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Interaction of cadmium sulfide quantum dots with jacalin for specific recognition of cancer cells

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ABSTRACT

Surface functionalizations of quantum dots (QDs) are expected to improve their optoelectronic properties and advance their targeting nature, which is highly warranted for application in molecular imaging and biomedical diagnostics. Therefore, an understanding the interaction between surface functionalization agents and QDs is a prerequisite for their application. Herein, we investigated the interaction between cadmium sulfide (CdS) QDs and dietary T-antigen binding lectin (jacalin) isolated from Indian jack fruit seeds. Fluorescence spectroscopy study showed that CdS QDs effectively quenched the intrinsic fluorescence of jacalin. Analysis of fluorescence quenching at a different temperature indicated that the mechanism of interaction is static and a non-radiation energy transfer occurred within the molecules. The obtained binding constant, K_a value in the order of $10^4\ M^{-1}$ at the tested temperature range suggested that the binding affinity between jacalin and CdS QDs is in the same range as those obtained for the interaction of lectin with carbohydrate. Thermodynamic analysis of the binding data, $\Delta H^0 = -44.24 \pm 1.21$ kJ mol^{-1}, $\Delta S^0 = -60.92 \pm 4.10$ J mol^{-1} K^{-1} and $\Delta G^0 = -26.21 \pm 0.1$ kJ mol^{-1} suggested that the binding reaction is enthalpy-driven spontaneous process. Hemagglutination activity of jacalin is well preserved even after binding to CdS QDs, indicating that the jacalin-CdS QDs complex can recognize T-antigens of the malignant tissues. To support the claim, we demonstrated selective fluorescence labeling of chronic myeloid leukemia cells, K562 with jacalin-CdS QDs complex.

1. Introduction

Distinguishing healthy and cancer cells were the major challenge in the cancer diagnostics and therapeutics. Targeted therapies are highly attractive to lower the overall dosage of administered drug without compromising the anti-cancer efficacy [1]. Active targeting of a drug can be accomplished through labeling with antibodies, carbohydrates, folic acids, peptides, aptamers, a monoclonal antibody of specific surface receptor expressed by cancer cells and so forth [2–7]. Thomsen-Friedenreich disaccharide antigen (T-antigen) is one of the appealing molecular targets for cancer therapies. T-antigen disaccharide is 2-acetamido,2-deoxy,3-O-β-D galactopyranosyl-α-D-galactopyranoside (Galβ1-3GalNAc-O-serine/threonine), which is over-expressed in more than 90% of primary human carcinomas, but is usually concealed in healthy cells [8].

Jacalin, a tetrameric lectin isolated from the seeds of jackfruit (*Artocarpus integrifolia*) bind specifically to the T-antigen expressed on the surface of HT-29 cells with a dissociation constant (K_d) of 500 ± 50 nM [9–11]. The mechanism of interaction and specificity of jacalin and carbohydrate has already been studied in detail [12–15]. In addition to carbohydrates, jacalin also interacts with porphyrins, phycocyanin, ruthenium complexes, gold and silver nanoparticles with considerable affinity [16–20]. Single-crystal X-ray diffraction studies of jacalin-methyl-α-D-galactopyranoside complex [12–15] and jacalin-porphyrin complex reveals that the carbohydrate binding site of jacalin exhibits structural plasticity to recognize other hydrophobic molecules [21]. Obaid et al. directly coupled jacalin and gold nanoparticles stabilized with thiolated polyethylene glycol and ocataalkyl-substituted zinc (II) phthalocyanin photosensitizer to target the T-antigen on the surface of HT-29 human colorectal adenocarcinoma cells [22]. Marangoni et al. demonstrated jacalin-gold nanoparticles conjugates has the higher affinity for leukemic K562 cells [20]. Recently, we showed that jacalin capped silver nanoparticles loaded with acetylshikonin kill K562 cells at lower concentration [23]. These studies clearly suggest that jacalin has high potential to encapsulate active drug and also useful in the development of targeted therapy.

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Recent years, quantum dots (QDs) are widely investigated for the development of bioimaging agents [24]. The efficacy of the QDs could be enhanced through coupling with targeting agents [25]. Therefore, an understanding the interaction of QDs and targeting agents is important to determine the diagnostic effectiveness of the QDs. In this study, we investigated the binding between cadmium sulfide (CdS) quantum dots and jacalin and delineated the thermodynamics forces involved in the interaction. Synchronous fluorescence spectroscopy study showed that the microenvironment of tryptophan residues was altered upon binding to CdS QDs. Hemagglutination assay revealed that the CdS QDs binds to jacalin at a site that is different from the carbohydrate binding site. As a proof of concept, an application of jacalin-CdS QDs in selective imaging of cancer cells has been demonstrated.

2. Materials and methods

2.1. Materials

Jackfruit (*Artocarpus integrifolia*) seeds were obtained from local seed vendors. Guar gum was obtained from Loba, India. Sodium phosphate dibasic and monobasic, sodium chloride, epichlorohydrin, pectin and taurine were purchased from Merck. Acrylamide, bis-acrylamide and sodium dodecyl sulfate were purchased from Sigma, India. All other reagents were of analytical grade.

