

Design of an Aptamer-Amphiphile for the Detection of β -Lactoglobulin on a Liquid Crystal Interface

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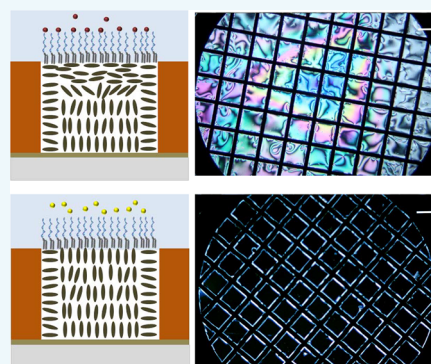
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ABSTRACT: An aptamer-amphiphile was designed that binds to β -lactoglobulin (β -LG), a major allergen from cow's milk. For this work, a 23-nucleotide ssDNA aptamer β -LG-23, capable of forming antiparallel G-quadruplexes was used, and its specificity and binding affinity of 22 ± 2 nM for β -LG were evaluated via enzyme-linked apta-sorbent assay (ELASA). The β -LG-23 aptamer was synthesized as an amphiphile by conjugating it to a C_{16} double tail via different spacers, and the effect of the spacers on the binding affinity and secondary structure of the aptamer was investigated. From all amphiphiles tested, direct conjugation of the aptamer to the tail gave the lowest binding affinity to β -LG (37 ± 2 nM), while maintaining the antiparallel G-quadruplex secondary structure of the aptamer. As a proof of concept, the β -LG-23 aptamer-amphiphile was used to decorate the interface of a liquid crystal (LC) and effectively detected 10 nM or 0.18 ppm of β -LG with a 20 min equilibration time, thus demonstrating that it has the potential to be used for fast and label-free detection of β -LG.



Food allergies pose a significant public health problem. Although there are more than 170 foods that can initiate allergic reactions, eight of them are responsible for more than 90% of food reactions: milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans. Allergy to cow's milk is the most common food allergy in infants and young children, and studies have shown that children affected by allergies are more likely to develop other atopic diseases in later life.¹ In addition, recalls related to undeclared allergens in foods are on the rise, with milk and milk products being the predominant undeclared allergens in food product recalls.^{2,3} Therefore, testing for allergens on a regular basis is critical in order to minimize the cost and impact of a food recall.

To detect allergens, food companies use different methods with enzyme-linked immunosorbent assay (ELISA) being the most popular, as it can provide in general a limit of detection in the parts per million range.⁴ Whereas ELISA has its advantages, it also has some limitations such as being time-consuming, requiring a trained analyst to perform the test, and variable results being obtained if different antibodies and calibrants are used.^{5,6}

The focus of this study is to design an aptamer-amphiphile that can be used for the detection of β -lactoglobulin (β -LG) with a sensor that is fast, cheap, and easy to use in food processing facilities. β -LG was chosen as it is identified as the major allergen from cow's milk along with α -lactalbumin and caseins.⁷ β -LG is a main whey protein in cow, buffalo, sheep, goat, mare, and donkey milk, while human milk is free of β -LG.⁸ There are two

single-stranded DNA (ssDNA) aptamers reported in the literature for β -LG. A 58-nucleotide aptamer (called BLG14) with a dissociation constant of 82 ± 30 nM for β -LG A and 80 ± 26 nM for β -LG B,⁹ where β -LG A and B are genetic variants of β -LG that differ by only two residues. The BLG14 was integrated in a graphene-based electrochemical biosensor for the detection of 100 pg/mL of β -LG with a 20 min wait time.⁹ Electrochemical sensors though have their disadvantages as, in general, there can be a gradual passivation of the electrode during continued operation, signal reduction from fouling agents, and interference from chemicals in the sample; in addition, they can be complex, requiring trained personnel for their operation, and they are expensive.¹⁰

A 23-nucleotide aptamer has also been used in the literature for the separation of β -LG A and β -LG B with capillary electrochromatography, even though its binding affinity for the proteins was not evaluated.¹¹ This 23-nucleotide aptamer (that we will be calling β -LG-23) has not been utilized before for the design of a biosensor and will be used in our study.

We first evaluated the binding affinity and specificity of β -LG-23 for β -LG with an enzyme-linked apta-sorbent assay (ELASA). β -LG-23 was then synthesized as an amphiphile by conjugating it to a C_{16} dialkyl tail via different spacers, as spacers

