Achieving Large Color Changes in Response to the Presence of Amino Acids: A Molecular Sensing Ensemble with Selectivity for Aspartate

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Colorimetric assays are currently being sought for practical applications in single analyte and multianalyte sensing.[1] The vast majority of these sensing systems have the chromophore covalently attached to the recognition moiety. Unlike fluorescence,[2] it can be difficult to perturb the microenvironment sufficiently to cause a spectral modulation when absorbance spectroscopy is used. However, one excellent method for dramatically changing the microenvironment around a chromophore (indicator) is its complete displacement from the receptor.[3] By virtue of making ionic or hydrogen bonding interactions to a charged receptor, pH indicators are particularly useful in this regard. They have different protonation states and colors when bound to the receptor or free in solution.[4] It seems logical that the use of a metal ion to coordinate with the indicator would not only change the indicators ionization state, but also give metal/ligand visual transitions that lead to even larger color changes.

To demonstrate this use of metal coordination complexes, we targeted amino acids as analytes, which are well-known to bind metals by cooperative chelation between the carboxylate and the amine.[5] Specifically, we targeted the neurotransmitters aspartate and glutamate by combining organic and inorganic molecular recognition motifs.[6,7] Therefore, there were two goals for the study reported here: (1) demonstrating a method for achieving large color changes in indicator displacement assays and (2) studying the extent to which cooperativity between coordination chemistry and organic molecular recognition could control selectivity.

2,2′:6,2″-Terpyridine derivatives are known to be universal ligands for many transition metals.[8] We took advantage of the facile synthetic access to such structures to develop receptor **1** (eq 1).[9,10] A Zn(II) complex of the ligand was used since such

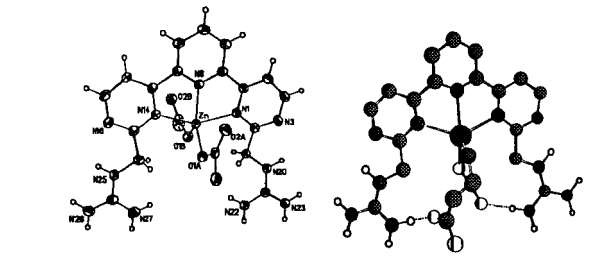
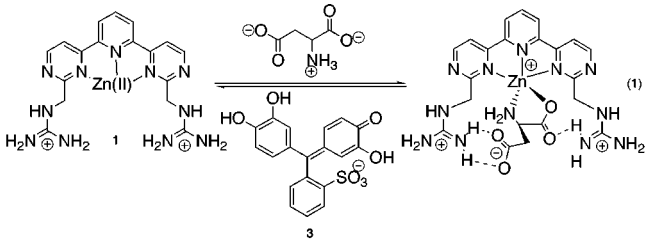


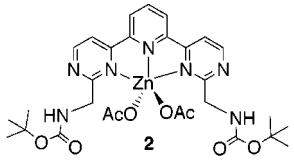
Figure 1. (Left) View of **1(OAc)$_2$** with the partial atom labeling scheme. Displacement ellipsoids are scaled to the 30% probability level. (Right) Energy-minimized structures of **1**:aspartic acid (some atoms are omited for clarity).



complexes have very little color to start. The two guanidinium groups were incorporated to impart selectivity for the side chains of aspartate and glutamate due to their proven efficient binding of carboxylate anions.[11]

An X-ray crystal structure of **1(OAc)$_2$** showed that the Zn(II) ion adopts a distorted trigonal bipyramidal geometry (Figure 1). The guanidinium groups are roughly located in the same plane as the ligand. They are preorganized such that one NH_2 from each lines a cleft that places the Zn(II) ion at the center. Upon ligation of an amino acid to the metal ion via a carboxylate and an amine, the guanidinium groups are placed in proximity to the aspartate or glutamate side chains, such that the hydrogen bonding interactions as given in eq 1 are probable. This possible geometry is supported by the structure generated by molecular modeling that accompanies Figure 1.

Pyrocatechol violet, **3**, was chosen for the displacement assay due to its ability to chelate the Zn(II) in **1** or **2**. This indicator



has been previously used to signal the presence of different metals such as bismuth and tin.[12] The color changed from yellow

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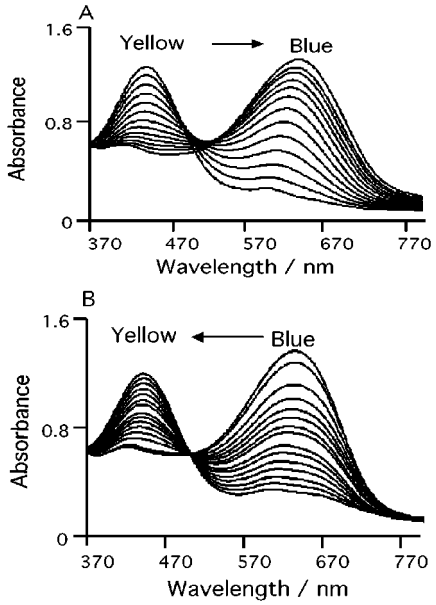

Figure 2. (A) Addition of **1** to a constant concentration of **3** (0.06 mM). (B) Addition of aspartic acid to a solution containing both **1** and **3** (0.08 and 0.06 mM) at a constant concentration.

