Colorimetric Recognition of Different Enzymology-concerning Transition Metals Based on a Hybrid Cluster Complex

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A hybrid cluster complex, formed by chelating a chromogenic ligand to a [2Fe-2S] cluster, sensitively exhibited differential colorimetric responses towards Hg²⁺, Cd²⁺, Cr³⁺, Pb²⁺, Sn²⁺, Cu²⁺, Zn²⁺, Fe³⁺ and Co²⁺ in water at physiological pH. Speciation of some of these metal elements, such as Cr(III) and Sn(IV), was also studied by UV/Vis absorption.

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We have recently developed a [2Fe-2S] cluster-containing hybrid complex as a multi-functional artificial enzyme, acting as both horseradish peroxidase and dihydroxy acid dehydratase.¹ It has been learned that the structure of the [2Fe-2S] cluster can be changed by binding different metal atoms to its di-sulfur anions,² which can be taken advantage of as a differential receptor for different transition metals. Because iron-sulfur clusters are prosthetic groups commonly found in proteins that participate in oxidation-reduction reactions and catalysis,^{3,4} the responses of the [2Fe-2S] cluster-containing hybrid complex towards different transition metals may reflect the different enzymological effects that they introduce in the concerned biological processes. Herein, we have developed another chromogenic [2Fe-2S] cluster-containing hybrid complex, which exhibited differential optical responses towards Hg²⁺, Cd²⁺, Cr³⁺, Pb²⁺, Sn²⁺, Cu²⁺, Zn²⁺, Fe³⁺ and Co²⁺ in water at physiological pH. Speciation of some of these metal elements, such as Cr(III) and Sn(IV), could also be done by a UV/Vis absorption study.

For assembling a molecular probe, a new kind of complex, Na₅[(μ-S)₂Fe₂]HQSAC (Scheme 1), was adopted, which composes a [2Fe-2S] cluster center and a conformation-labile azo ligand, 2-[(8-hydroxy-5-sulfo-7-quinoline)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (HQSAC).⁵ The preparation of Na₅[(μ-S)₂Fe₂]HQSAC·6H₂O was based on the

high reactivity of NO groups of Roussin's red salt, which were proved to be ready for release by photodecomposition (313 – 546 nm) or displacement due to external chelants.⁶ Roussin's red sodium salt, Na₅[(μ-S)₂Fe₂(NO)₄]·8H₂O,^{2,7} and HQSAC were dissolved in aqueous solution at pH 5.0 – 8.0 with a molar ratio of 1:1. The red mixture solution was stirred at 60°C in a water bath, and a disgusting gas was released. After stirring for 6.0 h, the solution was dried by rotary evaporation. After the dried solid was extracted with methanol, the methanol solution was dried under vacuum to give Na₅[(μ-S)₂Fe₂]HQSAC·6H₂O as a red solid. The obtained product was stored under a nitrogen atmosphere in a desiccator, where it was found to be stable for one month. *Anal.* Calcd for Na₅[(μ-S)₂Fe₂]HQSAC·6H₂O, C₁₉H₁₉Fe₂N₃Na₅O₁₈S₅: C, 23.67; H, 1.99; N, 4.38; S, 16.63. Found: C, 23.61; H, 2.05; N, 4.40; S, 16.53. The number of crystal waters was further determined by thermogravimetry. The biological mass spectrum displayed the characteristic peak at *m/z* 742 (*M* + 1), the FT-IR spectrum of the solid in KBr displayed no ν_{NO} bands at around 1800 (w), 1715 (s) or 1675 (s) cm⁻¹, which were usually found in the IR spectra of Roussin's red or Roussin's black salts.⁶

The UV/Vis absorption responses of Na₅[(μ-S)₂Fe₂]HQSAC sensing solution towards different metal ions (added with

Scheme 1 Chemical structure and sensing principle of [(μ-S)₂Fe₂]HQSAC.

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Fig. 1 Absorption responses of Na₅[(μ-S)₂Fe₂]HQSAC (3.40 × 10⁻⁵ M; buffered at pH 5.0 with 0.02 M NaAc-HAc solution) towards different transition metal ions (1.0 × 10⁻⁴ M, chloride as the counterions).

Fig. 2 UV/Vis spectra of $\text{Na}_5[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}$ solutions titrated with a standard solution of metal ions (a, Cu^{2+} ; b, Cr^{3+}). The final concentration of $[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}$ is 2.50×10^{-5} M. Test solutions were buffered at pH 7.40 with a 0.05 M Tris-HCl solution.

