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Author(s): Melinda R. Stephens, Caroline D. Geary, Stephen G. Weber Source: Photochemistry and Photobiology, 75(3):211-220. Published By: American Society for Photobiology https://doi.org/10.1562/0031-8655(2002)075<0211:KAOAPC>2.0.CO;2 URL: http://www.bioone.org/doi/full/10.1562/0031-8655%282002%29075%3C0211%3AKAOAPC %3E2.0.CO%3B2

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Kinetic Analysis of a Photosensitive Chelator and its Complex with Zn(II)¹

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Received 27 July 2001; accepted 20 December 2001

ABSTRACT

The reversible sequestration and release of metal ions is an important objective in biological and environmental research. Unfortunately, although there have been dramatic examples of metal ion activity control, there are very few quantitative investigations of stoichiometry, equilibria and kinetics. A significant contributor to this lack of quantitative work is the complexity of many photochromic systems. Therefore, we have attempted to create a simple, reversible photochromic metal-ion chelator that can be analyzed quantitatively. The chelator should have certain other attributes as well, namely, that it binds to divalent metal ions (because of their extreme biological importance) and that it binds metal ions in the dark so that light is used to release metal ions rather than sequester them. The photochromic chelator (1) binds to divalent metal ions [Zn(II), Cu(II), Pb(II), Hg(II), Fe(II), Co(II) and Cd(II); other metal ions have not yet been tested] in the dark with a significant binding strength. In both methanol (by spectrophotometry) and methanolwater (by voltammetry), the stoichiometry of the 1-Zn(II) complex is 2:1. The binding constant (K_1K_2) is on the order of 10^{12} – $10^{14} M^{-2}$ in methanol and $5.0 \times 10^8 M^{-2}$ in 50% aqueous methanol. The chelator 1 is photolabile, yielding 2 with a quantum efficiency of 0.91. In a solution containing excess Zn(II), so that over 99% of the ligand exists as the monodentate complex, photolysis produces 2 with a quantum efficiency of 0.15. A kinetic analysis leads to the conclusion that the complex itself is photolabile.

INTRODUCTION

Photochromic compounds have been used to control protein and enzyme activity (1–12) and metal-ion binding (13–31). In metal-ion binding systems, light can be used to drive photochromic molecules from one stable or metastable avid binding structure to another stable or metastable loosely binding structure. Such reversible, switchable metal-ion binding activity will lead to systems that can sequester metal ions while in the avid form and to systems that can exchange metal ions rapidly while in the other. These systems have applications in biological research, sensors and extraction. A variety of metal-binding photochromics have been investigated, including thioindigos (5,32,33), azobenzenes (13,15,34–36), spirooxazines (30,37– 40), spiropyrans (20,21,41–50), chromenes (23,24), styryl dyes (31) and triphenylmethanes (14,16,18,51). Despite the many systems analyzed, few are intended to bind rapidly with metal ions in the dark (14,16–18,23), a characteristic that is a practical necessity if these compounds are to be used in biological research, sensors or extraction processes.

An important question that has not been answered for any divalent metal-binding systems and has only recently been asked for crowned systems binding to alkali metals involves the photosensitivity of the *complexes* formed between metal ion and photosensitive ligand. If the complexes are photolabile, then light can be used to force the dissociation of the complex. However, if only the ligand is photosensitive, then light can only influence the metal-ligand binding indirectly. The latter case is not technically attractive. By a kinetic analysis, we indirectly show that, for a newly designed and synthesized photoactive chelator, the complex itself can be photolyzed.

Often, the measurement of binding constants and stoichiometry of these systems is complicated. Thus, a quantitative analysis of these photochromic complexes has been limited in number and in the types of systems analyzed. The majority of quantitative reports have involved azobenzene, styryl or thioindigo structures, which undergo a cis-trans isomerization when photolyzed. (13,25,31,34,52-54). These systems are relatively easy to understand because only two photoproducts exist, cis and trans. In all cases thus far, metal-binding occurs only in the cis form and is controlled only by changes in geometric conformation. Although several metal-binding spirooxazines, spiropyrans and chromenes have been reported (4,17,21,24,49,55-60), only a few have been analyzed quantitatively (24,46), a result of the complicated photochemistry of these compounds. In contrast, triphenylmethanes exhibit very simple photochromism. For example, when malachite green (MG) is irradiated with UV light of 225-300 nm, heterolytic cleavage of the C-R bond occurs, and a charged photoproduct is formed with minimal changes in geometric conformation (Scheme 1).

As our goal is to photodissociate tightly bound metal ions, we thought that the photochromic chelator 1 would be a

Posted on the website on December 31, 2001.

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Abbreviations: DI, deionized; MG, malachite green; NMR, nuclear magnetic resonance; THF, tetrahydrofuran; TMP, tetramethylpyridine; TETA, triethylenetetraamine; UV, ultraviolet.

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R = OH, CN, OMe

Scheme 1.

good target. With only one heteroatom participating in resonance stabilization, the effect of the photolysis of the triarylmethanol on the binding constant will be maximal (Scheme 2).

We will discuss the synthesis, photochromism and metalbinding ability of **1**. Because of **1**'s simple photochromism and unique design, we were able to observe binding of metal ions to **1** rapidly in the dark, analyze **1** quantitatively and determine the photosensitivity of a 1-Zn(II) complex. Each of these analyses are rare in themselves, but, to our knowledge, have never been accomplished in one system.

