IMMUNOCONJUGATION OF QUANTUM DOTS MEDIATED BY SYNTHETIC PEPTIDE

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Abstract

The bioconjugation of quantum dots (QDs) is a key process in their application for bioanalysis as well as imaging. The coupling of QDs with biologically active molecules such as peptides, nucleic acids and/or antibodies enables their fluorescent labeling and therefore selective and sensitive tracking during the bioanalytical process, however the efficiency of the labeling and preservation of the biological activity of the bioconjugate have to be considered. In this study, a new approach of the bioconjugation of CdTe QDs and human immunoglobulin employing a small peptide is described. The heptapeptide (HWRGWVC) was synthesized and characterized by mass spectrometry, liquid chromatography and capillary electrophoresis. Moreover, the peptide was used as a capping agent for QDs synthesis. The CdTe QDs were synthesized by microwave synthesis (600 W, 20 min) using 3.2 mM $CdCl_2$ and 0.8 mM Na_2TeO_3. The bioconjugation of QDs capped by this peptide with immunoglobulin was investigated by capillary electrophoresis and magnetic immunoextraction coupled with electrochemical detection by differential pulse voltammetry. Furthermore, the applicability of prepared bioconjugates for fluorescent immunodetection was verified using immobilized goat anti-human IgG antibody.

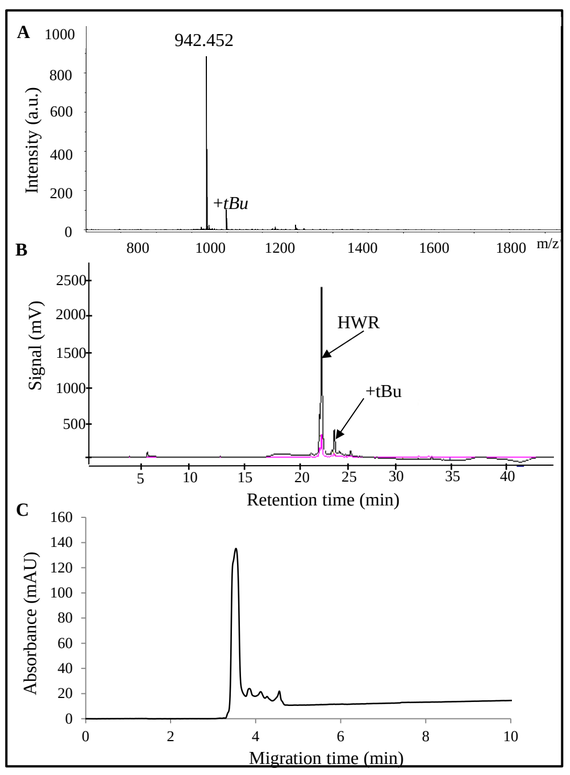

Figure 1
Characterization of synthetic HWR peptide. (**A**) Mass spectrum of the peptide. (B) HPLC characterization of the peptide. (**C**) CE characterization of the peptide. CE-UV, conditions – detection: 214 nm, capillary: 75 µm id., 60 cm/50cm, background electrolyte (BGE): 20 mM sodium borate, pH 9.2, voltage: +20 kV, injection: 3.4 kPa, 20 s. MS and HPLC conditions are in Experimental section.

