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Fluorescent sensor for redox environment: a redox controlled molecular device based on the reversible mercury mediated folded structure formation of oligothymidylate

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Abstract—We report the synthesis of a novel molecular sensor that changes fluorescence emission intensity according to redox environments. The sensor is based on the reversible mercury mediated folded structure formation of oligothymidylates.

The design and synthesis of organic derivatives for molecular devices such as sensors, switches, electrical conductors, ferromagnets, electronic circuits, and nonlinear optical materials has been actively pursued in recent years.[1] DNA has a wire-like structure composed of repeatedly connected structural units such as nucleobases, deoxyriboses, and phosphodiesters.[2] Thus, functional groups can be arrayed along DNA strands by attaching them to the structural units.[3] Recently, synthetic oligodeoxyribonucleotides containing artificial bases have been used for forming metal-mediated base pairs in which hydrogen bonds in Watson–Crick (W–C) type base pairs in natural DNA are replaced by metal–base bonds.[4] We reported the alternative method for generating metal-mediated base pairs in DNA duplexes using only naturally occurring pyrimidine bases.[5] Namely, thymine–thymine (T–T) miss pairs in DNA duplexes selectively capture Hg^{II} ion and the metal mediated base pairs, T–Hg–T, are formed in DNA duplexes (Fig. 1a). Furthermore, Hg^{II} mediated double helical structure formation of two DNA sequences which containing several T residues (Fig. 1b) has been used for developing DNA-based sensors selectively detecting Hg^{II} ions in solutions.[6] This report describes a novel DNA based fluorescent sensor for redox environment which is based on the reversible mercury mediated folded structure formation (Fig. 1c) (supplementary data).

The structure of a redox sensor is shown in Figure 2. The sensor, **D–(T)$_{21}$–F**, consists of 21-mer thymidylate carrying a fluorescent residue (fluorescein, **F**) and a quencher (Dabcyl, **D**) at the 3′- and the 5′-ends, respectively.[6] Both residues are available commercially and are stable under the reaction conditions used for the chemical synthesis and deprotection of oligodeoxyribonucleotides.[7] The hairpin structure forming oligonucleotides containing thymine, guanine, and cytosine bases, which were used for developing the Hg^{II} sensor,[6] was not used for redox sensors since it is well known that guanine residues are easily degraded in oxidative environments.[2,8] A mechanism for detecting redox environment using **D–(T)$_{21}$–F** is shown schematically in Figure 2, lower. In the presence of Hg^{II} ion, Hg^{II}-mediated T–Hg–T pairs are formed between T-residues in the $(T)_{21}$ sequence of **D–(T)$_{21}$–F**, which causes the formation of folded structures. Both termini of the **D–(T)$_{21}$–F** are brought close to each other on formation of the folded structures in the presence of Hg^{II} ions which enhances fluorescence resonance energy transfer (FRET) between the **F** and **D** residues and results in significant quenching of the fluorescent emissions. At the concentrations of the sensor used in the experiments in this article, an intermolecular double helical structure formation may not be a major cause of the quenching of the fluorescence intensities (see supplementary data). In a reductive environment (in the presence of Na_2SO_3), Hg^{II} is converted

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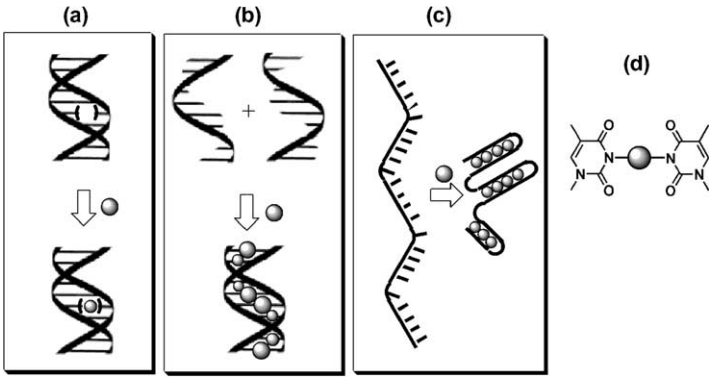