MATERIALS AND METHODS

General methods. UV–Vis absorption spectra were recorded on an HP 8452 Diode Array Spectrometer. To allow simultaneous photolysis and UV–Vis observations, a 1 mm cuvette was placed at an angle of 45°. Photolysis was accomplished using a 150 W Xenon lamp filtered by water for IR and a CVI UG-5 bandpass filter. (Transmission in the UV is maximal at 370 nm [17.6% T] and falls to 8% T at 240 and 404 nm and to 2% T at 229 and 428 nm.) Electrochemical measurements were performed on a Cypress Systems Model CS-1087 potentiostat controlled by a DTK Keen-2530 computer with Model CS-1087 software. ¹H- and ¹³C–nuclear magnetic resonance (NMR) were measured using a Bruker 300 NMR spectrometer.

Chemicals. Anhydrous ether was prepared with stirring over sodium and distillation over Na–benzophenone. Zinc perchlorate (GFS Chemicals, Powell, OH) and sodium perchlorate (GFS Chemicals) were recrystallized from water before use. Water for use in all experiments was doubly deionized (DI) with a Millipore Milli-Q system.

4-[Bis(carboxymethyl-amino)-phenyl] diphenyl methanol (H_2I) via 4-aminophenyldiphenyl methanol. 4-Aminophenyldiphenyl methanol (3.11 g, 11.3 mmol) was placed in a 250 mL flask equipped with a reflux condenser and a stirbar. DI H₂O (100 mL), potassium iodide (0.59 g, 3.5 mmol), sodium hydroxide (1.83 g, 45.8 mmol) and bromoacetic acid (3.45 g, 24.8 mmol) were added. After refluxing for 1.5 h, additional bromoacetic acid (3.85 g, 27.7 mmol) was added to ensure complete alkylation of the amine. As the solution was refluxed. NaOH was added to maintain the pH above 7. This can be monitored by maintaining a yellow color because the solution turns red as it becomes acidic. Once the pH remained constant for 30 min, the basic solution was decanted off, and several milliliters of concentrated HCl were added to the hot solution. A dark-red product was extracted with dichloromethane. During the extraction, a white precipitate formed. This was recovered by filtration, analyzed by NMR and found to be pure H₂1 (394.7 mg, 9% yield). The combined organic layers were dried with sodium sulfate and evaporated to yield crude $H_2 \mathbf{1}$ as a flaky red solid (700 mg), which dissolved in methanol and water to yield a colorless solution.



Scheme 2.

¹H-NMR (300 MHz, C₃D₆O): δ = 7.2–7.33 (m, 10H), 7.11 (d, 2H), 6.54 (d, 2H), 4.31 (s, 4H). ¹³C-NMR (70 MHz, C₃D₆O): δ = 176, 130, 129, 128, 127, 111, 70, 58.

4-[Bis(carboxymethyl-amino)-phenyl] diphenyl methanol (H₂1) via 4-[bis(ethoxycarbonylmethyl-amino)-phenyl] diphenyl methanol (5). Ethyl ester 5 (0.42 g, 0.94 mmol) was dissolved in 20 mL tetrahydrofuran (THF) and 6 mL DI H2O. The solution was cooled on ice, and lithium hydroxide monohydrate (88.2 mg, 2.1 mmol) was added. The reaction mixture was allowed to warm to room temperature and react for 2 h. The THF was removed in vacuo, and the cloudy solution that remained was diluted with CH2Cl2 and poured into a separatory funnel. Slowly, 0.5 N HCl was added until a dark-red precipitate formed. The product was extracted several times with CH2Cl2. The organic layers were combined, dried over Na2SO4, filtered and concentrated in vacuo to yield a shiny orangered solid. This solid was dissolved in a small amount of CH₂Cl₂ and precipitated with hexanes. After removal of the solvent in vacuo, H₂1 remained as a fluffy bright-orange solid (0.2318 g, 63% yield), which dissolved in methanol and water to yield a colorless solution. ¹H-NMR (300 MHz C_3D_6O): $\delta = 7.2-7.32$ (m, 10H), 7.11 (d, 2H), 6.56 (d, 2H), 4.31 (s, 4H). MS-fast atom bombardment (negative) meta-nitrobenzoic acid 391.

4-[Bis(trimethylsilyl-amino)-phenyl] diphenyl methanol (3). 4-Bromo-N,N-bis(trimethylsilyl)aniline (Aldrich, St. Louis, MO; 3.8 mL, 13.4 mmol) was added to 40.0 mL anhydrous ether in a 250 mL flask equipped with a reflux condenser, a stirbar and a nitrogen inlet. A solution of 1.6 M BuLi in hexanes (Aldrich; 8.4 mL, 13.4 mmol) was added, and the mixture was stirred at room temperature for 1 h. Benzophenone (EM Science, Gibbstown, NJ; 2.27 g, 12.5 mmol) was dissolved in anhydrous ether and added slowly. The solution was stirred with gentle reflux for 45 min and was then quenched with 30 mL DI water. The product was extracted with ether, dried with Na2SO4 and evaporated to yield a yellow oil (crude weight, 5.44 g; crude yield, $\sim 100\%$). Crude 3 could be used in the next step without purification or could be purified by column chromatography. During purification, some cleavage of the silyl groups occurs, but the deprotected amine 3 can be collected. ¹H-NMR (300 MHz, CDCl₃): $\delta = 7.20$ (m, 10H), 7.00 (d, 2H), 6.77 (d, 2H), 2.80 (s, 1H), 0.00 (s, 18H).

