Photoinduced Electron Transfer in a Phenothiazine−Riboflavin Dyad Assembled by Zinc−Imide Coordination in Water

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Abstract: The known electron acceptor systems whereby the redox centers are linked by reversible noncovalent interactions are in most cases restricted to *organic solvents*. A kinetically labile coordinative bond has been designed for self-assembly of an electron donor (phenothiazine) and a photoinducible electron acceptor (riboflavin) *in water at neutral pH*. The pH dependent formation of the donor−acceptor complex in water was investigated by potentiometric titrations showing a binding constant of log $K = 5.9$. The strong binding constant supports the observed large fluorescence deactivation of the riboflavin emission by the phenothiazine zinc complex. The riboflavin fluorescence lifetime was found to be constant ($\tau = 4.7$ ns) whatever the quencher concentration, clear evidence for a static quenching mechanism. A strong thermodynamical driving force and the observation of the riboflavin radical anion and phenothiazine radical cation by transient spectroscopy provide evidence for intramolecular electron transfer as the likely mechanism for the fluorescence quenching.

Introduction

The transfer of an electron between redox centers is the essential step in many important biological processes and catalytic cycles. But although it is the most simple chemical reaction,[1] not all details are fully understood. A large number of artificial systems with covalently linked electron donor−acceptor dyads or triads have been synthesized to mimic the biological processes.[2] More recently electron donor−acceptor arrays which are tethered by reversible interactions, such as hydrogen bonds, salt bridges, or charge-transfer interactions, have been prepared[3] because the self-assembled aggregates more closely resemble the situation of electron transfer in biology where most redox cofactors are associated reversibly.[4] However, artificial electron donor−acceptor dyads tethered by a small number of hydrogen bonds or salt bridges immediately dissociate in methanol or water. Using cyclophanes an assembly in water was achieved, but the observed binding constant is still very small.[5] To improve the binding while keeping the reversibility, we have designed a photoinducible electron donor−acceptor dyad linked by a kinetically labile coordinative bond, which provides sufficient binding strength for assembly in water under physiological conditions.

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Experimental Section

Materials and Techniques. Compounds **1**,[6] **3a**,[7] **3b**,[8] **4**,[9] **6**,[10] and **7**[9] were synthesized according to known procedures. Melting points were taken on a hot-plate microscope apparatus and are not corrected. NMR spectra were recorded at 400 (^{1}H) and 100 MHz (^{13}C) in [D]-chloroform solutions unless otherwise stated. The multiplicity of the ^{13}C signals was determined with the DEPT technique and quoted as (+) for CH_3 or CH, (−) for CH_2, and (C_{quat}) for quaternary carbons. CC means column chromatography on silica gel. UV and fluorescence spectra were recorded on HP 8452A, Perkin-Elmer MPF 44, and Hitachi F-4500 spectrometers. The fluorescence quantum yields were measured at OD = 0.1 with quinine sulfate in 1 N sulfuric acid as the standard ($\phi_F = 0.55$). For details of potentiometric measurements and sample preparations, see Supporting Information. A laser-flash-photolysis system with 5 ns response time and a frequency tripled mode-locked Nd-laser (Quantel, 355 nm, 200 ps, 30 mJ/pulse) for excitation perpendicular to the analytical beam was used for transient absorption spectroscopy and kinetics measurements.

Synthesis. 10-(3-Phenothiazin-1-ylpropionyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (5b): A mixture of 3-phenothiazine-10-ylpropionic acid (**4**) (312 mg, 1.15 mmol), **3b** (472 mg, 1.15 mmol), dicyclohexyl carbodiimide (237 mg, 1.15 mmol), and 4-(*N*,*N*-dimethylamino)pyridine (140 mg, 1.15 mmol) in 12 mL of dichloromethane was stirred at room temperature overnight. Precipitated dicyclohexyl urea was removed by filtration, and the red solution was extracted with 2 N NaOH, washed with water, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by HPLC on RP-18 with acetonitrile (TLC: EE, $R_f = 0.7$) to yield 760 mg (91%) of **5b**, as a white solid, mp 78 °C; ^{1}H NMR (400 MHz, [D_6]-acetone) δ 1.33 (s, 9 H), 1.50 (s, 18 H), 2.05 (m, 2 H), 3.42 (m, 16 H), 4.20 (m, 2 H), 6.98 (m, 2 H), 7.03 (m, 2 H), 7.07 (m, 2 H), 7.11 (m, 2 H); ^{13}C NMR (100 MHz, [D_6]-acetone) δ 28.6 (+), 29.6 (+), 31.6 (−), 43.9 (−), 50.2 (−), 50.5 (−), 51.0 (−), 51.7 (−), 80.3 (C_{quat}), 116.2 (+), 123.4 (+), 125.2 (C_{quat}), 128.0 (+), 128.4 (+), 145.9 (C_{quat}), 155.9 (C_{quat}), 157.6 (C_{quat}); IR (KBr) ν 3446, 2975, 1646 cm^{-1}; UV/VIS (MeCN) λ_{max} (log ϵ) 192 (4.715), 256 (4.522), 308 (3.615); MS (70 eV, EI) *m*/*z* 725 (100) [M^+], 212 (70) [methyl phenothiazine], 57 (30) [butyl]; $C_{38}H_{55}N_5O_7S$ calcd C 62.87, H 7.64, N 9.65, S 4.4, found C 62.59, H 7.87, N 9.12, S 4.4.

