

A ground-state complex between methyl viologen and the fluorescent whitening agent 4,4'-bis(2-sulfoxyethyl)-biphenyl disodium salt: a fluorescence spectroscopy study

Emily J. Armstrong, Henry Galas, R. Stephen Wylie, Shiva Zohrehvand, Jan van Stam, and Christopher H. Evans

Abstract: This study explores the quenching of the dianionic fluorescent whitening agent, NFW, by various substances, including methyl viologen (MV), in water and in the presence of beta-cyclodextrin (β -CD). Results of a fluorescence spectroscopic examination of the β -CD–NFW system are presented. It was found that NFW forms a 1:1 inclusion complex with β -CD with an association constant of $2540 \pm 380 \text{ M}^{-1}$. The included NFW fluorescent state is protected by the β -CD cavity from a range of water-based quenchers (neutral, anionic, and cationic). Quenching proceeds near the diffusion-controlled limit in water for the quenchers tested with the exception of the dicationic MV. Methyl viologen is an extremely efficient quencher of NFW fluorescence with a nominal $K_{SV} \sim 5.0 \times 10^3 \text{ M}^{-1}$ in water alone, corresponding to a nominal k_q of $\sim 4 \times 10^{12} \text{ M}^{-1} \text{ s}^{-1}$, which exceeds the diffusion-controlled limit in this solvent. The quenching efficiency of MV is strongly suppressed in the presence of 10 mM β -CD ($K_{SV} = 105 \pm 12 \text{ M}^{-1}$) and in the presence of NaCl ($K_{SV} = 106 \pm 9 \text{ M}^{-1}$ at 0.5 M salt). In the absence of CD or salt, there is a strong contribution from static quenching in the MV system; the presence of these additives suppresses the static quenching. Various results suggest the static quenching is due to formation of a ground-state complex between the dianion NFW and the dication MV.

Key words: cyclodextrin, fluorescent whitening agent, distyrylbiphenyl, fluorescence quenching, methyl viologen, ground-state complex.

Résumé : Cette étude porte sur l'extinction de fluorescence de l'agent de blanchiment fluorescent dianionique NFW par diverses substances, notamment le méthylviologène (MV), dans l'eau et en présence de bêtacyclodextrine (β -CD). Nous présentons les résultats d'une analyse par spectroscopie de fluorescence du système β -CD–NFW. Nous avons constaté que l'agent NFW forme un complexe d'inclusion 1:1 avec la β -CD, dont la constante d'association est de $2\,540 \pm 380 \text{ M}^{-1}$. L'état fluorescent de l'agent NFW inclus dans la cavité de la β -CD est protégé par celle-ci des divers extincteurs de fluorescence (neutres, anioniques et cationiques) à base d'eau. L'extinction par les extincteurs que nous avons évalués se produit dans l'eau près de la limite imposée par la diffusion, à l'exception du MV dicationique. Le MV est un extincteur extrêmement efficace de la fluorescence de l'agent NFW, la valeur nominale K_{SV} dans l'eau uniquement étant d'environ $5,0 \times 10^3 \text{ M}^{-1}$, qui correspond à une valeur nominale k_q d'environ $4 \times 10^{12} \text{ M}^{-1} \text{ s}^{-1}$, ce qui est au-delà de la limite imposée par la diffusion dans ce solvant. L'efficacité d'extinction du MV est fortement réprimée en présence de 10 mM de β -CD ($K_{SV} = 105 \pm 12 \text{ M}^{-1}$) et en présence de NaCl ($K_{SV} = 106 \pm 9 \text{ M}^{-1}$ à 0,5 M de sel). En l'absence de CD ou de sel, on observe une forte contribution de l'extinction statique dans le système MV; la présence de ces additifs empêchant l'extinction statique. Ces résultats laissent supposer que l'extinction statique est due à la formation d'un complexe à l'état fondamental entre le dianion NFW et le dication MV. [Traduit par la Rédaction]

Mots-clés : cyclodextrine, agent de blanchiment fluorescent, distyrylbiphényle, extinction de fluorescence, méthylviologène, complexe à l'état fondamental.

1. Introduction

Fluorescent whitening agents (FWAs)^{1–6} — also known as optical brighteners — are organic compounds that convert a portion of the ultraviolet spectral region into visible blue light. FWAs have applications in different industries such as textiles, papers, detergents, plastics, and coatings. Optical brighteners fluoresce under exposure to an UV lamp, and they are often used as tracers, e.g., monitoring of plastic laminates for uniformity and flaws.