4-Aminophenyldiphenyl methanol (4). Crude 3 (~27 mmol) was placed in a flask equipped with a stirbar, nitrogen inlet and reflux condenser. Deprotection of the amine was accomplished by refluxing in 35 mL methanol for 2 h. The reaction was allowed to cool, and the methanol was evaporated to yield an orange fluffy solid (6.9 g, 100% yield). ¹H-NMR (300 MHz, CDCl₃): $\delta = 7.28$ (m, 10H), 7.01 (d, 2H), 6.60 (d, 2H), 3.6 (broad, 2H), 2.8 (broad, 1H).

4-[Bis(ethoxycarbonylmethyl-amino)-phenyl] diphenyl methanol (5). Compound 4 (0.3869 g, 1.4 mmol) was placed in a 25 mL flask equipped with a reflux condenser, stirbar and nitrogen inlet. Sodium iodide (115.5 mg), 1,8-bis(dimethyl-amino) naphthalene (0.67 g, 3.1 mmol) and 5 mL dry acetonitrile were added. Ethyl bromoacetate $(0.35 \text{ mL}; \text{d} = 1.506 \text{ g mL}^{-1}, 1.6 \text{ mmol})$ was also added, and the reaction was heated to reflux for 3 days. The reaction was diluted with toluene and washed with phosphate buffer (pH = 2) until the pH remained 2. The organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo to yield 0.6339 g of a tan oil. The crude product was purified on silica gel with a mobile phase of 20% ethyl acetate in hexanes to give pure 5 (0.4207 g, 67%) as a light-tan oil. ¹H-NMR (300 MHz, $CDCl_3$): $\delta = 7.26$ (s, 10H), 7.07 (d, 2H), 6.52 (d, 2H), 4.22 (q, 4H), 4.1 (s, 4H), 2.7 (s, 1H), 1.27 (t, 6H). ¹H-NMR $(300 \text{ MHz}, \text{ C}_2\text{D}_6\text{O}): \delta = 7.26 \text{ (m, 10H)}, 7.04 \text{ (d, 2H)}, 6.53 \text{ (d, 2H)},$ 4.2 (s, 4H), 4.14 (q, 4H), 2.8 (s, 1H), 1.2 (t, 6H). ¹³C-NMR (70 MHz, CDCl₃): $\delta = 171$, 147.4, 147.2, 136.9, 129.3, 128.1, 128.0, 127.2, 112.0, 81.9, 61.4, 53.6, 14.4. MS-EI m/z (M+) 447. Found 447.2053, Calculated 447.2046 for C₂₇H₂₉N₁O₅.



Scheme 3.

Electrochemistry. The reduction of Zn^{+2} at a hanging mercury drop electrode was used to determine the formation constant and stoichiometry of **1** with Zn(II). A BAS RE-5 Ag–AgCl reference electrode and Pt grid auxiliary electrode were used in all experiments. The solution analyzed was 0.1 *M* NaNO₃ in 50% aqueous methanol, 20 μ M Zn(NO₃)₂ and 5 mM N-2-hydroxythylpiperazine-N'-2-ethane-sulfonic acid (HEPES) (SigmaUltra, St. Louis, MO) buffer (pH 7.0). Before voltammograms were collected, the electrolyte solution was degassed with N₂ for 20 min. Between scans, the solutions were degassed with N₂ for 5 min. The square wave parameters were as follows: step height (Δ E) = 2 mV, pulse amplitude (E_{sw}) = 30 mV and square wave period (τ) = 100 ms. For the determination of the rate of dissociation, data were acquired with the square wave period set to 30 ms, with varying concentrations of **1**.

Job Plot experiment. Stock solutions of 133 μ M tetramethylpyridine (TMP), 133 μ M H₂1 and 133 μ M Zn(ClO₄)₂·6H₂O were prepared in 0.1 M NaClO₄-methanol. A series of solutions containing H₂1 and Zn(II) were prepared with varying quantities of each stock solution but a total volume of 500 μ L. An additional set of solutions was prepared using the same amount of 1 but no Zn(II). Two equivalents of TMP (based on the triphenylmethanol concentration) were added to every solution, and the solutions were diluted to 5 mL with 0.1 M NaClO₄ in methanol. The difference in absorbance between the Zn(II)-containing and the Zn(II)-free solutions was plotted vs the mole fraction of the ligand.

Preparation of MG cyanide. MG cyanide or MG leuconitrile was prepared following the method of Holmes (61). Almost white crystals (very light green) were collected as pure MG cyanide. Melting point: 180–182°C. ¹H-NMR (300 MHz, CDCl₃): δ = 7.3 (m, 5H), 7.05 (d, 4H), 6.7 (br.s 4H), 2.95 (s, 12H). MS–EI *m/z* (*M*⁺) 355. Found 355.2050, Calculated 355.2048 for C₂₄H₂₅N₃.

Chemical actinometry. From the point of view of actinometry, it is best to have the source wavelength range smaller than the absorption wavelength range of the dye. Then, an accurate measurement of all of the light output by the source can be made. On the other hand, from the point of view of speed, a broad source that is capable of providing a large flux of absorbable photons is required. In an effort to speed up the photochemical reactions, we have chosen to use a source wavelength range that is wider than the absorption band of H₂1 or Zn1. Therefore, we have used MG, the spectrum of which is almost identical to that of H_21 and Zn1, for actinometry. MG stock solutions (1 mM in 1 mM HCl in ethanol) were kept frozen and in the dark when not in use and were used within 1 day of preparation. A 2 mm × 5 mm mask was placed in front of a 1 cm cuvette in an HP 8452 diode array spectrophotometer. A reference solution of 1 mM HCl in ethanol was placed in the cuvette, and spectra were acquired for 90 s with UG-5 filtered light illumination and stirring. The MG stock solution was diluted with 1 mM HCl in ethanol until the absorbance at the desired wavelength(s) was 1.5. Absorption spectra of the illuminated stirred solutions were acquired for 90 s. The initial linear region of absorbance (at 622 nm) increase was used to calculate I_o . A plot of absorbance (A) versus time (t) produced a regression line of A = 0.0731t + 0.1516. Thus, the rate of production of MG⁺ is 0.0731 s⁻¹ in absorbance units. The intercept corresponds to the presence of a small amount of photoproduct at the start of the experiment. Dividing by the molar absorptivity ($G_{622} = 1.06 \times 10^5 \text{ cm}^{-1} M^{-1}$) (62) and the cell length (1 cm), converts the rate of formation of MG+ to 6.9 \times 10^{-7} mol MG+

