J|A|C|S
COMMUNICATIONS

Published on Web 09/18/2004

Modified DNA Analogues That Sense Light Exposure with Color Changes

Jianmin Gao, Soichiro Watanabe, and Eric T. Kool*

Department of Chemistry, Stanford University, Stanford, California 94305-5080

Received May 25, 2004; E-mail: kool@stanford.edu

Molecular sensors are becoming increasingly useful for detection and identification of biomolecules and biophysical conditions in aqueous solution.[1] The large majority of sensors are designed compounds that include a recognition function and a reporting functionality.[2] While such design-based approaches are attractive and have enjoyed considerable successes, the trial-and-error, one-at-a-time approach can be heavily time consuming. For this reason, combinatorial approaches to sensor discovery may offer significant advantages.[2c,3] Here we report on the rapid combinatorial screening and discovery of color-changing sensors of light exposure from a library of over 14 000 DNA-analogous compounds. Their oligonucleotide-based structure and synthesis allows for ready conjugation to DNAs and RNAs for biochemical and biophysical applications.

Our combinatorial approach uses the DNA phosphodiester backbone as a scaffold for arranging fluorescent aromatic compounds in a potentially stacked oligonucleotide-like arrangement. This close proximity of fluorophores results in useful changes in fluorescence properties such as large Stokes shifts and tuned absorption and emission properties.[4] In addition, the DNA backbone maintains water solubility for compounds that otherwise would be poorly soluble, and the automated synthesizer makes preparation of such oligomeric compounds quite simple. We previously reported static fluorescence properties of a small test library of 256 compounds.[4] We now describe a new application of this approach, with the synthesis of a much larger library, and demonstrate a screening strategy that can be applied to changes in fluorescence rather than to static properties alone. This enables sensing of biophysical conditions in solution (in the present case, exposure to light).

We prepared 11 monomeric deoxyribosides as components of a library (Figure 1 and Supporting Information). The monomers were chosen to have varied redox and fluorescence properties. Both α- and *β*-glycosidic anomers of fluorophores on deoxyribose were included, for synthetic convenience and structural diversity. These were then assembled into tetrameric strands on PEG-polystyrene beads using a DNA synthesizer; standard split-and-mix methods were used to yield all possible combinations (11^4, 14 641 total) of the 11 monomers. The chemical encoding methodology of Still[6] was used to aid in later identification of sequences. Based on the weight and loading of the library, we estimate >10x overcoverage of the combinations. Images of the library under the microscope revealed a large variation in fluorescence color and intensity (Figure 2).

To evaluate the ability of these various molecules on beads to respond to light, we used a mercury light source combined with filters to illuminate the beads at 340−380 nm over a period of 1 h and gathered digital images of the visible emission over time. Digital subtraction of later images from the time-zero image revealed beads that were strongly sensitive to light exposure (Figure 2b), whereas others were more resistant. Selected beads of the most sensitive ones were decoded to yield sequences of the tetrameric fluorophores. We then resynthesized a number of these candidate sequences on a preparative scale and characterized them for their chemical integrity, their static fluorescence, and their responsiveness to light exposure. Interestingly, at least three of these were found to respond to light exposure by apparent color changes, rather than by simple photobleaching.

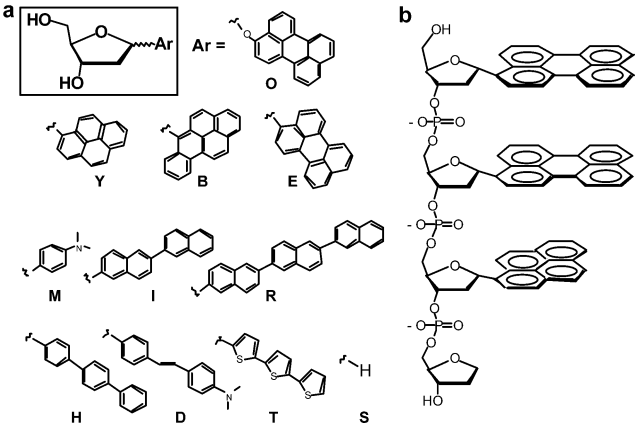

Figure 1. (a) Structures of the deoxyriboside monomers in this study. (b) Structure of one tetramer (5′-EEYS) assembled on a DNA backbone.

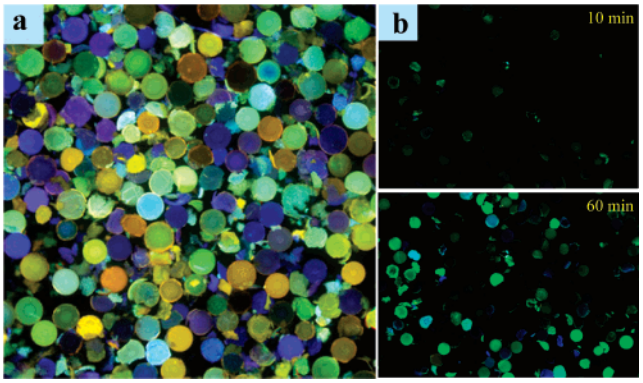

Figure 2. (a) Sample image of a tetrafluor library composed of 14 641 members (excitation 340−380 nm). (b) Digital subtraction images of the tetrafluor library after 10 min (top) and 60 min (bottom) of light exposure. Bright beads indicate a drop in emission intensity.

