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A General Strategy to Convert the MerR Family Proteins into Highly Sensitive and Selective Fluorescent Biosensors for Metal Ions

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Selective sensors for detection and quantification of various types of metal ions and organic structures are very important in many applications including environmental and security monitoring, waste management, nutrition, and clinical toxicology. An ideal sensory system would contain multiple members that provide rapid detection of a broad range of analytes. Each member should respond to its target with high sensitivity and selectivity. The combination of these properties is difficult to achieve with chemically designed sensors, although progress has been made.[1] Nature has evolved numerous sensory proteins to control the concentrations of beneficial or toxic metal ions and organic molecules with high sensitivity and selectivity. Coupling of these biological sensory events with detectable signals could afford practical biosensors that have all the desired properties.[2]

We describe here a novel, general approach to construct biosensors that can detect various types of metal ions and some organic molecules by using the MerR family proteins as the platform. The MerR family proteins are transcriptional factors that are widespread in nature, sensing and controlling the concentrations of metal ions such as Hg^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{+}, and Ag^{+} with up to picomolar−femtomolar sensitivity and high selectivity.[3,4] Metal ion binding to the protein induces a conformational change that sends a signal to activate metal detoxification or efflux systems. Some MerR-type proteins also regulate efflux of "toxic" organic molecules and act against oxygen radicals in bacteria.[5,6]

All MerR proteins are stable dimers that bind specific promoter DNA sequences. In the absence of their target molecules, the MerR proteins bind DNA without distorting the duplex structure. Upon binding to analytes, the MerR proteins *unwind DNA and break two central base pairs* in the duplex DNA.[7] The distortion remodels the DNA and triggers transcriptional initiation.

The untwisting and base-unpairing of the duplex DNA are unique to the MerR-type proteins. We envision that if this distortion of DNA structure exerted by the binding of analytes to the MerR proteins can be coupled to a fluorescent signal, then highly sensitive and selective biosensors can be obtained for the detection of a broad range of metal ions and small molecules.

Escherichia coli CueR, a MerR-type metal-regulatory protein that controls intracellular Cu^{+} concentration, was chosen as the first example for the study.[8,9] This protein was cloned, overexpressed, and purified. Pyrrolo-C, a fluorescent base analogue of cytosine which can emit fluorescence at ~445 nm upon excitation at 350 nm, was chosen as the fluorescent reporter.[10] This fluorescent reporter forms a tight base pair with G (Figure 1A). When pyrrolo-C is base-paired with G in a duplex DNA, the fluorescence of this base is significantly quenched through base stacking and hydrogen bonding. Under base-unpairing conditions pyrrolo-C exhibits a much more intense fluorescence signal (Figure 1B).

A 21-mer duplex DNA containing the CueR binding sequence was synthesized (Figure 1).[8,9] A pyrrolo-C base was incorporated into the central part of this sequence. The duplex DNA exhibits

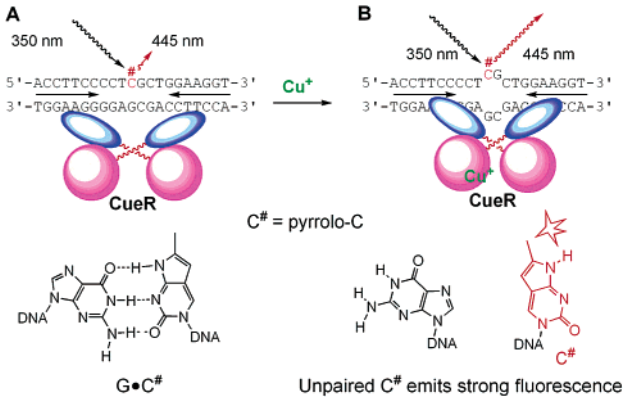

Figure 1. (A) Pyrrolo-C can form a stable base pair with G. (B) The fluorescence intensity of pyrrolo-C is quenched in the duplex DNA. The promoter sequence that CueR binds is shown; the dyad symmetrical sequence is marked with arrows. (C) Binding of the analyte Cu^{+} to CueR induces base-unpairing of pyrrolo-C, which emits strong fluorescence at ~445 nm.

weak fluorescence as expected (Figure 2A). Addition of the CueR dimer caused very small change of the fluorescence intensity (Figure 2A). This change could be due to association of a trace amount of Cu^{+} with CueR after the purification, or simply due to the binding of CueR. Addition of 1 equiv of Cu^{+} triggered an over 3-fold fluorescence enhancement within seconds (Figure 2A). The increase of the fluorescence intensity is proportional to the amount of metal ions present in the solution. Addition of Ag^{+} or Au^{+} triggered similar fluorescence responses as with Cu^{+} (Figures 2B, S1 and S2), which supports a previous finding that both Ag^{+} and Au^{+} can be recognized by *E. coli* CueR.[11,12]