 L^{-1} s⁻¹. Because the volume of the cell was 3 mL, the total moles of MG⁺ produced per unit time is 2.1×10^{-9} mol MG⁺ s⁻¹. The total moles of MG⁺ produced can be used to calculate the total number of photons by dividing the previous value by the quantum yield ($\phi = 0.91$) (62) to give 2.3×10^{-9} einstein s⁻¹.

RESULTS AND DISCUSSION

Synthesis

Compound H_21 was synthesized by the methods outlined in Scheme 3. The amine 4 was prepared by organometallic addition to benzophenone, followed by reaction with methanol as described by Walton (63) and Hellwinkel and Fristch (64). Alkylation of the amine with bromoacetic acid *via* the method of Cox and Smith (65) gave H_21 in 9% yield in three steps. Alternatively, H_21 could be prepared *via* alkylation of 4 to form the ethyl ester 5, followed by hydrolysis with lithium hydroxide. By this path, H_21 is synthesized in 42% yield in four steps.

Photochromism

A typical photochromic response for **1** in methanol is shown in Fig. 1. Initially, the UV-Vis spectrum of 1 has a large absorbance band from 230 to 280 nm (A). During irradiation with UV light, the C-O bond breaks heterolytically to form a colored species 2. The UV spectrum of the colored photoproduct alone is often difficult to obtain because both triphenylmethanol and its photoproduct exist at the same time in solution. We can, however, conclude that the UV-Vis spectrum for 2 is characterized by a visible absorption band at 464 nm and a decrease in molar absorptivity in the 260 nm band (B). When the UV light is removed, the hydroxide ion and 2 recombine slowly. We observe that 3.5 min after the light is turned off, the band at 464 nm has disappeared, whereas that at 260 nm has regained 94% of its initial absorbance (C). The small difference in absorbance between initial and final conditions is most likely a result of photodegradation. A lack of photostability is a common disadvantage for the triarylmethanols (66).

For biological and sensor applications, measurements will likely be performed in aqueous solutions; so it is necessary to design chelators that will photoreversibly bind metal ions in water. Although **1** is soluble and photochromic in aqueous solutions, it degrades to 53% of the initial absorbance after only five cycles, probably because of the hydrolytic instability of the aryliminium functional group (2). This is a disadvantage accompanying our design that places a significant positive charge on the nitrogen in the product. Such photodegradation is not as evident in methanol, in which only a 9% reduction in photochromism is seen after nine cycles (*i.e.* about 1% per cycle). In methanol-water (50:50), we see about a 5% decrease in absorbance over 30 s in the absence of the Zn(II) and only 0.1% decrease over 30 s of photoylsis in the presence of excess Zn(II). Thus, methanol and methanol-water (50:50) were selected as suitable alternatives to water. Because of their solubility in methanol, the perchlorate salts of metal ions were used in all metal-binding studies.

Metal-binding ability

The formation of a 1–Zn(II) complex was confirmed in both methanol and water by a hypsochromic shift from 260 to



Figure 1. Photochromism of **1** in methanol. 100 μM H₂**1** and 10 m*M* NaClO₄ in methanol. (A) in the dark, (B) after 30 s of UV-light and (C) 3.5 min after the light is removed.

244 nm. This blue shift is a common result of metal-ion binding and is caused by stabilization of the nitrogen lone pair, which increases the energy that is required for the $n \rightarrow \pi^*$ transition. Addition of a competitive chelator such as triethylenetetraamine (TETA) should disrupt the metal-ion complex and cause a bathochromic shift of the 244 nm band. Indeed, addition of a 10-fold excess of TETA to a 1:1 solution of 1 and Zn(II) resulted in a shift of the 244 nm band to longer wavelengths (260 nm). Zn(II) is used to minimize spectroscopic competition; 1 also binds to Cu(II), Pb(II), Hg(II), Fe(II), Co(II) and Cd(II). Other metal ions have not yet been tested.

The stoichiometry of the 1–Zn(II) complex was determined with a Job Plot to be 2:1 in methanol (Fig. 2). To eliminate complications that may result from the presence of the carboxylic acid protons, two equivalents of the base TMP were added to the Job Plot solutions. For this ligand, the binding constant is too large to obtain an accurate value from the Job Plot data. Curve fitting using Eqs. 1–5 indicates that K_1K_2 is on the order of 10^{12} – $10^{14} M^{-2}$, and K_1 is equal to or slightly greater than K_2 , where $K_2 = ML_2/ML\cdot L$ and $K_1 = ML/M\cdot L$.

