use of the organic fluoreophore still remains formidable due to the photobleaching issue.

To circumvent this limitation, we describe herein a chip-based system for the detection of protein glycosylation using the energy transfer between quantum dots (QDs) and gold nanoparticles (AuNPs). QD-based Förster resonance energy transfer (FRET) system is of particular interest owing to several advantageous properties, including high quantum yield, multiplexed analysis in a single excitation wavelength, and high resistance to chemical and photodegradation [8, 9]. In addition, it has been reported that the combined use of QDs and AuNPs in a FRET-based system can be very effective for detecting biomolecular interactions in solution [10, 11]. Yet, little attention has been paid to chip-based system, which could have great potential for the high-throughput screening of glycoproteins.

Compared with solution-based systems, our chip-based format could afford more reliable analyses with no aggregation of nanoparticles, requiring a much smaller amount of reagents. Moreover, the energy transfer mechanism taking place between the nanoparticles could allow high sensitivity for the detection of glycoproteins.

2 EXPERIMENTAL

Dextran-conjugated AuNPs were synthesized by mixing a thiolated dextran and the AuNPs. Thiolated dextran was synthesized by covalent modification of the amino groups of dextran with 2-iminothiolane. The final concentration of the conjugate in solution was calculated using the molar extinction coefficient (1.0×10^7 M^{-1} cm^{-1}) for the 5-nm AuNPs at 513 nm. Carboxyl QD and lectin were conjugated by EDC modification. The final concentration of the QDs (200 nM) was determined by measuring the absorbance of the solution at 488 nm and using molar extinction coefficients of 1.3×10^5 M^{-1} cm^{-1} (QD525) and 1.1×10^6 M^{-1} cm^{-1} (QD605) (from the manufacturer's specifications for the carboxyl QDs at 488 nm). For the synthesis of neoglycoproteins such as Man-BSA or Gal-BSA, a portion of the surface lysine residues of BSA were modified with α-D-mannopyranosyl phenyl isothiocyanate (MPI) or β-D-galactopyranosyl phenyl isothiocyanate (GPI) through covalent bonding between isothiocyanate and ε-amine groups. For the chip-based analyses, a multiwell-type chambered silicon cover-slip (ϕ 3mm × H 1 mm, Sigma) was overlaid onto an NHS-derivatized hydrogel glass slide (Nexterion). A solution (10 μL) containing 10 nM ConA-QDs in HEPES buffer (pH 7.4) was dropped into the wells formed by the chambered silicon cover-slip. The slide was incubated for 1 h at room temperature, and the QD-immobilized wells then were immersed in a solution of 2 % BSA (in 50 mM HEPES buffer) for 1 h to block the remaining NHS groups. After rinsing with distilled water, the wells were incubated either in a solution of Dex-AuNPs (10 μL at 100 nM) or in a mixture of Dex-AuNPs and glycoproteins (Man-BSA, Gal-BSA, or fetuin). Unless otherwise stated, the reaction solutions contained Ca^{2+},

Mn^{2+}, and Mg^{2+} at a final concentration of 100 μM to enhance the binding affinity between ConA and carbohydrate groups. Next, the slide was incubated for an additional 1 h at room temperature. The wells on the slide were then rinsed three times with distilled water and dried with a stream of nitrogen (N_2). An equal amount of BSA was used as another control. After measuring the PL intensity in each well, changes in energy transfer efficiency with respect to different concentrations of glycoprotein or varying numbers of mannose groups per BSA molecule were calculated along with the resulting IC_{50} values. Fluorescence scanning was carried out by using an arrayWoRx^e slide scanner (Applied Precision, USA). After scanning, spot fluorescence intensities were analyzed by using imaging software (GenePix Pro 4.0, Axon), and the mean signal intensities and standard deviations of the respective spots were calculated from two independent experiments.

The energy transfer between the QDs and AuNPs can be determined from the quenching efficiency (Q_E) of the experimentally obtained PL data. Considering multiple acceptors per quantum dot, the overall efficiency can be represented as:

$$Q_E = 1 - \frac{PL_{DA}}{PL_D} = \frac{nR_0^6}{nR_0^6 + r^6} \quad (1)$$

Where PL_D is the PL intensity of the donor alone, PL_{DA} is the PL intensity of the donor in the presence of acceptor(s), R_0 is the Förster distance, r is the separation distance from the center of the QD to the acceptor, and n is the number of surface-bound acceptors.