The sensor is selective as addition of 10-fold excess of Zn^{2+}, Co^{2+}, Hg^{2+}, Pb^{2+}, and Cd^{2+} caused less than 1/20 of the fluorescence increase at 445 nm compared to that for Cu^{+} or Ag^{+} (Figure 2B). The fluorescence response of the CueR-based sensor to Cu^{+} or Ag^{+} is over 200-fold higher than that of other metal ions. Nanomolar levels of Ag^{+} or Cu^{+} can be detected with this sensor. The results also indicate that *E. coli* CueR may have a very high affinity toward Cu^{+} since large excess amounts of chelator dithiothreitol (DTT) were used to stabilize Cu^{+} in the experiments.[13] The high selectivity and sensitivity of CueR revealed by our studies agree with the recent structural result of *E. coli* CueR.[12] It was found that the protein possesses a well-designed pocket that can specifically recognize +1 transition metal ions.

To show that this strategy is applicable to other MerR proteins, we also examined the *E. coli* MerR protein. This protein was overexpressed and purified by following a previously published procedure.[14a] A 31-mer duplex DNA containing a pyrrolo-C in the central part of the MerR binding sequence was prepared (Figure S3).[3] Dramatic enhancement of the fluorescence signal was observed when 1 equiv of Hg^{2+} ion was added to the MerR/DNA biosensor under the assay conditions described previously (Figure 3).[14b] This

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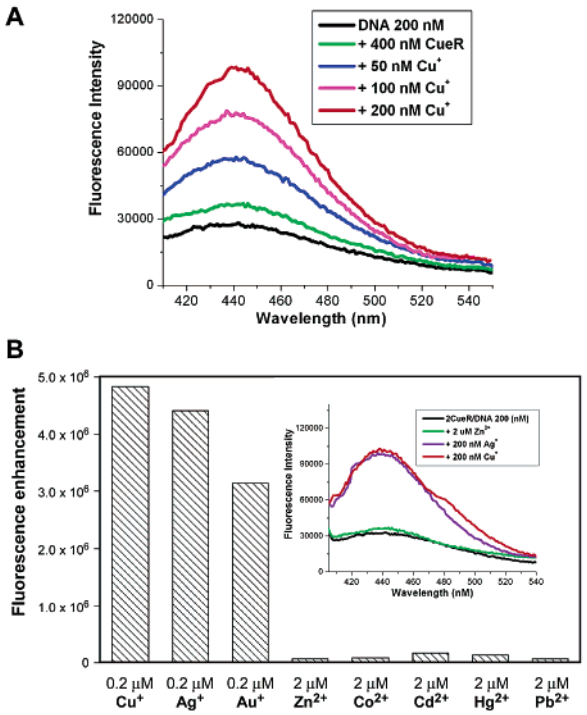

Figure 2. Fluorescence responses of the *E. coli* CueR-based biosensor toward metal ions. (A) Fluorescence spectra of the *E. coli* CueR-based biosensor in the absence and presence of various amounts of Cu^{+} ion. (B) The fluorescence enhancement integrated from 410 to 550 nm in the presence of different metal ions. The insert presents the fluorescence spectra of the sensor in the presence of 200 nM of Cu^{+}, 200 nM of Ag^{+} and 2 μM of Zn^{2+}.

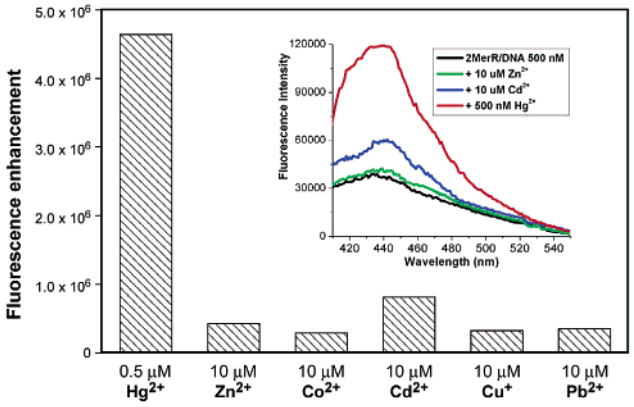

Figure 3. Fluorescence responses of the *E. coli* MerR-based biosensor toward metal ions (the integrated fluorescence enhancements from 410 to 550 nm are shown). The insert presents the fluorescence spectra of the sensor in the absence and presence of 500 nM of Hg^{2+}, 10 μM of Zn^{2+} and 10 μM of Cd^{2+}.

MerR-based biosensor is at least 100-fold more sensitive to Hg^{2+} ion than to the other metal ions tested, with Cd^{2+} having highest response besides Hg^{2+}. Only in the presence of a large excess amount of Cd^{2+} (0.5 mM, 1000 equiv) fluorescence enhancement comparable (~80%) to that obtained for the addition of 1 equiv of Hg^{2+} was observed, which agrees with previous biochemical studies on the MerR protein.[14,15] The selective response of the MerR-based biosensor toward Hg^{2+} further demonstrated that this approach is general for the MerR proteins.