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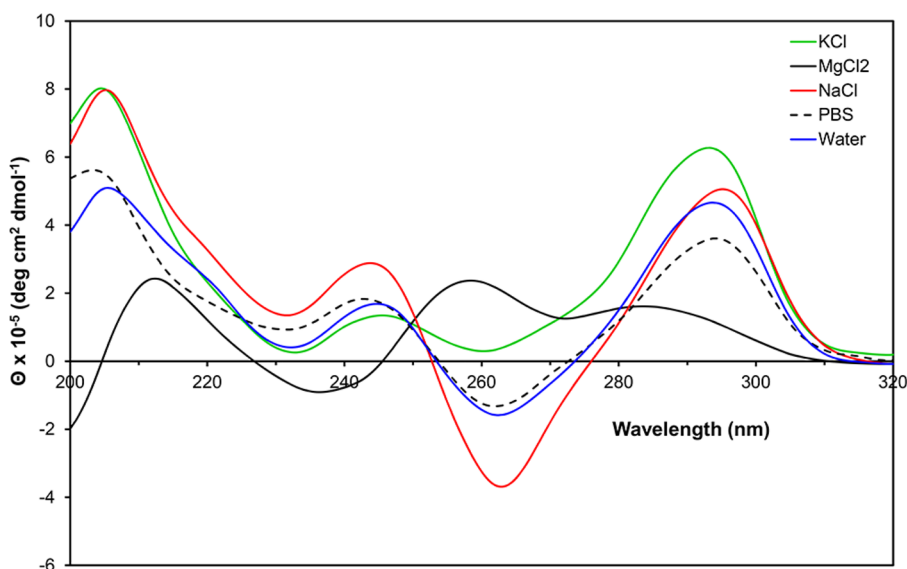


Figure 1. CD spectra of the free β -LG-23 aptamer in 10 mM NaCl, 10 mM KCl, 10 mM MgCl₂, PBS, and water. Data show the mean ($n = 3$).

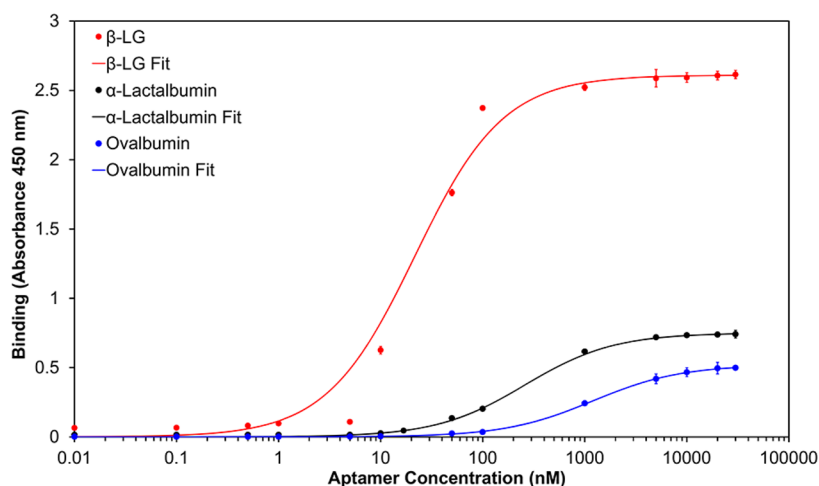


Figure 2. Binding curves of the free β -LG-23 aptamer to the β -LG protein and control proteins, α -lactalbumin and ovalbumin. Data are presented as mean $\pm$ SD ($n = 3$), and the lines show the fit to the data.

have been shown to affect the binding and secondary structure of an aptamer within an aptamer-amphiphile design.¹² The resulting β -LG-23 aptamer-amphiphiles were characterized for their binding affinity to β -LG with ELASA, and their secondary structure was evaluated with circular dichroism (CD). Control ssDNA-amphiphiles were also included. The self-assembly properties of the β -LG-23 aptamer-amphiphile with the highest binding affinity for β -LG were further evaluated with cryogenic transmission electron microscopy (cryo-TEM).

LCs have received considerable attention as sensors due to their sensitivity to external stimuli and ease of detection with a polarized optical microscope without the need of molecular labeling. LCs have been used for the detection of different biomolecules and events such as DNA, enzymes, oligopeptides, pathogens, proteins, DNA hybridization, catalytic processes, and others.^{6,13,14} While aptamer-amphiphiles have not been used in the literature with LCs, other adsorbates have been spread extensively at the aqueous–LC interface, and examples include surfactants, lipids, peptide-amphiphiles, and polymers.¹⁵ In this study, the β -LG-23 aptamer-amphiphile was adsorbed on an LC interface, and the resulting biosensor was evaluated for its

sensitivity and specificity to the detection of β -LG using a polarized optical microscope.

RESULTS AND DISCUSSION

Aptamers can adopt a variety of secondary structures including the standard B-form DNA formed from Watson–Crick base pairing between two strands of complementary DNA or within one strand forming a hairpin structure. B-DNA structures are characterized by a positive long wavelength band or bands at 260–280 nm and a negative band around 245 nm.¹⁶ Guanine (G)-rich sequences can form G-quadruplexes that are either parallel (a positive CD peak at 258–265 nm, and a negative peak around 240 nm) or antiparallel (two positive peaks, around 245 and 295 nm, and a negative peak around 265 nm).^{16,17} Both types of G-quadruplex display an additional positive peak around 210 nm.¹⁶ The β -LG-23 aptamer forms an antiparallel G-quadruplex, and this secondary structure is important for its binding to β -LG, as a control sequence that did not form G-quadruplex did not bind to the protein.¹¹ Therefore, we first examined the secondary structure of the β -LG-23 aptamer via CD in 10 mM NaCl, 10 mM KCl, 10 mM MgCl₂, phosphate