3 RESULTS AND DISCUSSION

Basic principle relies on modulation in the energy transfer efficiency between lectin-conjugated QDs (Lec-QDs) and carbohydrate-conjugated AuNPs (Carbo-AuNPs) (Figure 1). While Lec-QDs are immobilized on an amine-reactive glass surface, Carbo-AuNPs function as a quencher in close proximity. (Figure 1A). In case that glycoproteins co-exist in a solution of Carbo-AuNP (Figure 1B), the energy transfer between Lec-QD and Carbo-AuNP is reduced by a competitive inhibition, which results in the increase of the photoluminescence (PL) of QD.

We attempted to quantify the glycoproteins in terms of their concentration and the number of glycan moieties. As a model system, concanavalin A-conjugated QDs (ConA-QDs) and dextran-conjugated AuNPs (Dex-AuNPs) were used as energy donors and acceptors, respectively. The carboxy QDs were conjugated with ConA by the EDC modification, and the Dex-AuNPs were sysnthesized by attaching thiolated dextran on the surface of 5nm-AuNP. While the Dex-AuNPs quenched the ConA-QDs immobilized on glass, mannosylated BSA resulted in the change in the PL emission of QD-AuNP conjugates. This is

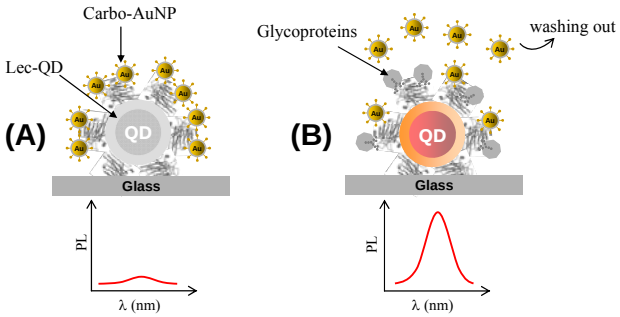

Figure 1: General principle for detection of the glycoprotein based on the energy transfer between lectin-conjugated QDs (Lec-QDs) and carbohydrate-conjugated AuNPs (Carbo-AuNP) on a glass slide; (A) without glycoprotein and (B) with glycoprotein

mainly because ConA can specifically bind with mannose or glucose moiety of glycoproteins. Typically, the PL emission of ConA-QDs increased as the concentration of Man-BSA increased, but the detection range and limit were different with the number of mannose moiety; that is, the IC_{50} values of 5-Man-BSA (BSA with 5 mannose residues) and 22-Man-BSA (BSA with 22 mannose residues) were estimated to be 39 μM and 820 nM, respectively (Figure 2A). This result indicates that BSA with a high degree of mannosylation enhanced the detection limit in this energy transfer system. To further test this possibility, we examined the PL emission of conA-QD by the different numbers of mannose residues conjugated to BSA. As a result, the increasing occupancy number of mannose residues on the BSA led to the increasing PL emission (Figure 2B). Based on these results, our system is expected to be effectively used for the quantitative analyses of the degree of glycan.

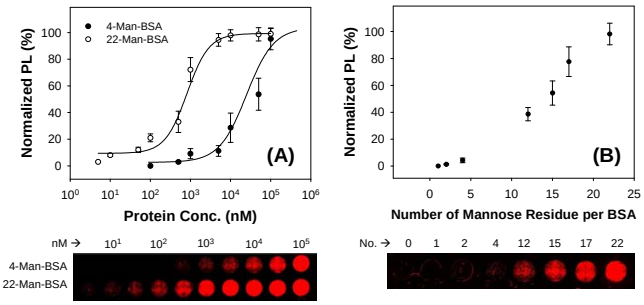

Figure 3: Changes in the PL intensity for the ConA-QD and Dex-AuNP systems as a function of either (A) the concentration of mannosylated BSA (5-Man-BSA and 22-Man-BSA) or (B) the number of conjugated mannose residues per BSA molecule. Error bars indicate the standard deviation derived from duplicate experiments.

