Conjugated Quantum Dots as a Labeling Tool for Dough and Bread

S. Ansari* and J. L. Kokini**

*University of Illinois at Urbana-Champaign, Urbana, IL, USA, sansari3@illinois.edu
** University of Illinois at Urbana-Champaign, Urbana, IL, USA, kokini@illinois.edu

ABSTRACT

Quantum dots (QDs) are commonly used in medical applications as a probe for diagnostic procedures and imaging both microscopic and nano scale structures. They have also been used for designing biosensors. However, the application of quantum dots is very new in food research. In this study, a novel application of QDs in food science was investigated. QDs were conjugated to gliadin protein antibodies and used as fluorescent probes to track gliadin protein in dough and baked bread samples. CLSM was used to monitor QDs-gliadin antibody conjugates and obtain images. The mean intensity value of gliadin for each sample was calculated and plotted. ANOVA was applied to compare the mean intensity value of gliadin in different samples and regions.

Keywords: *gliadin, quantum dots (QDs), anti-gliadin antibody, conjugation, CLSM*

1 INTRODUCTION

The wheat storage proteins, glutens, can be classified into two groups, gliadin and glutenin. Glutenin subunits impart elasticity to gluten protein doughs and are subdivided into low-molecular weight (LMW) and high-molecular weight (HMW) subunit glutenin [1]. Gliadins are the low molecular weight subunits and generally contribute to the viscosity of gluten proteins. They are soluble in aqueous alcohols. Gliadins are subdivided into four groups, α, β, γ and ω gliadins according to their relative mobilities [2].

The composition and quantity of gliadins and glutenins are very important in wheat quality. Gliadin accounts for about 50 % of wheat grain and glutenin accounts for 35 % of total proteins [3].

Gliadin proteins are primarily responsible for celiac disease (CD). Celiac disease is an inflammatory disease of small intestine which prevents absorption of parts of foods that are important for human health. Therefore, developing immunoassays which are able to exactly determine the content of gluten proteins are very important and remains a difficult task.

There are a series of enzyme-linked immunoadsorbent assays (ELISAs) that have been developed for the analysis of gliadin in food. ELISA is a test that uses antibodies and color change to identify typically a particular protein. However, there are some concerns about the usefulness of immunoassays. The use of reducing agents in these methods improves the extraction of prolamins but affects immunochemical quantification [4].

Fluorescence labeling technique was also used to investigate the dynamic surface and rheological properties of purified gliadin and glutenin with confocal laser scanning microscopy (CLSM) [5]. Varriale et al. (2007) labeled gliadin peptides with fluorescein-5-isothiocyanate (FITC) to measure fluctuations of fluorescein-labeled gliadin peptides with fluorescence correlation spectroscopy (FCS) in the absence and presence of anti-gliadin antibodies [4].

In all previous studies to investigate and locate gliadin proteins in food organic dyes such as RhB or FITC were used as a labeling tool. However, one of the main disadvantages of using organic dyes is their high sensitivity to laser illumination which causes bleaching in time. Also, during imaging of food structure by organic dyes multiple illuminations are ineffective [6]. Therefore, developments in molecular biology, cell biology and medical immunology have resulted in great improvement in fluorescent labeling techniques.

In this study, we developed a novel immunoassay for food by using fluorescent semiconductor nanocrystal quantum dots (QDs) as a labeling tool. QDs were conjugated to gliadin antibody and visualized by confocal laser scanning microscopy (CLSM) to identify the distribution of gliadin proteins in dough and flat bread samples.

2 QUANTUM DOTS

2.1 Quantum Dot Characterization

Quantum dots, nanometer sized semiconductor crystals have recently gained much attention due to their unique properties in fluorescent labeling techniques. Currently, the major types of QDs consist of II-IV, IV-IV or III-V semiconductor core which surrounded by a semiconductor shell such as ZnS to minimize the surface deficiency and enhance the quantum yield [7]. The QDs are small and in the range of 4 to 12 nm in diameter. The size

of QDs determines the emission wavelength which varies from ultraviolet to infrared.

