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ONE-STEP PREPARATION OF PROTEIN A/G-CONJUGATED CdSe/MSA QUANTUM DOTS FOR ULTRAFAST WESTERN BLOT VISUALIZATION

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ABSTRACT

Protein A/G (pA/G) has high affinity with the fragment crystallizable (Fc) region of antibodies so is used as a linker to attach antibodies to quantum dots (QDs), thereby helps the antigen-binding fragment (Fab) to expose outwardly. The present study deduced the interactive nature between pA/G and mercaptosuccinic acid-coated CdSe (CdSe/MSA) QDs by using different reagents to interfere the binding formation. Besides, the use of pA/G-conjugated QDs (QDs-pA/G) in Western blot was tested by using QDs-pA/G associated with anti-GST antibodies to label mPrPc-GST protein in *Escherichia coli* lysates. The results showed that most of the pA/G could not bind to QDs in presence of 2% sodium dodecyl sulfate, 0.1 M 1,4-dithiothreitol and 1% tween 20. The study revealed that pA/G was mostly bound to CdSe/MSA QDs by non-covalent interactions. The QDs-pA/G was successfully applied in direct Western blot with a stronger signal and half-time consuming as compared to conventional technique.

Keywords: CdSe/MSA, protein A/G (pA/G), Quantum Dots (QDs), Western blot

INTRODUCTION

Quantum dots (QDs), the semiconductor nanocrystals, have been applied in biology as potential fluorophores because of their unique photophysical properties including tunable size and narrow emission spectra, large absorption coefficients, high fluorescence quantum yields, photochemical robust, and photo-bleaching resistance (Rosenthal *et al.*, 2011). Biocompatible QDs are designed by three main steps: core synthesis, surface modification, and bioconjugation with antibodies, peptides or small molecules (Valizadeh *et al.*, 2012). Using antibody-QDs conjugate is the simplest and quickest labeling route. Antibodies can be directly conjugated to QDs by biotin-streptavidin interactions, or covalent bonds (Rosenthal *et al.*, 2011). However, antibodies are randomly bound to QDs surface if using these methods. An efficient strategy is to use linker proteins such as protein A, protein G, or protein A/G (pA/G), which specifically bind to the fragment crystallizable (Fc) region of antibodies (Foubert *et al.*, 2016). In our previous studies, mercaptosuccinic acid-coated CdSe (CdSe/MSA) QDs were synthesized in one-step and conjugated with pA/G without the support of crosslinkers (Vo *et al.*, 2020). These CdSe/MSA-pA/G conjugates were successfully associated with antibodies and applied in immunocytochemistry and immunohistochemistry (Tran *et al.*, 2019, 2020; Vo *et al.*, 2020). However, the interaction between pA/G and CdSe/MSA QDs and in many other non-covalent bioconjugation approaches have not yet been confirmed (Zrazhevskiy and Gao, 2013; Tsuboi *et al.*, 2017). The present work was aimed to study the interaction between pA/G and CdSe/MSA QDs and then apply the pA/G-conjugated QDs in Western blot.

MATERIALS AND METHODS

Deduction of interactive nature between QDs and pA/G

The 500 µL reaction contained 250 µL CdSe/MSA QDs (1 mg mL^{-1}) (provided by Department of Applied Physics, University of Science, Hochiminh City, Vietnam); 8 µL of pA/G (5 mg mL) (Bio Basic, Markham Ontario, Canada); one of these reagent groups: 2% sodium dodecyl sulfate (SDS), 2% SDS + 0.1 M 1,4-dithiothreitol (DTT), 2% SDS + 5% 2-mercaptoethanol (2-ME), 2% SDS + 0.5 M imidazole, 2% SDS + 0.5% triton X100, 2% SDS + 0.5% tween 20, or 2% SDS + 4% CHAPS; and 1X phosphate-buffered saline (PBS) pH 7.4 was rotated over-night at 4^{o}C. The control sample was prepared maintaining similar conditions but without reagents. The results were analyzed by SDS-PAGE and silver staining, which were conducted with some modifications (de Moreno *et al.*, 1985; Mortz *et al.*, 2001). Then 12 µL of each sample, after heating at 100^{o}C for 5 min, was loaded into each well of 12.5% separating gel, and separated by applying 2 mA/well and 220 V for 60 min. In control sample, pA/G-bound to QDs and the QDs-pA/G were bigger than the gel holes, so they stuck in the wells and no band of pA/G was shown in the gel. In contrast, there was one band of pA/G in the gel when the interaction was disrupted.

