Fluorescent Chemosensors for Cu^{2+} Ions: Fast, Selective, and Highly Sensitive

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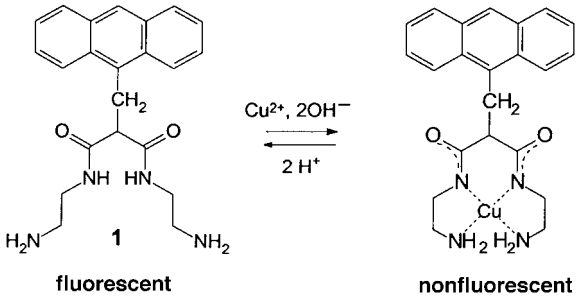
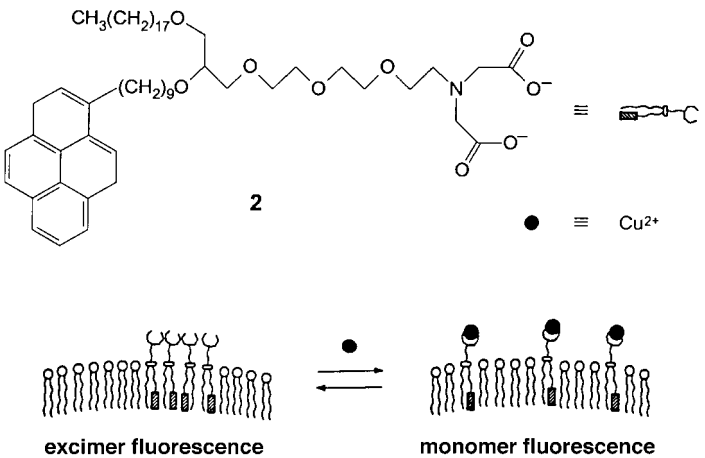
The design of chelating ligands for the selective complexation of metal ions has been an important goal of coordination and supramolecular chemistry for several decades. Chemical sensors for metal ions become accessible by combining a recognition event with an easily quantifiable signal event. The properties of an "ideal" sensor include high selectivity for one metal ion only, high sensitivity, fast and reversible (real-time) response, real-space response down to the micrometer level, and easy handling. Fluorescence signaling[1] offers the advantage of high sensitivity and can be directly used for sensors with fiber optic systems. A new generation of fluorescent reagents in which a chelating group and a fluorophore are discrete subunits of the same molecule[2] has the potential to fulfil the above-mentioned requirements. Recent attempts to apply these two-component systems to the detection of transition metal ions have been particularly successful in case of Cu^{2+} ions. This is not surprising, since among the more relevant transition metal ions, copper(II) has a particularly high thermodynamic affinity for typical N,O-chelate ligands and fast metal-to-ligand binding kinetics. Copper is a significant metal pollutant due to its widespread use, but it is also an essential trace element in biological systems. Whereas copper toxicity for humans is rather low compared with other heavy metals, certain microorganisms are affected by submicromolar concentrations. Compared with classical fluorescence reagents for Cu^{2+} detection in which the donor atoms are part of the fluorophore π-system,[3] the spatial separation of chelating group and fluorophore offers considerable flexibility in design. This is a prerequisite for the straightforward optimization of sensor properties with a view to specific applications.

The fluorosensor **1** of Fabrizzi et al. consists of a metal-binding dioxotetraaza unit linked to a light-emitting anthracene fragment (Scheme 1).[4] Compared with other copper(II) fluorosensors of this type described in recent years,[5] **1** combines a reversible response to nanomolar concentrations of Cu^{2+} in aqueous medium and low cross-sensitivity to certain transition metal ions and protons. When irradiated at 372 nm **1** displays an intense emission band at 415 nm. This fluorescence is quenched completely upon coordination of Cu^{2+} ions, presumably through a photoinduced metal-to-fluorophore electron-transfer mechanism. The copper concentration is determined by measurement of the fluorescence intensity with a fluorimeter. Binding and signaling are fast and fully reversible. The lower detection limit is 100 nM Cu^{2+}. At pH 7.1, equimolar concentrations of Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} do not affect the response for Cu^{2+}, since they do not bind to the receptor. Selectivity is due to the high affinity of Cu^{2+} for nitrogen donors and to the strong tendency to promote deprotonation of amide nitrogen atoms during complex formation. Metal-ion selectivity, binding kinetics, and proton cross-sensitivity are strongly dependent on the structure of the chelating group of the fluorescent sensor.[4]



Scheme 1. The binding of Cu^{2+} ions by **1**.

The membrane-based fluorosensor described by Arnold and coworkers is even more sensitive to Cu^{2+}.[6] Lipid **2** is functionalized with a fluorescent pyrene residue and a chelating iminodiacetate group. When added to distearoylphosphatidylcholine vesicles at pH 7.5, **2** forms aggregates within the parent membrane (Scheme 2). Upon excitation at



Scheme 2. Cu^{2+}-induced change in the fluorescence properties of a phospholipid membrane containing **2**.

