DNA Hydrogels

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Capture and Release of Protein by a Reversible DNA-Induced Sol–Gel Transition System**

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Aptamers are folded oligonucleotides that bind to specific targets (for example, proteins) and are usually created by an in vitro selection process.[1] Aptamers not only have similar target-binding specificities to antibody–antigen interactions, but they also exhibit good thermostability and feasibility of automatic selection, properties that lead to potential applications in biosensors.[2–6] Polyacrylamide can form a thermosensitive hydrogel, which makes it a desirable material for therapeutic applications such as regenerative medicine and drug release.[7] For this application, the investigation of the transitional responses of polymer–drug complexes to external stimuli has led to new understanding about polymer hydrogels.[8] The physical properties (viscosity, density, etc.) of polymer hydrogels are responsive to changes in pH value,[9] temperature,[10] electric fields,[11] and saccharide levels.[12] However, it should be remembered that external stimuli may denature proteins. Langrana and co-workers took advantage of DNA complementarity and developed a new method of creating a polyacrylamide sol–gel transition system.[13–15] This method utilizes the concept of DNA-strand displacement by base pairing, which is the driving force of DNA nanomachines for achieving detectable mechanical movements.[16–19] It provides the possibility of cyclically manipulating polyacrylamide sol–gel transitions by addition of DNA strands at constant temperature and under unchanged buffer conditions.

In this study, a DNA-induced sol–gel transition system was combined with a protein-binding aptamer in order to capture and release proteins. Human α-thrombin was selected as the target protein and an 85-mer cross-linking strand, A, was designed with the inclusion of a specific thrombin-binding aptamer (GGTTGGTGTGGTTGG), which was able to form a double-stacked G quadruplex[20] with a high affinity to α-thrombin. The polyacrylamide hydrogel was prepared in two steps: 1) Two kinds of 20-mer DNA strands (G1 and G2) were grafted on to the main chains of polyacrylamide; 2) the polyacrylamide main chains were cross-linked into a hydrogel by 85-mer DNA strand A with the two ends being complementary to strands G1 and G2, respectively. Upon addition of another 85-mer DNA strand, D, which was fully complementary to strand A, the hydrogel was dissolved and the thrombin was released.

The DNA-attached acrydite (Integrated DNA Technologies, Inc., IA, USA) is reported to have similar activity to those of acrylamide monomers.[21] When acrydites with strands G1 and G2 attached are copolymerized with acrylamide monomers, strands G1 and G2 can be grafted on to the main chain of the polyacrylamide. The average distance between the grafted DNA strands (G1 or G2) is 30 acrylamide monomers. Scheme 1a shows the capture of thrombin by the thrombin-binding aptamer segment of strand A. Since strand A has two segments complementary to G1 and G2, respectively, the hydrogel was formed by addition of the thrombin-bound strand A into the system, as shown in Scheme 1b. It should be noted that strand A has a toehold at the 3′ end, which acts as a recognition tag for strand displacement. The process of gel dissolution and thrombin release can be operated by addition of strand D, which is fully complementary to strand A, as shown in Scheme 1c.

The capture and release of thrombin by strand displacement were examined by native polyacrylamide-gel electrophoresis (PAGE). The gel image is shown in Figure 1. Lane 1 was loaded with a 100-base pair DNA ladder (Fermentas International Inc., CA, USA). Lane 2 was loaded with strand A. Lane 3 was loaded with the mixture of thrombin and strand A hybridized at 37 °C for 4 h. The band shift of strand A in lane 3 relative to lane 2 is due to the thrombin binding. Lane 4 was loaded with the thrombin-bound strand A and strand D. Lane 5 was loaded with a mixture of strands A and D, which acted as a positive control for the displacement. It can be clearly seen that the band of the duplex of strands A and D in lane 4 corresponds very well with the control experiment in lane 5, a result that demonstrates the successful design of the release mechanism.