3-Phenothiazine-10-yl-1-(1,4,7,10-tetraazacyclododecan-1-yl)propan-1-one (5c): A mixture of **5b** (726 mg, 1 mmol) and 5 mL of trifluoro acetic acid in 5 mL of dry dichloromethane was stirred for 1.5 h at room temperature. The reaction mixture was diluted with dichloromethane (30 mL) and aqueous NaOH (6 N, 5 mL), washed with aqueous NaOH (2 N), and dried over Na_2SO_4, and the solvent was removed in vacuo to yield **5c** (308 mg, 72%) as a white solid, mp 79 °C; ^{1}H NMR (400 MHz, [D_6]-acetone) δ 2.80 (m, 16 H), 2.90 (m, 2 H), 3.50 (m, 2 H), 6.95 (m, 2 H), 7.08 (m, 4 H), 7.20 (m, 2 H); ^{13}C NMR (100 MHz, [D_6]-acetone) δ 31.8 (−), 44.4 (−), 45.6 (−), 46.6 (−), 48.2 (−), 48.4 (−), 48.6 (−), 48.7 (−), 49.8 (−), 50.5 (−), 116.3 (+), 123.2 (+), 124.7 (C_{quat}), 127.8 (+), 128.4 (+), 145.8 (C_{quat}), 173.2 (C_{quat}); IR (KBr) ν 3428, 2894, 1631, 750 cm^{-1}; UV/VIS (MeCN) λ_{max} (log ϵ) 192 (4.382), 256 (4.222), 308 (3.324); MS (70 eV, EI) *m*/*z* 425 (20) [M^+], 212 (100) [methyl phenothiazine]; HRMS $C_{23}H_{31}N_5O_1S$ calcd 425.2249, found 425.224.

3-Phenothiazine-10-yl-1-(1,4,7,10-tetraaza-cyclododecan-1-yl)-propan-1-one zinc(II) perchlorate (2): Solutions of $Zn(ClO_4)_2 \cdot 6H_2O$ (88 mg, 0.24 mmol) in 2 mL of methanol and **5c** (100 mg, 0.24 mmol) in 3 mL of methanol were combined and refluxed for 1 h under nitrogen. Most of the solvent was removed in vacuo, and the precipitated product was collected by filtration and dried in vacuo to yield **2** (165 mg, 100%), as a slightly red solid, mp 164 °C. Samples for photophysical experiments were purified by HPLC on RP-18 column with acetonitrile. ^{1}H NMR (400 MHz, [D_6]-DMSO) δ 2.60−3.60 (m, 20 H), 6.80−7.40 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.0 (−), 42.7 (−), 43.2 (−), 43.3 (−), 44.8 (−), 45.5 (−), 115.1 (+), 115.6 (+), 122.5 (+), 122.6 (+), 127.0 (+), 127.1 (+), 127.6 (+), 127.7 (+), 144.2 (C_{quat}), 144.5 (C_{quat}), 173.2 (C_{quat}); IR (KBr) ν 3450, 3444, 2929, 1118 cm^{-1}; UV/VIS (MeCN) λ_{max} (log ϵ) 198 (4.369), 256 (4.335), 304 (3.490); MS (FAB) *m*/*z* 590 (7) [M^{2+} + ClO_4^-], 212 (36) [methyl phenothiazine], 55 (100).

Results and Discussion

Design and Synthesis. The binding of imides to Lewis-acidic zinc(II) azamacrocycles has been studied by Kimura and coworkers thoroughly.[11] As confirmed by several X-ray structures,[11] the deprotonated imide nitrogen coordinates to the zinc(II) ion, which is complexed by tetraazacyclododecane. The interaction of imide and metal complex is supported by hydrogen bonds between imide carbonyl oxygen and azamacrocycle N−H, whereby it remains unclear if this is a direct interaction or water molecules bridge the structure.[11a] A characteristic feature of the zinc(II) effected by the macrocyclic coordination is the promotion of proton dissociation at physiological pH from carboxamides and sulfonamides which is rendered more favorable by the strong interaction of zinc(II) and the anionic ligand.[12] Therefore, depending on the structure of the zinc−azamacrocycle, imide coordination is observed to a large extent in weakly basic aqueous solutions and even at neutral pH. The binding motif has been used as receptors for the recognition of anions and neutral molecules in water,[11,13] but it is also perfectly suited for the assembly of functionalized aggregates in water. In particular, the redox potential of zinc(II) tetraazacyclododecane (−1.77 V vs Ag/AgCl)[14] allows straight electron transfer between the attached redox partners. To demonstrate the formation of a photoinducible electron donor−acceptor dyad in water at physiological conditions we have used tetraazacyclododecane zinc(II)−phenothiazine complex **2** as a strong electron donor with the Lewis-acidic binding site tethered by a flexible spacer. Riboflavin tetraacetate **1**[6,15] was selected as the coordinating imide ligand and electron acceptor, because it becomes a strong oxidant upon irradiation and its high fluorescence quantum yield facilitates the detection of emission quenching.[16] We have employed the tetraacetate derivative **1** for its higher photostability[17] compared to riboflavin. The coordination of riboflavin to Zn(II) tetraazacyclododecane via imide coordination has been previously reported.[11a] Therefore in neutral aqueous solutions containing both components **1** and **2** the electron donor (phenothiazine) and the acceptor (riboflavin

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(14) Anodic peak potential. For experimental details, see Table 1 caption.

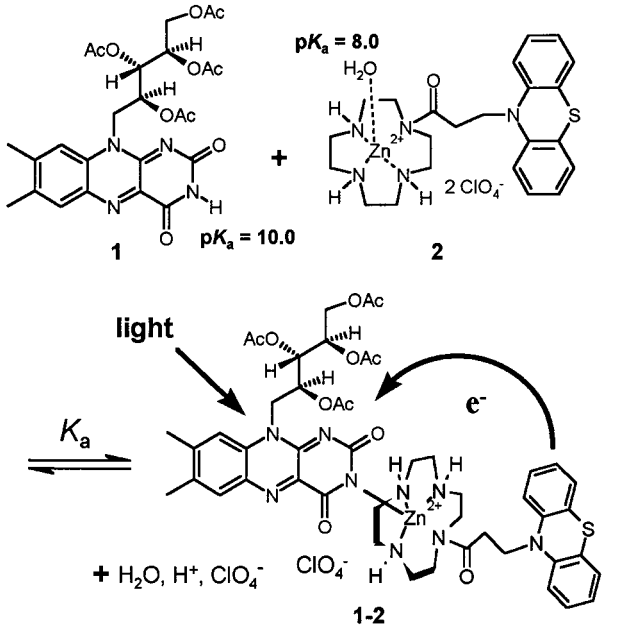
tetraacetate) are expected to associate tightly and pave the way for an efficient electron transfer even at low concentrations.

