Reversible sol–gel signaling system with epMB for the study of enzyme- and pH-triggered oligonucleotide release from a biotin hydrogel†

Young Jun Seo, Sankarprasad Bhuniya and Byeang Hyean Kim*

Received (in Cambridge, UK) 19th December 2006, Accepted 26th January 2007
First published as an Advance Article on the web 13th February 2007
DOI: 10.1039/b618528b

The biotin-based low molecular weight hydrogel (G1) is able to entrap the epMB and as a consequence, the color of the epMB changes from green to blue; the color change depends on the state of the gelator, *i.e.* upon proceeding from sol to gel and *vice versa*.

Hydrogels are very useful soft materials for many applications, including tissue implanting,[1] bio-sensing,[2] and drug carrying.[3] Fluorescently tagged oligonucleotides, such as molecular beacons (MBs),[4] are increasingly being used as diverse bio-sensing materials for single nucleotide polymorphism (SNP) typing and to detect target DNA. If a hydrogel can carry and release a DNA probe or a DNA drug by recognizing a specific environment, such as pH, enzyme, temperature, and salt concentration, this hydrogel might be useful as an "intelligent" release material; subsequent release at the target site occurs upon decay of the gel.

A sol–gel signaling system was developed to study DNA release from a hydrogel. Although many kinds of gel systems have been reported,[5] to the best of our knowledge there has been no previous report of the sol–gel transition of supramolecular hydrogels being signaled through the use of a MB. The purpose of the sol–gel fluorescence signaling system using MB is to study the DNA release from the hydrogel under particular pH and presence of enzyme. In this study, we examined an ethynylpyrene-labeled molecular beacon (epMB) as an oligonucleotide signal unit (Fig. 1).[6] We used the biotin derivative[7] as a gelator to entrap the DNA; biotin is biocompatible and non cytotoxic, and thus suitable for use *in vivo*.

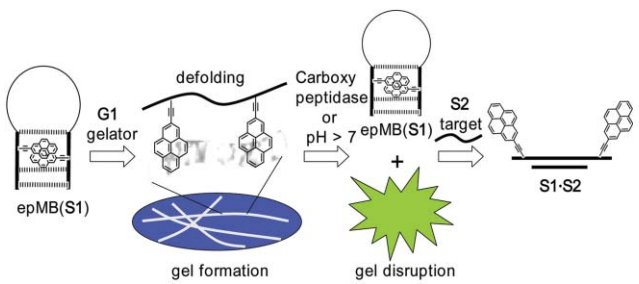

Fig. 1 Cartoon representation of a pH- and enzyme-dependent gel–sol transition system incorporating a molecular beacon.

Department of Chemistry, BK School of Molecular Science, Pohang University of Science and Technology, Pohang 790-784, Korea. E-mail: bhkim@postech.ac.kr; Fax: (82) 54-279-3399; Tel: (82) 54-279-2115

† Electronic supplementary information (ESI) available: MALDI-TOF mass data, photographic images, SEM images, fluorescence spectra, FTIR spectra, HPLC data after enzyme treatment, and the preparation and deformation of gels. See DOI: 10.1039/b618528b

S1 5'd-GCGAA^{Py}G AAGTTAGAACCTATG CA^{Py}TCGC
S2 3'd- TTCAATCTTGGATAC

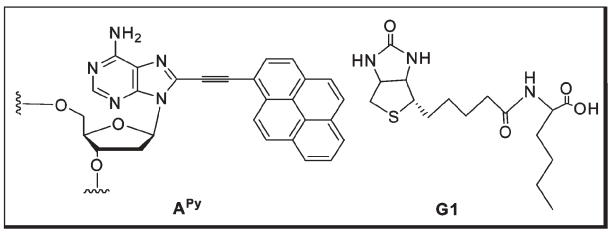

Fig. 2 Structures of the fluorophore nucleoside, oligonucleotide, and gelator used in this study.

To prepare the epMB probing system, we incorporated two pyrene-labeled phosphoramidite building blocks into the stem region of an oligonucleotide strand by using standard phosphoramidite methods and a DNA synthesizer (Fig. 2).[8] This MB-like probing system operates through self-assembly of the pyrene units in the stem region of a hairpin oligonucleotide; the stacking of these units leads to a new red-shifted band (green color) in a hairpin state and, if there is target DNA, the stacking of the pyrenes is broken: the red-shifted band changes to the blue band region. The signal of the epMB is reversible and reproducible.