Figure 1. Schematic representations of the interaction modes of DNA and Hg^{II}: (a) T–T miss pairs in DNA duplexes selectively capture Hg^{II} ion and the metal mediated base pairs, T–Hg–T, are formed in DNA duplexes; (b) a double helical structure consisting of Hg^{II} and the two DNA strands which containing several T residues; (c) Hg^{II} mediated folded structure consisting of an oligothymidylate and Hg^{II} ions; (d) an expected structure of the T–Hg–T pair.[5b]

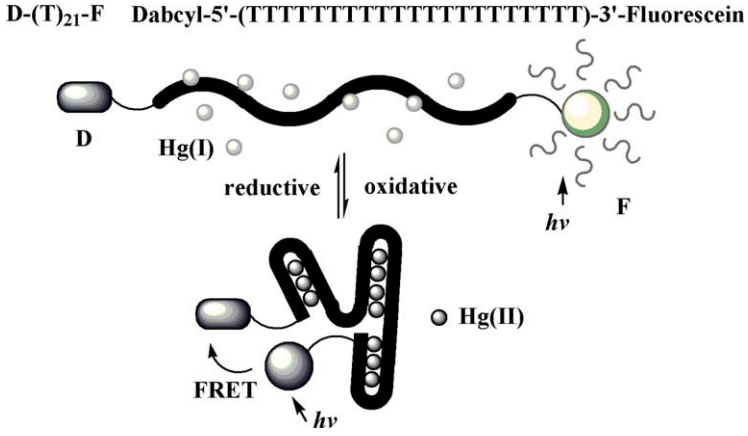

Figure 2. Upper: structure of the fluorescent sensor for redox environment. Lower: a schematic representation of the folded structure and the extended structure conversion induced by $Hg^{II} \leftrightarrow Hg^{I}$ conversion in oxidative and reductive environments.

into Hg^{I}, which cannot form T–Hg^{I}–T pairs, thus the folded structure is converted into the extended structures. Both termini of the **D–(T)$_{21}$–F** are moved far from each other on formation of the extended structure in the presence of Hg^{I} ions (i.e., in the absence of Hg^{II} ion), which reduces FRET between the **F** and **D** residues and results in significant enhancement of the fluorescent emissions.

As shown in Figure 3, the fluorescence emission intensity of a solution containing **D–(T)$_{21}$–F** decreased by adding Hg^{II} ion into the solution (arrow a).[9] The decreased emission intensity was recovered by adding a Na_2SO_3 solution (arrow b). Again, the emission intensity decreased by adding $KMnO_4$ solution (arrow c). Except for the significant changes in their intensities, the shapes of the fluorescence spectra were identical (see supplementary data). The emission intensity of a solution containing **D–(T)$_{21}$–F** in the absence of Hg ions was insensitive to the addition of the Na_2SO_3 and $KMnO_4$ solutions (see supplementary data).

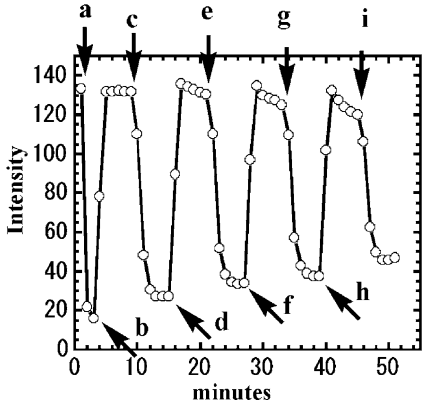

Figure 3. A fluorescence–redox diagram. To a solution containing **D–(T)$_{21}$–F** (0.5×10^{-3} OD at 260 nm), 20 mM NaCl, and 10 mM Mops (pH 7.06) (2 mL) was added following solutions, (a) 1 mM $Hg(ClO_4)_2$ solution, 2 μL; (b) 1 mM Na_2SO_3 solution, 7 μL; (c) 1 mM $KMnO_4$ solution, 7 μL; (d) 1 mM Na_2SO_3 solution, 8 μL; (e) 1 mM $KMnO_4$ solution, 8 μL; (f) 1 mM Na_2SO_3 solution, 9 μL; (g) 1 mM $KMnO_4$ solution, 9 μL; (h) 1 mM Na_2SO_3 solution, 10 μL; (i) 1 mM $KMnO_4$ solution, 10 μL.