Fig. 3 Speciation of Cr (a) in a pH 7.4 aqueous Tris-HCl buffer solution (final concentration: $C_{[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}} = 3.40 \times 10^{-5}$ M; $C_{\text{Cr}} = 2.00 \times 10^{-4}$ M) and Sn(IV) (b) in 85.0% ethanol aqueous solution (final concentration: $C_{[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}} = 3.10 \times 10^{-5}$ M; $C_{\text{Sn}} = 8.65 \times 10^{-4}$ M).

chlorides of metals) in aqueous solution at pH 5.0 are shown in Fig. 1; the tested ions included K^+ , Na^+ , Li^+ , Ca^{2+} , Ba^{2+} , Al^{3+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , Hg^{2+} , Cd^{2+} , Cr^{3+} , Pb^{2+} , Sn^{2+} , Cu^{2+} , Zn^{2+} , Fe^{3+} and Co^{2+} . Only a relatively low pH was used in order to ensure that the metal ions are not affected by OH^- anions in water. Figure 1 illustrates that obvious color shifts were induced by the addition of Hg^{2+} , Cd^{2+} , Cr^{3+} , Pb^{2+} , Sn^{2+} , Cu^{2+} , Zn^{2+} , Fe^{3+} or Co^{2+} . This phenomenon occurred over a wide pH range from 4.5 to 9.0 without any distinct changes in the absorption spectra. It is interesting that all of the color-responding ions are known to be toxic or to be essential to some physiological processes. Since iron-sulfur clusters are often involved in these processes, these phenomena, which reflect the structural changes of the clusters, are useful for toxicological evaluations of transition-metal ions. Further study also indicated that different species formed by different molar ratios of metal/ $[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}$ with the same metal element induced different UV/Vis absorption spectra. This diversity of the interaction modes between metal and $[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}$ made it difficult to measure the coordination constants of different metal ions binding to $[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}$. However, in a biological sense, this is true to the nature of biological systems.

The coordination constants of different metal ions binding to $[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}$ are expected to be high due to the known highly favorable interaction between these metal ions and sulfur anions. This was proved by the UV/Vis spectra gained by titrating a $\text{Na}_5[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}$ solution with standard

solutions of transition-metal ions, such as Cu^{2+} and Cr^{3+} (Fig. 2). There were good linearities between the characteristic absorbances and the concentrations of the corresponding metal ions, which indicated that metal ions had been completely "captured", even at low concentration levels. That is, a quantitative determination of these transition metal ions by spectrophotometry is ready to be performed at considerably low concentration levels. For example, the concentrations of Cu^{2+} and Cr^{3+} can be easily measured when their molar concentrations are below one third and half of that of $[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}$, respectively, a concentration condition that ensures the target metal ions are bound to the $[2\text{Fe-2S}]$ centers at an identical molar ratio.

Some applications of the developed sensor to speciation recognition of transition metals were also studied. Figure 3a shows the spectral responses of $\text{Na}_5[(\mu\text{-S})_2\text{Fe}_2]\text{HQSAC}$ towards $\text{K}_2\text{Cr}_2\text{O}_7$, K_2CrO_4 and CrCl_3 in water. Cr(III) ($\lambda_{\text{max}} = 354$ nm) and Cr(VI) ($\lambda_{\text{max}} = 606, 671$ nm) were clearly distinguished in the UV/Vis spectra. Obviously, HQSAC had been oxidized by Cr(VI) anions. It is interesting that the new species induced by the reaction with $\text{Cr}_2\text{O}_7^{2-}$ ($\epsilon_{354\text{nm}} = 2.42 \times 10^4 \text{ cm}^{-1} \text{ mol}^{-1} \text{ L}$) exhibited a near twofold molar absorption coefficient compared to CrO_4^{2-} ($\epsilon_{354\text{nm}} = 1.33 \times 10^4 \text{ cm}^{-1} \text{ mol}^{-1} \text{ L}$). Thus, Cr(III) and Cr(VI) can be well qualitatively and quantitatively analyzed using this sensing system. Developing optical molecular chemosensors for speciation of organic transition metal compounds is still a challenging task. Since Roussin's red

Table 1 Absorption maximum wavelength and molar absorption coefficient of products formed by $[(\mu-S)_2Fe_2]HQSAC$ and different transition metal ions according to Fig. 1

	Blank	Cr ³⁺	Sn ²⁺	Co ²⁺	Pb ²⁺	Cu ²⁺	Zn ²⁺	Cd ²⁺	Hg ²⁺
λ_{max} (nm)	532	620	573	577	571	547	5571a, 5276b	529	512
ϵ (cm ⁻¹ mol ⁻¹ L)	5000	6838	7438	6874	5247	9550	528a, 565b	5103	4282

sodium salt has been proved to react efficiently with a wide range of organic halides, such as R_3SnX ($R = Ph, Me$), Ph_3PbBr and $RHgX$ ($R = Ph, Me$)², some different organic Sn(IV)-containing compounds were also tested in a mixed ethanol/water solvent. The result showed that these different species of Sn(IV) induced different new UV/Vis spectra of the sensing solution (Fig. 3b). We think this will lead to its further important applications in the speciation analysis and toxicological evaluation of Hg(II), Sn (IV), Pb(II) and Cd(II). Compared to the traditional chromatogram-based method, this sensing system offers the possibility of performing *in-situ* measurements, since freely mobile chromogenic sensing molecules can be easily employed in microscopical analysis.

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