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Communication—DNA Quadruplex Hydrogel Beads Showing Peroxidase Activity

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Biodegradable, intelligent, and self-healing hydrogel beads showing peroxidase activity has been prepared by adding hemin to "DNA quadruplex hydrogel" beads prepared by assembling dG_4-PEG-dG_4 triblock copolymers through G quadruplex formation between the dG_4 portion. Efficient binding of hemin to parallel G-quadruplexes in hydrogels was confirmed by observing hyperchromicity of hemin absorbance. The enzymatic activity of resulting hemin peroxidase was visualized both with fluorogenic and chromogenic substrates. These observations serve as the first direct evidence of the presence of G-quadruplexes in DNA quadruplex hydrogels utilizing G-quadruplexes.

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Recent development of various hydrogels with unique properties is attracting scientists not only from material chemistry but also from broad research fields such as medical science.[1,2] DNA is one of popular materials used to prepare hydrogels since various functional DNA motifs are already known, and easily employed to achieve intelligent hydrogels.[3–5] In spite that most of such studies employ typical and popular DNA duplexes in the systems, we have recently developed a new class of DNA hydrogels by utilizing unique tetrameric higher-order structures such as G-quadruplexes and i-motifs.[6,7] The system employing G-quadruplex formation particularly has various advantages (Figure 1).[6] A solution of DNA-PEG-DNA triblock conjugate with only four deoxyguanosine (dG) residues in both DNA segment (L4.6k-dG4), which can be prepared in tens of grams in standard labs through modified high-efficiency liquid phase (HELP) synthesis of oligonucleotides,[8] turns into hydrogel quite rapidly in the presence of physiological Na^+ ion. The resulting "G-quadruplex hydrogel" is biodegradable, intelligent, and self-healable. It is thus considered as an injectable polymer hydrogel,[9] a strong candidate for future DDS carriers.

Although the above properties and advantages of G-quadruplex hydrogels had been carefully characterized already, the study on the mechanism of hydrogel formation has been scarcely carried out. In this study, we introduced hemin, an iron-containing porphyrin, to G-quadruplex hydrogel to construct hydrogels showing peroxidase activity,[10–15] and proved the existence of G-quadruplexes in bulk hydrogels for the first time.

Experimental

Materials.—L4.6k-dG4 were prepared according to the method described in Ref. 6. Control DNA strand d(GpGpGpG) (dG_4) was purchased from IDT DNA (Singapore). Poly(ethylene glycol) (Mw: 4600, $PEG_{4.6k}$) and 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate (6)] (ABTS) were purchased from Sigma-Aldrich. Hemin, 10 × PBS(-), and 1 × PBS(-) were purchased from Wako Pure Chemical. H_2O_2 was purchased from Tokyo Chemical Industry. 10H-Phenoxazine-3,7-diol, 10-acetyl- (Amplex Red) was purchased from Thermo Fisher Scientific.

UV-VIS spectroscopy.—UV-Vis absorbance spectra of 12.5, 25, 50, 125, or 250 μM L4.6k-dG4 ([dG_4 segments] = 25, 50, 100, 250, and 500 μM, respectively), or 25, 50, 100, 250, or 500 μM dG_4 DNA solution in 1 × PBS(-) with 50 μM hemin were collected using a Jasco V-630Bio spectrophotometer equipped with a Peltier temperature controller. Spectra at 25°C were collected between 340 and 440 nm with 2 nm steps and standard sensitivity. Scan rate was set to 100 nm/min with 1 second integration time. A total of three scans were collected and averaged.

CD measurements.—Circular dichroism (CD) of 250 μM, L4.6k-dG4 solution in 1 × PBS(-) with or without 50 μM hemin were collected using a Jasco J-1500 spectropolarimeter equipped with a Peltier temperature controller at 25°C. Scan rate was set to 20 nm/min with 1 second integration time. A total of three scans were collected and averaged.

Hemin-DNAzyme activity assay in hydrogel beads.—For preparation of 10wt% L4.6k-dG4 gel beads, 10 μL of milliQ water, 0.4 μL of 50 μM hemin (in DMSO), 2.6 μL of 3.9 mM Amplex Red (or 1.8 mM ABTS), and 8.88 mg of 25wt% L4.6k-dG4 stock solution were mixed. Then 10 μL of the mixture was added to 10 × PBS(-) containing 20wt% $PEG_{4.6k}$. Prepared gel beads were transferred into 5 ml of 1 × PBS(-) containing 20wt% $PEG_{4.6k}$, 1 μM hemin, and 125 μM H_2O_2, then the system were incubated at room temperature.