To obtain an insight into energy transfer, we examined the quenching efficiency (Eq. 1) of AuNPs to QDs. Compared with the fluorophore-labeled dextran (Dex-Alexa647), Dex-AuNPs showed much higher quenching efficiency (78.2% in Figure 3A and 35.4% in Figure 3B). The strong quenching ability and multiple binding pattern of the AuNPs to QDs might enable the present energy transfer system to become effective beyond the traditional FRET distance. Indeed, it should be noted that the use of AuNPs as an energy acceptor has been shown to extend the effective energy transfer distance up to 22 nm [12].

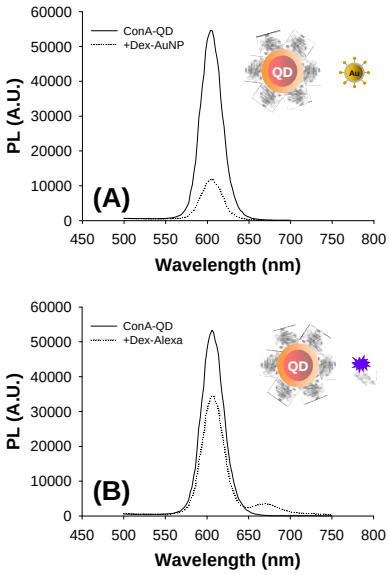

Figure 3: Quenching spectra of donor ConA-QD605 toward different acceptors: (A) Dex-AuNP and (B) Dex-Alexa647. The PL emissions of ConA-QD605 were displayed in the absence (solid line) and presence (dotted line) of energy acceptor. The molar ratio of acceptor to donor was 10:1.

To check whether our chip-based system has specificity among glycoproteins, different glycoproteins (Man-BSA, Gal-BSA, and Futuin) and non-glycoprotein (native BSA) were tested on the conjugate of ConA-QD and Dex-AuNPs (Figure 4). Since BSA with mannose moiety has a relatively high binding affinity to ConA-QD, addition of Man-BSA led to the notoble change in PL intesntiy, compared to other proteins. In constrast, there were no strong intensities in additions of native BSA, galactose-attached BSA (Gal-BSA), or sialic acid-rich fetuin. This result indicates that our chip-based system is specific for the mannose-conjugated proteins. The existence of metal ions (Ca^{2+}, Mn^{2+}, and Mg^{2+}) in the reaction solution enhanced the binding affinity between ConA-QD and Dex-AuNP, resulting in a higher degree of quenching and further improving the detection sensitivity for target glycoproteins (data not shown). Furthermore, the use of QDs with different colors (QD525; Figure 4A and QD605; Figure 4B) gave rise to similar changes in PL intensity as a result of specific binding events. This result clearly indicates that the Dex-AuNPs can be employed as common energy acceptors for different kinds of ConA-QDs, and thus our system might be suitable for multiplexed assay system to detect glycoproteins.

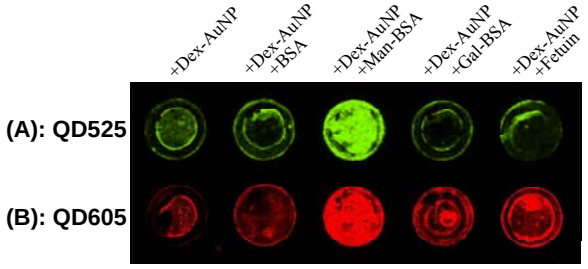

Figure 4: On-chip detection of glycoproteins using concanavalin A-modified QDs (ConA-QDs) and dextran-conjugated AuNPs (Dex-AuNPs). The PL intensities of (A) ConA-QD525 and (B) ConA-QD605 were observed in the presence or absence BSA, mannosylated BSA, galactosylated BSA, and fetuin. The concentrations of ConA-QDs, Dex-AuNPs, and glycoproteins were 10, 100, and 500 nM, respectively.

4 CONCLUSIONS

In conclusion, the chip-based detection of glycoproteins was developed based on the energy transfer between biofunctionalized QDs and AuNPs. Our system enabled a rapid and sensitive analysis of protein glycosylation in terms of the concentration as well as the number of glycan moiety. Since the AuNPs can be commonly employed as energy acceptors with different QDs, our system will contribute to develop a multiplexed assay of glycoproteins using various combinations of lectins and carbohydrate ligands in a high-throughput manner.

ACKNOWLEDGMENTS

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