Exploiting Polypeptide Motifs for the Design of Selective Cu(II) Ion Chemosensors

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The production of fluorescent devices for the sensing and reporting of chemical events is currently of significant importance for both chemistry and biology.[1] More specifically, sensors directed toward the detection and measurement divalent copper have enjoyed particular attention.[2] Our focus has been on the production of robust, small-molecule chemosensors that exhibit reversible signaling and operate in aqueous solution at neutral pH, without the requirement for organic cosolvents. Because the intended use for such a sensor is ultimately for the measurement of environmental or biological samples, sufficient detection selectivity is of prime concern to ensure that a measurement in the appropriate concentration range can be made, even in the presence of elevated levels of competing divalent cations.

To achieve highly selective binding of Cu(II) within aqueous solution, we sought to exploit the metal binding properties of the amino terminal Cu(II)- and Ni(II)-binding (ATCUN) motif found in the serum albumins.[3] These proteins bind both Cu(II) and Ni(II) avidly, with intrinsic affinity constants on the order of 10^{11} M^{-1} for the protein−Cu(II) complex.[4] Moreover, the simple tripeptide sequence Gly-Gly-His- effectively mimics these binding characteristics, competing with the intact protein for Cu(II) binding.[5] Crystallographic and potentiometric studies have shown that the histidine-derived imidazole δ-nitrogen, the N-terminal α-amine, and two intervening (deprotonated) amide nitrogens are recruited as ligands at neutral pH to form a tetradentate 1:1 peptide:metal complex with slightly distorted square-planar geometry.[3b] As this peptidyl motif selectively targets Ni(II) and Cu(II) ions, intramolecular quenching of an appropriately incorporated fluorophore represents a convenient mechanism for signaling cation binding.[6] Importantly, the N-terminal amine is stringently required for high-affinity binding; thus, the ATCUN motif for metal binding is limited to the N-terminus of naturally occurring peptides. However, substitution of the amino-terminal glycine for a residue with an amine-containing side chain allows elaboration of the motif using standard solid-phase peptide synthesis techniques while preserving high-affinity divalent metal binding.[7] By applying this strategy, the preparation of fluorescently labeled peptides that exhibit avid metal cation binding and effect optical signal transduction has been accomplished.

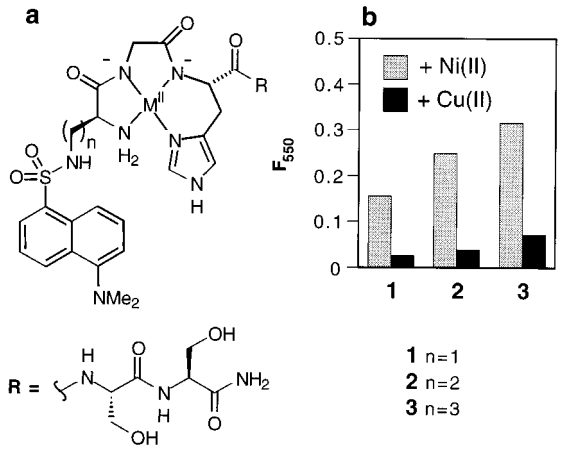

Figure 1. Proposed metal binding geometry of **1**−**3** and effect of fluorophore linker length on quenching. Data were acquired with 10 μM peptide in 150 mM NaCl and 50 mM Hepes (pH 7.0) with 1 equiv of the appropriate divalent metal. Excitation was performed at 333 nm. The data have been normalized such that, in the absence of divalent metal cations, the fluorescence emission at 550 nm is equal to 1.

A family of pentapeptides based upon the ATCUN motif has been prepared that contain a 5-(dimethylamino)naphthalene-1-sulfonamide (Dns) fluorophore. The N-terminal amino acid has been substituted with a homologous series of residues which contain a side chain amine for fluorophore attachment. The representative amino acids incorporated at this position are L-β-aminoalanine (Baa), L-(α,γ)-diaminobutyric acid (Amb), and L-ornithine (Orn) (Figure 1a). In addition, two C-terminal serine residues were appended to ensure aqueous solubility.[8] Fluorescence emission spectra of peptides **1**−**3** were acquired in 50 mM Hepes and 0.15 M NaCl (pH 7.0) with 10 μM peptide, and the effect of an added 1 equiv of Ni(II) or Cu(II) was determined (Figure 1b). In all cases, the titrations of divalent metal stocks into solutions of chemosensor were indicative of tight binding; a linear increase in fluorescence quenching was observed until the chemosensor was saturated at 1 equiv of added metal, and further addition of divalent metal produced no further quenching (data not shown).

As illustrated in Figure 1b, a shorter linker from the peptidyl backbone to the fluorophore results in more efficient quenching, and Cu(II) produces a greater change in fluorescence than Ni(II). For example, 1 equiv of Cu(II) leaves <7% of the initial emission intensity (measured at 550 nm), while for Ni(II), ~15−35% of the original emission intensity remains. Addition of excess EDTA to the assay mixture reverses the metal ion induced quenching. These results suggest that an intramolecular, distance-dependent quenching mechanism is in operation. Furthermore, 100 μM concentrations of Mn(II) or Co(II) and millimolar levels of Mg(II), Ca(II), Zn(II), or Cd(II) have no effect on the fluorescence of these chemosensors. The Fe(II) ion has a slight quenching effect; for 10 μM chemosensor, 1 equiv of Fe(II) causes <10% quenching. This motif is quite suitable for sensing ions

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(8) Peptides were synthesized using standard Fmoc-based chemistry, purified by reversed phase (C_{18}) HPLC, and confirmed by electrospray mass spectroscopy. Full experimental details can be found in the Supporting Information.