Results and Discussion

The structure of DNA-PEG-DNA triblock conjugate used in this study (L4.6k-dG4) is presented in Figure 1a. In modified HELP synthesis, $PEG_{4.6k}$ was used as a semi-solid-phase substrate, which is highly soluble in acetonitrile, a typical solution for most of the reactions in phosphoramidite DNA chemistry, and other solvents, and at the same time, easily precipitates upon addition to poor solvents such as ethers. To both of the ends of $PEG_{4.6k}$, four consecutive dG residues were coupled to provide dG_4 segments as a quaternary component of G-quadruplex. When Na^+ or K^+ is added to the solution of L4.6k-dG4, the system immediately turns into stiff hydrogels sufficiently stable even above 37°C (Figure 1b).[6]

As a probe for G-quadruplex formation in the G-quadruplex hydrogels, we selected hemin in this study. It is widely known that hemin strongly binds to G-quadruplex structures, and that the resulting hemin/G-quadruplex complexes show peroxidase-like activity in the presence of H_2O_2 (Figure 1c).[10–13] The hemin/G-quadruplex complexes is thus called hemin-DNAzyme, and often used to construct enzymatically active hydrogels.[14,15]

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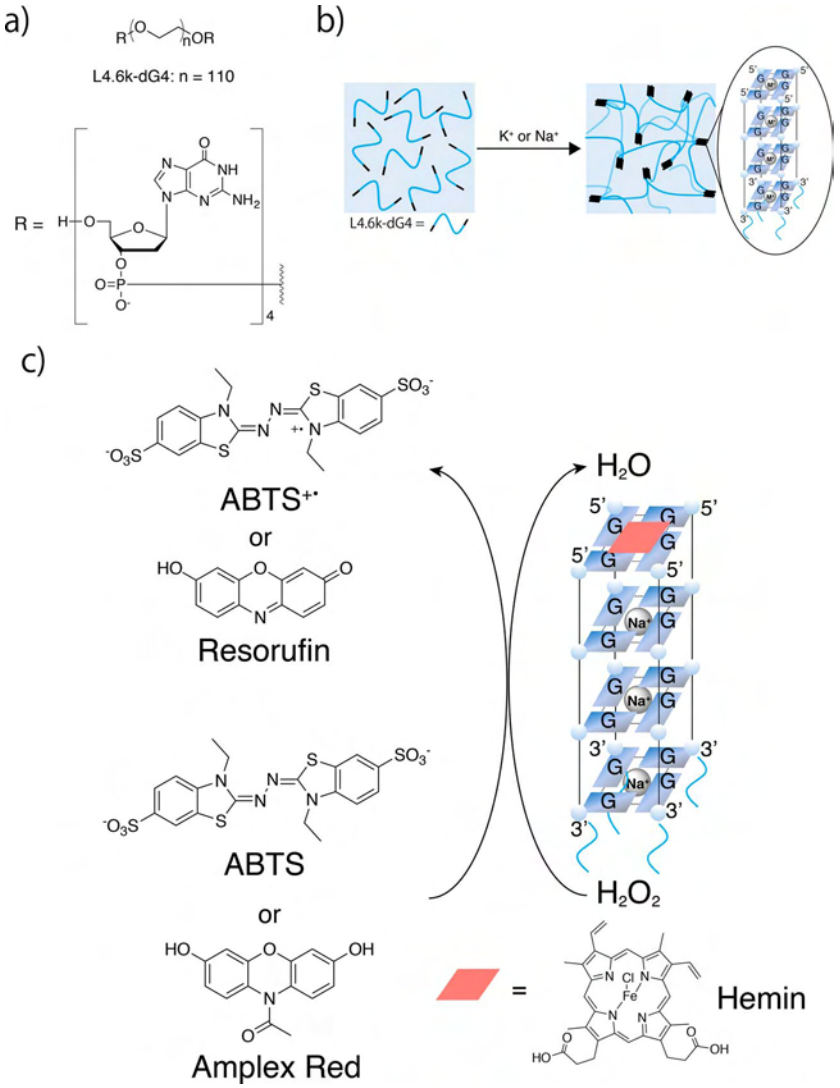

Figure 1. (a) The structure of the DNA-PEG-DNA conjugate used in this study. (b) Schematic illustration of the gelation mechanism of G-quadruplex hydrogels. (c) Hemin/G-quadruplex-catalyzed reaction of Amplex Red or ABTS.