The synthesis of **2** requires selective functionalization of one nitrogen atom of the azamacrocycle. Protecting groups, such as Cbz (benzyloxycarbonyl) or Boc (*tert*-butoxycarbonyl), were used to shield the other three amino groups.[6,7] The free amino group of **3a** or **3b** was coupled with 3-phenothiazin-10-ylpropionic acid (**4**)[8] by using standard peptide procedures.[18] Amides **5a** and **5b** were obtained in 87% and 91% yield, respectively. The connectivity of **5b** was confirmed by X-ray structure analysis.[19] Removal of the Cbz protecting groups from **5a** proved difficult,[20] but Boc protecting groups of **5b** were cleaved cleanly with TFA in dichloromethane to give **5c** in 72% yield. The substituted azamacrocycle **5c** was converted into zinc complex **2** by treatment with zinc perchlorate in refluxing methanol for 1 h.

To investigate binding and protonation constants of the equilibrium of **1** and **2** potentiometric titrations[21] were performed. The obtained data show that the Lewis acidity of complex **2** is similar to that of zinc(II) tetraazacyclododecane (**6**) (Scheme 3) as shown by the pK_a values of coordinated water (8.0 for **2**; 7.9 for **6**). A similar ability of **2** and **6** to coordinate the imide moiety of riboflavin is likely and was confirmed by titration of mixtures of **2** and riboflavin. The observed pH profile corresponds to the formation of a 1:1 complex with an association constant of log $K = 5.9$.

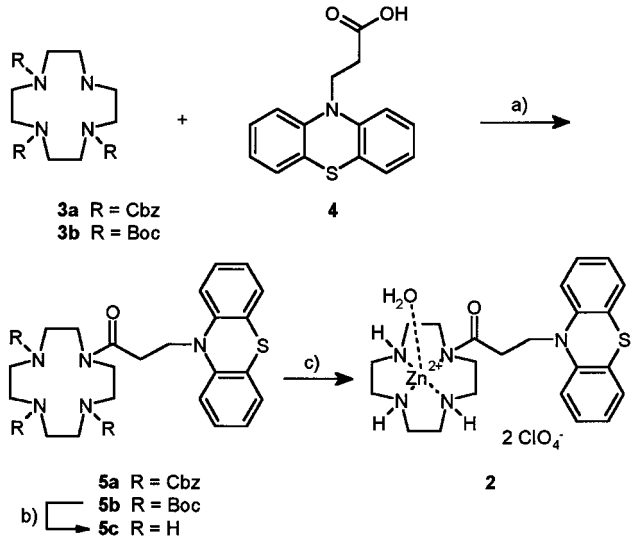
Cyclovoltammetry. A fast photoinduced electron transfer requires a sufficient driving force provided by the difference in redox potential of photoexcited electron acceptor and electron donor. To investigate if **1** and **2** fulfill this criterion, their redox potentials were determined by cyclovoltammetry.[22] The measured redox potentials allow an estimate[23] of the value of ΔG for electron transfer from phenothiazine to excited riboflavin. The significant redox potentials for the electron-transfer process are given in Table 1. By neglecting the entropy changes from ground to excited state, a driving force for electron transfer from phenothiazine to photoexcited **1** of $\Delta G \approx -1.15$ eV or -26 kcal/mol can be derived.[23] The Rehm−Weller[24] relation predicts

Scheme 1. Assembly of a Riboflavin−Phenothiazine Electron Donor−Acceptor Dyad via Zinc(II) Imide Coordination[a]



[a] Hydrogen bonds between carbonyl oxygen and N−H groups may support the assembly.[11a]

Scheme 2. Synthesis of **2**[a]



[a] (a) DCC (dicyclohexylcarbodiimide), DMAP (*N*,*N*-(dimethylamino)pyridine), CH_2Cl_2, 12 h, 87% (**5a**), 91% (**5b**). (b) TFA (trifluoroacetic acid), CH_2Cl_2, 1.5 h, 72%. (c) $Zn(ClO_4)_2 \cdot 6H_2O$, MeOH, reflux, 1 h, quantitative.

an outer-sphere[1c] fast electron transfer with $k_q \approx 10^{10}$ M^{-1} s^{-1} for this value. Therefore efficient intramolecular electron transfer

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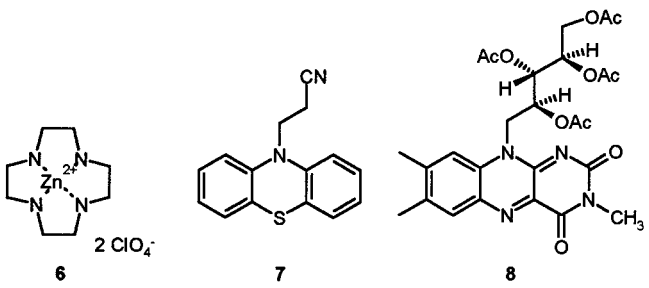
(22) The measurements were performed in acetonitrile and methanol as solvents to allow scanning over a wide electrochemical window. The actual redox potentials in water may differ slightly, which will lead to a different value of ΔG. However, the general conclusion that photoinduced electron transfer from phenothiazine to riboflavin is a strongly exothermic process will not be affected. For details of the experimental conditions, see Table 1 caption.