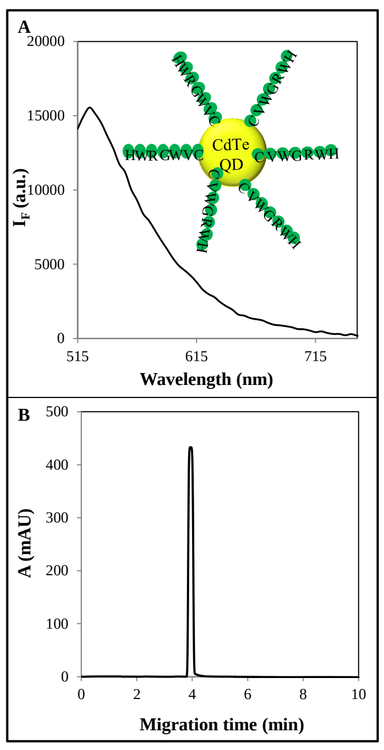

Figure 2
Characterization of HWR-QDs. (**A**) Emission spectrum of HWR-QDs (excitation 480 nm); inset: scheme of ideal structure of HWR-QDs. (**B**) CE-UV of HWR-QDs. Experimental conditions – detection wavelength: 214 nm, capillary: 75 µm id, 60 cm/50cm, BGE: 20 mM sodium borate, pH 9.2, voltage: +20kV, injection: 3.4 kPa, 20 s.

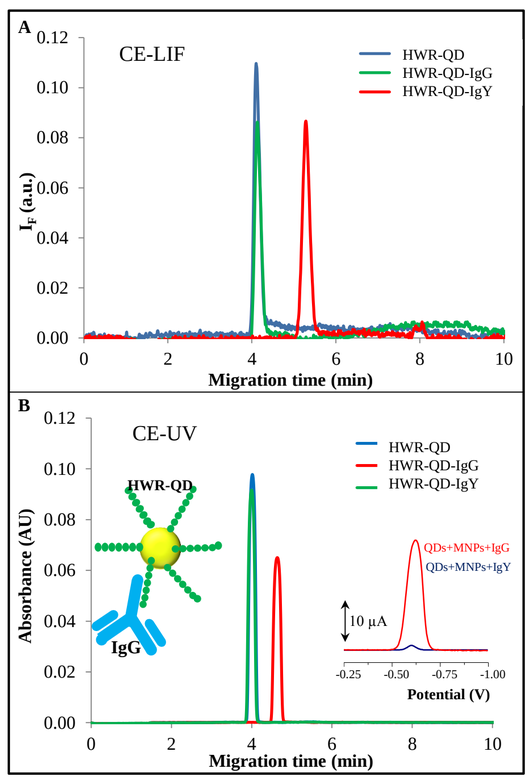

Figure 3
CE of HWR-QD and their bioconjugates with immunoglobulins. (**A**) CE-LIF, conditions - excitation: 488 nm, emission: 520 nm, capillary: 75 µm id, 47.5 cm/40cm, BGE: 20 mM sodium borate, pH 9.2, voltage: +20kV, injection: 3.4 kPa, 20 s. (**B**) CE-UV, conditions – detection: 214 nm, capillary: 75 µm id, 60 cm/50cm, BGE: 20 mM sodium borate, pH 9.2, voltage: +20 kV, injection: 3.4 kPa, 20 s; left inset: scheme of HWR-QD-IgG; right inset: differential pulse voltammograms of Cd determination extracted by magnetic particles coated with IgG and IgY. Experimental conditions – dilution: 1:1000, electrolyte: 0.2 M acetate buffer (0.2 M CH_3COOH + 0.2 M CH_3COONa, pH 5.0) at 25 °C, initial potential: -0.9 V; end potential: -0.1 V; deposition potential: -0.9 V; duration: 600 s; equilibration time: 5 s; modulation time: 0.057; time interval: 0.2 s; potential step: 0.00195 V; modulation amplitude: 0.02505.