QDs are inorganic and thus photochemically robust. Also, the high brightness, long-lasting, size-tunable and narrow luminescence of QDs set them apart from organic dyes [8]. For instance, the fluorescence intensity of a single CdSe QD is about 20 times more than organic dye. However, the most important advantage of using QDs is that the different QD with different emission wavelengths can be stimulated with just one specific excitation wavelength, simultaneously which is very difficult to achieve with organic dyes [9].

QDs are hydrophobic and must be functionalized to make them hydrophilic before they can be used in biology. Therefore numerous effective methods have been developed for creating hydrophilic QDs, which can be divided into two main categories [10].

The first route is commonly designated as "cap exchange". The hydrophobic layer of organic solvent can be replaced with biofunctional molecules containing a soft acidic group (usually a thiol, e.g. sodium thioglycolate) and hydrophilic groups (for example carboxylic or aminic groups) which point outward from the QD surface towards bulk water molecules.

The second route is native surface modification, for example, adding a silica shell to the nanoparticles by using a silica precursor (usually alkoxysilanes such as tetraethylorthosilicate, TEOS) during the polycondensation.

2.2 Quantum Dots Applications in Biology

QDs are developed to use in variety of applications in biology such as disease detection, single protein tracking and intracellular reporting and fluorescent assays for drug discovery [9]. For biological applications, quantum dots must be conjugated to target molecules. Since in this study QDs-antibody conjugate was used, the previous studies regarding the conjugation of QDs to antibody in biological system are very relevant.

There are three primary ways to label QDs directly: antibodies, peptides and small molecules. The simplest labeling strategy is using an antibody and the complicated strategy is using a small molecule. Each approach has its advantages and disadvantages. However, there is no universal approach for all applications.

Antibody-QD conjugates have been used in various applications. Bentzen et al. (2005) used antibodies for F and G proteins on two QD sizes to detect and follow the progression of respiratory syncytial viral infection in vitro [11, 8].

Gao et al (2004) used antibody-QD conjugates to target a prostate-specific membrane antigen in tumors in vivo in mice [12]. Also, the use of anti-HER2 QD conjugates have been reported in imaging of breast cancer cells in vitro [13] and in vivo [14].

Antibodies can be biotinylated and used with streptavidin-coated QDs or they can be directly conjugated to QDs. A wide selection of antibody-QD conjugates is also commercially available. However, the availability of antibodies, their selectivity and affinity are the disadvantages of this method [15].

Figure 1 shows a schematic of a quantum dot conjugate used for in vivo imaging. The image represents a cross section where the quantum dot semiconductor core is coated for biocompatibility and solubility. The diagram also shows how the antibodies can be biotinylated and used with streptavidin-coated QDs or how they can be directly conjugated to QDs [15].

Hua et al (2006) performed the conjugation of QDs with goat anti-human immunoglobulin antibodies (Abs). They used column separation with circular dichroism (CD) and UV-vis absorbance to characterize and quantify the QD labeling process. They showed that proteins can be comfortably labeled with QDs which opens a very powerful avenue in life sciences for probing tissue not available before the availability of QD techniques [9].

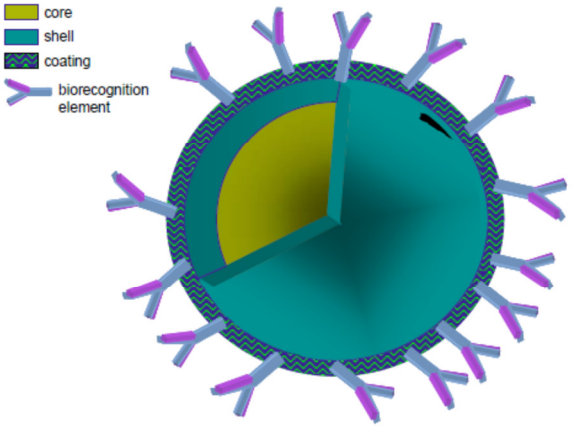

Figure 1. Schematic of a QDs-antibody conjugation [15].

3 MATERIALS AND METHODS

3.1 Materials

For preparation of dough and bread samples bleached wheat flour from General Mills was used. Commercially available polyclonal gliadin antibody was purchased from Sigma-Aldrich. For labeling of the samples CdSe QDs coated with ZnS and polyethylene glycol (PEG) and amine activated for crosslinking were conjugated to gliadin antibody. The quantum dot antibody conjugation kit was purchased from Invitrogen Company.

