A Metal-Ion-Insensitive Polyanion Chemosensor

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The synthesis of fluorescent chemosensors that detect ions in aqueous solution is requisite to the development of sensing schemes and devices. We have shown previously that anthrylpolyamines signal monomeric anions via chelation-enhanced fluorescence (CHEF)[1] and polyanions via template-directed excimer formation (TDEF).[2] However, such chemical sensors also complex tightly to transition-metal ions, decreasing their practical usefulness and negating their employ in metal-catalyzed reactions. We now report the synthesis and evaluation of a conceptually distinct fluorescent chemosensor that signals anions without interference by transition metals.

While polyamines protonate and thereby complex anions,[3] their free base forms complex transition metals with high binding affinities.[4] Thus, we envisioned sensor **5** as a species embodying all essential structure elements; yet, because the nonbenzylic nitrogens are quaternary, **5** cannot exist in a chelating free base form. Surprisingly, there are few general methods reported for the chemoselective construction of consecutively quaternarized polyamines. Our synthesis (Scheme I) generates this functional group using a variation on the method of Gabbay.[5] The reaction of tertiary amine **3** with (bromopropyl)trimethylammonium bromide[6] in isopropanol affords **4** in 27%. Removal of the *t*-BOC protecting groups with aqueous HBr, which might have led to hydrolysis or Hofmann elimination, instead gave sensor **5** in 47% after recrystallization from ethanol/acetone.[7]

Consistent with the intramolecular photoinduced electron transfer (PET) quenching mechanism,[8] sensor **5**'s fluorescence intensity at 422 nm decreases 55-fold from pH 4 to 12 with an apparent single pK_a of 5.8. While responding to changing proton concentration, the emission intensity of 0.75 μM **5** at pH 7 (0.1 M HEPES) is *unaffected by up to 1000 equiv of* Cd^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Mg^{2+}, *or* Ca^{2+}. By comparison, unquaternerized anthrylpolyamines demonstrate nearly stoichiometric CHEF or CHEQ titrations with the first six of these metal ions.[9] Nevertheless, sensor **5** signals an interaction with both ds and ss DNA[10] in a manner consistent with TDEF.[2] Addition of ds calf thymus DNA to a 1.5 μM solution of **5** results in decreasing fluorescence (excitation, 354 nm; emission, 422 nm) up to the titrated minimum of seven base pair equivalents ($I/I_0 = 0.35$), after which increasing DNA:**5** ratios yield gradually enhanced fluorescence. This behavior and stoichiometry is wholly consistent with that observed using unquaternarized anthrylpolyamines.[2]

Scheme I

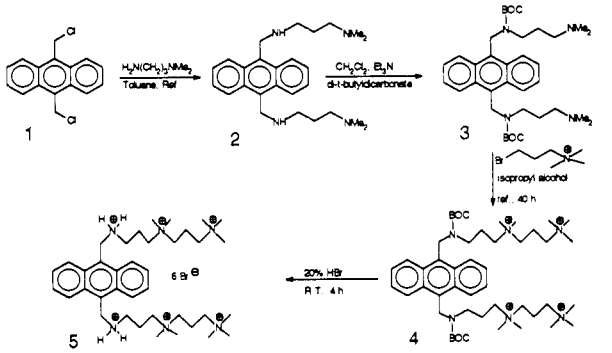

Table I. Relative Initial Rate of Fluorescence Enhancement as a Function of Divalent Metal Ion[a] activation of *Bal31* Nuclease

	Ca^{2+}	Ni^{2+}	Co^{2+}	Mg^{2+}
Mg^{2+}	1.00	0.21	0.13	0.21
Co^{2+}	0.26	≤0.1	≤0.1	
Ni^{2+}	0.32	≤0.1		
Ca^{2+}	≤0.05			

[a] All reactions contained calf thymus ss DNA (5.6 μg/mL), **5** (2.5 μM), Bal31 nuclease (5.0 units/mL), and M^{2+} (4 mM each, total = 8 mM) in 25 mM Tris buffer (pH 7.0) at 25 °C.