(23) (a) When the change in shape, size, and solvation after electronic excitation is small, the entropy difference between ground and excited state can be neglected. With this assumption the redox potential of excited molecules can be derived from their ground-state redox potential and the zero spectroscopic energy of the excited state. Julliard, M.; Chanon, M. *Chem. Rev*. **1983**, *83*, 425−506. (b) $\Delta G° \approx 0.23$ V (phenothiazine/phenothiazine$^{\bullet+}$) + 1.18 V (riboflavin/riboflavin$^{\bullet-}$) − 2.48 eV (zero spectroscopic energy riboflavin) − 0.08 (coulomb term) ≈ − 1.15 eV. The 0−0 transition should be taken at ca. 500 nm from consideration of absorption and emission spectra of **2**. We suggest therefore to use $\Delta E_{00} \approx$ 2.48 eV. We have to add the Coulombic term $-e^2/\epsilon r$, which for intermolecular quenching with $r = 5$ Å gives ca. −0.08 eV. From the measurements in methanol a similar value of $\Delta G° \approx -1.27$ eV (−28 kcal/mol) can be derived.

Scheme 3. Structures of Compounds Which Have Been Used for Comparison



6 7 8

Table 1. Redox Potentials of **1**, **2**, **5c**, **6**, and **7**, Determined by Cyclic Voltammetry

compd	redox potentials E^a			
1	$\mathbf{1}^0/\mathbf{1}^{\bullet-}$: -1.18^b (-0.89)^c	$\mathbf{1}^{\bullet-}/\mathbf{1}^{2-}$: -1.87		
2	$\mathbf{2}^{2+}/\mathbf{2}^{\bullet+}$: 0.96^d	$\mathbf{2}^{\bullet+}/\mathbf{2}$: 0.23^e	-0.94^f	-2.04^g
5c	$\mathbf{5c}^{2+}/\mathbf{5c}^{\bullet+}$: 0.98^d	$\mathbf{5c}^{\bullet+}/\mathbf{5c}$: 0.30^e		-2.69^g
7	$\mathbf{7}^{2+}/\mathbf{7}^{\bullet+}$: 0.78^h	$\mathbf{7}^{\bullet+}/\mathbf{7}$: $0.44^h/0.36^i$		
6			-0.93^f	-2.31^g

[a] Values quoted in *V*, at a scan rate of 50 mV/s in 3×10^{-3} M solutions of the compounds in 0.1 M $Bu_4NPF_6-CH_3CN$ at 20 °C vs ferrocene/ferrocenium. [b] $\mathbf{1}/\mathbf{1}^{\bullet-}$ give as formal potential $E_f = \nu_2(E_{p,c} + E_{p,a})$; $E_{p,c}$ = cathodic peak potential. [c] In 0.1 M $LiClO_4$−methanol at a scan rate of 0.1 V/s and 20 °C vs ferrocene/ferrocenium. [d] Phenothiazine^{2+}/phenothiazine$^{\bullet+}$. [e] Phenothiazine$^{\bullet+}$/phenothiazine. [f] This potential corresponds to the cathodic peak potential for the dissolution of metallic zinc that was deposited in the reduction cycle. [g] Anodic peak potential ($E_{p,a}$). [h] In 0.1 M $LiClO_4$−methanol at a scan rate of 0.1 V/s and 20 °C vs ferrocene/ferrocenium; cathodic peak potential. [i] In 0.1 M $LiClO_4$−methanol at a scan rate of 0.1 V/s and 20 °C vs ferrocene/ferrocenium; anodic peak potential.

can be expected for the fluorescence quenching in the complex of **1** and **2**.

The electrochemical investigation of mixtures of **1** and **6** in 0.1 M $LiClO_4$ in methanol solution[25] clearly shows a significant stabilization of the radical anion of **1** by coordination to the zinc complex. The peak potential observed for the oxidation of $\mathbf{1}^{\bullet-}$ shifts from -0.81 V[26] to -0.73 V[26] upon addition of 1 equiv of **6** and to -0.60 V[26] upon addition of 10 equiv of **6**. From the corresponding change of the formal redox potential E_f a small increase ($\approx$2 kcal/mol) of the thermodynamic driving force ΔG for photoinduced electron transfer can be estimated.[27]

UV-Absorption Spectroscopy. To examine interactions between the ground state of **1** and **2** UV spectra of the pure compounds and mixtures were recorded in acetonitrile, methanol, and water buffered at pH 7.4. Solvochromic effects[28] were found to be very small. In all solvents the UV absorption of mixtures of **1** and **2** up to a ratio of 1:20 did not show any significant deviation from the sum of the individual spectra. The same result was observed if **2** was replaced by compound **7**, which has a similar absorption spectrum, but does not possess a binding site. As an example, the UV spectra of **7** and **1** in acetonitrile are represented in Figure 3.[29] It is clear that at the wavelength selected for excitation (442 nm), only **1** will be excited whereas **2**, **7**, and other reference molecules do not absorb light. The measurements lead to the conclusions that (1) there is no significant interaction between the ground state of riboflavin and phenothiazine, neither in the complex nor intermolecularly, and (2) a selective excitation of the riboflavin chromophore is possible in the complex **1**−**2**.

Fluorescence Spectroscopy. Figure 3 shows the known emission spectrum of **1** (quantum yield $\Phi_F = 0.37$ in acetonitrile). Steady-state fluorescence quenching studies with electron donor compounds that do not have binding sites, such as **5c** or **7**, yielded linear Stern−Volmer plots, indicating a diffusional mechanism of fluorescence quenching.[30] Accordingly, tetraazacyclododecane, zinc(II) perchlorate, and **6** were investigated for their ability to quench the fluorescence of **1**. In all cases a large excess of the quencher molecule was necessary for a decrease of fluorescence intensity.[31] *The situation changes dramatically when **2** is used as quencher*. Whereas a ca. 1000-fold excess of **7** is needed to quench half of the fluorescence intensity of **1,** ca. 1.3 equiv of **2** is enough to reduce the fluorescence intensity of **1** by nearly 50%. The addition of 2 equiv of **2** decreases the fluorescence intensity of **1** to 5% of its initial value. From the experiments in acetonitrile it was concluded that phenothiazine derivatives without a binding site for **1**, such as **5c** or **7**, quench the emission of **1** by a *diffusional mechanism* (zinc(II) perchlorate or **6** do not significantly quench the emission of **1**) whereas a very efficient quenching,[32] mainly via *static mechanism*, is observed with **2** (vide infra). However, the main intention of the work was the development of a functional structure that shows PET *in water at neutral pH*. Therefore all further experiments were performed in buffered water.[33] With **1** as the only emitting species (vide infra) in the equilibrium, the fitting of fluorescence titration data to a 1:1 binding isotherm provides a tentative value of $\log K = 3.8$ (± 0.1) for the binding constant (Figure 4).[34] The stoichiometry of the complex was confirmed by a Job's plot,[34] as shown in Figure 5. The results confirm that the formation of a defined donor−acceptor complex occurs in water at physiological conditions.