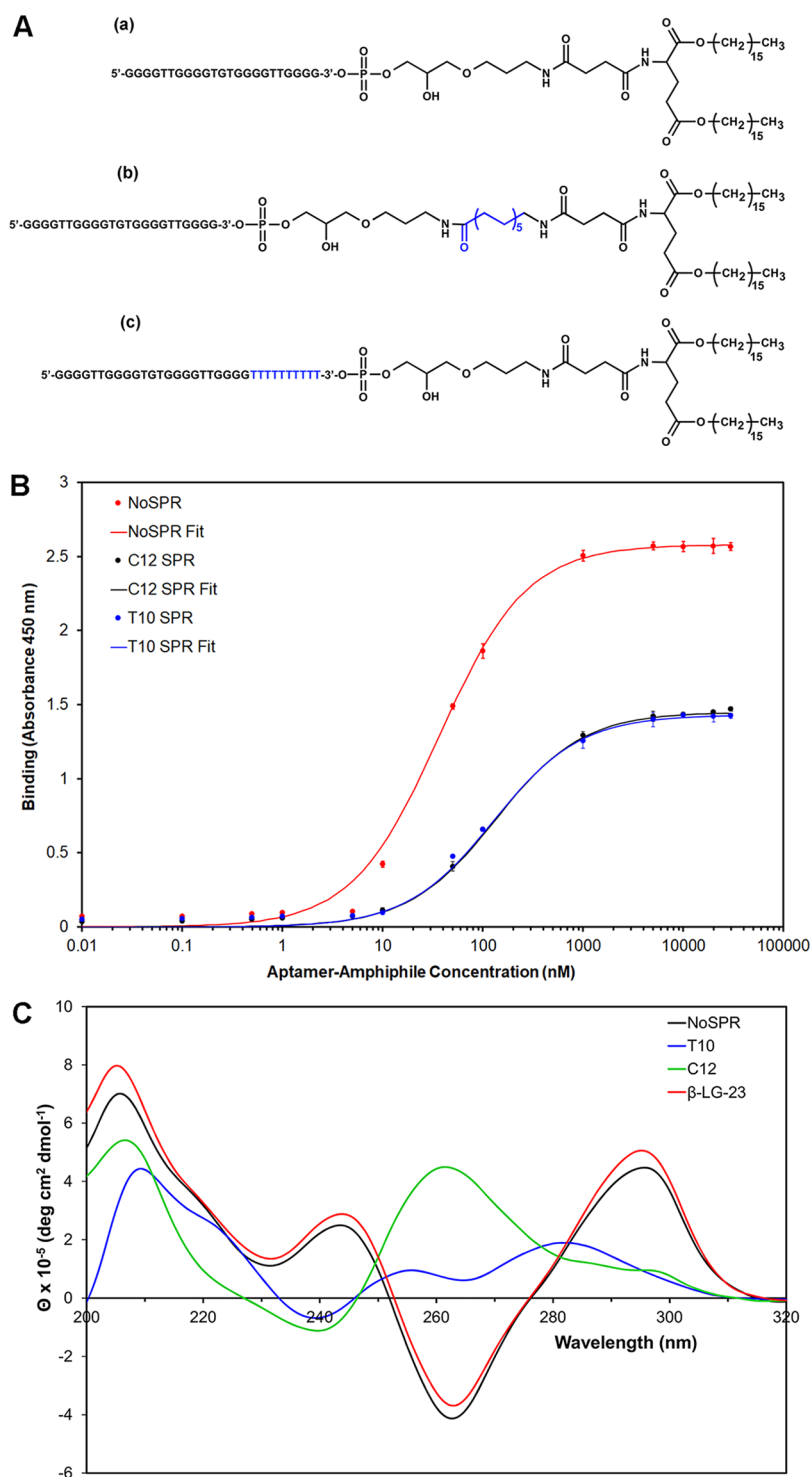


Figure 3. A. Chemical structure of the β -LG-23 aptamer-amphiphile without a spacer (a), with a C₁₂ alkyl spacer (b), or with a T₁₀ oligonucleotide spacer (c). B. Binding curves of the β -LG-23 aptamer-amphiphile with different spacers (no spacer, C₁₂ and T₁₀), to β -LG protein. Data are presented as mean $\pm$ SD ($n = 3$), and the lines show the fit to the data. C. CD spectra of aptamer-amphiphiles with different spacers (no spacer, C₁₂ and T₁₀). The CD of the free β -LG-23 aptamer is included for comparison. Data show the mean ($n = 3$).

buffered saline (PBS), and water in order to determine the best binding buffer for our studies. Figure 1 shows that β -LG-23 formed an antiparallel G-quadruplex in NaCl, KCl, PBS, and water, with the strongest signal measured in NaCl. Therefore, all the following experiments were performed in 10 mM NaCl.

