Cooperative Chemical Sensing with Bis-tritylacetylenes: Pinwheel Receptors with Metal Ion Recognition Properties

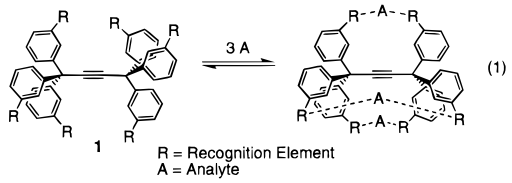
Timothy E. Glass*

Department of Chemistry
The Pennsylvania State University
University Park, Pennsylvania 16802

Received December 16, 1999

Chemical sensors can play a critical role in the elucidation of cellular mechanisms by giving real-time information about the environment of a cell in a non-destructive manner.[1] For example, selective fluorescent Ca^{2+} sensors have provided a convenient way to monitor changes in Ca^{2+} concentrations during cellular processes.[2] For these applications, sensor affinity and selectivity are of utmost concern. A useful sensor must recognize its analyte with high specificity and possess an affinity which is commensurate with the average concentration of the analyte in solution. The desired affinity and selectivity can be achieved, in some cases, by using biosensors.[3] Nevertheless, small molecule chemical sensors remain an attractive approach to such problems, given their ease of modification and cell permeability properties. However, the highly complex and competitive nature of the aqueous cellular environment coupled with the low concentration at which some analytes are found presents a substantial challenge to the design of small molecule sensors that would be effective for biochemical applications.

The issues of affinity and selectivity could be addressed by the use of cooperative recognition.[4] The cooperative binding of multiple analytes can, in principle, impart a higher affinity and greater selectivity to a given sensor relative to a similar non-cooperative system. This type of recognition is virtually unexplored in the field of chemical sensors[5] since cooperativity is typically associated with a sharp transition between the unbound and bound state of the receptor. This sharp transition restricts the range of concentrations over which the analyte can be detected. Nevertheless, in the context of applications in which sensitivity and selectivity are limiting factors, a smaller dynamic range should be acceptable. Herein is described the first application of cooperative recognition for enhancement of binding affinity to fluorescent chemical sensing.



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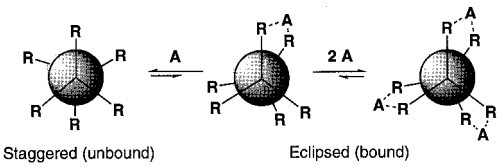
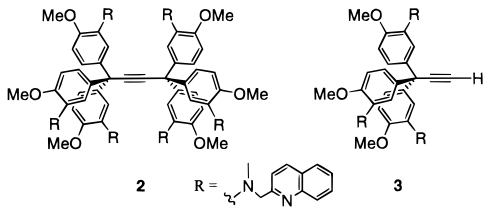


Figure 1. Newman-type representation of compound **1**.

There are several examples of homoallosteric receptors in the literature,[6] but none appeared to be sufficiently general for use as a sensor framework. Therefore, a cooperative receptor framework was designed on the basis of a novel molecular architecture having three interacting sites (eq 1). This "pinwheel"-shaped receptor consists of two trityl groups connected by a linear acetylene spacer.[7] Each phenyl ring of the trityl groups is substituted at the meta position with a recognition element (R). A pair of such recognition elements can bind an analyte across the acetylene axis, creating a set of three identical interacting binding pockets.

This framework is expected to exhibit cooperativity since, in the absence of analyte, the trityl groups rotate freely about the acetylene axis.[8] Binding the first analyte forces the sensor into an eclipsed rotamer (Figure 1). The loss of rotational freedom, as well as the introduction of unfavorable steric interactions, disfavors the first binding event. However, on the basis of the symmetry of the molecule, binding the first analyte forces the remaining recognition elements to align for binding the next two analytes. The result is a cooperative binding event.[9] For the proper operation of this receptor, two recognition elements on the same trityl group cannot chelate an analyte between them. Molecular modeling of compound **1** (Macromodel 6.5) indicates that only analytes of large dimension could interact with two recognition elements on the same trityl group due to its propeller shape.[10]



A simple metal binding assay was devised using compound **2** in order to evaluate the cooperative nature of the framework. A quinoline-amine group was utilized as the recognition element since it has appropriate metal binding and fluorescent properties. A pair of these groups can chelate one metal ion, creating a receptor with three tetrahedral metal binding sites (cf. eq 1). Compound **3** was used as a functionally deficient control. Both compounds **2** and **3** show a decrease in fluorescence upon addition of metal ions such as Zn(II), Ag(I), Ni(II), Co(II), and Hg(II).[11]

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10.1021/ja994398e CCC: $19.00
Published on Web 04/21/2000

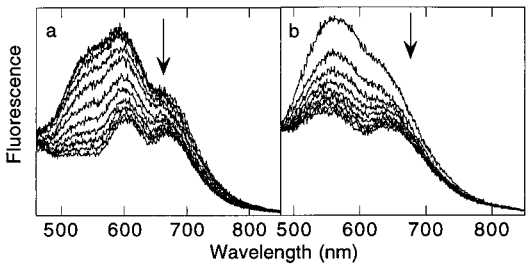

Figure 2. Fluorescence emission spectra (λ_{ex} = 235 nm) for: (a) Compound **2** (0.333 μM with 1.67 mM Me_4NClO_4 in acetonitrile). The spectra resulting from addition of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 equiv of Ag(I) are shown. (b) Compound **3** (1 μM with 5 mM Me_4NClO_4 in acetonitrile). The spectra resulting from addition of 0, 40, 80, 120, 160, 200, 240, 280, and 320 equiv of Ag(I) are shown.