(24) Rehm, D.; Weller, A. *Ber. Bunsen-Ges. Phys. Chem.* **1969**, *73*, 834−839.

(25) A measurement in buffered water is not possible, because of the very narrow electrochemical window.

(26) All potentials vs ferrocene/ferrocenium in 0.1 M $LiClO_4$ at 20 °C and a scan rate of 0.1 V/s.

(27) E_f(riboflavin/riboflavin$^{\bullet-}$): -0.89 V. E_f (+ 1 equiv **6**): -0.87 V. E_f(+ 5 equiv **6**): -0.83 V. E_f(+ 10 equiv. **6**): -0.80 V. All potentials vs ferrocene/ferrocenium in 0.1 M $LiClO_4$ at 20 °C and a scan rate of 0.1 V/s. For a recent investigation of the binding properties of riboflavin and its radical anion, see: Breinlinger, E. C.; Keenan, C. J.; Rotello, V. M. *J. Am. Chem. Soc.* **1998**, *120*, 8606−9609 and cited references.

(28) Reichardt, C. *Solvent and Solvent Effects in Organic Chemistry;* VCH: Weinheim, Germany, 1988.

(29) See Supporting Information for UV spectra of compounds **1, 2, 7,** and **8** in different solvents. The identical UV spectra of **1** and **8** reveal that exchange of the imide proton by methylation or deprotonation does not change the properties of the chromophore.

(30) A k_q of 1.4×10^{10} M^{-1} s^{-1} for diffusional quenching of the fluorescence of **1** by phenothiazine was derived from the slope of the Stern−Volmer plot (k_{SV}) of the fluorescence intensity ratios of mixtures of **1** and **7** ($k_{SV} = k_q\tau$). This value is in good agreement with $k_q = 1.8 \times 10^{10}$ L mol^{-1} s^{-1}, derived from the Debye estimation of diffusional quenching in acetonitrile at room temperature. Turro, N. J. *Modern Molecular Photochemistry*; Benjamin/Cummings: Menlo Park, **1978**; p 314.

(31) The results are summarized in the Supporting Information. With tetraazacyclododecane, zinc(II) perchlorate, and **6** the observed changes in fluorescence intensity of **1** were very small. Due to the limits of resolution of the spectrometer the inaccuracy of the calculated k_q values is high.

(32) A detailed analysis of the fluorescence data was performed with the program LETAGROP-SPEFO indicating a 1:1 stoichiometry and yielding a binding constant of 4.7×10^4 ($\pm$8000) M^{-1}. Sillen, L. G.; Warnquist, B. *Ark. Kemi* **1968**, *31*, 315−339, 377−390.

(33) Complex formation and emission quenching was also studied in methanol. See Supporting Information for results.

(34) To get an order of magnitude of the binding constant, it was derived by nonlinear fitting with algorithms provided by the program Sigma plot. Connors, K. A. *Binding Constants*; Wiley: New York, **1987**. This value is underestimated as the quenching mechanism is more complex than assumed in the calculation.