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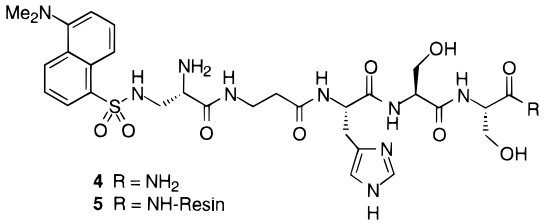
such as Cu(II) in aqueous media; complex formation is not affected by protonation between pH 6 and 10.[9]

Although peptides **1**−**3** may be used to perform sensing of Ni(II) and Cu(II), it is advantageous to enhance the metal-binding selectivity of the ATCUN motif by altering the presentation of ligands. Previous studies have demonstrated that modifications to the side chains of the residues in the ATCUN motif produce only modest changes in the thermodynamic binding affinity for Ni(II) and Cu(II).[10] The metal-binding properties of the motif were therefore altered via modification of the peptide backbone. Replacement of the second residue of **1** (glycine) with β-alanine was effected and the peptide Baa(Dns)-β-Ala-His-Ser-Ser (**4**)



prepared. The fluorescence emission properties of a 1 μM solution of **4** upon the addition of increasing concentrations of Cu(II) are presented in Figure 2a.[11] Under these conditions, incremental additions of divalent copper are easily discernible, with a linear fluorescence response in the range 100−1000 nM. Significantly, the cross reactivity of 10 μM **4** with 1 equiv each of Mn(II), Fe(II), Co(II), Ni(II), Zn(II), Cd(II), Mg(II), and Ca(II) is also minimal with less than 4% quenching observed in the presence of all these cations (Figure 2b). Additionally the Cu(II)-induced quenching is reversible with an excess of EDTA.

To expand the utility of these chemosensors, we have prepared immobilized analogues of **1**−**4** for study on the solid phase. This modification, in contrast to the solution-state chemosensors described above, now allows chemosensor regeneration through simple washing procedures. For the first demonstration of these principles, chemosensor **4** was synthesized on PEGA-1900 resin (selected for its enviable water swelling characteristics) to yield the chemosensor−PEGA adduct **5**.[12] A typical assay of this chemosensor with various divalent metal cations is shown in Figure 3. Measurements are made in 50 mM Hepes, 150 mM NaCl (pH 7.0), and 10% v/v methanol with observations made after ≤2 h.[13] Most importantly, this methodology enables strategies for facile metal ion screening through combinatorial methods. By exploiting the inherently modular nature of the peptidyl architecture, we are now employing a program of combinatorial synthesis and rapid solid-phase screening to identify additional fluorescent chemosensors for Cu(II), as well as other metal cations.

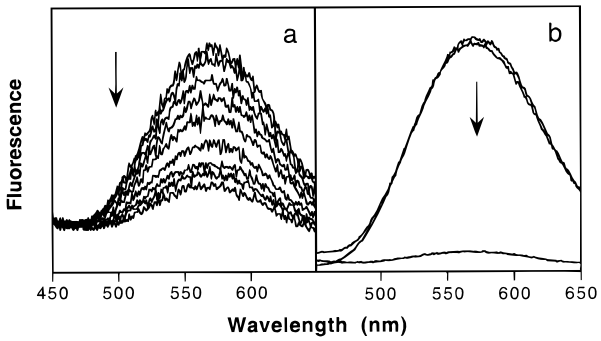


Figure 2. Fluorescence emission spectra of **4** in 50 mM Hepes and 150 mM NaCl (pH 7.0), excitation at 333 nm. (a) 1 μM **4** with increasing concentrations of Cu(II). The effects of 0, 0.12, 0.25, 0.37, 0.50, 0.64, 0.76, 0.88, and 1.0 equiv of added Cu(II) are shown. (b) 10 μM **4** with no added divalent metal; after the addition of 10 μm each Mn(II), Fe(II), Co(II), Ni(II), Zn(II), Cd(II), Mg(II), and Ca(II), and finally, after the addition of 1 equiv of Cu(II).

Figure 3. Typical solid-phase quenching assay of **5**. The samples shown are in 50 mM Hepes, 150 mM NaCl, and 10% v/v methanol (pH 7.0) and contain 100 μM of the appropriate divalent metal cation or EDTA. Fluorescence excitation was provided with a handheld low-wattage mineral lamp set on "long wavelength" (365 nm). The image was acquired after 2 h of incubation.

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Supporting Information Available: Experimental details (2 pages). See any current masthead page for ordering and Internet access instructions.

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(11) The **4**−Cu(II) complex has also been characterized by UV−vis spectroscopy (ϵ = 65 M^{-1} cm^{-1}; λ_{max} = 545 nm; 50 mM Hepes, 150 mM NaCl, pH 7.0).

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(13) Assays performed without the addition of organic cosolvent required extended incubations (>12 h).