$$T = M_T + L_T \tag{1}$$

$$M_{\rm T} = M + ML + ML_2 \tag{2}$$

$$L_{\rm T} = L + ML + 2 \times ML_2 \tag{3}$$

$$ML = K_1 \times M \times L \tag{4}$$

$$ML_2 = K_2 \times ML \times L \tag{5}$$

A more quantitative analysis of the formation constant of the 1–Zn(II) complex was accomplished with square wave voltammetry. With an increase in concentration of 1 from 0 to 30 equivalents, the reduction potential of zinc shifts from -963 to -1021 mV, which is consistent with complex formation (Fig. 3). A plot of the change in potential (ΔE) *versus* log[1] confirmed the stoichiometry of the 1–Zn(II) complex to be 2:1. With these data, a binding constant, K, of 5.0 × 10⁸ M^{-2} (log K = 8.70) in 50% aqueous methanol was calculated. As expected, the formation constant is significantly



Figure 2. Job Plots of 1–Zn(II). The difference in absorbance between solutions (in methanol 0.1 *M* NaClO₄) containing varying ratios of 1 and Zn(II) and similar solutions without Zn(II). (\blacklozenge) Experimental and (A) theoretical results for 1 concentrations ranging from 1.33 to 13.3 μ *M*. (**■**) Experimental and (B) theoretical results based on 1 concentrations from 0.53 to 5.32 μ *M*. K₁K₂ = 10¹³ *M*⁻²; K₁ = 10⁷ *M*⁻¹; K₂ = 10⁶ *M*⁻¹.

lower in the more polar solvent. However, binding in both media is avid.

There are two pathways for the formation of **2** from photolysis of the 1–Zn(II) complex (Scheme 4): Path "A" is indirect and is possible if the rate of dissociation of the ML complex is rapid. Path "B" leads to direct dissociation as the *complex* itself is photolyzed. The second pathway is the preferred one and requires that the complex be photosensitive.

The photosensitivity of the complex can be determined by examining its photochemical response at large excess concentrations of metal ion, which diminishes the concentration of free L and discourages path A. The photochemical yield of **2** (represented by the absorbance at 464 nm), resulting from 10 s of photolysis of solutions containing **1**, decreases as the concentration of metal ion increases (Fig. 4). Data were collected for solutions containing 0–0.997 mole fraction zinc, which corresponds to M/L ratios from 0 to 332. Even when the ligand exists as greater than 99% complex, there is a significant amount of **2** resulting from photolysis.

Because we know the stoichiometry and have an estimate of the binding constant $(10^{13} M^{-2})$ for this system in methanol, we can estimate the contribution from paths A and B in Scheme 4. If only free 1 is photolyzed (path A), the production of 2 will be proportional to the amount of free 1, assuming that the rate of dissociation is slow (curve A in Fig. 4). Thus, the absorbance will be determined by Eq. 6, where k is an adjustable constant and [L] is the concentration of free 1. If the 1-Zn(II) complex is also photolyzed, even when zinc is in excess, there will be significant production of 2 (curve B). In this case, the absorbance will be described by Eq. 7, where k, k' and k'' are adjustable constants, [ML] is the concentration of the 1:1 complex and [ML₂] is the concentration of the 2:1 complex. Initially, it appears that the complex is photolyzed with about one-tenth of the efficiency of the free ligand.

$$\mathbf{A} = k[\mathbf{L}] \tag{6}$$

$$A = k[L] + k'[ML] + k''[ML_2]$$
(7)



Figure 3. Electrochemical evidence of zinc binding to **1.** The reduction of 20 μ *M* Zn(II) in 50% aqueous methanol with addition of (A) 0, (B) 1, (C) 5, (D) 10 or (E) 30 equivalents of **1.** Details are in Materials and Methods.

Kinetic analysis

In the preceding analysis, we assumed that the rate of dissociation of the metal complex is slow. We have performed a detailed kinetic analysis of this system to examine the validity of this assumption and determine the photosensitivity of **2.** Ultimately, we will simulate the photolysis of **1** with a 100-fold excess of Zn(II) in 50% aqueous methanol. This solvent is chosen because we need a rate that is determined by electrochemistry, and 100% methanolic solutions are not compatible with the hanging mercury drop working electrode used in the determination.

The set of Eqs. 8–19 describe our system. All the rate constants can be calculated directly from experimental data, except those for the sets of Eqs. 14 and 15 and Eqs. 18 and 19. These pairs of equations describe the unknown quantum efficiency for formation of the colored photoproduct from ML and ML_2 . The individual rate constants are so fast that only the relative values of the constants matter in the current analysis. In the following paragraphs, we will describe the determination of the rate constants and the kinetic analysis.

$$L \xrightarrow{k_{h\nu}} L^* \tag{8}$$

$$L \xrightarrow{k_h} L^+ + OH^- \tag{9}$$

$$L^* \xrightarrow{k_{nr}} L \tag{10}$$

$$L^{+} + OH^{-} \xrightarrow{k_{r}} L$$
 (11)

$$\mathbf{M} + \mathbf{L} \stackrel{k_1}{\underset{k_{-1}}{\rightleftharpoons}} \mathbf{M} \mathbf{L}$$
(12)

$$ML \xrightarrow{\kappa_{h\nu l}} ML^*$$
 (13)

$$ML^* \xrightarrow{\kappa_{h1}} M + L^+ + OH^-$$
(14)

$$ML^* \xrightarrow{\kappa_{nr1}} ML \tag{15}$$

$$ML + L \stackrel{k_2}{\underset{k_{-2}}{\rightleftharpoons}} ML_2 \tag{16}$$



Scheme 4.

$$\mathrm{ML}_2 \xrightarrow{\kappa_{h\nu^2}} \mathrm{ML}_2^*$$
 (17)

$$\mathrm{ML}_{2}^{*} \xrightarrow{\kappa_{h2}} \mathrm{ML} + \mathrm{L}^{+} + \mathrm{OH}^{-}$$
(18)

$$\mathsf{ML}_2^* \xrightarrow{\star_{nr^2}} \mathsf{ML}_2 \tag{19}$$

Calculation of $k_{\mu\nu}$ by chemical actinometry

N

Equations 8, 13 and 17 describe the formation of an excited state species with rates $k_{h\nu}$, $k_{h\nu 1}$ and $k_{h\nu 2}$. These rates depend on the total number of photons incident on the sample (I_o) , which was determined by actinometry (see Materials and Methods).