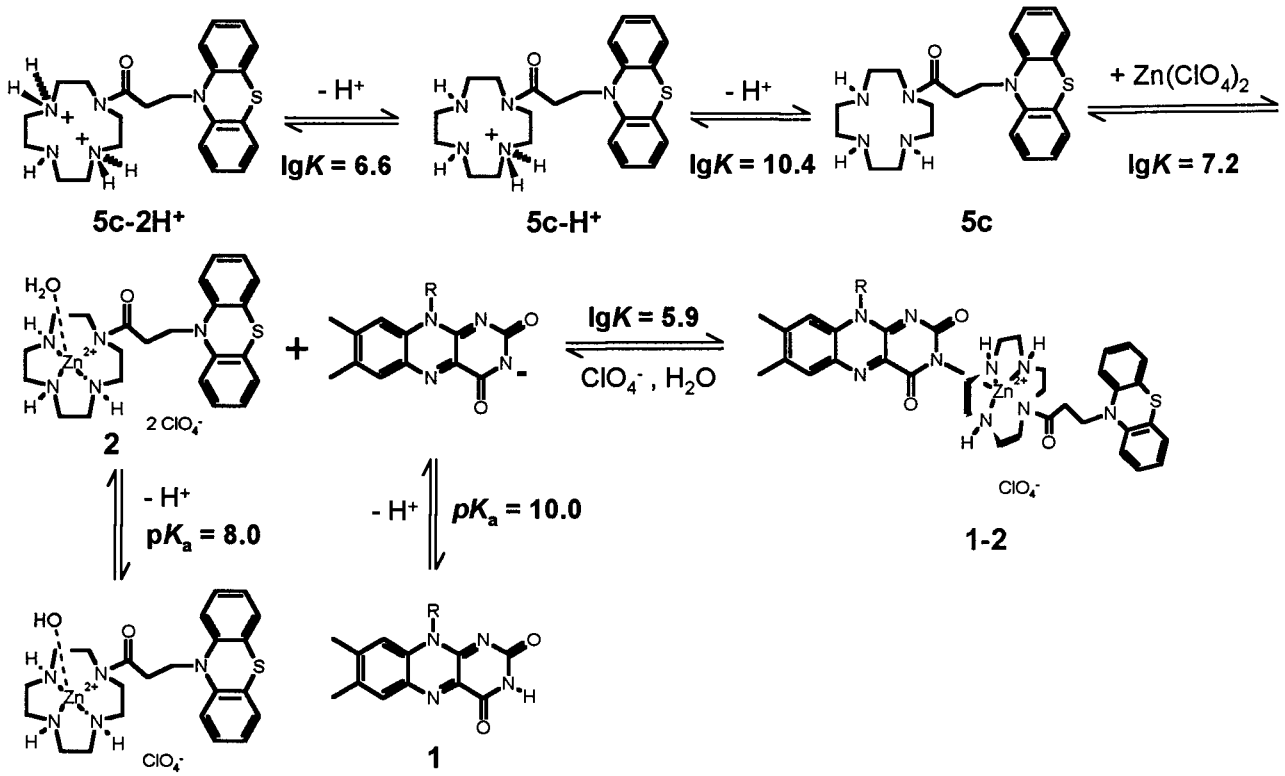


Figure 1. Equilibrium constants of **1** and **2** as determined by potentiometric titration in water (I = 0.1 mol/L $Et_4N\,ClO_4$, 25 °C) with Et_4NOH. For experimental details, see Supporting Information (R = ribityl).

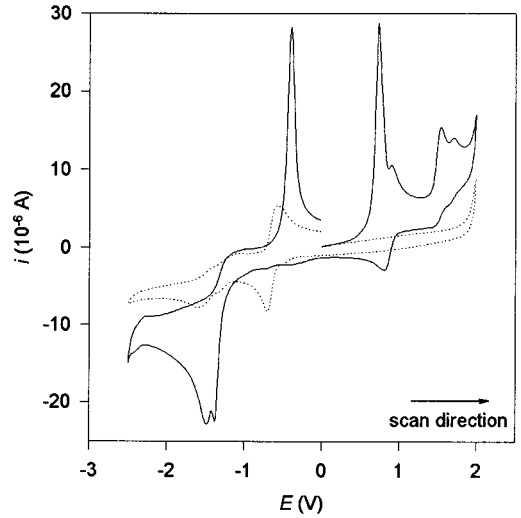


Figure 2. Cyclic voltammogram of **1** (···) and **2** (—) in acetonitrile. For conditions see Table 1.

Fluorescence decay measurements were performed in different solvents to investigate the emission properties of the complex.[35] Solutions of **1** and mixtures of **1** and **2** in acetonitrile or water (pH 7.4, buffered with Tris−HCl, 50 mmol NaCl) showed single exponential decays corresponding to lifetimes of the excited state of **1** of τ = 6.8 (acetonitrile) and 4.7 ns (buffered water). The observed lifetime τ is constant and independent of the amount of added **2**. This leads to the conclusion that the complex **1**−**2** is not emitting.

(35) For details of fluorescence decay measurements, see Supporting Information. Fluorescence decays were recorded with use of the single photon timing technique as already described (Desvergne, J.-P.; Castellan, A.; Bouas-Laurent, H.; Soulignac, J. C. *J. Luminescence* **1987**, *37*, 175−181); the exponential decays profiles were fitted by using the DECAN 1.0 program (De Roeck, T.; Boens, N.; Docks, J. DECAN 1.0, Leuwen, Belgium).

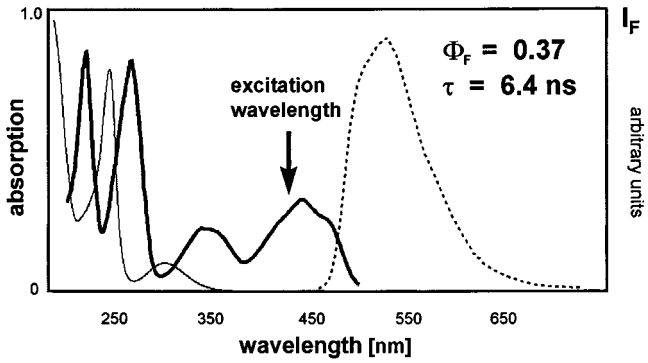


Figure 3. UV absorption spectra of **7** (—) and **1** (—) in acetonitrile (concentration ≅ 10^{-5}M). **5c** and **2** have similar spectra to that of **7**. Fluorescence spectrum of **1** (---). ϕ_F: Riboflavin fluorescence quantum yield. τ: fluorescence lifetime of riboflavin in CH_3CN (single-exponential decay).

To provide evidence that deprotonation of the imide N−H is indispensable for complex formation, fluorescence quenching experiments were performed with the *N*-methylated derivative **8**.[36] The addition of 55 equiv of **2** to an aqueous solution of **1** reduces the emission intensity by nearly 90%, whereas the same amount of **2** added to an aqueous solution of **8** decreases the emission intensity only slightly (Figure 6). This result clearly underlines the importance of the imide nitrogen and its deprotonation for complex formation.

Laser Flash Photolysis (LFP). Transient spectroscopy was employed in an attempt to detect the charge separated state thus providing clear evidence for electron transfer as the mechanism of quenching of the excited singlet state of the coordinated riboflavin.

(a) Measurements in the Time Range of 100 ns to 100 ms. LFP (354 nm, 200 ps) of **1** (0.2 mmol/L) in degassed

(36) Foery, F.; Hemmerich, P. *Helv. Chim. Acta* **1967**, *50*, 1766−1774.

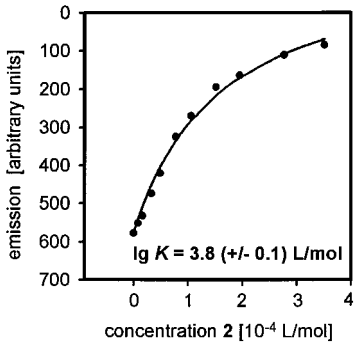


Figure 4. Fluorescence titration data and fitted binding isotherm of **1** and **2** in water buffered to pH 7.4 (Tris−HCl, 10 mmol/L), 50 mmol/L NaCl.