QDs-pA/G-anti-GST preparation

QDs-pA/G was prepared as described above. QDs-pA/G then was blocked by 5% bovine serum albumin (BSA) for 16 h at 4^{o}C, 3 μL^{-1} of anti-GST mouse antibody (200 µg mL^{-1}) (Santa Cruz, Texas, USA) was added and rotated for 1 h at room temperature.

Semi-dry electrophoretic transfer and preparation for Western blotting

The lysates of mPrPc-GST protein (48 kDa) expressed in *E. coli* cells and *E. coli* BL21 cells were separated by SDS-PAGE and confirmed by Coomassie blue staining (Blancher and Jones, 2001; Truong *et al.*, 2019). Proteins in gels were transferred to nitrocellulose membranes (GE Healthcare, USA) by semi-dry transfer method in 37 min at 10 V and 150 mA (Blancher and Jones, 2001).

Applying QDs-pA/G-IgG in Western blot

The membrane was shaken with 200 μL^{-1} QDs-pA/G-anti-GST for 1 min. at room temperature. After washing the membrane with 1X PBS, 0.05% tween 20, the signal was observed under 480 nm wavelength light.

Conventional Western blot

The conventional Western blot was performed as described with some modification (Blancher and Jones, 2001). Anti-GST antibody (0.6 µg) and 1:20000 dilution of anti-mouse HRP-conjugated antibody (Proteintech, Rosemont, USA) were used. The signal was observed by incubating with TMB substrate for 10 min.

RESULTS AND DISCUSSION

Deduction of interactive nature between QDs and pA/G

The interaction between QDs and pA/G showed variable results and was influenced by different reagents (Fig. 1A). The pA/G successfully bound to QDs and stuck in the well with QDs without any reagents which was indicated by a thinner band of pA/G (50 - 5 kDa) (Fig. 1A, lane 2) as compared to the quantity of pA/G before mixing (Fig. 1A, lane 1). Most of the pA/G was unbound when added with 2% SDS combined with 0.1 M DTT, or 5% 2-ME, or 0.5% tween 20 (Fig. 1A, lanes 4, 5 & 9). The 5% 2-ME showed the least impact. Different concentrations of DTT and tween 20 were tested (Fig. 1B). 0.1 M DTT and 1-2% tween 20 were significantly most effective. The combination of 2% SDS, 0.1 M DTT, and 1% tween 20 showed the best results (lane 2, Fig. 1C).

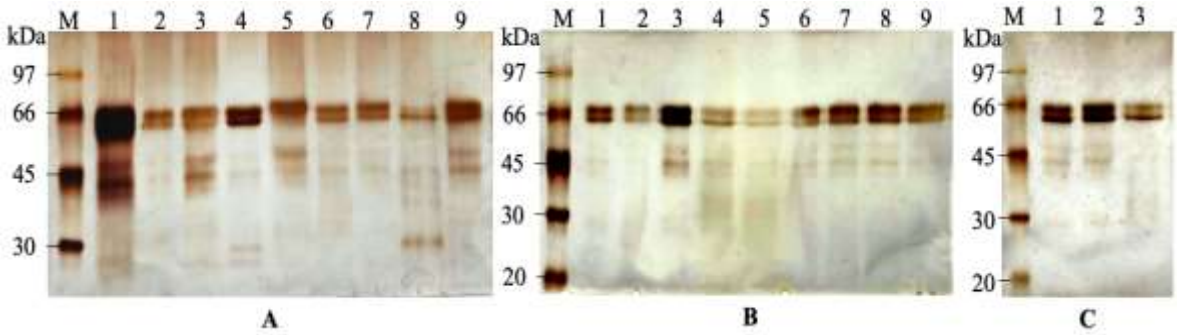

Fig. 1: The unbound pA/G after mixing with QDs in the presence of inhibitors. (A) M: protein marker; 1: before mixing; 2: non-inhibitors; 3: 2% SDS; 4: 2% SDS + 0.1 M DTT; 5: 2% SDS + 5% 2-ME; 6: 2% SDS + 0.5 M imidazole; 7: 2% SDS + 0.5% triton X100; 8: 2% SDS + 4% CHAPS; 9: 2% SDS + 0.5% tween 20. (B) M: protein marker; 1: 2% SDS; 2: non-inhibitor; 3-5: 0.1, 0.2, and 0.4 M DTT; 6-9: 0.5, 1, 2, and 4% tween 20. (C) M: protein marker; 1: 0.1 M DTT + 0.5% Tween 20; 2: 0.1 M DTT + 1% tween 20; 3: 0.1 M DTT + 2% tween 20.