2.2. Purification of jacalin

Jacalin was purified by affinity chromatography on cross-linked guar gum as described previously [26]. In order to free from the eluting sugar (galactose) the purified jacalin was thoroughly dialyzed against 10 mM sodium phosphate buffer, pH 7.4, containing 150 mM sodium chloride (PBS). The purity of the protein was assessed by polyacrylamide gel electrophoresis in the absence as well as in the presence of sodium dodecylsulfate. The concentration of jacalin was determined by Lowry assay using bovine serum albumin as the standard [27].

2.3. Binding of CdS QDs to jacalin

CdS QDs were prepared as mentioned previously [28]. Briefly, 50 mg of pectin, 1 mM of cadmium chloride and 10 mM of taurine was dissolved in 50 ml distilled water. The solution has been heated for 1 h at 80 °C. Then, the solution of Na_2S (2 mM final concentration) was added to the mixed solution. Finally, this solution is fitted to an air-tight condenser and heated at 100 °C for 8 h. The obtained yellow color solution showed bright blue fluorescence when illuminated with UV lamp at 360 nm. Fluorescence spectra were recorded on a Jasco-FP8200 spectrofluorimeter. The spectral slit width was set to 2.5 nm for both excitation and emission monochromators. The intrinsic fluorescence spectra of jacalin were recorded in 300–400 nm at an excitation wavelength of 280 nm. A fixed volume of jacalin solution (3.0 ml, 5 μM) was titrated by adding small aliquots of the CdS QDs from a concentrated stock solution (1 mM) and the fluorescence intensity was recorded after an equilibration period of 2 min. To determine the binding of CdS QDs interferes with the natural saccharide binding characteristic of jacalin, we performed QDs interaction studies by preincubating jacalin with a high concentration (50 mM) of galactose. All binding experiments were performed in PBS (phosphate buffered saline) buffer. All titrations were repeated at least three times to arrive at average values. Fluorescence intensities were corrected for volume changes before further analysis.

2.4. Lectin activity assay

Jacalin activity was checked by hemagglutination and hemagglutination inhibition assays as described in Ref. [29]. To determine whether CdS QDs binding, altered the sugar-binding activity of the lectin, the hemagglutination experiments were conducted by preincubating jacalin with a high concentration of CdS QDs as used in the fluorescence studies.

2.5. Fluorescence imaging

A fresh confluent culture of K562 cells wase used as model cells of Chronic Myeloid Leukemia (CML) and human PBMC were used as normal control cells. 1×10^4 cells were seeded in 8 well cover glass slide (Genetix) in complete culture medium [RPMI1640, supplemented with 10% FBS and 1X pen-strep (Gibco)]. The overnight culture of the seeded cells was washed with PBS three times and fixed with 4% Paraformaldehyde (freshly prepared in 0.1 M phosphate buffer) followed by a wash with PBS to remove the paraformaldehyde. After that, 50 μl of stock jacalin-CdS QDs-complex (1:20) was added to the cover slide in dark. After 30 min of incubation at room temperature, the QDs solution was removed and washed the cell with PBS two times. Cells were observed under phase contrast fluorescence microscope (Nikon) at excitation/emission 350/470.

3. Results and discussion

The interaction of jacalin with CdS QDs was elucidated by following the change in fluorescence. The intrinsic fluorophores of jacalin are deeply buried in the hydrophobic core and showed characteristic fluorescence emission maximum at 330 nm [16]. Titration of jacalin with CdS QDs resulted in quenching in the fluorescence intensity in the wavelength range of ca. 300–400 nm, with the maximum change in fluorescence intensity around 330 nm, suggesting an interaction between jacalin and CdS QDs (Fig. 1A).

Tryptophan and tyrosine are the main fluorophores responsible for the protein fluorescence, whose microenvironments may undergo modification upon drug binding as a result fluorescence quenching can be observed [30]. Synchronous fluorescence spectroscopy was widely applied to infer the microenvironment

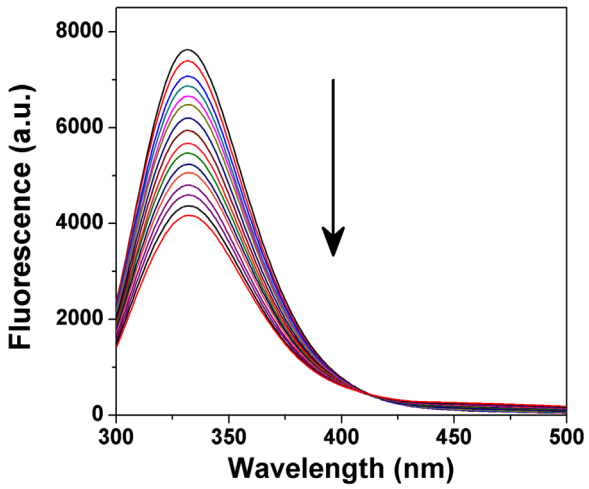

Fig. 1. Jacalin fluorescence emission spectra monitored after addition of increasing concentrations of CdS QDs (0–100 μM). The upper spectrum in each panel corresponds to free jacalin and the remaining spectra with decreasing fluorescence emission were recorded in the presence of increasing concentrations of CdS QDs.