The binding affinity of the β -LG-23 aptamer for the β -LG protein and control proteins α -lactalbumin (primary milk

protein) and ovalbumin (main protein in egg white) was evaluated via ELISA (Figure 2). A dissociation constant, K_D , of 22 ± 2 nM was calculated for the binding of β -LG-23 aptamer to β -LG protein, 0.26 ± 0.01 μ M for the binding of the aptamer to α -lactalbumin, a 12-fold reduction in affinity compared to the affinity of the aptamer for the β -LG protein, and 1.20 ± 0.09 μ M for the aptamer–ovalbumin binding (about 55-fold decrease in

affinity). The specificity of the β -LG-23 aptamer for β -LG was comparable to that reported in the literature for other systems where, for example, the binding of lactoferrin aptamers to control proteins was 4- to 6-fold less compared to the binding of the aptamers to the lactoferrin milk protein,^{18,19} and the dissociation constant of the bisphenol A (BPA) aptamer to control molecules was 17- to 25-fold higher compared to the dissociation constant for the binding of the BPA aptamer to BPA.²⁰

ssDNA-amphiphiles can be synthesized by conjugating different hydrophobic tails, such as linear lipophilic chains, cholesterol derivatives, or block copolymers, to either terminus of the ssDNA or between two consecutive nucleotides.^{21,22} It has been shown that direct conjugation of a C_{16} dialkyl tail, such as the one used in this study, to the 3' end of an aptamer reduced the aptamer's binding affinity for the target protein by about 8-fold. The presence of an alkyl (C_{12}) spacer between the aptamer and the tail resulted in further loss of affinity (9-fold reduction compared to the free aptamer), while a T_{10} spacer had less impact (about 3-fold reduction).¹² Motivated by these findings, for the design of the β -LG-23 aptamer-amphiphile, the β -LG-23 aptamer was conjugated to a C_{16} dialkyl tail either directly or through a C_{12} or T_{10} spacer (Figure 3A). Successful synthesis was verified via liquid chromatography–mass spectrometry (LC-MS), and Table 1 gives the expected and measured masses of all ssDNA-amphiphiles used in this study.

Table 1. Expected and Measured Masses of ssDNA-Amphiphiles as Determined by LC-MS

ssDNA-amphiphiles	expected mass (M-H)	observed mass (M-H)
β -LG-23- $(C_{16})_2$	8248.9	8248.0
β -LG-23- T_{10} - $(C_{16})_2$	11290.9	11290.2
β -LG-23- C_{12} - $(C_{16})_2$	8446.3	8444.9
Biotin- β -LG-23- $(C_{16})_2$	8642.4	8641.6
Biotin- β -LG-23- T_{10} - $(C_{16})_2$	11684.4	11682.5
Biotin- β -LG-23- C_{12} - $(C_{16})_2$	8839.7	8839.0
Biotin- T_{23} - $(C_{16})_2$	8217.2	8218.9
Biotin-Ctrl1- $(C_{16})_2$	8544.4	8546.4
Biotin-Ctrl2- $(C_{16})_2$	8525.3	8528.7

The binding affinities of the β -LG-23 aptamer-amphiphiles for the β -LG protein were evaluated via ELISA (Figure 3B), and the dissociation constant was 37 ± 2 nM for the amphiphile with no spacer, 0.134 ± 0.008 μ M, and 0.127 ± 0.005 μ M for the amphiphile with the C_{12} and T_{10} spacer, respectively. Results showed that conjugation of the aptamer to a tail, with or without a spacer, reduced its affinity for the protein, as shown before in the literature.¹² The secondary structure of the β -LG-23 aptamer-amphiphiles was evaluated via CD. As shown in Figure 3C, the aptamer and the β -LG-23 aptamer-amphiphile with no spacer formed an antiparallel G-quadruplex. The β -LG-23 aptamer-amphiphile with the C_{12} spacer formed a predominantly parallel G-quadruplex, whereas the spectrum of the amphiphile with the T_{10} spacer showed contributions from parallel G-quadruplex and a hairpin structure that may be attributed to a hairpin loop of a G-quadruplex.²³ Therefore, only the β -LG-23 aptamer-amphiphile with no spacer adopted the same secondary structure as the free aptamer. This finding may explain why the β -LG-23 aptamer-amphiphile with no spacer had the highest binding affinity for β -LG among all the amphiphiles tested. Therefore, ssDNA-amphiphiles with no spacer were used for further studies.

After evaluating the binding of the β -LG-23 aptamer to different control proteins (Figure 2), the binding of control ssDNA sequences to the β -LG protein was also examined, with the control sequences used as amphiphiles, as they are most relevant to the focus of this study. A scrambled oligonucleotide sequence that has the same base composition as the aptamer but not the same structural features is not possible, as 74% of the β -LG-23 nucleotides are G with the other 26% being only T, and such a sequence will form G-quadruplexes. Therefore, for the control sequences we used a T_{23} sequence that is completely unstructured, and two sequences from the literature that form G-quadruplexes but have different base compositions from the aptamer. Figure 4A shows that conjugation of the Ctrl1²⁴ sequence to the tail did not affect its antiparallel G-quadruplex structure, and the T_{23} remained unstructured²⁵ as an amphiphile. However, Ctrl2²⁶ formed parallel G-quadruplex as free ssDNA and a hybrid type parallel/antiparallel G-quadruplex²⁷ as an amphiphile. All control amphiphiles gave weak binding to β -LG (Figure 4B), with a dissociation constant of 0.32 ± 0.02 μ M for the T_{23} -amphiphile (about 9-fold reduction in binding compared to the aptamer-amphiphile), 1.63 ± 0.13 μ M for Ctrl1-amphiphile (44-fold reduction), and 0.64 ± 0.02 μ M for Ctrl2-amphiphile (17-fold reduction). The binding of the T_{23} -amphiphile to β -LG can be explained if we consider that although the ssDNA is unstructured, it shares 26% of the aptamer's nucleotides. The results for the control amphiphiles are in agreement with other reports in the literature where the dissociation constant of thrombin for the thrombin-binding aptamer M08, with a hybrid antiparallel G-quadruplex structure with a duplex, was reduced 22-fold when another ssDNA sequence was used with a parallel G-quadruplex structure.²⁸

