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Short Communication

Biotin-modified glutathione as a functionalized coating for bioconjugation of CdTe-based quantum dots

In this study, biotin-conjugated glutathione was synthesized using peptide bonding of the biotin carboxy group and amino group of the γ-glutamic acid to prepare an alternative coating for CdTe quantum dots (QDs). This type of coating combines the functionality of the biotin with the fluorescent properties of the QDs to create a specific, high-affinity fluorescent probe able to react with avidin, streptavidin and/or neutravidin. Biotin-functionalized glutathione-coated CdTe QDs were prepared by a simple one-step method using Na_2TeO_3 and $CdCl_2$. Obtained QDs were separated from the excess of the biotin-conjugated glutathione by CE employing 300 mM borate buffer with pH 7.8 as a background electrolyte. The detection of sample components was performed by the photometric detection at 214 nm and LIF employing Ar^+ ion laser (488 nm).

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Quantum dots (QDs) with the dimensions in the range of 2–10 nm belong to the family of nanomaterials having a significant impact on chemical as well as on biological research. QDs are semiconductor nanocrystals with unique spectral properties featured mainly by the size-tunable emissions due to quantum size effects and high resistance toward photobleaching. The emission spectra of homogenously sized QDs are narrower than typical fluorophores and a variety of QDs types can be produced covering almost the whole spectral range [1, 2]. Since their first appearance in the late 1980s [3–5],] the interest in QDs has increased extremely; however, the application of QDs as fluorescent probes has been triggered by their bioconjugation to the target compounds enabling the specific fluorescent labeling of biological samples. Currently, QDs play an important role mainly in the imaging and as fluorescent probes for biological sensing [2]. The most popular types of QDs include CdTe, CdSe, ZnSe and ZnS; however, other semiconductor metals such as In, Ga and many others also can be used [6, 7]. Majority of sensing techniques employing QDs in biological systems are applied in solution (colloidal form) [8–10]. To date, two original approaches have been reported for the synthesis of colloidal QDs. The organometallic way produces QDs, which are generally capped with hydrophobic ligands (e.g. trioctylphosphine oxide (TOPO)) and hence cannot be directly employed in bioapplications. The second way is the aqueous synthesis route, producing QDs with excellent water solubility, biological compatibility and stability. Thiol-capped QDs could be prepared directly in aqueous solution with thiols as efficient stabilizers. Cysteine [11, 12], mercaptopropionic acid [9, 13] and reduced glutathione (GSH) [14–16] are the most popular coatings among thiols; however, quantum yields only up to 10% were typically obtained without any following treatment [17]. On the other hand, GSH due to its key function in detoxification of heavy metals in organisms [18] provides an additional functionality to the QDs. The fluorescence is considerably quenched at the presence of heavy metals and therefore glutathione-coated QDs (GSH-QDs) were successfully employed for determination of heavy metals [19, 20]. In addition, GSH-QDs exhibit high sensitivity to H_2O_2 produced from the glucose oxidase catalyzing oxidation of glucose and therefore glucose can be sensitively detected by the quenching of the GSH-QDs fluorescence [21]. Beside the application as simple sensors, QDs have much higher impact as unique fluorescent labels. Various specific labeling strategies are known and most of these approaches are based on bioconjugation with other biomolecules exhibiting some specific affinity to the target compound. Summary of these approaches was recently presented in a review article published by Algar et al. [2]. One of these strategies utilizes the biotin–avidin (respectively, streptavidin and neutravidin) interaction, exhibiting very high specificity. Modification of QDs by the streptavidin proved

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Abbreviations: B-GSH, biotinylated glutathione**; B-GSH-QDs,** QDs coated with B-GSH**; GSH,** reduced glutathione**; GSH-QDs,** quantum dots coated with reduced glutathione**; QDs,** quantum dots

Colour Online: See the article online to view Figs. 2 and 3 in colour

to be a very successful method evaluated in various publications [22–24] and due to this success streptavidin-QDs are nowadays also commercially available. Also, biotin-functionalized QDs have been developed to exploit the same interaction [25–28]. However, so-called multicolor QDs, which means that particles modified by several different molecules, are now of great interest.

Therefore, the aim of this study was to prepare QDs applicable in organisms based on the biocompatible properties due to the presence of GSH and also with the possibility to be employed in modern biotechnological biotin–avidin (or its homologues) applications. The first aim was to prepare biotinylated GSH, which would be subsequently used concurrently as a stabilizer of QDs and bioreactive layer.