3.2 Methods

3.2.1 Preparation of Dough sample

Dough samples were prepared by mixing 50 g of wheat flour with 32 mL of distilled water. The dough samples were hand kneaded and sheeted to a thickness of 1.87 mm. Then the dough samples were stored in closed glass plates in a freezer. Figure 2 shows the dough sample. The dough samples were cut in their frozen state and were thawed and prepared for QD conjugation experiments.

3.2.2 Preparation of Bread Samples

Bread samples were prepared by making dough similar to previous step. When the dough sample's thickness reaches to 1.87 mm, it was cooked at an oven temperature of 375 °F at two different baking times of 5 and 9 minutes.

3.2.3 Sectioning of Samples

Each sample was cut to four regions for visualizations under confocal laser scanning microscopy (CLSM). Then from each region square pieces were cut from the center of the sample. Also, baked bread samples were cut using a razor into three different layers consisting of the top, center and bottom. Top layer in baked breads means the layer which is far from the direct heat, then center layer and the closest layer to the heat source is the bottom layer of baked breads. Figure 3 shows the three different layers of baked bread samples.

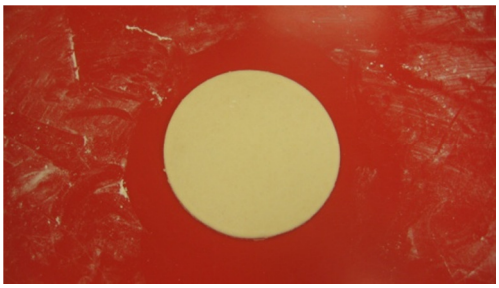

Figure 2. Dough sample

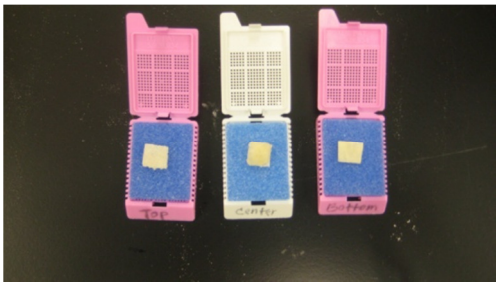

Figure 3. Three layers of top, center and bottom in bread.

3.2.4 Conjugation of QDs to Gliadin Antibody

Conjugation of gliadin antibody to quantum dots (QDs) was conducted based on the protocol which Invitrogen Company provided. The conjugation process was through covalent linkages in the presence of SMCC (Succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate) cross linker. SMCC is a popular cross linker that has an amine-reactive NHS-ester group at one end which reacts with QDs and a sulfhydryl reactive maleimide group which reacts with gliadin antibody. This allows for sequential, two-step conjugation process.

3.2.5 Confocal Laser Scanning Microscopy

The confocal laser scanning microscopy (Zeiss LSM 700) with Carl Zeiss objective Plan-Apochromat 20X/0.8 M27 was used. Digital image files in 1024X1024 pixels resolution were recorded with the Zen LSM software. The samples were loaded on a motorized xy stage. For each sample nine images at the same constant distance from nine different regions were taken.

Each sample was scanned in 2*2 tile scan mode and a z-stack of 8 slices to obtain a whole 3D scanned area of target sample. The image is created pixel by pixel and then line by line. Therefore, the brightness of sample image pixel represents the relative intensity of the detected light originating from QDs. In order to do the interactive measurement on each image, Axiovision software was applied. Representative area for intensity from each image was chosen manually and it was constant for all images. The mean intensity values of gliadin for each sample were calculated.

3.2.6 Analysis of Variance (ANOVA)

To consider whether the differences in distribution of gliadin protein are significant with regard to different samples and different regions studied, analysis of variance (ANOVA) was conducted. The ANOVA test was performed using target significance level $\alpha=0.05$ and $\alpha=0.01$. If the probability (p-value) is less than a significance level (α), different treatments result in statistically significant differences. In contrast, if the p-value is greater than a significance level (α) this indicates that different treatments are not statistically different in their outcome.

The QDs conjugated to gliadin antibody bind to gliadin, specifically. CLSM images of QDs-gliadin antibody were taken by confocal microscope LSM 700. In order to avoid of under-saturation and/or over saturation of images the laser power was selected based on the intensity level of QDs-gliadin antibody conjugates. However, the mean intensity for all samples was calculated at a constant laser power.