With this system, we could identify the sol–gel transitions of the biotin-based gelator by monitoring the fluorescence changes of the epMB probe molecule. We expected that sol–gel transition would provide a new stable microenvironment for the epMB; *i.e.* one not involving π-stacking, and, hence, induce a color change. Fig. 3

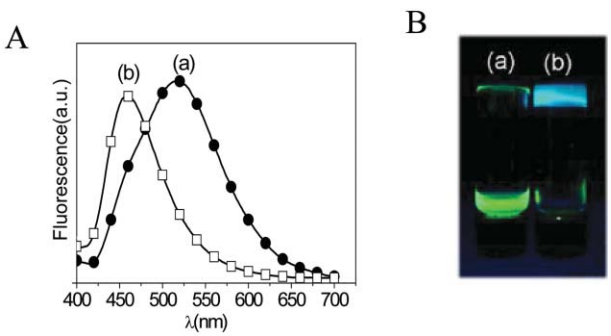

Fig. 3 A. Emission spectra of (a) **S1** (1.5 μM, 200 μL), (b) a mixture of **S1** (1.5 μM, 200 μL) and **G1** (0.3%) after heating and cooling (gel formation). B. Photographic image of A. All samples were prepared in buffer (100 mM Tris–HCl (pH 7.2), 10 mM $MgCl_2$, 100 mM NaCl) and imaged at 20 °C under irradiation at 386 nm.

shows the fluorescence changes of the epMB that occurred during the sol–gel transitions of a biotin gel. In the solution state [*i.e.* below the minimum gelation concentration (MGC) of the biotin gelator] the epMB exhibited its original color (green), whereas in the gel state (0.3% MGC), interestingly, it exhibited a blue color. We believe that the stacking that occurs between the two pyrene units in the sol state must be disrupted in the gel state because of strong interactions between the gelator and the epMB.

To confirm if this epMB system can be used generally in other gel systems, we screened several kinds of gels, such as polyacrylamide, hydrophilic agarose, and other amino acid-attached biotin gelators (Fig. S1). As expected, gels containing polyacrylamide and biotin also show the color change. However, agarose gel, which is hydrophilic and does not have a hydrophobic region, did not show any color change. On the basis of these data, we think that this epMB can be used as a probe for detecting general sol–gel transitions.

We envisioned that this kind of system might be useful for studying the delivery of oligodeoxynucleotide (ODN) strands to a specific region of bio-organ.[9] To examine the release of an epMB from the gel phase into solution under specific conditions, we monitored the fluorescence signal at various pH values (Fig. 4). At pH 2 and pH 4 (acidic conditions), no phase change occurred and the signals appeared at a λ_{max} of 445 nm; at pH 7 (neutral conditions), we observed only a slight change in the appearance of the signal. However, at pH 7–10 (basic conditions), the gel was disrupted quickly and the signal shifted from a value of λ_{max} of 445 nm to 492 nm (blue to green), indicating that epMB has reformed the hairpin conformation (Fig. 4A).

The self-assembly of biotin-based hydrogels occurs mainly through hydrogen bonding between the carboxylic acid group of the amino acid unit with the ureido moiety of the biotin unit.[7] Under alkaline conditions, however, the carboxylic acid unit is ionized and self-assembly is disrupted. As a consequence, the rate of release of the epMB is increased.

The structure of our biotin gel moiety consists of normal leucine peptide containing the carboxylic acid. Carboxypeptidase can cleave the peptide bond by recognition of the carboxylic acid. Carboxypeptidase Y was used for disruption of the biotin gel by enzymatic cleavage. We added carboxypeptidase Y to the biotin hydrogel containing epMB and incubated it for 10 min at room

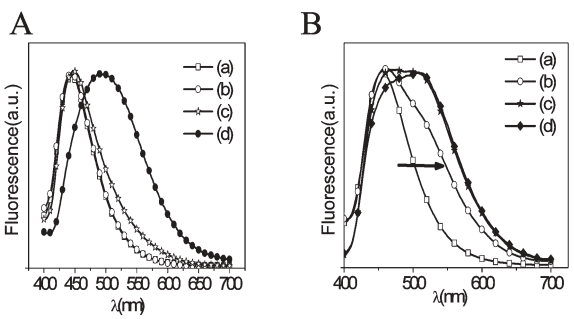

Fig. 4 A. Emission spectra of pH-sensitive controlled-release of entrapped ODN **S1** from **G1** gels at (a) pH 2, (b) pH 4, (c) pH 7, and (d) pH 10 after 1 h. B. Normalized emission spectra of release of entrapped ODN **S1** from **G1** gels with (a) 1 mg, (b) 2 mg, (c) 3 mg, (d) 4 mg of carboxypeptidase Y. The spectra were recorded under the conditions described for Fig. 3.

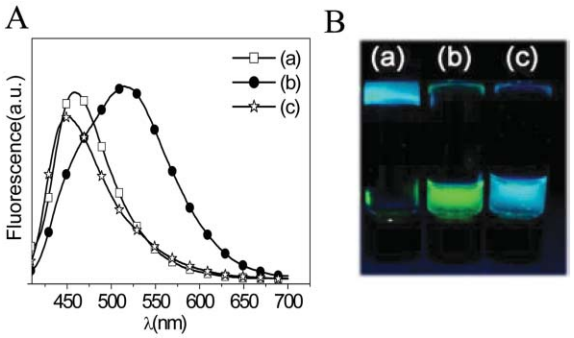

Fig. 5 Emission spectra (A) and photographic image (B) of (a) **G1** gel (entrapped with ODN **S1**), (b) after adding 5% $NaHCO_3$ (100 μL) to **G1** gel, and (c) after adding **S2** (1.5 μM, 30 μL) into the solution (b). The spectra were recorded under the conditions described for Fig. 3.

temperature with shaking. The change of fluorescence from blue to green was observed with disruption of the hydrogel (Fig. 4B). An HPLC experiment demonstrated peptide bond cleavage; we observed the normal leucine after treating the biotin hydrogel with carboxypeptidase Y (Fig. S2).