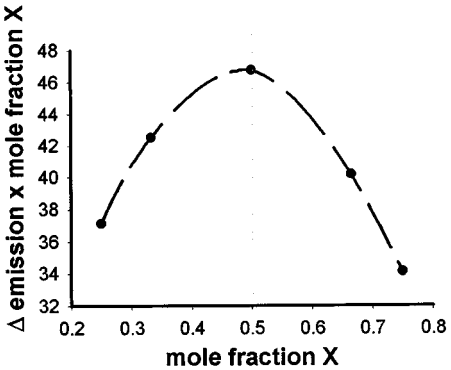


Figure 5. Job's plot of **1** and **2** confirming the 1:1 stoichiometry. Solvent: water buffered to pH 7.4 (Tris−HCl, 10 mmol/L), 50 mmol/L NaCl.

methanol without added phenothiazine yields the triplet state of **1** as the only transient species characterized by the TT absorption at 650 nm.[37] Addition of **2** or **7** reduces the triplet lifetime, but only addition of **2** reduces the amount of triplet state formed. In solutions containing **1** and **2**, the triplet absorption is reduced in the same proportion as the amount of noncoordinated **1**, estimated from the binding constant (log $K \approx 4$) and concentrations of **1** and **2**. This confirms the results obtained from fluorescence lifetime measurements: the excited singlet state of the coordinated riboflavin is quenched so efficiently that it does not fluoresce nor cross to the triplet.

(b) Measurements in the Time Range of 0 to 750 ms. Traces of optical transmission of methanolic solutions containing **1** (0.2 mmol/L) and **2** or **7** (1 mmol/L) have been recorded vs time at a series of wavelengths between 490 and 650 nm and were converted into curves of absorbance vs time as shown in Figure 7. Two different transient species absorb in the investigated region. The first one, with an absorption between 490 and 550 nm and an (approximately) first-order decay with a lifetime around 30 μs, is clearly the *phenothiazine radical cation* as indicated by its spectrum.[38] The transient species is not observed when solutions of **2** or **7** are irradiated without added **1**. The second transient species shows weak absorption in the region 500−650 nm and a much slower decay time, in the millisecond time scale. This second transient species is, very likely, the *radical anion of riboflavin* that is known to absorb in this region.[39] While the experiments confirm electron transfer from the phenothiazine to riboflavin, the different lifetimes of cation and anion radicals indicate that besides charge recombination another mechanism is responsible for the shorter lifetime of the phenothiazine radical cation. This is most probably a reaction of the phenothiazine radical cation with the solvent methanol.

Actually, LFP of the system **1** (0.2 mmol/L) and **2** (1 mmol/L) in acetonitrile gives quite different kinetic results: the decays at 520 nm ($\mathbf{2}^{\bullet+}$) and 610 nm ($\mathbf{1}^{\bullet-}$) are both in the millisecond range and the transient absorption at 520 nm decays according a second-order process with $k_2/\epsilon \approx 10^5$ cm·s^{-1}. With $\epsilon = 9300$ mol^{-1}·L·cm^{-1} [38b] this yields $k_2 \approx 10^9$ mol^{-1}·L·s^{-1}. Although the small size of the transient absorption at 610 nm does not allow a precise kinetic analysis, it is clear that the decays at 520 and 610 nm are closely related, both absorptions going to zero (i.e. within the noise) about 3 to 4 ms after excitation. A second-order kinetics for the decay of the radical ion species indicates that these species are diffusing in the solution. The fact that similar results are obtained with the systems **1** + **2** and **1** + **7** although the latter does not give any stable complex yields the same conclusion. Under excitation of the system **1** + **7**, the radical ions result mainly from the encounter of $^3\mathbf{1}^*$ with **7** since $^1\mathbf{1}^*$ has too short a lifetime to react efficiently with **7** at 1 mmol/L concentration. It could thus be thought that the radical ion species observed under excitation of the system **1** + **2** result from the reaction of the triplet state of some uncomplexed **1** with **2**. This is not the case since the amplitudes of the transient absorption at 520 nm are about the same for the systems **1** + **2** and **1** + **7** whereas it can be calculated that, with a complexation constant $K \approx 4.7 \times 10^4$ M^{-1} in acetonitrile[32] and the concentrations used in these LFP experiments, ca. 3% of **1** is uncomplexed so that ≈97% of the excitation light is absorbed by the complex. Therefore the radical ions, freely diffusing in the solution, observed under excitation of the system **1** + **2** originate (almost exclusively) from the complex.

The whole set of LFP results may be rationalized by the following sequence: (1) excitation of the riboflavin moiety in the complex; (2) very fast electron transfer from the phenothiazine to the excited riboflavin singlet state so that fluorescence and intersystem crossing are completely quenched; (3) dissociation of the charge separated complex into free radical ions in competition with back electron transfer; and (4) slow recovery of the system by back electron transfer between the radical ions during diffusional encounters (see Scheme 4).

The competition between back electron transfer and dissociation into radical ions (step 3) can explain why the yields of radical ions are low and similar for both the **1** + **2** and **1** + **7** systems whereas one would expect this yield close to unity for the **1** + **2** system if the dissociation were quantitative. The processes involved in step 3 could not be monitored or even detected with our LFP system: they probably occur in the nanosecond time scale and would require a system with picosecond time resolution.[40]

Conclusions

Riboflavin, as photoinducible electron acceptor, can be assembled with electron donor **2** in water at neutral pH forming

(37) (a) Grodowski, M. S.; Veyret, B.; Weiss, K. *Photochem. Photobiol.* **1977**, *26*, 341−352. (b) At 530 nm the same trace is observed with similar decay time but an amplitude twice lower in agreement with the TT absorption spectrum of **1**. (c) Methanol was used as solvent to achieve high complex formation.

(38) (a) Lewis, G. N.; Bigeleisen, J. *J. Am. Chem. Soc.* **1943**, *65*, 2419. (b) Alkaitis, S. A.; Beck, G.; Graetzel, M. *J. Am. Chem. Soc.* **1975**, *97*, 5723−5729.