Only a fraction of the total I_o is absorbed by 1 because the average absorbance (\overline{A}) for the photochromic band (224 to 284 nm) was less than 2 when 1 was photolyzed. I_{abs} can be calculated from Eq. 20, where b = 1 cm and \overline{A} is the average absorbance from 224 to 284 nm. The \overline{A} for both 1 and the 1–Zn(II) complex is approximately 0.35. This results in a value of 4.2×10^{-7} mol photons L⁻¹ s⁻¹.

$$I_{\rm abs} = [I_o(1 - \exp(2.303\bar{\rm A}))]/b$$
(20)

Finally, we can calculate a rate of formation of excited **1**, $k_{\rm h\nu}$, from Eq. 21 where $I_{\rm abs} = 4.2 \times 10^{-7}$ mol photons L⁻¹ s⁻¹ and [**1**] is 40 μ *M*. Because the $I_{\rm abs}$ for the complex is the same as that for **1**, we have $k_{\rm h\nu} = k_{\rm h\nu 1} = k_{\rm h\nu 2} = 0.011$ s⁻¹

$$k_{\rm h\nu} = I_{\rm abs} / [1] \tag{21}$$

Calculation of k_r

We first need to determine the molar absorptivity of the product **2.** This is a transient species; so a direct measure-



Figure 4. Photosensitivity of the 1–Zn(II) complex. (\blacklozenge) Experimental data for photolysis of 44 μM 1 in 0.1 *M* NaClO₄ in methanol with increasing Zn(II) (0–17 m*M*). Solid lines are calculated based upon K = 10¹³ M^{-2} for (A) Path A: A = k[L] and (B) Path B: A = $k[L] + k'[ML] + k''[ML_2]$, where $k = 900 M^{-1}$, $k' = 100 M^{-1}$ and $k'' = 100 M^{-1}$.

ment of the molar absorptivity ε_{258}^1 of **2** is precluded. This value can be obtained in two ways. First, **2** can be generated independently of photolysis by titration with acid. In this case, complete ionization of **1** occurs; so the concentration of **2** can be calculated based on the initial number of moles of **1**. A molar absorptivity of 22 000 cm⁻¹ M^{-1} is determined with this method. This number agrees well with the molar absorptivity of an analog, the salt of 4-*N*,*N*-dimethylaminophenyldiphenyl methanol, which is solvent sensitive but in the range of about $1.5-3.5 \times 10^4$ cm⁻¹ M^{-1} (67).

Alternatively, ε_{480} can be calculated from mass balance during photolysis. At short photolysis times, the decrease in concentration of **1** will correspond to the increase in concentration of **2.** After 10 s of UV photolysis, we see a decrease of 0.0245 in absorbance at 258 nm ($\varepsilon_{258}^1 = 15300$ cm⁻¹ M^{-1}) and an increase of 0.0354 in absorbance at 480 nm. Thus, a molar absorptivity of 22 100 cm⁻¹ M^{-1} is calculated. The two values agree well.

Equation 11 describes the recombination of **2** and hydroxide to regenerate **1**. The rate of change of absorbance, which is proportional to the rate of recombination (k_r) , is calculated from measurements of absorbance *vs* time following the cessation of photolysis. Because this is a bimolecular process, we anticipate a second-order reaction. A plot of 1/ A *versus* t gives a linear response ($r^2 = 0.999$) with a slope equal to 6.4823 s⁻¹ (absorbance units)⁻¹. The recombination rate is thus 143 000 M^{-1} s⁻¹.

Calculation of K₁ and K₂

As discussed earlier, the overall binding constant (K_1K_2) in 50% aqueous methanol is 5.0 × 10⁸ M^{-2} . Because K_1 is equal to or slightly greater than K_2 in methanol, the same relationship is likely to exist in 50% methanol. Fitting to experimental data suggests that K_1 is 5 × 10⁴ M^{-1} and K_2 is 1 × 10⁴ M^{-1} (Eqs. 12 and 16).

The rate of dissociation of the complex was determined with square wave voltammetry according to the method of Correia dos Santos *et al.* (68). A fit of experimental data to published model curves (68) results in a value of 2.24 for $k_{-1} \pm 0.14 \times 10^3$ s⁻¹. This requires that $k_1 = 1.36 \times 10^6$ M^{-1} s⁻¹. With such a large excess of metal ion (100:1), ML₂ and its related equations and rate constants are of no significant importance; however, they were included in the simulations. The "on" rate for ML₂ is assigned a nearly diffusion controlled limit of 10⁹ M^{-1} s⁻¹, with k_{-2} then equal to 10⁵ s⁻¹.