Biotin and GSH were conjugated via standard peptide bond using carboxy group of the biotin and amino group of the γ-glutamic acid. The biotinylation at the N-end of the tripeptide was the last step of the peptide synthesis. The purification of the product was carried out using high-performance liquid chromatography and the purity of 99% was reached. Final product (for structure, see Fig. 1) was analyzed by mass spectrometry (MS). Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) was carried out using an Ultraflex III instrument (Bruker Daltonik, Germany). Samples (0.6 μL) were pre-mixed with 2.4 μL of the matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid in a water/acetonitrile mixture 1:1, v/v) and 0.6 μL of this mixture was deposited on a stainless steel MALDI target. Measurements were carried out in a reflectron positive ion detection arrangement. The obtained MS spectrum is shown in Fig. 1. The major peak in the spectrum has the molecular mass of 532.185, which is in good agreement with the theoretical molecular mass of 532.2 Da calculated for the biotinylated glutathione (B-GSH). Then, B-GSH was used as an alternative coating for CdTe-based QDs (emission maximum at 504 nm). The procedure for synthesis of these dots was adapted from the work of Duan et al. [29]. Sodium tellurite was used as the Te source. Owing to the fact that sodium tellurite is air stable, all of the operations were performed in the air without requiring any inert atmosphere. The synthesis pathway is thus very simple using harmless aqueous solutions. The synthesis of CdTe QDs and their subsequent coating were as follows: 330 μL of the $CdCl_2$ solution (c = 0.04 mol/L) was diluted with 2.5 mL of water. During constant stirring, 8 mg of sodium citrate, 330 μL of Na_2TeO_3 solution ($c = 0.01$ mol/L), 15 mg of B-GSH and 3.3 mg of $NaBH_4$ were added into water–cadmium(II) solution. The mixture was kept at 95°C under the reflux cooling for 2.5 h. As a result, yellow solution of the QDs coated with B-GSH (B-GSH-QDs) was obtained. An inset in Fig. 2 shows the solution of the B-GSH-QDs under the ambient light (left) and the fluorescence under the illumination by the UV lamp is shown in the right.

Synthesized B-GSH-QDs were analyzed by CE (Beckman Coulter, PACE 5500) with absorbance detection at 214 nm and with the LIF detection (Ar^+, λ_{ex}-488 nm/λ_{em}-530 nm). Separation of the excess of B-GSH and GSH was carried out using uncoated fused-silica capillary with 50 m internal diameter and 375 m *b* outer diameter. Total length was 47 cm and the effective length was 40 cm. Borate buffer (300 mmol/L, pH 7.8) was used as a background electrolyte (BGE). The typical electropherogram of the B-GSH-QD solution is shown in Fig 2A. Signals of GSH, B-GSH as well as B-GSH-QDs were baseline separated with the resolutions of 1.9 (GSH–B-GSH) and 4.2 (B-GSH–B-GSH-QDs). The identification of GSH and B-GSH signals was done by the standard addition method and identification of the B-GSH-QDs signal was done by CE-LIF (Fig. 2B).

CE-LIF was also used for monitoring of the stability of B-GSH-QDs during storage in the dark at 4°C. QDs were

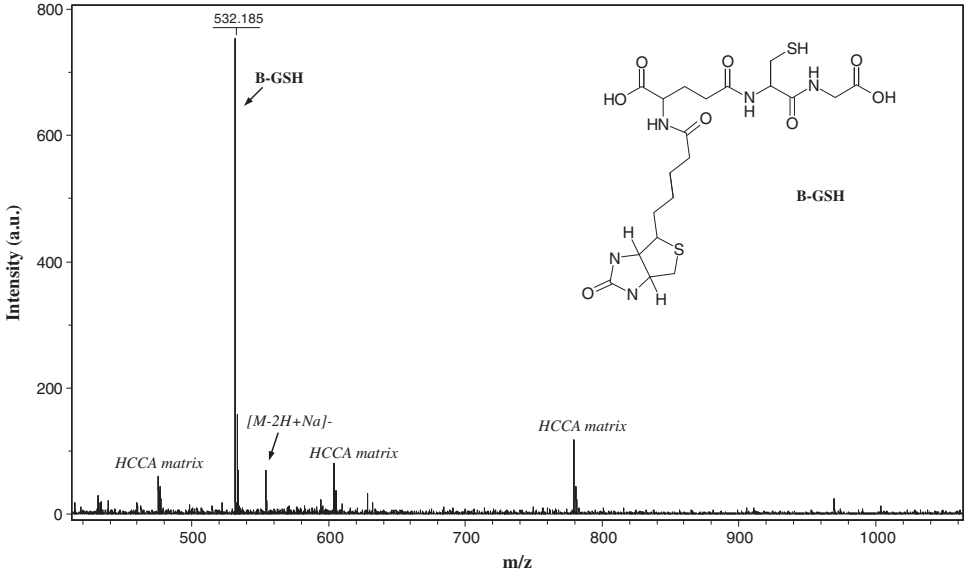

Figure 1. MALDI-TOF MS spectrum obtained for B-GSH (matrix: α-cyano-4-hydroxycinnamic acid; for more details, see text) and structure of the B-GSH.