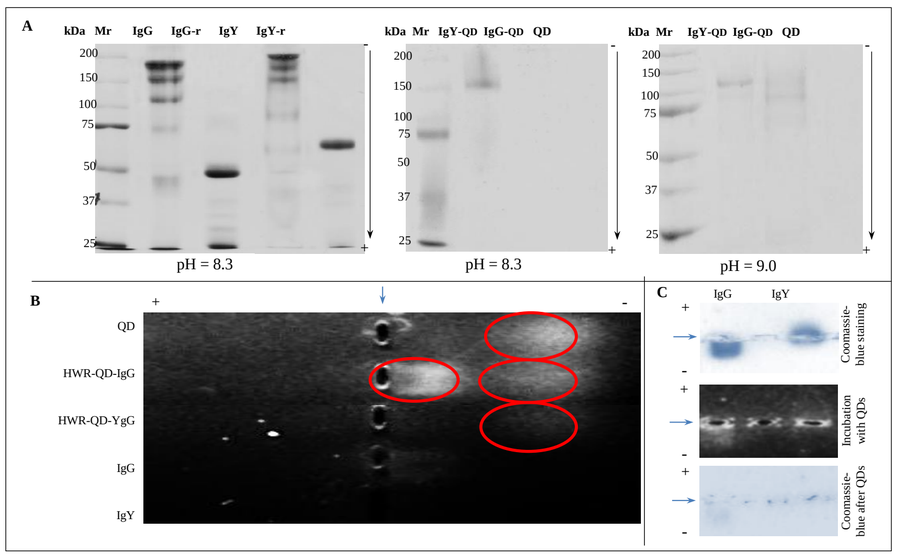

Figure 4
(**A**) SDS-PAGE electropherogram of 1000 ng of IgG and IgY standards under reducing (3.3% β-mercaptoethanol (*v*/*v*), labeled as -r) and nonreducing conditions in running electrolyte pH = 8.3 (left), SDS-PAGE electropherogram of QD-IgG and IgY conjugates in running electrolyte pH = 8.3 (in the middle), SDS-PAGE electropherogram of QD-IgG and IgY conjugates in running electrolyte pH = 9.0 (right). (**B**) Agarose gel electropherogram of QD-IgG and IgY conjugates, pH of the running buffer = 8.0. Arrow indicates wells position, + and – indicate poles orientation. (**C**) Agarose gel electropherogram of IgG and IgY stained either with Coomassie –blue (above) or incubated with QDs (in the middle) and consequently with Coomassie-blue (below), pH of the running buffer = 8.0. Arrows indicate wells positions, + and – indicate poles orientation.

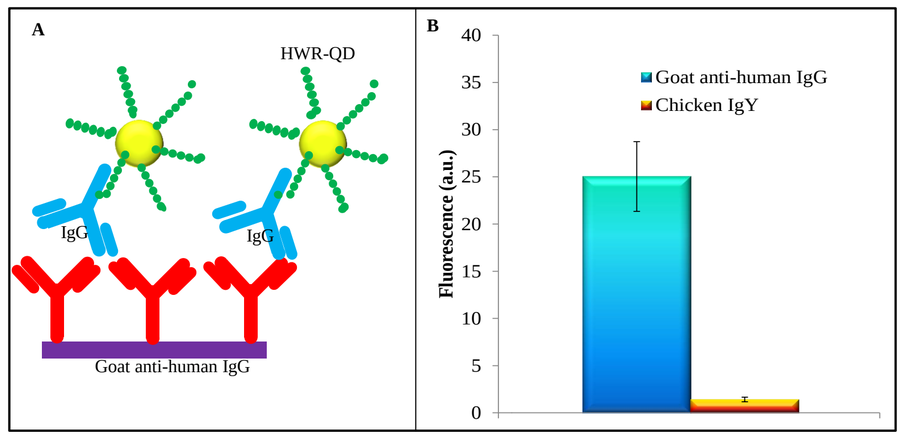

Figure 5
Fluorescent immunodetection. (**A**) Scheme of the possible interaction between HWR-QD-IgG and goat anti-human IgG. (**B**) Fluorescence intensity determined in the wells coated with goat anti-human IgG and chicken IgY (excitation: 480 nm, emission 525 nm).

Conclusion

The surface modification and functionalization of QDs is extensively studied due to the possibility of fine tuning of the properties according to the requirements. Their functionalization by antibodies enables the fluorescent visualization of interactions between the antigen and antibody as well as biodistribution. However, the bond between the antibody and QD has to exhibit correct sterical orientation to preserve the biological activity of the antibody and to provide required efficiency. It was demonstrated that small synthetic heptapeptide (HWRGWVC) can serve as a QD capping agent providing suitable surface properties for interaction with Fc fragment of human IgG.

ACKNOWLEDGMENT

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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