Three examples of the DNA-like color-changing photosensors were studied in further detail (Table 1). The data showed that the selected compounds were fluorescent and exhibited large Stokes shifts of ca. 130 nm. We evaluated color responses in aqueous solution, using the Xe lamp in a fluorescence spectrometer as the light source being sensed. Figure 3 shows spectra of the tetrafluors before and after light exposure; the data showed that all three changed their colors markedly. For example, compound 5′-SBBB originally showed a broad (green) emission band at 510 nm. This band decreased in intensity over a few minutes, and a shorter wavelength band (412 nm) increased simultaneously. This blue-

10.1021/ja046910o CCC: $27.50

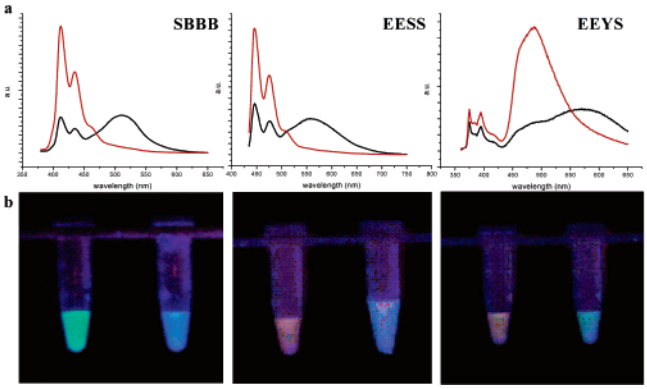

Figure 3. Three tetrafluors that respond to light exposure. (a) Fluorescence emission spectra of the polyfluors before (black lines) and after (red lines) light exposure. (b) Images of the same compounds in aqueous solution before (left) and after (right) light exposure.

Table 1. Static Photophysical Data for Color-Changing Oligomers in Aqueous Solution[a]

	λ_{abs} (nm)	λ_{em} (nm)	λ_{em} (nm) (after light exposure)	$\Phi_{(before)}$
SBBBSS	378, 397	412, 435, 510	412, 435	0.09
EEYSS	329, 346, 418, 445	375, 576	375, 488	0.07
EESSSS	418, 444	447, 476, 567	447, 476	0.03

[a] Extra spacers (S) were added to insure solubility. All samples were prepared in phosphate buffered saline, pH = 7.2.

violet band closely resembles the emission of the monomeric benzo[*a*]pyrene deoxyriboside (data not shown).

Subsequent experiments showed that addition of an oxygen scavenger, Trolox,[7] greatly diminished the rate of the color change, suggesting that an irreversible reaction with oxygen caused the drop in intensity of the longer wavelength band. We hypothesize that the original long-wavelength band arises from excited-state electronic interactions between adjacent benzo[*a*]pyrene monomers in the SBBB sequence.[8] Selective reaction of one of these monomers could render that monomer nonfluorescent, leaving other nonadjacent monomer(s) behind to emit only monomer fluorescence. Other mechanisms may also be possible; more work is underway to evaluate the mechanism of the color change in greater detail.

Similar results were also seen for sequences 5′-EESS and 5′-EEYS. The former changes emission color from orange to blue (Figure 3) while sensing irradiation at 418 nm, and the latter changes from orange to cyan-green with excitation at 346 nm. Thus, either UV or visible light can be sensed. Excimer- and exciplex-based mechanisms[8] are also possible for these latter cases, explaining the initial long-wavelength band. Selective reaction with oxygen might explain the loss of this band with emergence of shorter wavelength emissions. Clearly, this general explanation should be considered preliminary for all three compounds until more data can be obtained.

The clear separation of starting and final emission colors in these tetrafluors allows for a ratiometric measurement of light exposure over time for these light-sensing species. Using a ratio of two wavelengths would be much more accurate and reliable than simple photobleaching, where one band is lost over time. Such compounds could therefore be useful in quantitative measurement of integrated photon flux at tuned wavelengths in biological and materials applications.[9] One-time (read only) usage in DNA-based molecular memory storage is also conceivable. Simpler applications are also possible, including such uses as molecular timers or calendars (by measuring hours or days of light exposure), or even as color-changing indicators in applications such as sunscreens.