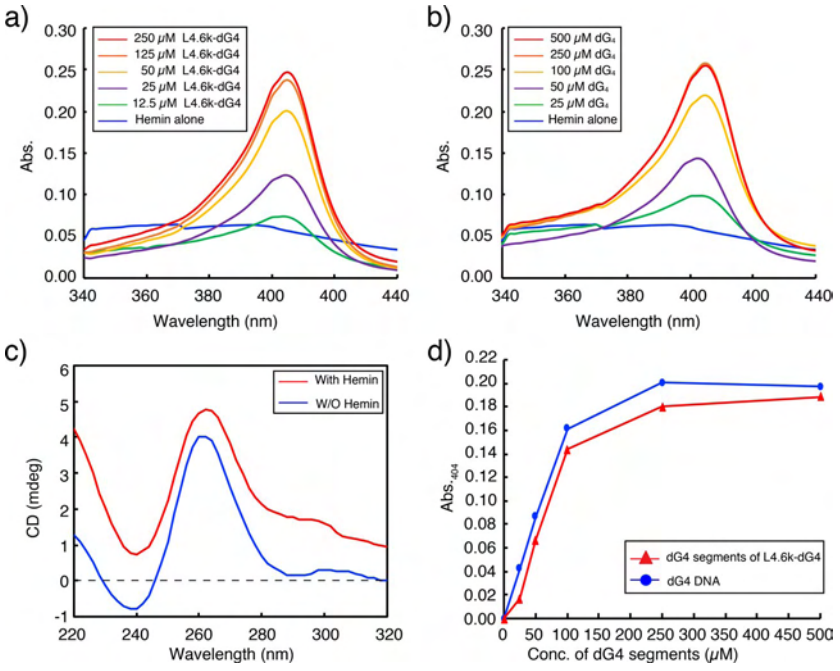

Figure 2. UV-vis absorption spectra (340–440 nm) in 1 × PBS(-) of 50 μM hemin alone (blue), with 25 (green), 50 (purple), 100 (yellow), 250 (orange), or with 500 μM (red) dG_4 segment in L4.6k-dG4 (a) or in dG_4 control strand (b). (c) CD spectra of 250 μM L4.6k-dG4 in the absence (blue) or the presence (red) of 50 μM hemin. (d) Concentration-dependent increase of absorbance at 404 nm against dG4 segments in L4.6k-dG4 (red triangles) and dG4 DNA (blue circles).

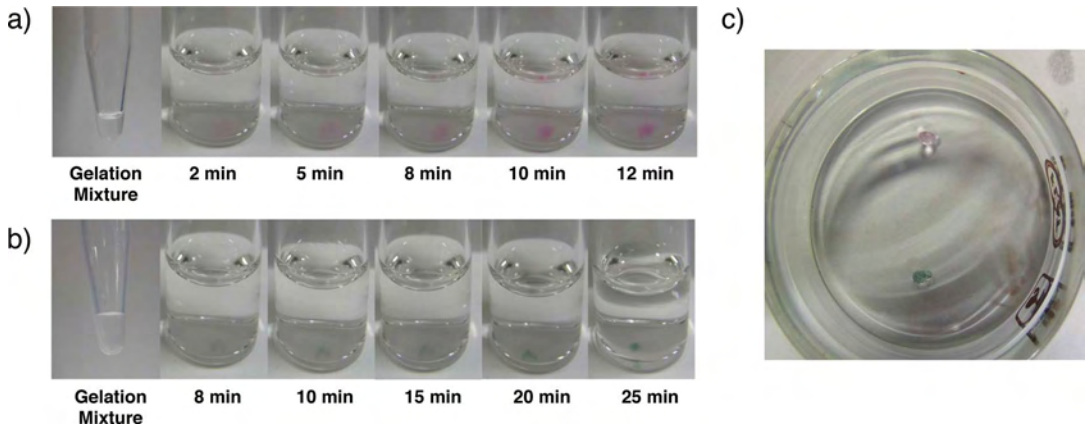

Figure 3. Reactivity of hemin/G-quadruplex complexes within hydrogel bead against Amplex Red (a) or ABTS (b) in reaction solution containing H_2O_2. (c) Hydrogel beads were taken out from the solution after reaction and put into a glass dish (top, with Amplex Red; bottom, with ABTS).

We first observed UV-VIS absorbance of hemin in the presence of dilute L4.6k-dG4, or free G-quadruplexes as a control (Figure 2). As the dG_4 segments in the solution increases, significant hyperchromicity with the maximum absorbance around 420 nm was observed both for L4.6k-dG4 and free G-quadruplexes solutions (Figures 2a and 2b). CD spectra of 250 μM L4.6k-dG4 showed typical Cotton effects for parallel G-quadruplexes both in the absence and presence of hemin (Figure 2c). Concentration-dependent profiles of the hyperchromicity for both of the systems were almost the same as each other (Figure 2d). When prepared from dilute solutions such as 0.1wt% ([dG_4 segment] ≈ 280 μM), L4.6k-dG4 gives mesoscale hydrogel particles of 100–200 nm in diameter (unpublished). The present observation confirms that, at least in mesoscale hydrogels, G-quadruplexes are indeed formed and bind hemin as efficiently as free G-quadruplexes without PEG segment.