All ssDNA-amphiphiles with no spacer, including the β -LG-23 aptamer-amphiphile, formed spherical micelles. Figure 5 shows the micelles for the β -LG-23 aptamer-amphiphile, as evaluated via cryo-TEM, with a diameter of 12 ± 1 nm. Dynamic light scattering gave diameters of 13.4 ± 1.5 nm for the β -LG-23 aptamer-amphiphile, 14.3 ± 2.5 nm for the T_{23} -amphiphile, 13.7 ± 1.9 nm for Ctrl1-amphiphile, and 12.0 ± 1.0 nm for Ctrl2-amphiphile.

The alignment of the LC biosensor in the presence and absence of the β -LG-23 aptamer-amphiphile and β -LG is shown in Figure 6. The 5CB LC in water has a bright optical appearance, as shown in Figure 6A, corresponding to a planar (parallel to the interface) anchoring of 5CB at the aqueous–LC interface.¹⁵ However, when a monolayer of surfactants or lipids, for example, is present at the aqueous–LC interface, 5CB assumes a homeotropic (normal to the interface) alignment and the LC appears dark.¹⁵ Relevant to this work, it was shown that when micelles of lipids and surfactants were introduced into the aqueous phase, the appearance of the 5CB LC changed from bright to dark as a result of lipid–surfactant coadsorption at the interface and the formation of a continuous lipid-rich monolayer at the aqueous–5CB interface.¹⁵ For the preparation of the amphiphile-laden interface, 10 μ M of β -LG-23 aptamer-amphiphile was added to the grids. This concentration is above the 50–100 nM critical micelle concentration of ssDNA-amphiphiles and aptamer-amphiphiles evaluated before.^{12,29} At concentrations above the critical micelle concentration, the β -LG-23 aptamer-amphiphiles self-assemble into small spherical micelles as observed from cryo-TEM. Based on previous studies, it is hypothesized that the β -LG-23 aptamer-amphiphiles adsorb from the micelles onto the LC interface as a monolayer causing a homeotropic anchoring of 5CB, as shown in Figure 6B. The