Our experiment confirms the utility of combinatorial fluorophore/sensor libraries built on the DNA backbone. The phosphodiester scaffold allows for ready water solubility in fluorophores that would otherwise not be useful in aqueous applications, and the DNA backbone allows for ease of construction and conjugation to molecules and surfaces. The DNA scaffold facilitates face-to-face interactions in the fluorescent monomers, yielding useful new wavelengths of emission and possibly leading to the origin of the sensing color change in the present application.

Finally, the results illustrate the ease of combinatorial screening not only for static fluorescence properties but also for sensing of a physical condition in solution. Future applications of such oligomeric molecules might be developed for responses to other physical properties such as temperature, pH, or ionic strength. In addition, combinatorial searches for responsiveness to molecular species might also be envisioned. A number of these experiments are underway.

Acknowledgment. This work was supported by the U.S. Army Research Office and the NIH (GM067201). J.G. acknowledges a Stanford Graduate Fellowship. S.W. acknowledges Yamada Science Foundation for financial support.

Supporting Information Available: Details of monomer and library synthesis and fluorescence methods (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Guardigli, M.; Michelini, E.; Mirasoli, M.; Pasini, P. *Anal. Chem.* **2003**, *75*, 462A−470A. (b) Pozzan, T.; Mongillo, M.; Rudolf, R. *Eur. J. Biochem.* **2003**, *270*, 2343−2352. (c) Lakey, J. H.; Raggett, E. M. *Curr. Opin. Struct. Biol.* **1998**, *8*, 119−123. (d) Chen, P.; He, C. *J. Am. Chem. Soc.* **2004**, *126*, 728−729. (e) Merino, E. J.; Weeks, K. M. *J. Am. Chem. Soc.* **2003**, *125*, 12370−12371. (f) Li, J.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10466−10467. (g) Fahlman, R. P.; Sen, D. *J. Am. Chem. Soc.* **2002**, *124*, 4610−4616. (h) Fang, X.; Sen, A.; Vicens, M.; Tan, W. *ChemBioChem* **2003**, *4*, 829−834.

(2) (a) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302−308. (b) Muzukami, S.; Nagano, T.; Urano, Y.; Odani, A.; Kikichi, K. *J. Am. Chem. Soc.* **2002**, *124*, 3920−3925. (c) Schneider, S. E.; O'Neil, S. N.; Anslyn, E. V. *J. Am. Chem. Soc.* **2000**, *122*, 542−543. (d) Stojanovic, M.; de Prada, P.; Landry, D. *J. Am. Chem. Soc.* **2000**, *122*, 11547−11548. (e) Hirano, T.; Kikuchi, K.; Urano, Y.; Higuchi, T.; Nagano, T. *J. Am. Chem. Soc.* **2000**, *122*, 12399−12400.

(3) (a) O'Sullivan, C. K. *Anal. Bioanal. Chem.* **2002**, *372*, 44−48. (b) Rosania, G. R.; Lee, J. W.; Ding, L.; Yoon, H. S.; Chang, Y. T. *J. Am. Chem. Soc.* **2003**, *125*, 1130−1131. (c) Lee, J. W.; Jung, M.; Rosania, G. R.; Chang, Y. T. *Chem. Commun.* **2003**, 1852−1853. (d) Apostolidis, A.; Klimant, I.; Andrzejewski, D.; Wolfbeis, O. S. *J. Comb. Chem.* **2004**, *6*, 325−331. (e) Jhaveri, S.; Rajendran, M.; Ellington, A. D. *Nat. Biotechnol.* **2000**, *18* (12), 1293−1297.

(4) Gao, J. M.; Strassler, C.; Tahmassebi, D.; Kool, E. T. *J. Am. Chem. Soc.* **2002**, *124*, 11590−11591.

(5) Strassler, C.; Davis, N. E.; Kool, E. T. *Helv. Chim. Acta* **1999**, *82*, 2160−2171.

(6) (a) Ohlmeyer, M. H. J.; Swanson, M.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922−10926. (b) Nestler, H. P.; Bartlett, P. A.; Still, W. C. *J. Org. Chem.* **1994**, *59*, 4723−4724.

(7) Grunwell, J. R.; Glass, J. L.; Lacoste, T. D.; Deniz, A. A.; Chemla, D. S.; Schultz, P. G. *J. Am. Chem. Soc.* 2001, *123*, 4295−4303.

(8) (a) Birks, J. B.; Christophorou, L. G. *Nature* **1962**, *194*, 442−444. (b) Birks, J. B.; Christophorou, L. G. *Nature* **1962**, *196*, 33−35.

(9) (a) Miron, A. S.; Grima, E. M.; Sevilla, J. M. F.; Christi, Y.; Camacho, F. G. *J. Appl. Phycol.* **2000**, *12*, 385−394. (b) Su, W. W.; Li, J.; Xu, N. S. *J. Biotechnol.* **2003**, *105*, 165−178. (c) Yguersbide, J. *Rev. Sci. Instrum.* **1968**, *39*, 1048−1052.

JA046910O