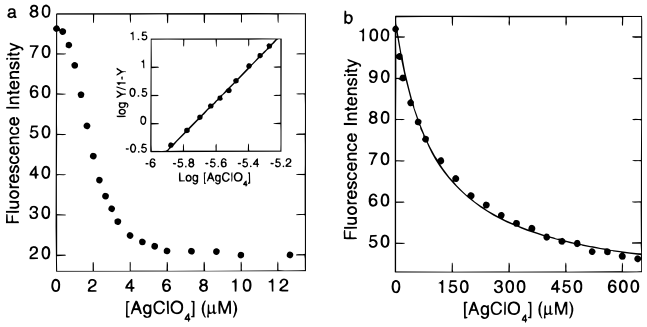

Figure 3. Plot of the fluorescence intensity at 550 nm as a function of added $AgClO_4$ for (a) Compound **2**. Inset is the Hill plot of the data using the equation $\log(Y/1 - Y) = n \log[\mathrm{Ag(I)}] + \log K_a$ where n is the Hill coefficient and Y is the fractional saturation. (b) Compound **3**. The solid line is a fit to the theoretical binding curve.

The emission spectra of compounds **2** and **3** with various concentrations of Ag(I) are presented in Figure 2. The spectra were recorded in acetonitrile using tetramethylammonium perchlorate as an ionic strength buffer. Compound **2** clearly demonstrated cooperative binding as the first few equivalents of added Ag(I) did not give a substantial change in the fluorescence spectrum. Addition of subsequent equivalents of Ag(I) gave a decrease in fluorescence which quickly saturated at 8 μM Ag(I).

Plots of the change in fluorescence at 550 nm as a function of added Ag(I) are presented in Figure 3. Compound **2** shows a sigmoidal binding isotherm (Figure 3a) which is indicative of a cooperative binding mode. Hill analysis[12] of the titration data (inset of Figure 3a) gives a Hill coefficient of 2.9 which is consistent with a highly cooperative system possessing three interacting sites ($K_a = 7.94 \times 10^{16}$ M^{-3}). These data establish that compound **2** is indeed a cooperative sensor. To gain insight into the mechanism of cooperativity, compound **3** was similarly analyzed. The binding isotherm of compound **3** (Figure 3b) fits to a non-interacting-site binding model with $K_a = 8.54 \times 10^3$ M^{-1} (per site, calculated with Associate 1.6[13]) and is clearly not cooperative. Taken together, these data are consistent with the proposed mechanism of cooperativity for compound **2** in which three analytes are bound across the acetylene axis as shown in eq 1 and Figure 1.[14] The control compound **3** which lacks the second trityl group is not capable of this type of recognition and is therefore not cooperative.

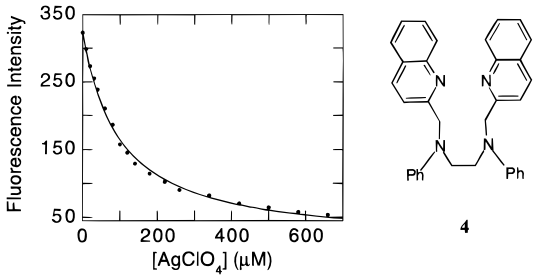

Figure 4. Plot of the fluorescence intensity for compound **4** (1 μM with 5 mM Me_4NClO_4 in acetonitrile) at 550 nm as a function of added $AgClO_4$. The solid line is a fit to the theoretical binding curve.

To compare the cooperative sensor to a similar noncooperative sensor, the single site ligand **4** was prepared (Figure 4). Titration of compound **4** with Ag(I) gave an association constant, $K_a = 1.04 \times 10^4$ M^{-1}. The best point of comparison for the affinities of the different sensors is the concentration of Ag(I) at which 50% saturation is achieved: 1.83 and 96 μM Ag(I) for compounds **2** and **4**, respectively.[15] Thus, the cooperative sensor binds Ag(I) 52 times more tightly than the single site sensor **4**. Compound **2** also has a relatively sharp transition from the unbound state to the saturated state over a range from 0.6 to 6 μM compared to the similar transition seen in compound **4** which covers almost 2 orders of magnitude in concentration of analyte (10 to 900 μM).[16] These data confirm the initial supposition that a compromise between higher affinity and smaller operational range will result from the cooperative effect. Thus, compound **2** can sense a lower concentration of Ag(I), but over a smaller range of concentrations.

In summary, a general molecular framework for cooperative recognition has been developed. It is anticipated that this cooperative effect will be applicable to detection of many types of analytes provided that they can be chelated between two recognition elements. The use of cooperative recognition in chemical sensing represents a new paradigm in sensor technology and promises to provide a general strategy for enhancing the affinity of a sensor for its analyte.

Acknowledgment. This work was supported by the Pennsylvania State University and the National Institutes of Health (GM 59245, RR 10524).

Supporting Information Available: Supporting Information Available: Synthesis and complete experimental details for compounds **2**-**4** (23 pages, print/PDF). This material is available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

JA994398E

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(14) Stoichiometry was established by spectroscopic analysis, see Supporting Information.
(15) The value for compound **2** was determined from Figure 3a by interpolation. The value for compound **4** was determined from the calculated K_a (half saturation value (K_d) = $1/K_a$).
(16) For the purpose of this comparison, the operational range of the sensor is assumed to be the concentration of analyte which gives between 5 and 95% saturation of the sensor.