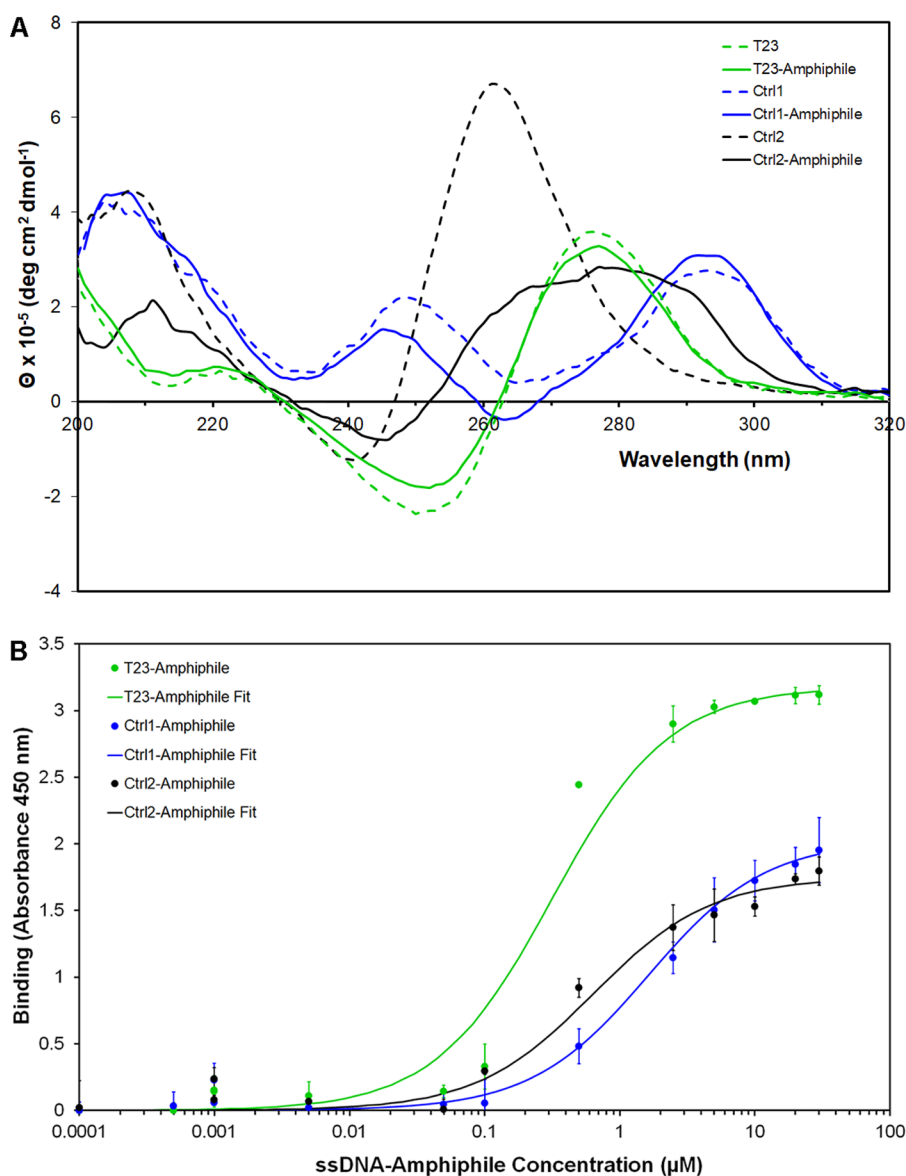


Figure 4. A. CD spectra of control amphiphiles. B. Binding curves of control amphiphiles to β -LG protein. Data are presented as mean $\pm$ SD ($n = 3$), and the lines show the fit to the data.

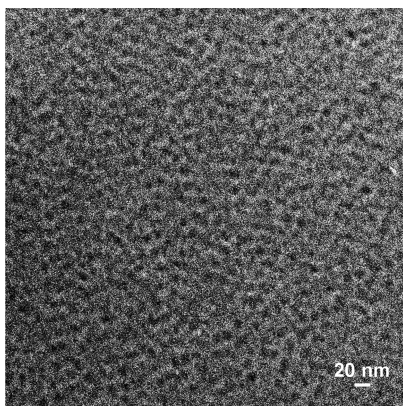


Figure 5. Cryo-TEM image of micelles (black dots) formed by the β -LG-23 aptamer-amphiphile.

bright areas near the edges of the squares are the result of SCB interacting with the grid and assuming a planar orientation, as

has been discussed before in the literature.^{15,30,31} Specific binding of a protein by a functionalized LC can lead to reordering of the LC and detection of the protein of interest.¹³ As can be seen in Figure 6C, the binding of the β -LG protein by the β -LG-23 aptamer-amphiphile decorated LC interface causes a transition from homeotropic to planar anchoring of SCB that gives rise to a bright image. Specificity of the LC β -LG-23 aptamer-amphiphile biosensor for the detection of β -LG was evaluated by exposing the biosensor to ovalbumin and a mixture of ovalbumin and β -LG. The presence of the random protein ovalbumin did not change the dark appearance of the LC (Figure 6D) and did not prevent detection of β -LG in the mixture (Figure 6E).

Different concentrations of the β -LG protein were presented at the LC β -LG-23 aptamer-amphiphile interface. As seen in Figure 7, the majority of the squares were bright at a protein concentration of 5 μM to 0.01 μM . Therefore, 10 nM or 0.18 ppm of the protein could be detected by this LC, with a 20 min equilibration time. This result is comparable to some

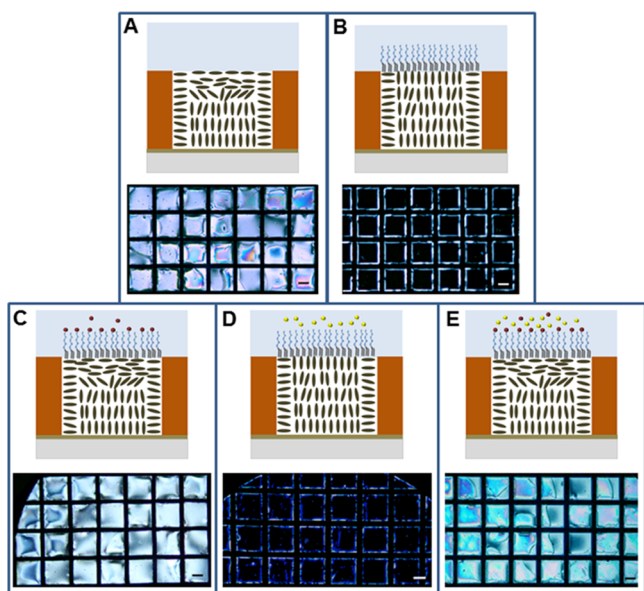


Figure 6. Schematic illustrations (adapted with permission from ref 32) and polarized light microscopy images of the SCB alignment (A) in water; (B) in the presence of 10 μM β -LG-23 aptamer-amphiphile, and with the β -LG-23 aptamer-amphiphile after interacting with (C) 10 μM of the β -LG protein, (D) 10 μM of ovalbumin, or (E) a mixture of 10 μM β -LG and 10 μM ovalbumin. The scale bars are 100 μm .

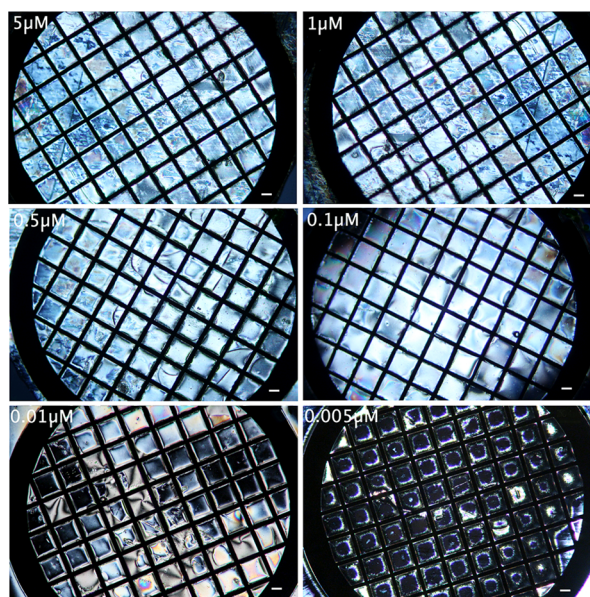


Figure 7. Polarized light microscopy images of the SCB alignment in the presence of 10 μM β -LG-23 aptamer-amphiphile with different concentrations of the β -LG protein. The scale bars are 100 μm .