The pA/G bound to CdSe/MSA QDs by non-covalent interactions because of the opposite charges. pA/G was positively charged at pH 7.4 because of its isoelectric point (4.65) and CdSe/MSA QDs was reported to be negatively charged due to the presence of -COOH groups in MSA (Vo *et al.*, 2020). Tween 20 and SDS hindered the interactions between QDs and pA/G by disrupting non-covalent interactions and negatively charging pA/G (Johnson, 2013). When DTT or 2-ME were added, the -SH group in DTT or 2-ME competed with the -SH group in MSA to interact with Cd^{2+}, which disrupted the bond between MSA and CdSe core (Krężel *et al.*, 2001; Vo *et al.*, 2020). Consequently, most of the pA/G bound to MSA were detached.

Amino acids played an important role in the adhesion of pA/G to the CdSe core regions that is not covered by MSA (Goede *et al.*, 2004). Amino acid histidines were sufficient to form stable bonds between pA/G and CdSe core while neighboring amino acids acted as modulators (Peelle *et al.*, 2005). These stable interactions between amino acid residues and II-VI semiconductors were also used to attach many kinds of protein to mercaptopropionic acid-conjugated $Zn_xHg_{1-x}Se$ QDs by one-step synthesis strategy (He *et al.*, 2013).

Applying QDs-pA/G in western blot

Coomassie blue staining revealed the overexpressing mPrPc-GST protein band (48 kDa) in positive sample while no band was found in negative sample (Fig. 2A). In conventional Western blot, the positive sample showed two bands, one of them was mPrPc-GST protein; however, no band was observed in negative sample (Fig. 2B). When using QDs-pA/G-anti-GST, the positive sample also had two bands of same size as those observed in conventional method, but no signal appeared in negative sample (Fig. 2C). The anti-GST antibody was not so specific, so there were two same bands in both methods.

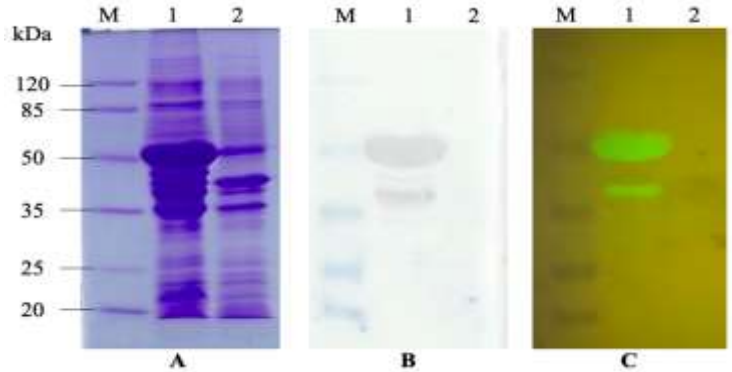

Fig. 2: mPrPc-GST protein in *E. coli* lysates when analyzed by SDS-PAGE (A), by western blot using anti-GST mouse antibody and HRP-conjugated anti-mouse antibody (B), and by western blot using QDs-pA/G-anti-GST (C). M: protein marker, 1: positive sample–mPrPc-GST expressing *E.coli* lysates; 2: negative sample–*E. coli* BL21 lysates.

The total time taken for labeling mPrPc-GST protein on the membrane using QDs-pA/G-anti-GST was one and a half hour, including 1 h for pre-binding with primary antibodies, 30 min for washing, and 1 min for signal visualization. In contrast, the traditional method took 3 h, including 1 h for incubation with each primary antibody and secondary antibody, 1 h for washing, and at least 10 min for reaction with substrate for signal visualization. Gilroy *et al.* (2010) reported that covalently cross-linking of pA/G to commercial carboxyl-functionalized QDs. However, their conjugation step was complicated and took almost three times the time required in our method. Similarly, Makride and Gasbarro (2005) reported attachment of biotinylated double-IgG-binding-Z domain to streptavidin-coated QDs; however, the process was complecated and required many times of centrifugation (Makride *et al.*, 2005). In contrast, the present reported strategy enables facile conjugation of antibodies to QDs in one step, thereby saving the chemicals and time.

In summary, we confirmed that the pA/G bound to CdSe/MSA QDs by non-covalent interactions between positively charged pA/G and negatively charged CdSe/MSA, and by interactions between pA/G's amino acid and the CdSe core. Additionally, pA/G-conjugated QDs were successfully applied in ultrafast Western blot visualization with a stronger signal in half of the time required in conventional method. The results obtained may facilitate further research for applying our pA/G-conjugated CdSe/MSA QDs in Multiplex Western blot.

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