MerR proteins have been used for constructing whole cell- and protein-based biosensors in the past with some success.[16] We introduce here a novel strategy to convert the MerR-type sensory proteins into fluorescent biosensors. Because it is mechanism based, the method is highly selective and sensitive. It gives a direct read rapidly and can provide quantitative measurements. The wide-spread MerR-type proteins offer the potential to construct a series of similar biosensors for the detection of different analytes by applying the same principle. The fluorescence technique may be used as a screening method to evolve new biosensors from the MerR protein templates to recognize unnatural analytes. The method also allows the evaluation of the substrate preferences of the MerR proteins and the study of the kinetics and thermodynamics of substrate binding. This research is under way in our laboratory.

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Supporting Information Available: Experimental details. Figures S1−S3 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Tsien, R. Y. In *Fluorescent Chemosensors for Ion and Molecule Recognization*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993; Vol. 538, pp 130−146. (b)Winkler, J. D.; Bowen, C. M.; Michelet, V. *J. Am. Chem. Soc.* **1998**, *120*, 3237−3242. (c) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968−969. (d) Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1996**, *118*, 3053−3054. (e) Godwin, H. A.; Berg, J. M. *J. Am. Chem. Soc.* **1996**, *118*, 6514−6515. (f) Deo, S.; Godwin, H. A. *J. Am. Chem. Soc.* **2000**, *122*, 174−175. (g) Li, J.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10466−10467. (h) Fahrni, C. J.; O'Halloran, T. V. *J. Am. Chem. Soc.* **1999**, *121*, 11448−11458. (i) Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831−7841.
(2) (a) Miyawaki, A.; Llopis, J.; Heim, R.; McCaffery, J. M.; Adams, J. A.; Ikura, M.; Tsien, R. Y. *Nature* **1997**, *388*, 882−887. (b) Thompson, R. B.; Maliwal, B. P.; Feliccia, V. L.; Fierke, C. A.; McCall, K. *Anal. Chem.* **1998**, *70*, 4717−4723. (c) Benson, D. E.; Conrad, D. W.; de Lorimer, R. M.; Trammell, S. A.; Hellinga, H. W. *Science* **2001**, *293*, 1641−1644.
(3) Brown, N. L.; Stoyanov, J. V.; Kidd, S. P.; Hobman, J. L. *FEMS Microbiol. Rev.* **2003**, *27*, 145−163.
(4) Silver, S. *Gene* **1996**, *179*, 9−19.
(5) Ahmed, M.; Borsch, C. M.; Taylor, S. S.; Vazquezlaslop, N.; Neyfakh, A. A. *J. Biol. Chem.* **1994**, *269*, 28506−28513.
(6) Hidalgo, E.; Ding, H. G.; Demple, B. *Trends Biochem. Sci.* **1997**, *22*, 207−210.
(7) Heldwein, E. E. Z.; Brennan, R. G. *Nature* **2001**, *409*, 378−382.
(8) Outten, F. W.; Outten, C. E.; Hale, J.; O'Halloran, T. V. *J. Biol. Chem.* **2000**, *275*, 31024−31029.
(9) Stoyanov, J. V.; Hobman, J. L.; Brown, N. L. *Mol. Microbiol.* **2001**, *39*, 502−511.
(10) Liu, C. H.; Martin, C. T. *J. Mol. Biol.* **2001**, *308*, 465−475.
(11) Stoyanov, J. V.; Brown, N. L. *J. Biol. Chem.* **2003**, *278*, 1407−1410.
(12) Changela, A.; Chen, K.; Xue, Y.; Holschen, J.; Outten, C. E.; O'Halloran, T. V.; Modgragón, A. *Science* **2003**, *301*, 1383−1387.
(13) Byrd, J.; Berger, R. M.; McMillin, D. R.; Wright, C. F.; Hamer, D.; Winge, D. R. *J. Biol. Chem.* **1988**, *263*, 6688−6694.
(14) (a) Zeng, Q. D.; Stalhandske, C.; Anderson, M. C.; Scott, R. A.; Summers, A. O. *Biochemistry* **1998**, *37*, 15885−15895. (b) Ralston, D. M.; O'Halloran, T. V. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3846−3850. (c) Shewchuk, L. M.; Verdine, G. L.; Walsh, C. T. *Biochemistry* **1989**, *28*, 2331−2339.
(15) Caguiat, J. J.; Watson, A. L.; Summers, A. O. *J. Bacteriol.* **1999**, *181*, 3462−3471.
(16) (a) Corbisier, P.; van der Lelie, D.; Borremans, B.; Provoost, A.; de Lorenzo, V.; Brown, N. L.; Lloyd, J. R.; Hobman, J. L.; Csöregi, E.; Johansson, G.; Mattiasson, B. *Anal. Chim. Acta* **1999**, *387*, 235−244. (b) Bontidean, I.; Lloyd, J. R.; Hobman, J. L.; Wilson, J. R.; Csöregi, E.; Mattiasson, B.; Brown, N. L. *J. Inorg. Biochem.* **2000**, *79*, 225−229 and references therein.

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