commercially available ELISA tests for β -LG. For example, the ELISA kit by Crystal Chem currently has a 0.31 ppm limit of detection with an assay time of about 3 h. However, the LC detection limit of 10 nM is higher compared to the 100 pg/mL of β -LG using a different aptamer (BLG14) and a label-free electrochemical biosensor with graphene electrodes.⁹ However, as mentioned in the introduction, electrochemical sensors have their disadvantages. The main advantage of the LC evaluated in this work is that the detection method is fast and simple and can be easily used in food processing facilities, for example, without the need of a trained analyst to perform the test. In addition,

even though in this work detection was focused on visual observation, quantitative detection can be done by creating a calibration curve of the area ratio of the bright LC regions to the whole image versus the concentration of the protein that will be detected.³⁵

CONCLUSIONS

The binding affinity of the β -LG-23 aptamer for the β -LG protein was evaluated via ELASA to be 22 ± 2 nM and was shown to be specific for β -LG. It was synthesized as an amphiphile by conjugating it to a $(\text{C}_{16})_2$ tail, and its binding affinity for β -LG was 37 ± 2 nM, while both the β -LG-23 aptamer and the amphiphile formed antiparallel G-quadruplexes. The β -LG-23 aptamer-amphiphile was used to decorate the surface of a SCB LC for the detection of β -LG. The resulting biosensor effectively detected 10 nM or 0.18 ppm of β -LG with a 20 min equilibration time, thus making it a promising detection method for β -LG in food processing facilities.

EXPERIMENTAL PROCEDURES

Materials. ssDNA sequences and nuclease free water were purchased from Integrated DNA Technologies (Coralville, IA), polystyrene nontreated 96-well EIA/RIA clear flat-bottom plates from Corning (Pittston, PA), β -LG from Davisco Foods International (Eden Prairie, MN) and LSBio (Seattle, WA), α -lactalbumin from Davisco Foods International, ovalbumin from Fisher Scientific (Hanover Park, IL), hexafluoroisopropanol (HFIP), and triethylamine (TEA) from Oakwood Products Inc. (West Columbia, SC), lacey Formvar/carbon, 100 and 200 mesh, copper grids from Ted Pella Inc. (Redding, CA), and 3,3', 5,5'-tetramethylbenzidine (TMB) stabilized substrate for horseradish peroxidase (HRP) from Promega (Madison, WI). All other materials were purchased from Thermo Fisher Scientific (Hanover Park, IL) or Sigma-Aldrich (St. Louis, MO) and used without further purification or modification unless otherwise stated.

ssDNA-Amphiphile Synthesis. The ssDNA-amphiphiles were synthesized as discussed previously in the literature.^{12,29} Briefly, the tails were synthesized by conjugating two cetyl alcohols to the carboxyl groups of L-glutamic acid and adding a succinic acid to the amino group of L-glutamic acid.^{34,35} For the synthesis of the amphiphiles, the ssDNA sequences were purchased with an amino linker at their 3' end and conjugated to the N-hydroxysuccinimide (NHS) activated tails. The following ssDNA sequences were used: β -LG-23 (5'-GGG-GTTGGGGTGTGGGGTTGGGG/3AmMO/-3'), T_{23} (5'- T_{23} /3AmMO/-3'), Ctrl1 (5'-GGGTTAGGGTTAGGG-TTAGGGTT/3AmMO/-3'), and Ctrl2 (5'-GGCGAGGAGG-GGCGTGGCCGGTT/3AmMO/-3'). For the ELASA binding studies, the ssDNA had an additional biotin at the 5' end, and the amino linker was at the 3' for the tail conjugation. For the synthesis of the β -LG-23 aptamer-amphiphiles with different spacers, the alkyl (C_{12}) spacer was added to the carboxyl group of the succinic acid of the tail using the NHS chemistry, while the oligonucleotide (T_{10}) spacer was ordered as part of the ssDNA sequence at its 3'.¹² The ssDNA-amphiphile products were purified by reverse-phase high performance liquid chromatography on an Agilent Zorbax C3 300 Å SB column, and molecular weights were verified by liquid chromatography–mass spectrometry (LC-MS). LC-MS data were acquired with an Agilent Zorbax C8 300 Å SB column with a linear gradient from 50% to 80% buffer B over 30 min at 15 $\mu\text{L}/\text{min}$. Buffer A: 15 mM

ammonium acetate in Milli-Q water. Buffer B: Acetonitrile. An Agilent MSD ion trap was used for the determination of the mass.