(λ_{max} = 445 nm) to a deep blue (λ_{max}= 647 nm) upon addition of **1(OAc)$_2$** to a solution of the indicator (Figure 2A) in a water/methanol mixture (1:1; buffered with 10 mM HEPES at pH 7.4). The lack of a perfect isosbestic point indicates that a simple 1:1 binding scenario is not operative, and it is likely there is some aggregation of **1** at these concentrations. Most important for this study, full deprotonation of **3** (pH 11.0) results in a λ_{max} of only 598 nm and the indicator's characteristic violet color. Therefore, a full 49 nm larger shift is obtained with the use of **1**. Moreover, complete binding of this indicator to receptors which possess only guanidinium groups[13] gives color changes only approaching that of full deprotonation. Therefore, a metal ion in a synthetic receptor used in an indicator displacement assay leads to larger color changes than receptors possessing only hydrogen bonding groups.

By following the absorption at 647 nm (Figure 2A) and analyzing the data with a 1:1 binding algorithm,[14] binding constants of 3.75×10^5 and 6.0×10^4 M^{-1} for **1**:**3** and **2**:**3** were obtained, respectively. Analysis of the ^{1}H NMR of a **1**:**3** mixture showed resonances that were indicative of an intact Zn(II) complex, confirming that the indicator did not simply strip the metal from the ligand.

The addition of various amino acids to an ensemble of **1**:**3** or **2**:**3** resulted in a color change from deep blue to yellow (Figure 2B shows the results for aspartate), indicating the expected displacement of the indicator. Now, a sharp isosbestic point was found, pointing out a simple competition between complexes both having 1:1 stoichiometries. The change in absorbance can be analyzed by using standard algorithms for competitive binding to extract affinity constants for the various amino acids and controls (Table 1).[15]

Benzoic acid bound **1** with an affinity constant of 4.0×10^3 M^{-1}, but benzylamine showed a dramatically reduced affinity. However, the binding constants of **1** with hydrophobic amino acids such as glycine, valine, and phenylalanine are in the range of 1×10^4 M^{-1}, consistently above that of a single carboxylic acid. These values give the baseline affinity for the chelate that is formed between α-amino acids and **1**. A slightly enhanced affinity is found for asparagine. Moreover, a similar affinity is found for succinate, indicating that chelation of the Zn(II) by two carboxylates does not significantly alter the affinity over simple chelation of an α-amino acid.

Table 1. Binding Constants (M^{-1}) of **1** and **2** with Different Amino Acids as Determined by the Sensing Ensemble (**1**:**3** or **2**:**3**)[a,b]

run	analyte	host	binding constant[c]
1	benzoic acid	1	4.0×10^3
2	benzylamine	1	2.5×10^2
3	L-phenylalanine	1	8.0×10^3
4	L-valine	1	1.1×10^4
5	glycine	1	1.3×10^4
6	L-asparagine	1	2.3×10^4
7	succinic acid	1	2.3×10^4
8	l-aspartic acid	1	1.5×10^5
9	l-glutamic acid	1	2.2×10^4
10	l-aspartic acid	2	2.8×10^4
11	l-glutamic acid	2	2.9×10^4
12	L-phenylalanine	2	2.4×10^4

[a] The error in these numbers is at less than 10%, as determined by multiple analyses. [b] The binding constants are measured in water/methanol (1:1; buffered with 10 mM of HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, at pH 7.4). [c] Pyrocatechol violet (0.06 mM) and **1** or **2** (0.08 mM) were used as a sensing ensemble.

The highest affinity was found for aspartate ($K_a = 1.5 \times 10^5$ M^{-1}). In comparison, a binding constant of 2.2×10^4 M^{-1} for glutamic acid was measured. The fact that succinate does not bind as well as aspartate indicates that the closer positioning of the two carboxylates in aspartate relative to glutamate is not the reason for the higher aspartate affinity. The difference is a factor of 7 between aspartate and succinate, or glutamate, and a factor of near 15 over the hydrophobic amino acids. The selectivity factors between 7 and 15 (between 1 and 2 kcal/mol), are approximately what would be expected for a solvent exposed ion pair/hydrogen bonding interaction in a highly competitive media such as 50/50 H_2O/MeOH.[15] Exposed ion pairs on the surfaces of proteins are commonly quoted to contribute only 0 to 1 or 2 kcal/mol in stabilization to a folded structure.[16]

A comparison between **1** and **2** is also instructive. Unlike **1**, the binding constants for **2** with aspartate, glutamate, and phenylalanine are almost identical. Additionally, their affinity is nearly identical with that obtained for the guests that cannot interact with an appended guanidinium. Therefore, **1** gives an interaction with aspartate that is not present with **2**, and not present with the other amino acids. All these studies support a contribution of the guanidinium arms in the observed selectivity for aspartic acid.

In summary, the affinity of **1** for amino acids is dominated by the interaction with the Zn(II), but in the case of aspartate, the appended guanidinium groups give an extra boost, thereby imparting some selectivity for this single guest. In addition, we have shown that the use of metals in receptors can lead to larger color changes in indicator displacement assays. This is due to a shift in absorbance of the bound indicator that cannot be achieved with receptors that simply rely upon hydrogen bonding and ion pairing for perturbing the ionization state of a bound indicator.

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Supporting Information Available: Experimental details of the synthesis of **1** and **2**, λ_{max} of **3** vs pH, X-ray diffraction data for **1**, and ^{1}H NMR spectra of **1(OAc)$_2$**, **1**:**3**, aspartic acid and **1**:aspartic acid (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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