Calculation of $k_{\rm h}$ and $k_{\rm nr}$

The quantum yield can be calculated from Eq. 22 where k_r is the rate of recombination, $k_{h\nu}$ is the rate of formation of excited L, $[L^+]_{ss}$ is the concentration of L⁺ at steady state conditions and $[L_T]$ is the total concentration of ligand (1). In this case, $k_r = 143\,000 \, M^{-1} \, s^{-1}$, $k_{h\nu} = 0.011 \, s^{-1}$, $L_T = 40 \, \mu M$ and L⁺ = 1.65 μM . The value of L⁺ is obtained from experimental data for 40 μM 1 in 50% aqueous methanol after 60 s photolysis (Fig. 5). Thus, we calculate a quantum yield of 0.91.

$$\phi = \frac{k_r [L^+]_{ss}^2}{k_h ([L_T] - [L^+]_{ss})}$$
(22)



Figure 5. Steady state absorbance and recombination. 40 $\mu M \mathbf{1}$ with two equivalents of TMP and 0.1 *M* NaClO₄ in 50% aqueous methanol after 60 s photolysis while stirring (b = 1 cm).

Using Eq. 23, we can assign values for the last two rate constants. Equations 9 and 10 describe the fraction of excited molecules that undergo heterolysis *versus* nonradiative decay. With a quantum yield of 0.91, we can determine that the ratio of $k_{\rm h}$ to $k_{\rm nr}$ must be 10. Because we know that these processes are rapid, we will assign arbitrary, but large, rates of 10⁶ s⁻¹ to $k_{\rm h}$ and 10⁵ s⁻¹ to $k_{\rm nr}$.

$$\phi = \frac{k_h}{k_h + k_{nr}} \tag{23}$$

Simulation results

Simulations were completed using the chemical kinetics simulator (IBM Inc., White Plains, NY). This simulator does not integrate sets of coupled differential equations, but uses stochastic algorithms, to predict product concentrations (69). Figure 6 shows the results of the simulations of Eqs. 8–19. In the top figure, the experimental data for photolysis of **1** in 50% aqueous methanol are plotted against the simulated results, where $L = 4 \times 10^{-5} M$ and M = 0. In this case, only Eqs. 8–11 are actually necessary. Thus, all the rate constants are known. Although there is some noise in the data because of the stirring of the solution while collecting the spectra, the match is quite good.

The pairs of equations, Eqs. 14 and 15 and Eqs. 18 and 19, describe quantum yields for ML and ML₂. With a 100-fold excess of Zn(II), the concentration of ML₂ will be negligible; so we need to concern ourselves with the quantum yield from ML only. Simulation of experimental results for photolysis of **1** with 100-fold excess Zn(II) in 50% aqueous methanol, with adjustment of the rate constants in Eqs. 14 and 15, indicates that the quantum yield for the complex is 0.15 (Fig. 6B). The constants are estimated as follows: $k_{\rm hl} = 1.8 \times 10^5 \, {\rm s}^{-1}$ and $k_{\rm nrl} = 10^6 \, {\rm s}^{-1}$ (*i.e.* $k_{\rm hl} = 0.18 \times k_{\rm nrl}$).

There are two side reactions that we are justified in ignoring. Photodegradation in the presence of Zn(II) is negligible on the timescale of these experiments; so it is not included. The recombination of **2** with methoxide is possible. However, we have not seen differences in the UV spectra resulting from photolysis and relaxation in the dark. Some rough calculations show that the effect of the potential formation of the methoxy compound is small. The excited state production rate is 0.011 s⁻¹, and the quantum efficiency in the presence of Zn²⁺ [Zn(II)] is 0.15. Thus, the overall first-



Figure 6. Simulation of photoionization of **1.** The solid lines are the simulation results, and the diamonds are the experimental data of (A) photolysis in 50% aqueous methanol of 40 μ M **1** with two equivalents of TMP and 0.1 M NaClO₄ (B) with 100-fold excess Zn(ClO₄)₂. Simulation rate constants are: $k_{h\nu} = 0.011 \text{ s}^{-1}$, $k_h = 10^6 \text{ s}^{-1}$, $k_{nr} = 10^5 \text{ s}^1$, $k_r = 143\,000 M^{-1} \text{ s}^{-1}$, $k_{nr1} = 10^6 \text{ s}^{-1}$, $k_{nr2} = 10^6 \text{ s}^{-1}$, $k_{1} = 1.34 \times 10^6 M^{-1} \text{ s}^{-1}$, $k_{1} = 2236.2 \text{ s}^{-1}$, $k_{h1} = 180\,000 \text{ s}^{-1}$, $k_{2} = 10^9 M^{-1} \text{ s}^{-1}$, $k_{-2} = 10^5 \text{ s}^{-1}$, $k_{h2} = 170\,000 \text{ s}^{-1}$.

order rate constant for production of the product L^+ is 0.0017 s⁻¹. From this, we can estimate that about 10% of the original complex has been photolyzed in 60 s. The preponderant form of L at the end of the 60 s measurement, even if all L⁺ reacted to form the methoxy compound, which is unlikely, is the hydroxy form.

We have hypothesized that the production of L⁺ results from photolysis of the ML complex (path B). If this is so, then using only path A (by eliminating photolysis of ML and ML₂), it will not be possible to simulate the experimental results with reasonable parameters. We can turn off complex photolysis in the simulation by making log k_{h1} and log $k_{h2} \ll 0$. With these changes, we can test the productivity of path A by adjusting the values for the rates of dissociation and the binding constants, K₁ and K₂. The results of these simulations are shown in Fig. 7. The same experimental data and simulated results for L⁺ from Fig. 6B are shown for reference (curve A). When we remove k_{h1} and k_{h2} from the simulation, we are specifying that all excited complexes re-



