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Design of an Emission Ratiometric Biosensor from MerR Family Proteins: A Sensitive and Selective Sensor for Hg^{2+}

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Fluorescent detection and quantification of heavy-metal ions is of great interest because of their environmental and biological importance. For most biological samples, many different metals are present in low concentrations in complex matrices, increasing the need for highly sensitive, selective, and robust sensors that are water compatible and give fast responses. Many small molecules have been designed with some success but for a limited number of metals.[1] Most small molecules suffer from low selectivity and sensitivity as well as low water solubility. As for Hg^{2+} a number of small molecule fluorescent sensors have been designed, but only very few work in water,[1f,g,j,l] have turn on response,[1f−i] and have relatively high sensitivity,[1j,k] and there are even less ratiometric Hg^{2+} sensors reported.[1m,l] The selectivities of these small molecule-based Hg^{2+} binders are still far less than that exhibited by proteins such as MerR (at least 100-fold toward Hg^{2+} over any other metal ion). To overcome these limitations the use of target specific proteins as biosensors is a promising approach,[2] and this study intends to combine all the merits mentioned above.

While most metals are toxic at high concentrations, some are needed for various life processes, and therefore nature has evolved a number of tight regulatory proteins, which are a good platform to achieve high selectivity and sensitivity in water.[3]

MerR family proteins are transcriptional regulators that tightly control the efflux systems of a number of metals such as Hg^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{+}, and Ag^{+} and a number of organic molecules.[4,5] MerR proteins exist as stable dimers in solution and bind sequence specifically to the corresponding promoter sequences in the absence of their specific targets. When a MerR-type protein recognizes its specific target metal ion or organic molecule the protein causes a distortion of the bound duplex DNA. Specifically, the central base pairs of the palindromic sequence of the promoter DNA are broken, and the duplex DNA is untwisted. This action sends the signal for transcriptional initiation.[6,7] In previous work, we have taken advantage of this DNA distortion mechanism and converted different members of the MerR family proteins into fluorescent reporters, such as MerR for Hg^{2+}, CueR for Cu^{+}, and PbrR for Pb^{2+}.[8] A fluorescent DNA base analogue, pyrrolo-C, was placed into the middle of the protein-binding sequence. The fluorescence of pyrrolo-C is quenched when base paired with G in a duplex DNA. DNA distortion, caused by target binding to the MerR proteins, restores the fluorescence of pyrrolo-C.

The pyrrolo-based fluorescent reporter has disadvantages: (i) pyrrolo-C has a low quantum yield (0.07), (ii) the system has a high background and a relatively low fluorescence increase upon DNA distortion, and (iii) the fluorescent response is not ratiometric. We hope to overcome these limitations with a new design. In addition, we also wish to develop simple and reliable methods that can readily report protein binding, dissociation, and distortion of DNA. We decided to rely on dye−dye interactions in the duplex DNA to report protein−DNA interactions.[9] We chose pyrene as the dye because it has a high quantum yield (0.65) and forms an excited-state dimer, termed excimer, resulting in a large stock shift of ∼100 nm. The pyrene excimer is very sensitive to the distance between and the geometry of two close pyrene units, and the response is ratiometric, making this a highly attractive method to probe biological interactions.[10] Furthermore, pyrene has been incorporated into DNA before, and DNA has proven an excellent scaffold to bring dyes close to each other.[11]

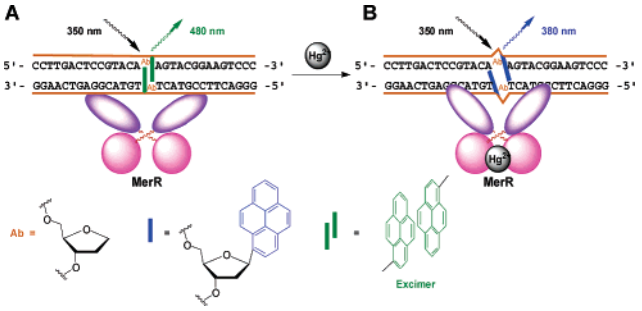

Figure 1. (A) A pyrene excimer is inserted inside a duplex DNA containing the MerR protein binding sequence. With MerR protein bound, fluorescence emission from the excimer at 480 nm is expected. (B) The binding of Hg^{2+} to MerR induces DNA distortion and causes emission at 380 nm from the monomers.