346 nm, the vesicles show two distinct bands in the fluorescence spectrum: a weak emission at 377 nm attributed to a small number of isolated monomers of **2**, and a broad and intense band at 470 nm attributed to aggregates of **2** (pyrene excimers). The addition of Cu^{2+} ions has a dramatic effect on the fluorescence spectrum. The monomer band increases, and the excimer band decreases. Unlike the previous example, copper ions are not directly involved in the fluorescence quenching mechanism. Complexation of copper by the iminodiacetate moiety induces a reorganization of the lipids (Scheme 2) by dispersion of **2** in the matrix, which results in an

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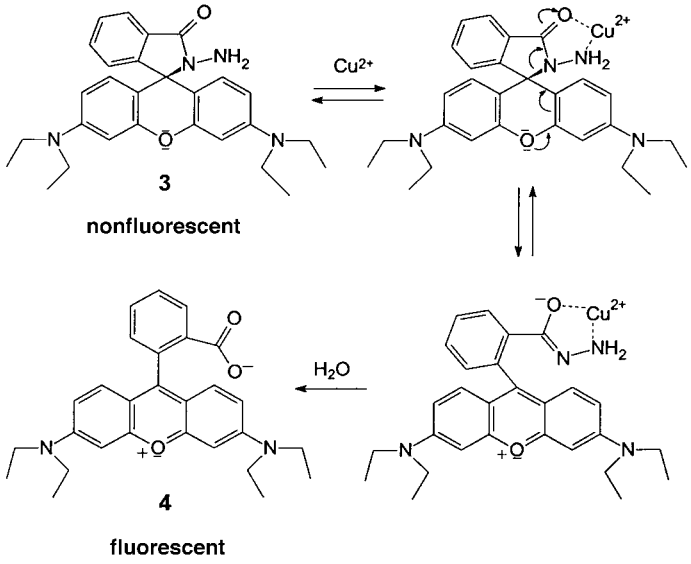
increase of the monomer-to-excimer ratio. The copper-induced changes in the fluorescence spectrum are fast and reversible, and can be used for quantification of Cu^{2+} down to a concentration of 5 nM. The sensor is at least 10 times more sensitive for Cu^{2+} than for Co^{2+}, Ni^{2+}, Mn^{2+}, and Ca^{2+}. Once again, selectivity is attributed to the high thermodynamic stability of the copper-iminodiacetate complex. Optimization of the system should soon lead to analytical applications such as monitoring Cu^{2+} concentrations by continuous-flow fluorimetry.

The rhodamine B hydrazide **3**[7] of Czarnik et al. is a "chemodosimeter" rather than a sensor in the strict sense, since the fluorimetric signaling is not reversible. Compound **3** is a nonfluorescent substance. Addition of Cu^{2+} ions results in N,O chelation by the hydrazide moiety and subsequently in a redox hydrolysis with release of fluorescent rhodamine B (**4**) (Scheme 3). Stoichiometric reagents for the fluorimetric



Scheme 3. Reaction of **3** with Cu^{2+} ions.

detection of copper are mainly redox-based and have been known for 30 years,[8] but Czarnik's dosimeter is the first based on a hydrolysis reaction.

The process is induced by the unique ability of Cu^{2+} ions to efficiently hydrolyze carboxylic acid derivatives with anchoring groups, such as α-amino esters, hydroxamic acids, and hydrazides. Analyte recognition and signaling are stoichiometric, irreversible processes. In a typical assay, excess **3** is added to the aqueous Cu^{2+} sample at pH 7.5 and the amount of released **4** is determined by fluorescence measurement (excitation 510 nm, emission 578 nm) after 2 minutes, when hydrolysis is complete. Copper concentrations down to 10 nM can be detected accurately with this method. Although the chemodosimeter method does not allow real-time monitoring, it is highly selective for copper due to a double selection process: response to the target metal ion requires both high affinity for the sensor molecule and hydrolytic reactivity of the metal ion. Many other metal ions (such as Mn^{2+}, Fe^{3+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+}, Ag^{+}, Ca^{2+}, and Mg^{2+}) in equimolar amounts do not produce any increase in fluorescence intensity after 2 minutes.

A paper by B. Imperiali and co-workers[9] shows the potential of combinatorial methods for the optimization of sensor properties. Prompted by the Cu^{2+} binding site of serumalbumin a series of pentapeptides was synthesized. The metal ion was bound by the amino-terminal NH_2 group and the deprotonated amide-N atoms. The side chain of the N-terminal amino acid contains as fluorophore a 5-(dimethylamino)naphthalene-1-sulfonamide group, which does not participate in the metal coordination. Sensitivity and selectivity for Cu^{2+} are comparable with that for the fluorosensor **1**. The sensor properties can be modified in a simple way by variation of the amino acids. For example, the exchange of a glycin residue by β-alanine gives a distinctly better selectivity for Cu^{2+} in the presence of Ni^{2+}. The immobilization of the fluorescing peptide by covalent binding to a poly(ethylene glycol) matrix enables combinatorial peptide synthesis on a solid phase and a rapid screening of the metal-ion selectivity by observation of fluorescence quenching directly in the matrix.

The fluorescent Cu^{2+} sensors described here display outstanding sensitivity and selectivity. For many other metal ions, the development of highly selective reagents of this type is still a challenge. The combination of fluorophores with hydrolyzable groups may provide new systems for the specific determination of metal ions with high hydrolytic reactivity, for example lanthanide(III) and Pb^{2+} ions which efficiently cleave phosphate ester bonds.

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