Figure 7. Simulation of photoionization of the Zn(II)-complex of **1.** (\blacklozenge) Experimental data for photolysis of 40 μ *M* **1** with two equivalents of TMP and 0.1 *M* NaClO₄. The solid lines are simulation results with M = 10⁻³, L = 4 × 10⁻⁵ and the following rate constants: $k_{h\nu} = 0.011 \text{ s}^{-1}$, $k_{h} = 10^{6} \text{ s}^{-1}$, $k_{nr} = 10^{5} \text{ s}^{-1}$, $k_{r} = 143\,000$ $M^{-1} \text{ s}^{-1}$, $k_{nr1} = 10^{6} \text{ s}^{-1}$, $k_{nr2} = 10^{6} \text{ s}^{-1}$ and (A) $k_{1} = 1.34 \times 10^{6} M^{-1} \text{ s}^{-1}$, $k_{h2} = 170\,000 \text{ s}^{-1}$ (B) $k_{1} = 1.34 \times 10^{6} M^{-1} \text{ s}^{-1}$, $k_{h2} = 10^{7} \text{ s}^{-1}$, $k_{h1} = 10^{7} \text{ s}^{-1}$, $k_{h2} = 10^{9} M^{-1} \text{ s}^{-1}$, $k_{h2} = 10^{7} \text{ s}^{-1}$, $k_{h1} = 10^{7} \text{ s}^{-1}$, $k_{h2} = 10^{9} M^{-1} \text{ s}^{-1}$, $k_{h2} = 10^{9} M^{-1} \text{ s}^{-1}$, $k_{h2} = 10^{7} \text{ s}^{-1}$, $k_{h1} = 10^{9} M^{-1} \text{ s}^{-1}$, $k_{h2} = 10^{6} \text{ s}^{-1}$, $k_{h1} = 10^{9} M^{-1} \text{ s}^{-1}$, $k_{h2} = 10^{6} \text{ s}^{-1}$, $k_{h1} = 10^{9} M^{-1} \text{ s}^{-1}$, $k_{h2} = 10^{6} \text{ s}^{-1}$, $k_{h2} = 10^{9} M^{-1} \text{ s}^{-1}$, $k_{h2} = 10^{9} M^{-1} \text{ s}^{-1}$, $k_{h2} = 10^{9} M^{-1} \text{ s}^{-1}$, $k_{h2} = 10^{9} \text{ s}^{-1}$, $k_{h2} = 10^{-6} \text{ s}^{-1}$.

lax nonradiatively. Thus, the quantum yield for nonradiative decay is 1 and k_{nr1} and k_{nr2} need only be set to large values. Therefore, there are no adjustable parameters for this system. By maintaining the calculated rate constants and eliminating photolysis of the complexes, we see that barely any L⁺ is produced (curve B).

Our previous conclusion that the metal complex is photosensitive relied on the assumption that the rate of dissociation is slow. Even though the measured rate of dissociation is $2.24 \pm 0.14 \times 10^3 \text{ s}^{-1}$, we have simulated the results with rates of dissociation that are large $(k_{-1} = k_{-2} = 10^5 \text{ s}^{-1})$ and rates of association that are nearly diffusion controlled $(k_1 = k_2 = 10^9 M^{-1} \text{ s}^{-1})$. The results are shown in curve C. Even in this case, insufficient L⁺ is produced.

From these simulations, we must conclude that photolysis of the complex is the only method to achieve sufficient production of **2.** Thus, the complex is photosensitive.

There is one lingering question that we have not answered satisfactorily with these experiments. That is the question of whether the Zn(II) is truly liberated from the complex or whether it remains associated, e.g. as an ion pair with a carboxylate. We have tried to minimize this possibility by using a high concentration of salt, NaClO₄, in the experiments. The presence of the visible band in this class of molecules is associated with a significant positive charge on nitrogens para to the methanol carbon. Therefore, in compound 2, there is no driving force for metal-ion coordination by the nitrogen. In fact, an electrostatic repulsion between the nitrogen and zinc would be expected. In recent studies in a similar system, Allgeier et al. (70) placed ligands near the iron atom of a ferrocene. The +1 change in charge caused by oxidation of the iron center led to a 10^6 to 10^7 decrease in binding of Rh(I) and nearly a 1010-fold decrease in binding of Pd(II) to the adjacent ligands. Kimura et al. (16) showed a similar decrease in a cation's affinity for a positively charged ligand in comparing a crowned leuconitrile with an azacrowned leuconitrile. As in 1 and 2, the nitrogen in Kimura's azacrown, by virtue of using its lone pair to relax the positive charge formed at the central carbon atom after photolysis of the leuconitrile, did not bind metal ions, whereas the crowned analog (no nitrogen) did. Their compounds were stable in the colored form, allowing, for example, NMR experiments to reveal the metal-free photoproduct. Thus, photolysis of 1-Zn(II) should result in a considerable decrease of the affinity of the iminodiacetic acid moiety for the metal ion. This is hard to confirm, however. Product 2 is not stable, reverting to 1 rapidly. It's steady state concentration is a mere few percent of the starting concentration of 1. The visible absorption spectrum should not be particularly sensitive to changes in Zn(II) concentration. That is because the carboxylate functional groups are not part of the chromophore, and the nitrogen, which is part of the chromophore, is no longer basic. We do see a 12 nm shift (468 to 456 nm) in the visible band's wavelength of maximum absorbance (λ_{max}) from a change in Zn(II) concentration from 0 to 14.7 mM ([1] = 44.0 μ M). On the other hand, protonation of 2 gives a λ_{max} of 484 nm. Thus, the spectroscopic shift is small and bathochromic for protonation of the carboxylates and small and hypsochromic with increasing Zn(II) ion concentration. Although the evidence is not conclusive, it does not support a strong interaction of Zn(II) with the carboxylates in 2.

Acknowledgement—The authors would like to thank the Office of Naval Research for support of this work.

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