Because sensor **5** functions in the presence of metal ions, it can be used to monitor metal-catalyzed reactions. To exemplify this utility, we monitored the activity of the metallophosphatase DNase I[11] as a function of the essential metal ion cofactor.[12] As reported previously by this laboratory,[2] we expect that the fluorescence of a solution starting at the titrated fluorescence minimum should increase during the reaction. This is observed, and the rate varies as a function of metal ion ($Co^{2+} > Mn^{2+} > Mg^{2+} > Cd^{2+} > Ni^{2+}$, Zn^{2+}, $Ca^{2+} >$ EDTA). These findings are wholly consistent with the reported metal activating abilities of DNase I, although the current method utilized amounts of DNA 100 times less than used in the UV assay employed previously.[12] Controls with Co^{2+} but without DNA or enzyme or fluorophore yielded no fluorescence change over 1 h.

Chemosensor **5** was also employed to evaluate the heretofore unreported transition-metal activation of *Bal*31 nuclease from *Alteromonas espejiana*, a molecular biology tool with activity as an exonuclease toward ds DNA and as an endonuclease toward ss and supercoiled ds DNA.[13] The literature assay used to measure activity against ss DNA involves batch radiochemical quantitation of reactions using photometric determination of acid-soluble DNA nucleotides. We observed that the hydrolysis of calf thymus ss DNA could be monitored in real time using chemosensor **5** via fluorimetry. Initial rate data are shown in Table I, from which we conclude that both Co^{2+} and Ni^{2+} can substitute for Mg^{2+}, albeit less effectively, in the Ca^{2+}/Mg^{2+} diad; productive Ca^{2+} substitutions were not observed.

In summary, we report (1) a fluorescent polyanion chemosensor that is insensitive to metal ions; (2) a potentially generalizable scheme by which to synthesize such consecutively quaternarized polyamines; (3) a real-time assay with which to monitor the hydrolyses of ss and ds DNA that permits the rapid survey

(1) Huston, M. E.; Akkaya, E. U.; Czarnik, A. W. *J. Am. Chem. Soc.* **1989**, *111*, 8735.
(2) Van Arman, S. A.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 5376.
(3) For an overview of the design of polyammonium receptors, see: Schmidtchen, F. P. *Nachr. Chem., Tech. Lab.* **1988**, *8*, 10.
(4) For binding constants of various metal ions to parent polyamines, see: (a) Kodama, M.; Kimura, E.; Yamaguchi, S. *J. Chem. Soc., Dalton Trans.* **1980**, 2536. (b) Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1978**, 1081.
(5) Gabbay, E. J. *J. Am. Chem. Soc.* **1969**, *91*, 5136.
(6) All reagents were obtained from the Aldrich Chemical Company, Milwalkee, WI.
(7) Full synthetic details for compounds **2**–**5**, with characterization data, are included in the supplementary material.
(8) (a) de Silva, A. P.; Rupasinghe, R. A. D. D. *J. Chem. Soc., Chem. Commun.* **1985**, 1669. (b) Huston, M.; Haider, K.; Czarnik, A. W. *J. Am. Chem. Soc.* **1988**, *110*, 4460.
(9) Akkaya, E. U.; Huston, M. E.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 3590.
(10) Quantitative aspects of polyamine (Stewart, K. D.; Gray, T. A. *J. Phys. Org. Chem.* **1992**, *5*, 461) and quaternarized polyamine (Schneider, H.-J.; Blatter, T. *Angew. Chem.* **1992**, *104*, 1244) binding to DNA have been described recently.
(11) Calf thymus DNA and DNase I were purchased from Sigma Chemical Company, St. Louis, MO. *Bal*31 nuclease was purchased from U.S. Biochemical, Cleveland, OH.
(12) Junowicz, E.; Spencer, J. H. *Biochim. Biophys. Acta* **1973**, *312*, 72.
(13) See: (a) Hauser, C. R.; Gray, H. B., Jr. *Arch. Biochem. Biophys.* **1990**, *276*, 451 and references therein. (b) U. S. Biochemical catalog, Cleveland, OH, 1992, p 126.

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of metal activations; and, (4) that Co^{2+} and Ni^{2+} serve as surrogates for Mg^{2+} in the *Bal*31 nuclease-catalyzed hydrolysis of ss DNA.

Note Added in Proof: The *Bal 31* metal ion activating effects reported here prove to be in agreement with unpublished data, communicated privately by Prof. Gray (Zhou, X.-g., Ph.D. Dissertation, University of Houston).

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Supplementary Material Available: Experimental methods and characterization data for the syntheses of compounds **2–5** (3 pages). Ordering information is given on any current masthead page.