The capture and release of the thrombin were measured by the Bradford method,[22] which employed a Bio-Rad protein assay. After the gelatinization by thrombin-bound strand A, the hydrogel was immersed in tris(hydroxymethyl)aminomethane/acetic acid/ethylenediaminetetraacetate (TAE)/Mg^{2+} buffer at 4 °C for a week to leach out free thrombin. The thrombin concentration in the buffer solution was measured to be 2.3 μmol L^{-1}. The hydrogel was then taken out of the buffer and dissolved in a solution of strand D; the thrombin concentration was measured to be 29.7 μmol L^{-1}.

The reversible sol–gel transition was examined through the flow behavior and viscosity measurements. Figure 2a

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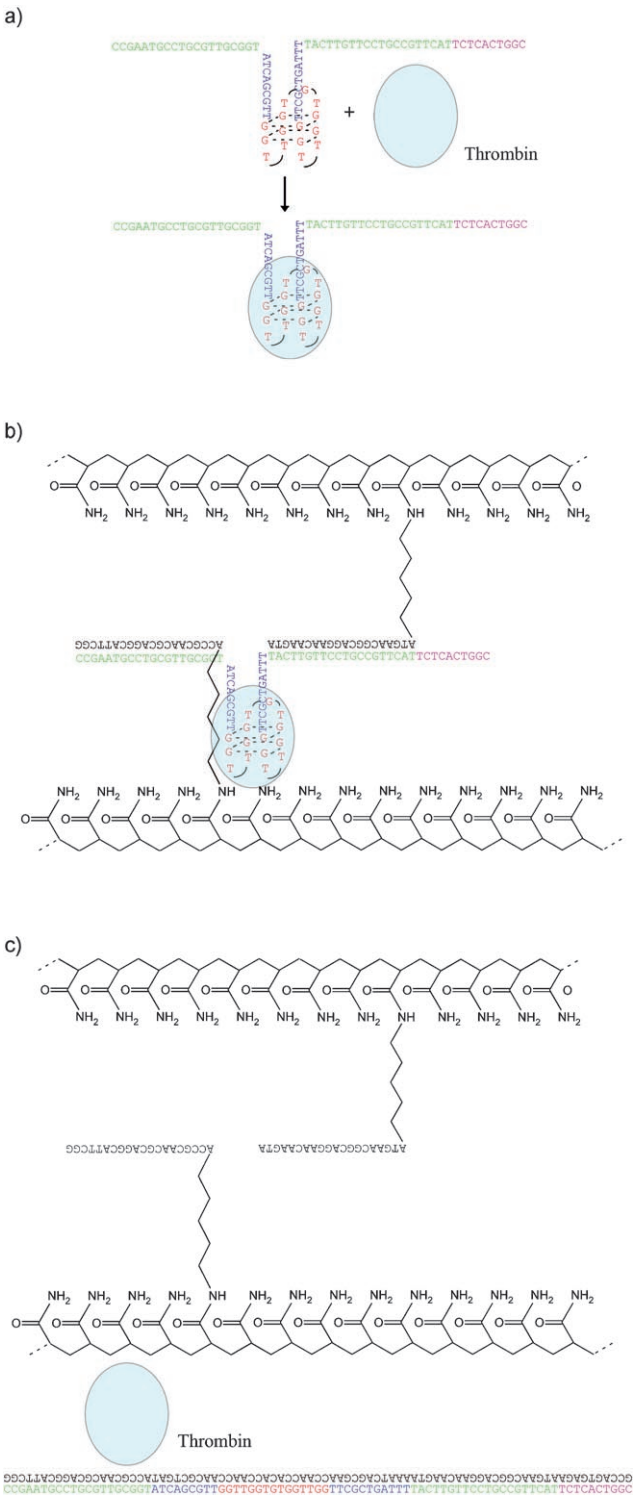

Scheme 1. Capture and release of thrombin by a sol–gel transition. a) The 15-mer segment of strand As red, forms a G quadruplex, which can bind strongly to thrombin in solution to form a thrombin–aptamer complex. b) When the thrombin–aptamer complex is mixed with polyacrylamide linked with strands G1 and G2, the G1- and G2-complementary segments of strand A bind to strands G1 and G2 to form the hydrogel and thrombin is captured in the hydrogel matrix. c) When strand D, which is fully complementary to strand A, is added to the hydrogel, the hydrogel is dissolved and thrombin is released.