The major commercial optical brighteners are based on three chemical structural frameworks: stilbene, coumarin, and pyrazoline.

In this study, the primary molecule of interest is the fluorescent whitening agent 4,4'-bis(2-sulfoxyethyl)-biphenyl disodium salt (NFW). NFW (Scheme 1) is a member of the substituted distyrylbiphenyl (DSBP) family of FWAs, which are highly fluorescent in the *trans* isomeric form.⁷ As derivatives of stilbene, DSBPs are subject to photo-induced *trans*–*cis* isomerization, which causes an overall decrease in fluorescent intensity.^{5,7} DSBPs such as

Received 17 June 2020. Accepted 8 January 2021.

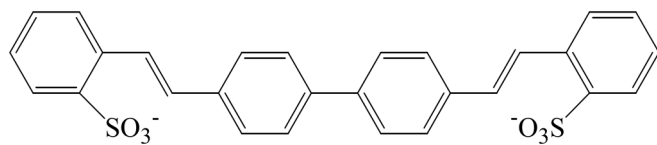
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Scheme 1. Structure of the fluorescent whitening agent 4,4'-bis(2-sulfoethyl)-biphenyl disodium salt.



NFW are also subject to photodegradation, which is both an industrial and an environmental concern.^{8–11}

It is well established that complexation of an organic guest by a cyclodextrin host such as β -cyclodextrin (β -CD) can be used to control both thermochemical¹² and photochemical^{13–15} reactivity. It is also well known that complexation by cyclodextrins (CDs) isolates the guest from reactive species such as excited state quenchers (e.g., O_2), present in the aqueous bulk medium.^{15,16} Therefore, complexation of stilbene-like chromophores by host molecules such as CDs is expected to reduce the extent of photoisomerization¹⁷ and limit photodegradation.

Cyclodextrins^{6,7} have received a great deal of attention for their ability to form host–guest complexes¹⁸ and for their potential use as building blocks for supramolecular structures.^{19,20} CDs are torus-shaped cyclic oligosaccharides containing 6–12 glucose units joined together by α -1,4 glycosidic linkages. The most common and well-studied CDs are α -CD, β -CD, and γ -CD, which consist of six, seven, and eight glucopyranose units, respectively.

Our long-term goal is to evaluate CDs as a possible tool to improve the fluorescent performance of NFW and related compounds, as well as to limit their photodegradation. The first step towards this goal is to establish whether there is a significant association between NFW and CD. In this article, we report a study of the interaction of β -CD with the DSBP type FWA molecule, Uvitex (NFW). We have used fluorescence spectroscopy to evaluate the association of NFW with β -CD in water, as well as fluorescence quenching of NFW by neutral and charged quenchers, to further explore the interaction between the fluorophore and the β -CD cavity. In the course of the quenching experiments, we discovered that one of the quenchers, methyl viologen (MV), behaved in an unexpected fashion. An important aim of this article is, therefore, to explore this unexpected quenching phenomenon. The ability of β -CD to improve NFW performance will be the subject of a subsequent report from our laboratory.

2. Materials and methods

2.1. Materials

β -Cyclodextrin hydrate (99.5%), NaI (99.9%), CsBr, succinamide (98%), MV, and 99.8% pure A.C.S. spectrophotometric grade dimethylformamide (DMF) were all Aldrich products. Sodium chloride and potassium nitrate were AnalR grade from BDH. The fluorescent whitening agent NFW (provided as a solution of NFW in 25% DMSO in water) was kindly donated by Ciba Geigy, Canada. A.C.S. grade dimethylsulphoxide (DMSO) was from Fisher Scientific. All these substances were used as received. Distilled water was deionized by a Milli-Q ion-exchange filtration system (academic model V2.04, Millipore).

2.2. Sample preparation

2.2.1. NFW stocks

Sample stock solutions of NFW were prepared by dissolving the undiluted NFW stock in water to yield a 200 μ M NFW concentration. This stock was then stirred overnight and diluted with water to produce aqueous NFW stocks of 0.2 μ M and 0.1 μ M, respectively. Next, 10 mM β -CD stock solutions were prepared by weighing an appropriate mass of β -CD into volumetric flasks and diluting them with a suitable NFW stock. These were subse-

Table 1. Molar absorptivity and λ_{\max} values for NFW in various solvents.