To examine the functional reproducibility of the epMB after release, we added the target complementary oligonucleotide (ODN **S2**) into the released solution (Fig. 5). The color of epMB changed to blue once again; *i.e.* this hairpin-type epMB changes its conformation into a duplex upon binding with the target sequence and, as such, it operates as a probe.

In summary, we have developed a novel reversible sol–gel signaling system incorporating an epMB. We demonstrated the controlled release of oligonucleotides from a hydrogel through monitoring the color change of the epMB (as evidenced by its fluorescence signal) that occurred upon its release from the gel into solution. The release of this oligonucleotide from the biotin hydrogel depends upon the presence of a specific enzyme and the pH of the buffer. We believe that this epMB can be used as a signal unit for general sol–gel transitions and such biotin hydrogels will be useful materials for delivering oligonucleotide probe and drugs to biological targets with physiological (pH) and enzymatic stimuli.

Notes and references

1 R. Dagani, *Chem. Eng. News*, 1997, **75**, 26.

2 (*a*) M. Shirakawa, N. Fujita, T. Tani, K. Kaneko and S. Shinkai, *Chem. Commun.*, 2005, 4149; (*b*) J. Eastoe, M. Sànchez-Dominguez, P. Wyatt and R. K. Heenan, *Chem. Commun.*, 2004, 2608; (*c*) C. Wartelle and F. Bedioui, *Chem. Commun.*, 2004, 1302; (*d*) I. Yoshimura, Y. Miyahara, N. Kasagi, H. Yamane, A. Ojida and I. Hamachi, *J. Am. Chem. Soc.*, 2004, **126**, 12204; (*e*) B. E. Rothenberg, B. K. Hayes, D. Toomre, A. E. Manzi and A. Varki, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 11939.

3 T. Okano, *Biorelated Polymers and Gels*, Academic Press, San Diego, 1998.

4 (*a*) S. Tyagi and F. R. Kramer, *Nat. Biotechnol.*, 1996, **14**, 303; (*b*) S. Tyagi, D. Bratu and F. R. Kramer, *Nat. Biotechnol.*, 1998, **16**, 49; (*c*) G. T. Hwang, Y. J. Seo and B. H. Kim, *J. Am. Chem. Soc.*, 2004, **126**, 6528; (*d*) Y. J. Seo, J. H. Ryu and B. H. Kim, *Org. Lett.*, 2005, **7**, 4931.

5 (*a*) R. Oda, I. Huc and S. J. Candau, *Angew. Chem., Int. Ed.*, 1998, **37**, 2689; (*b*) R. Oda, I. Huc, M. Schmutz, S. J. Chandu and F. C. Mackintosh, *Nature*, 1999, **399**, 566; (*c*) F. M. Menger, K. L. Caram, V. A. Seredyuk and R. P. Apkarian, *J. Am. Chem. Soc.*, 2002, **124**, 1140; (*d*) A. D. Hamilton and L. A. Estroff, *Angew. Chem., Int. Ed.*, 2000, **39**, 3447; (*e*) U. Maitra,

S. Mukhopadhyay, A. Sarkar, P. Rao and S. Indi, *Angew. Chem., Int. Ed.*, 2001, **40**, 2281; (*f*) A. R. Hirst, D. K. Smith, M. C. Feiters, H. P. M. Geurts and A. C. Wright, *J. Am. Chem. Soc.*, 2003, **125**, 9010; (*g*) S. M. Park, Y. S. Lee and B. H. Kim, *Chem. Commun.*, 2003, 2912; (*h*) K. Numata, M. Fujita, S. M. Park, Y. J. Yun, B. H. Kim and S. Shinkai, *Chem. Commun.*, 2004, 1996; (*i*) Z. Yang, H. W. Gu, Y. Zhang, L. Wang and B. Xu, *Chem. Commun.*, 2004, 208.

6 (*a*) Y. J. Seo, G. T. Hwang and B. H. Kim, *Tetrahedron Lett.*, 2006, **47**, 4037; (*b*) Y. J. Seo, H. S. Jeong, E.-K. Bang, G. T. Hwang, J. H. Jung, S. K. Jang and B. H. Kim, *Bioconjugate Chem.*, 2006, **17**, 1151–1155.
7 S. Bhuniya, S. M. Park and B. H. Kim, *Org. Lett.*, 2005, **7**, 1741.
8 M. J. Gait, *Oligonucleotide Synthesis: A Practical Approach*, IRL Press, Washington, DC, 1984.
9 G. E. Jones, *Human Cell Culture Protocols*, Humana Press, Totowa, New Jersey, 1996.