(39) (a) Holmström, B. *Arkiv Kemi* **1964**, *22*, 281. This paper is often quoted to justify the monitoring of the semireduced radical of flavins at 560 nm. (b) Navarro, J. A.; Roncel, M.; De la Rosa, M. A. *Photochem. Photobiol.* **1987**, *46*, 965−970. In this paper, the absorption spectrum of semireduced lumiflavin appears as a very broad band, nearly isosbestic from 530 to 600 nm and slowly decaying from 600 to 700 nm.

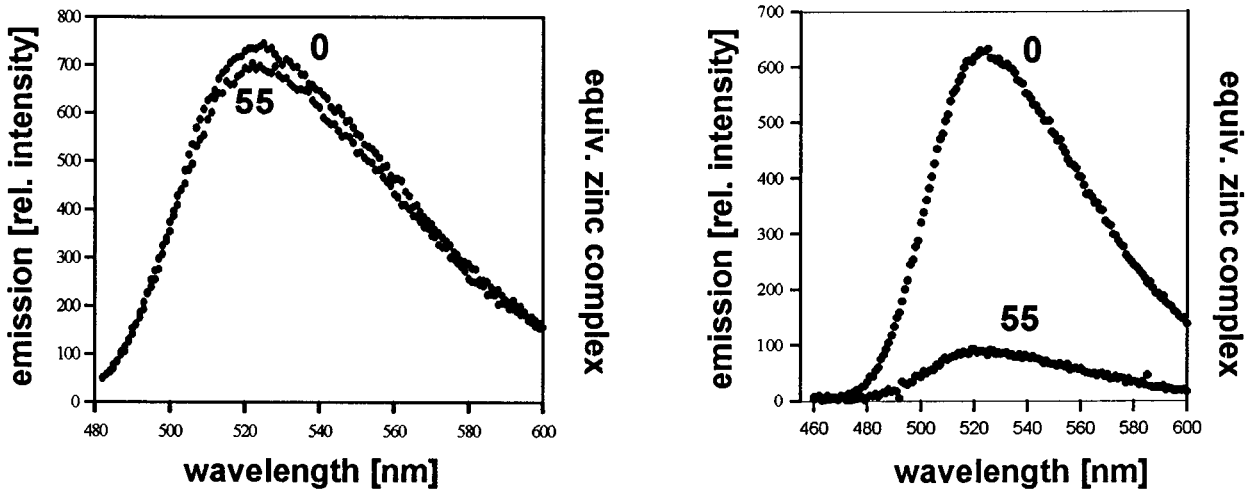


Figure 6. Change in emission intensity of **8** (10^{-5} mol/L, left) and **1** (10^{-5} mol/L, right) in water (pH 7.4, 10 mmol/L Tris-HCl, 50 mmol/L NaCl) upon addition of 55 equiv of **2**.

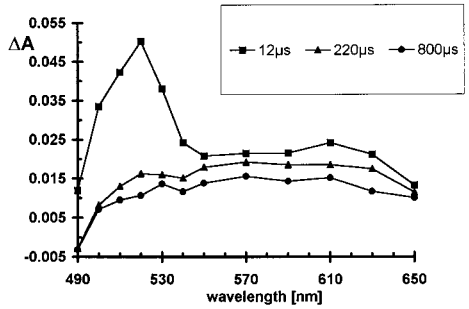
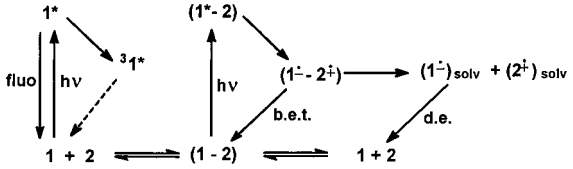


Figure 7. Differential optical absorption at various delays for **1** (2×10^{-4} mol/L) and **2** (10^{-3} mol/L) in methanol.

Scheme 4. Proposed Mechanism for the Formation of Cation and Anion Radicals Resulting from Intra-assembly Electron Transfer (b.e.t. = back electron transfer; d.e. = diffusional encounter).



a complex originating in zinc(II) cyclenimide interaction. The emission of riboflavin is quenched very efficiently in the complex by innersphere electron transfer, as confirmed by the observation of phenothiazine radical cation and riboflavin radical anion as transient species. By way of the zinc(II) cyclenimide coordination or similar binding motifs the selective interception of biological electron transfer pathways or the self-assembly of functional aggregates at physiological conditions in the presence of biomolecules might be envisaged.

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Supporting Information Available: Details of electrochemical measurements and potentiometric titrations; synthesis and characterization of compound **5a**; cyclic voltammogramme of mixtures of **1** and **6**; UV-absorption spectra of **1**, **2**, and mixtures in water; Stern−Volmer plots of emission quenching of **1** by **5c**, **7**, or **2** in methanol and acetonitrile; table of Stern−Volmer coefficients; details of the determination of binding constants of **1** and **2** in water and methanol; Job's plot of **1** and **2** in methanol; time-resolved absorption of mixtures of **1** and **2**, respectively, **1** and **7** in methanol and acetonitrile; transient spectra of **1** and **7** in methanol; analysis of the decay of transient absorption of a mixture of **1** and **2** in acetonitrile (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(40) A rational for the dissociation of **1**−**2** may be derived from the fact that the phenothiazine moiety, which becomes positively charged upon electron transfer, is repelled from the positively charged zinc complex to which it is tethered by a flexible alkyl chain. Due to the increased distance of riboflavin and phenothiazine the stabilization of the complex from $\pi-\pi$ stacking is lost which will facilitate dissociation. Moreover, a solvent separated ion pair between $\mathbf{1}^{-\cdot}$ and $\mathbf{2}^{+\cdot}$ (formed after electron transfer) will contribute to the dissociation of the complex allowing radical ions to diffuse into the solution.