ELASA. The protocol used was modified from a previous publication.³⁶ 50 ng of a protein solution (β -LG, α -lactalbumin, or ovalbumin) dissolved in PBS was added to each reaction well of a 96-well plate and incubated for 16–18 h at 4 °C. The plate was washed three times with 200 μ L of PBS with 0.05% Tween 20 in a Stat Fax 2600 microplate washer (Awareness Technology Inc., Palm City, FL). Surface blocking was performed by adding 200 μ L of 2% bovine serum albumin (BSA) in PBS in each well and incubated at room temperature for 2 h. The BSA solution was removed by inverting the plate. Biotinylated ssDNA, free or as amphiphile, (0–30 μ M) in 10 mM NaCl in nuclease free water was added to each reaction well, the plate was incubated at room temperature for 2 h on an orbital shaker (Fisher Scientific, Hanover Park, IL), and washed three times with 200 μ L of PBS with 0.05% Tween 20 in the Stat Fax 2600 microplate washer. NeutrAvidin HRP was added to each reaction well, 50 μ L of 1 μ g/mL solution in PBS; the plate was incubated for 30 min at room temperature and washed in the automatic plate washer. 100 μ L of substrate TMB was added in each well and the plate was allowed to oxidize for 20 min at room temperature. The stop solution, 1 M H₂SO₄, was added and the plate absorbance was read at 450 nm in a Bio-Tek Synergy HT (Winoski, VT) plate reader. Background binding of the ssDNA in the well without the protein was subtracted from the measurements. Binding data from individual experiments were averaged, followed by analysis using a nonlinear least-squares regression analysis fit to $B = B_{\max} \times [A]/(K_D + [A])$ to determine K_D and $B_{\max}$, where B is binding, $B_{\max}$ is saturated binding, $[A]$ is the ssDNA concentration, and K_D is the dissociation constant.³⁶

Circular Dichroism (CD). 10 μ M solutions of the aptamer in 10 mM NaCl, 10 mM KCl, 10 mM MgCl₂, PBS, and water were transferred to 0.1 cm path length cuvettes and their CD spectra were obtained using a Jasco J-815 spectropolarimeter (Biophysical Spectroscopy Center, University of Minnesota). Nuclease free water was used for all experiments. Data were collected from 320 to 200 at 50 nm/min read speed in 1 nm steps. Three independent measurements were collected for each sample ($n = 3$), the background spectrum from the electrolyte was subtracted, and the data averaged. Raw ellipticity data were converted to molar ellipticity (Θ) and smoothed with a Matlab filter. Similar experiments were performed with the ssDNA-amphiphiles (with or without the biotin tag) in 10 mM NaCl. The presence of the biotin tag did not affect the CD spectra.

Cryogenic Transmission Electron Microscopy (cryo-TEM). 4.5 μ L of 500 μ M amphiphiles were deposited onto lacey Formvar/carbon grids that were treated for 60 s with glow discharge and vitrified in liquid ethane by Vitrobot (Vitrobot parameters: 4 s blot time, 0 offset, 3 s wait time, 3 s relax time, 95% humidity). The grids were kept under liquid nitrogen and transferred to a Tecnai G2 Spirit TWIN 20–120 kV/LaB₆ TEM operated with an acceleration voltage of 120 kV. An Eagle 2k CCD camera was used to capture images.

Dynamic Light Scattering (DLS). DLS measurements were made using a Brookhaven ZetaPALS Particle Analyzer. ssDNA-amphiphile solutions were diluted to 20 μ M with Milli-Q water and transferred to a 1.0 cm path length cuvette for data collection. For each micelle sample, three independent measurements were collected ($n = 3$).

Preparation of LC-Filled Grids. LC-filled grids were prepared as discussed in the literature.³¹ Briefly, glass slides

were cleaned in a piranha solution (70% v/v sulfuric acid and 30% v/v hydrogen peroxide) for 1 h at 80 °C (Warning: piranha solution reacts strongly with organic compounds and should be handled with extreme caution. Do not store in closed containers). The slides were rinsed subsequently with Milli-Q water, ethanol, and methanol, and then dried under a stream of nitrogen gas followed by heating in an oven at 110 °C for 2 h. The slides were immersed in 0.5 mM octadecyltrichlorosilane (OTS) solution in heptane for 30 min, rinsed with dichloromethane, dried with nitrogen, and stored under vacuum. TEM grids were cleaned sequentially with dichloromethane, ethanol, and methanol, heated at 110 °C for 24 h, and placed on the OTS-treated glass slide. 1 μ L of 4-cyano-4'-pentylbiphenyl (SCB) was placed in the grid and the slide was heated for 30 min at 50 °C.

Preparation of LC Aptamer-Amphiphile Biosensor. 10 μ M of β -LG-23 aptamer-amphiphile in 10 mM NaCl was added to the grids and allowed to equilibrate for 20 min. Different concentrations of β -LG, 10 μ M of ovalbumin, or a mixture of 10 μ M β -LG and 10 μ M ovalbumin, all dissolved in 10 mM NaCl, were added to each grid and allowed to equilibrate for 20 more min before optical observation by a Nikon Optiphot light microscope with crossed polarizers in transmission mode (Characterization Facility, University of Minnesota). The source light intensity was set to 50% of full illumination and the aperture at 10%. Images were captured using a Canon SL1 digital camera.

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Notes

The authors declare no competing financial interest.

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