We then prepared G-quadruplex hydrogel beads in the presence of hemin, and examined their peroxidase activities using two distinct peroxidase probes (Figure 1c). A hydrogel bead of 10 μL in volume (ca. 4 mm in diameter) was prepared by dropping 10wt% L4.6k-dG4 solution containing both hemin and Amplex Red reagent into 10 × PBS(-), and it was transferred into reaction buffer containing H_2O_2 in 1 × PBS(-). Amplex Red is a highly sensitive and stable fluorogenic probe for H_2O_2 or peroxidase activity, and it reacts in a 1:1 stoichiometry with H_2O_2 in the presence of peroxidase to produce water-insoluble and red resorufin dye. Figure 3a shows the color change of G-quadruplex hydrogel bead in the presence of hemin together with Amplex Red reagent. The hydrogel bead rapidly turned into red within 15 min under room temperature, clearly showing that hemin/G-quadruplex complexes are formed in the hydrogel, and working as hemin-DNAzyme in the bead. Considering that the red color accumulated almost uniformly in the bead, G-quadruplexes may be evenly distributed in the hydrogel.

Similar coloring of G-quadruplex hydrogel bead was also observed with other chromogenic peroxidase substrate, ABTS (Figure 3b). As the ABTS-containing bead was submerged in 1 × PBS(-) with H_2O_2, green color corresponding to the reaction product ($ABTS^{\cdot+}$) gradually grew in the bead within 25 min under room temperature. In contrast with Amplex Red system, one-electron oxidation of ABTS gives water-soluble product. Significant accumulation of the green color in the hydrogel bead may represent relatively slow chemical diffusion in the hydrogel matrix, or, possibly, weak interactions between G-quadruplexes and $ABTS^{\cdot+}$.

Conclusions

In conclusion, hemin peroxidase has been successfully integrated into G-quadruplex hydrogels, and its activity was confirmed. The present observations serve as the first direct evidence of the existence of G-quadruplexes in G-quadruplex hydrogels. In addition, G-quadruplex hydrogels with enzymatic activity may realize new class of intelligent drug delivery systems that can convert prodrugs into target drugs in situ and release them according to the environment, as well as a sensing probe.[16]

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References

1. E. M. Ahmed, *J. Adv. Res.*, **6**, 105 (2015).
2. A. S. Hoffman, *Adv. Drug Deliver. Rev.*, **64**, 18 (2012).
3. X. Xiong, C. Wu, C. Zhou, G. Zhu, Z. Chen, and W. Tan, *Macromol. Rapid Comm.* **34**, 1271 (2013).
4. Y. Shao, H. Jia, T. Cao, and D. Liu, *Acc. Chem. Res.*, **50**, 659 (2017).
5. J. S. Kahn, Y. Hu, and I. Willner, *Acc. Chem. Res.*, **50**, 680 (2017).
6. S. Tanaka, K. Wakabayashi, K. Fukushima, S. Yukami, R. Maezawa, Y. Takeda, K. Tatsumi, Y. Ohya, and A. Kuzuya, *Chem. Asian J*, **12**, 2388 (2017).
7. S. Tanaka, S. Yukami, K. Fukushima, K. Wakabayashi, Y. Ohya, and A. Kuzuya, *ACS Macro Lett*. **7**, 295 (2018).
8. G. M. Bonora, S. Zaramella, and F. M. Veronese, *Biol. Proced. Online*, **1**, 59 (1998).
9. Y. Li, J. Rodrigues, and H. Tomás, *Chem. Soc. Rev.*, **41**, 2193 (2012).
10. T. Li, S. Dong, and E. Wang, *Chem. Commun*., 4209 (2007).
11. N. Lu, C. Shao, and Z. Deng, *Chem. Commun*., 6161 (2008).
12. S. Bi, J. Zhang, and S. Zhang, *Chem. Commun*., **46**, 5509 (2010).
13. R. Freeman, E. Sharon, C. Teller, A. Henning, Y. Tzfati, and I. Willner, *ChemBioChem*, **11**, 2362 (2010).
14. C. H. Lu, X. J. Qi, R. Orbach, H. H. Yang, I. Mironi-Harpaz, D. Seliktar, and I. Willner, *Nano Lett*, **13**, 1298 (2013).
15. C. H. Lu, W. Guo, X. J. Qi, A. Neubauer, Y. Paltiel, and I. Willner, *Chem. Sci.*, **6**, 6659 (2015).
16. B. Ruttkay-Nedecky, J. Kudr, L. Nejdl, D. Maskova, R. Kizek, and V. Adam, *Molecules*, **18**, 14760 (2013).