shows that the system of polyacrylamide grafted with strands G1 and G2 was in the liquid state since the system flowed downward when the test tube was turned upside down. However, after the thrombin-bound strand A was added, the system gelatinized, as demonstrated by the fact that the system did not flow downward when the test tube was turned upside down (Figure 2b). By immersion of the hydrogel in a solution of displacing strand D, the hydrogel was dissolved again (Figure 2c). We further confirmed this reversible sol–gel transition by measuring the viscosity change of the system at different stages. The results in Figure 2d show that, before thrombin-bound strand A was added, the viscosity of the system was lower than 1 poise. After thrombin-bound strand A was added, the viscosity of the system came to a value of around 20000 poise. After immersion of the gel in a

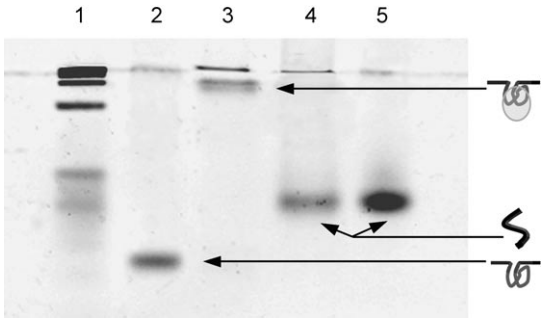

Figure 1. Native PAGE results of controlled thrombin capture and release. Lane 1: 100-base-pair DNA ladder; lane 2: strand A; lane 3: strand A and thrombin mixture at a ratio of 1:1; lane 4: strand A and thrombin complex displaced by strand D at a ratio of 1:1:1; lane 5: strand A/strand D duplex.

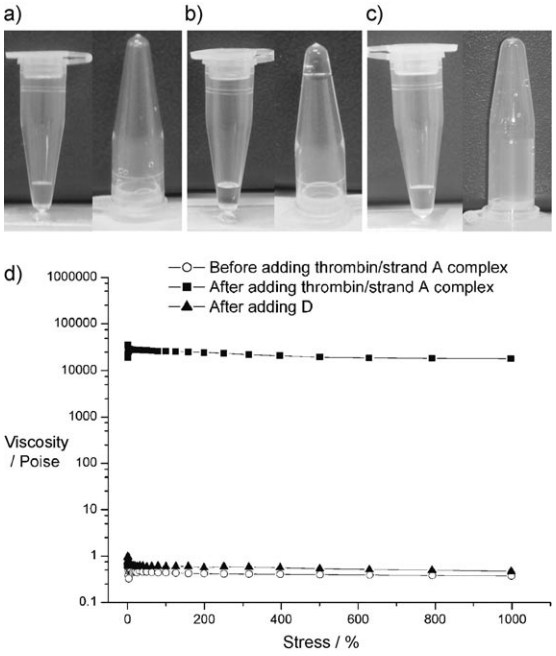

Figure 2. Examination of the sol–gel transition. a) The system was in the liquid state before thrombin-bound strand A was added. b) The system was in a hydrogel state after thrombin-bound strand A was added. c) The system resumed the liquid state after strand D was added. d) Graph showing the viscosity change of 10^4 times between the liquid and hydrogel states.

solution of strand D for a week, the viscosity resumed the value of about 1 poise, a result that shows that the sol–gel transition had occurred reversibly.

In summary, a novel DNA-based reversible hydrogel system was designed to capture and release a protein. Although thrombin was selected as the model protein in this study, other proteins and compounds could also be applied to this sol–gel transition system, provided that a proper binding agent (that is, aptamer) is engineered into the system. As a variety of aptamers can specifically bind to different compounds, this system provides a promising and potentially convenient tool for drug delivery and protein-detection biosensors.