4 RESULT AND DISCUSSION

CLSM images of gliadin protein which is labeled with QDs-gliadin antibody conjugate in all samples showed that the distribution of gliadin protein changes between the dough and bread samples with two different baking times. Also, the distribution of gliadin protein changes between the four regions in each sample and between the three different layers in baked bread samples. 3-D intensity profile of QDs-gliadin antibody labeled image of bread sample is shown in Figure 4. As shown in Figure 4, the baked bread sample contains a high level of conjugated QDs bound to gliadin. This indicates that a high level of gliadin protein exist in this sample.

In addition, ANOVA test at two significance levels α=0.05 and α=0.01 showed that with probability 95% (α=0.05) the mean intensity of gliadin protein are significant different between samples.

Also, in order to understand whether the mean intensity values of gliadin in 4 different regions are different in each sample, the ANOVA test with single factor was performed. The result indicated that different regions play an important role on the distribution of gliadin in bread samples.

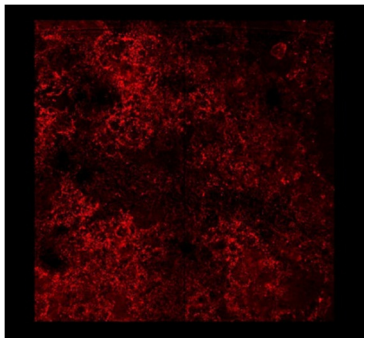

Figure 4. 3-D intensity profile of QDs-gliadin antibody labeled image of bread sample.

REFERENCES

[1] C. Loussert, Y. Popineau and C. Mangavel, J Cereal Sci. 47, 445, 2008.

[2] Q. Y. Li, K. M. Ji, X. Y. Song, E. Y. Zhang, Y. H. Pie and Y. M. Yan, Cereal Reaseach Communications, 36 (1), 117, 2008.

[3] J. Leszczynska, A. Lacka, M. Bryszewska and E. Brzezinska-Blaszczyk, Czech J. Food Sci, 26, 24, 2008.

[4] A. Varriale, M. Rossi, M. Staiano, E. Terpetschnig, B. Barbieri, M.D. Rossi and S. Auria, Anal. Chem, 79, 4687, 2007.

[5] W. Li, B.J. Dobraszczyk and P.J. Wilde, J Cereal Sci. 39, 403, 2004.

[6] N. Sozer, M. Sivaguru, and J.L. Kokini ,Submitted to J Food Engineering.

[7] S. Jin, Y. Hu, Z. Gu, L. Liu and H.C. Wu, H. C. J Nanomaterials, 2011, 1, 2011.

[8] S. J. Rosenthal, J.C. Chang, O. Kovtun, J.R. McBride and I.D. Tomlinson, Chemistry and Biology, 18, 10, 2011.

[9] X. F. Hua, T.C. Liu, Y.C. Cao, B. Liu, H.Q. Wang, J.H. Wang, Z.L. Huang and Y.D. Zhao, Anal Bioanal Chem, 386, 1665, 2006.

[10] J. Drbohlavova, V. Adam, R. Kizek and J. Hubalek, Int. J. Mol.Sci. 10,656,2009.

[11] E. L. Bentzen, I. D. Tomlinson, J. Mason, P. Gresch, M. R. Warnement, D. Wright, E. Sanders-Bush, R. Blakely and S.J.Rosental, Bioconjug.Chem, 16, 1488, 2005b.

[12] X. Gao. Y. Cui, R, M, Levenson, L.W.K. Chung and S. Nie, Nat. Biotech. 22, 969,2004.

[13] X.Wu, H. Liu, J. Liu, K. N. Haley, J. A. Treadway, J. P. Larson, N. Ge, F. Pealeand and M.P. Bruchez, Nat. Biotech. 21, 41, 2002.

[14] H. Tada, H. Higuchi, T. M. Wanatabe and N. Chuchi, Cancer Res. 67,1138, 2007.

[15] M. A. Walling, J. A. Novak and J. R. E. Shepard, Int, J. Mol, Sci. 10, 441, 2009.