Molecular devices that use different chelating properties of a certain metal ion in different redox states have been reported.[10] To the best of the author's knowledge, synthetic molecules and proteins but not nucleic acids have been used for developing such redox sensors. Similar to

these pre-existing devices, the redox sensor, **D–(T)$_{21}$–F**, has the properties of a molecular device[1] as it is sensitive to energy input (redox change) with its components reproducibly transforming between the folded structure ↔ the extended structure and the consequent change in fluorescence emission intensity performing the role of an output. Hence these results demonstrate the utility of nucleic acid structures as the bases of molecular devices.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.02.049.

References and notes

1. (a) Balzani, V.; Credi, A.; Raymo, F. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2000**, *39*, 3348–3391; (b) Balzani, V.; Venturi, M.; Credi, A. *Molecular Devices and Machines. A Journey into the Nanoworld*; Wiley-VCH: Weinheim, 2003; (c) *Molecular Switches*; Feringa, B. L., Ed.; Wiley-VCH: Weinheim, 2001.
2. Saenger, W. *Principal of Nucleic Acid Structure*; Springer: New York, 1984.
3. (a) Kosuge, M.; Kubota, M.; Ono, A. *Tetrahedron Lett.* **2004**, *45*, 3945–3947; (b) Kubota, M.; Ono, A. *Tetrahedron Lett.* **2004**, *45*, 5755–5758.
4. (a) Zimmermann, N.; Meggers, E.; Schultz, P. G. *Bioorg. Chem.* **2004**, *32*, 12–25; (b) Tanaka, K.; Tengeiji, A.; Kato, T.; Toyama, N.; Shionoya, M. *Science* **2003**, *299*, 1212–1213; (c) Zimmermann, N.; Meggers, E.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *124*, 13684–13685; (d) Tanaka, K.; Yamada, Y.; Shionoya, M. *J. Am. Chem. Soc.* **2002**, *124*, 8802–8803; (e) Tanaka, K.; Tengenji, A.; Kato, T.; Toyama, N.; Shiro, M.; Shionoya, M. *J. Am. Chem. Soc.* **2002**, *124*, 12494–12498; (f) Atwell, S.; Meggers, E.; Spraggon, G.; Schultz, P. G. *J. Am. Chem. Soc.* **2001**, *123*, 12364–12367; (g) Weizman, H.; Tor, Y. *J. Am. Chem. Soc.* **2001**, *123*, 3375–3376; (h) Meggers, E.; Holland, P. L.; Tolman, W. B.; Romesberg, F. E.; Schultz, P. G. *J. Am. Chem. Soc.* **2000**, *122*, 10714–10715.
5. (a) Ono, A.; Miyake, Y. *Nucleic Acids Res.* **2003**, *3*(Suppl.), 227–228; (b) Y. Miyake et al. Submitted for publication.
6. Ono, A.; Togashi, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4300–4302.
7. The ODN-based sensors were synthesized using phosphoramidite chemistry on a DNA synthesizer (Applied Biosystems DNA/RNA Synthesizer 392) with commercially available reagents, including a Dabcyl-amidite unit (Glen Research, VA) and fluorescein-attached-CPG (Glen Research). The ODNs were deprotected and purified using established procedures. Each ODN prepared in this study exhibited a sharp major peak in an HPLC analysis.
8. Singer, B.; Hang, B. *Chem. Res. Toxicol.* **1997**, *10*, 713–732.
9. Fluorescence spectra were measured on an RF-5300PC Spectrofluorophotometer (Shimadzu, Japan) at an excitation wavelength of 480 nm with an excitation slit width of 5 nm, and an emission slit width of 5 nm at 24 °C.
10. (a) Kalny, D.; Elhabiri, M.; Moav, T.; Vaskevich, A.; Rubinstein, I.; Shanze, A.; Albrecht-Gary, A.-M. *Chem. Commun.* **2002**, 1426–1427; (b) Heinze, J.; Willmann, C.; Bäuerle, P. *Angew. Chem., Int. Ed.* **2001**, *40*, 2861–2864; (c) Rathore, R.; Magueres, P. L.; Lindeman, S. V.; Kochi, J. K. *Angew. Chem., Int. Ed.* **2000**, *39*, 809–811; (d) Steenwinkel, P.; Grove, D. M.; Veldman, N.; Spek, A. L.; van Koten, G. *Organometallics* **1998**, *17*, 5647–5655; (e) Belle, C.; Pierre, J.-L.; Saint-Aman, E. *New J. Chem.* **1998**, 1399–1402.