Experimental Section

The DNA strands used in this study were purchased from Integrated DNA Technologies, Inc., IA, USA. All DNA sequences were generated by the sequence-generation program Uniqimer[23] and are shown in Table 1. Strands G1 and G2 are 20-mer strands modified by

Table 1: The sequences of the DNA strands used in the sol–gel transition system.

Name	Sequence
G1	/5′-acrydite/ACCGCAACGCAGGCATTCGG
G2	/5′-acrydite/ATGAACGGCAGGAACAAGTA
A	CCGAATGCCTGCGTTGCGGTATCAGCGTTGGTTGGTGTGGTTGG TTCGCTGATTTTACTTGTTCCTGCCGTTCATTCTCACTGGC
D	GCCAGTGAGAATGAACGGCAGGAACAAGTAAAATCAGCGAA CCAACCACACCAACCAACGCTGATACCGCAACGCAGGCATTCGG

acrydite at the 5′ end. Strand A is a cross-linking strand. The middle 15-mer segment in strand A, highlighted in red, is the thrombin-binding aptamer and the two 20-mer segments, highlighted in green, are complimentary to G1 and G2, respectively. The 10-mer segment at the 3′ end of strand A, highlighted in purple, acts as a toehold that facilitates strand displacement. Strand D, which is fully complementary to strand A, acts as the displacement strand for dissolving the hydrogel and for thrombin release.

The reversible hydrogel transition of polyacrylamide was controlled by DNA strands by using the protocol of Langrana and co-workers.[13] All four DNA strands were stored in stock solutions in 1 × TAE/Mg^{2+} buffer (40 mM tris(hydroxymethyl)aminomethane (Tris), 20 mM acetic acid, 2 mM ethylenediaminetetraacetate (EDTA), 12.5 mM magnesium acetate; pH 8.0) at a concentration of 3 mM. The G1/G2 solution was prepared in a 1.8 mM buffer (4% acrylamide, 1 × TAE/Mg^{2+}) with nitrogen bubbling through the solution for 5 minutes. A catalyst solution (ammonium persulphate at a concentration of 0.05 g in 0.5 mL, 5% (v/v) *N,N,N′,N′*-tetramethylethylenediamine) was added to the G1/G2 solution for polymerization at 1.4% volumetric ratio with nitrogen bubbling through the solution.

The α-thrombin, purchased from Sigma–Aldrich, was from human plasma lyophilized in saline sodium citrate buffer (pH 6.5). It was dissolved in deionized water at a concentration of 180 μM. A solution of thrombin and strand A (1:1 molar ratio) was prepared in the binding buffer (50 mM Tris-HCl, 100 mM NaCl, 3 mM KCl, 1 mM EDTA; pH 7.5) at 37 °C for 4 h.

In order to form the hydrogel, the thrombin-bound strand A and strands G1 and G2 were mixed in stoichiometric concentrations. After incubation at 37 °C for 10 minutes, the mixture became a hydrogel. The cross-linked hydrogel was dissociated by immersion in a solution of strand D with an excess amount relative to strand A. (As a negative control, the hydrogel was immersed in TAE/Mg^{2+} buffer.) The amount of thrombin release, after dissolution of the hydrogel in the strand D solution in TAE/Mg^{2+} buffer, was calculated from the protein concentration measured by the Bradford method,[22] which employed a Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA). The Rheometrics ARES 3 system was used to measure the viscosities of the G1/G2 solution, the hydrogel, and the dissolved hydrogel.

The capture and release of thrombin was examined by native PAGE. Thrombin and strand A were mixed at a 1:1 ratio (1 μM) in TAE/Mg^{2+} buffer. After incubation at 37 °C for 4 h, the solution was then stoichiometrically mixed with strand D (thrombin/strand A/strand D 1:1:1) at 37 °C. (As a positive control, strands A and D were also mixed in the absence of thrombin.) An aliquot (about 5 pmole) of sample for each step was taken for PAGE. A 12% native PAGE assay was run in TAE/Mg^{2+} buffer at 4 °C for 10 h at a constant voltage of 60 V. The gel was stained with ethidium bromide (Sigma–Aldrich).

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