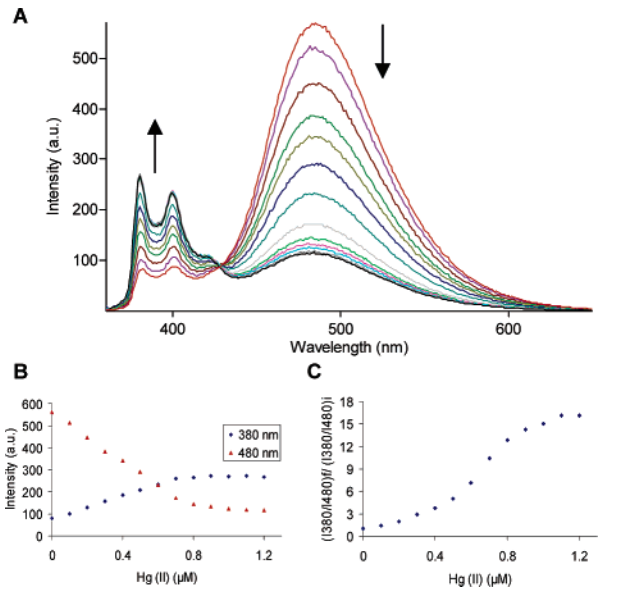

Figure 2. (A) Fluorescence response of MerR (dimer)−DNA complex (1 μM) to the addition of Hg^{2+} at 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, and 1.2 μM. The measurements were performed at room temperature in a nitrogen-purge buffer containing 10 mM Tris-HCl (pH 7.4), 100 mM potassium glutamate, 2 mM $MgCl_2$ and 5% glycerol. (B) Fluorescence intensity as a function of Hg^{2+} concentration. (C) Ratiometric calibration curve final I_{380}/I_{480} ratio over the initial I_{380}/I_{480} as a function of Hg^{2+} concentration.

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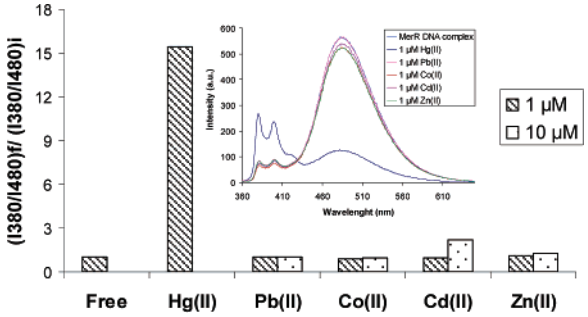

Figure 3. (A) Ratiometric response for different metal ions at 1 and 10 μM. The inset shows the fluorescence response of MerR (dimer) DNA complex to the addition of 1 μM of different metal ions. Measurement conditions are as above.

In the new design, two pyrenes will be incorporated next to each other into the separate strands of duplex DNA. The excimer will be positioned in the middle of a promoter DNA sequence recognized by a MerR protein. When the MerR protein binds to DNA, excimer emission at 480 nm will be observed (Figure 1A). Addition of the target molecule will cause a conformational change in MerR and distortion of the duplex DNA, which disturbs the excimer and increases the pyrene monomer's emission at 380 nm (Figure 1B). With this design, a ratiometric sensor could be achieved by taking advantage of the inherent sensitivity and selectivity of the MerR protein.

The Hg^{2+}-responsive MerR was chosen as a model protein for this approach. MerR protein was overexpressed and purified as described previously.[12] The 1-pyrene-substituted β anomer 5′-dimethoxytrityl-3′-phosphoramidite was synthesized as described in the literature.[13] Two complementary 31-mer oligonucleotides containing the specific MerR binding sequence were prepared, each containing one pyrene in the center of the sequence opposing an abasic site to ensure base stacking of pyrenes in DNA.[14] The two oligonucleotides were annealed to give the final DNA probe.

The MerR protein and DNA probe were mixed in a 1:1 MerR (dimer)/DNA ratio, and Hg^{2+} was added in 100 nM increments resulting in an instant change in the fluorescence signal.[15] The addition of 1 equiv of Hg^{2+} resulted in a 3.3-fold increase in the emission intensity of the pyrene monomer (380 nm) and a 5-fold decrease of the pyrene excimer (480 nm), resulting in an overall 15-fold ratiometric response (Figure 2A). The response is linear with respect to the concentration of Hg^{2+} in solution (Figure 2B) and the change in fluorescence can be seen visually under UV light (365 nm) after the addition of 1 μM Hg^{2+} (Supporting Information, Figure S1).

Sensors that rely only on changes in intensity are susceptible to errors caused by small changes in temperature, ionic strength, and other factors such as photobleaching, variations in the sensor concentration, instrumental artifacts, etc. These error sources can be overcome in ratiometric sensors since the intensity ratio of two wavelengths is taken, allowing for a built-in correlation for factors described above, thus, increasing the sensitivity and the robustness of the sensor. By inserting the pyrene excimer in to DNA, we were able to create a ratiometric biosensor based on the MerR family proteins. The ratiometric calibration curve allows Hg^{2+} concentrations in aqueous solutions to be determined with high accuracy and a lower detection limit of ~10 nM (Figure 2C).The inherent selectivity of MerR toward its specific target Hg^{2+} is also reflected in the designed sensor. The selectivity against other biologically relevant metals was tested. No significant signal was obtained at equal molar concentrations of Pb^{2+}, Cd^{2+}, Co^{2+}, or Zn^{2+}. More remarkably, even a 10-fold excess of Pb^{2+}, Co^{2+}, or Zn^{2+} did not result in any significant signal change; only a 10-fold excess of Cd^{2+} led to some signal change that is consistent with the native property of MerR (Figure S2).[16]

In summary, pyrene excimer incorporated into duplex DNA is an excellent probe to study protein−DNA interactions. The intense emission and ratiometric response provide high sensitivity and robustness of the probe. In the current study, by using the pyrene excimer strategy we can convert the DNA complex of the MerR protein into a ratiometric biosensor for Hg^{2+}. The basis for a highly sensitive, selective, ratiometric, water compatible sensor with fast response is demonstrated. Potentially, the same approach can be applied to other members of this protein family to construct sensitive and selective biosensors for other metal ions and organic molecules.

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Supporting Information Available: Experimental details; Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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