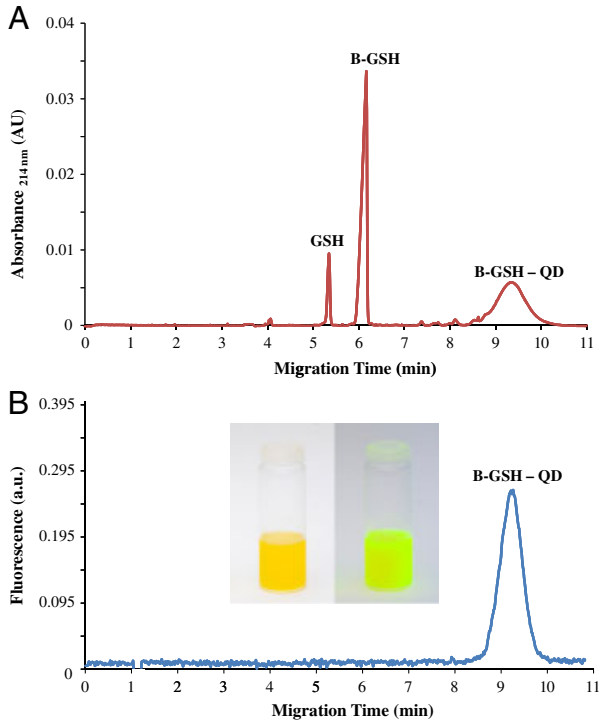

Figure 2. Electropherogram of the mixture of the B-GSH-QDs and excess B-GSH and GSH; BGE: 300 mmol/L sodium borate buffer, pH 7.8; U: +20 kV; injection: 0.5 psi for 20 s; (A) UV detection at 214 nm; (B) LIF detection (488 nm/530 nm); inset: B-GHS-QDs under ambient light (left), B-GSH-QDs under UV light illumination (right).

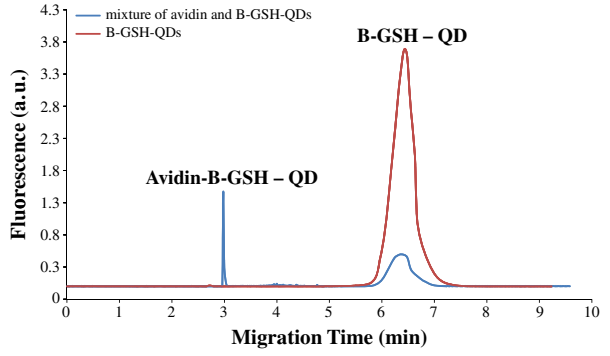

Figure 3. Electropherogram of the mixture of the B-GSH-QDs and avidin solution; BGE: 20 mmol/L sodium borate buffer, pH 9.5; U: +20 kV; injection: 0.5 psi for 20 s; LIF detection (488 nm/530 nm).

sampled per 6 h and the signal height of B-GSH-QDs was observed. The height of the signal steadily decreased with increasing time of the storage. After 3 days, the height decreased for more than 80%. Based on the results obtained, it can be concluded that the B-GSH-QDs are less stable in comparison with the QDs coated with unmodified GSH (GSH-QDs) created according to the same procedure [29].

The functionality of B-GSH-QDs was tested by addition of the avidin solution. The resulted mixture was analyzed by CE-LIF and typical electropherogram is shown in Fig. 3. The separation procedure was adapted from Huang et al. [30] and sodium borate buffer (20 mmol/L, pH 9.5) was used as a BGE. It was observed that the protein–QD complex was well separated from the QDs and the signal intensity of the avidin–B-GSH–QD complex was directly proportionate to the concentration of the avidin added and the signal of B-GSH-QDs decreased accordingly.

It follows from the results obtained that B-GSH is a suitable alternative coating for the elegant single-step synthesis of thiol-stabilized CdTe QDs. Obtained QDs are of good properties for the fluorimetric detection with the excitation by Ar^+ laser at the wavelength of 488 nm and emission of 530 nm. Moreover, we show that CE is an efficient method for the separation of the GSH and B-GSH excess from the B-GSH-QDs and for stability control.

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