Solvent	λ_{\max} (nm)	$\epsilon \times 10^{-3}$ ($M^{-1} cm^{-1}$)
Acetonitrile	342	187 ± 1
Methanol	348	91.4 ± 0.2
10 mM β -CD in water	348	116 ± 1
Water	349	84.5 ± 0.2
Water–DMF 1:1	352	87.0 ± 0.2
DMSO	356	98.4 ± 0.2

quently diluted with the appropriate NFW stock to obtain solutions with a constant NFW concentration but the desired variable concentration of β -CD.

2.2.2. Quencher stocks

Appropriate stock solutions of the quenchers (NaI, NaCl, CsBr, EDTA, succinamide, MV, and KNO_3) in the 0–1 M range were prepared just before use. Microlitre aliquots of these stock quenchers were added via μ L syringe (Hamilton) to the measurement cuvette (standard 1×1 cm² quartz, Hellma) containing the sample solution under continuous stirring conditions. The total cuvette volume was typically 3 mL. Exposure to light was kept to a minimum during all sample preparation and handling.

2.3. Instrumental measurements

Steady-state fluorescence measurements were carried out at a fixed temperature (normally 25 °C, but also at an elevated temperature in some cases; VWR–Polyscience Circulating water bath) with a Perkin Elmer LS50B luminescence spectrometer. Data were recorded with excitation at 350 nm, 5 nm (excitation slit), and 2.5 nm (emission slit) with a scan rate of 100 nm/min. Spectra were scanned between 360 and 600 nm. Absorption spectra were recorded with a Perkin Elmer Lambda 40 UV–vis spectrophotometer at room temperature.

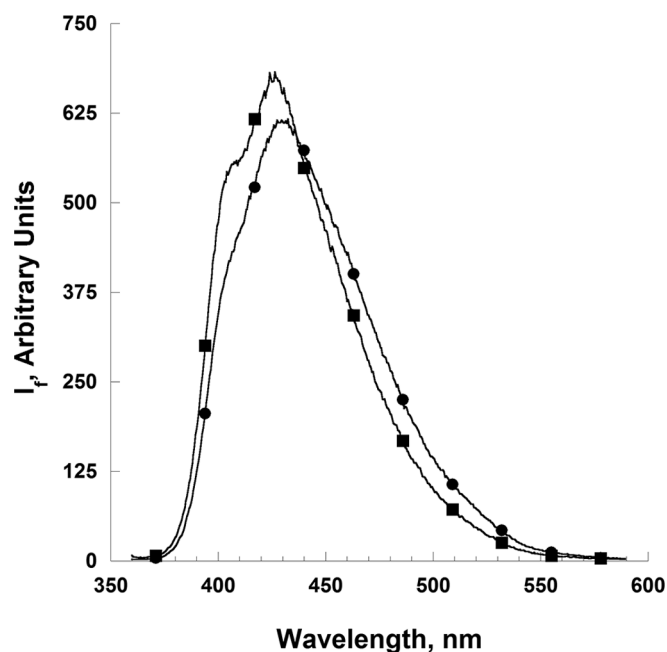
3. Results and discussion

3.1. Spectral properties of NFW in various solvents

The absorption spectrum of 0.2 μ M NFW was measured in a variety of solvents, including water and water containing 10 mM β -CD. In each case, the spectrum consists of one rather broad, featureless peak centered near 350 nm (Table 1). The shape of the spectrum does not change for the systems we have studied. The molar absorptivity (ϵ) and λ_{\max} values of NFW were determined in water, acetonitrile, 1:1 water–DMF (v/v), DMSO, and methanol, as well as in water containing 10 mM β -CD. The ϵ values are consistently large and range from $8.5 \times 10^4 M^{-1} cm^{-1}$ (water) to $18 \times 10^4 M^{-1} cm^{-1}$ (acetonitrile) (Table 1). The λ_{\max} value of NFW is not strongly dependent on solvent polarity. The high absorptivity and the weak dependence of transition energy on solvent are characteristic of a strongly allowed π – π^* transition. This is consistent with the nature of NFW as a substituted stilbene.

Figure 1 shows fluorescence spectra for 0.2 μ M NFW recorded in water and in 10 mM aqueous β -CD. The wavelength of maximum fluorescence emission was found to be near 425 nm and to be only slightly influenced by the fluorophore's environment. There is a modest intensity enhancement, a slight narrowing of the fluorescence band, and a slightly better defined vibronic structure in the presence of β -CD. These latter two results are consistent with the observations made by Smit and Ghiggino²¹ in their study of NFW in water and ethanol; the less polar solvent narrowed the fluorescence band and enhanced vibronic structure. Our observations support an interaction between β -CD and NFW (vide infra). Smit and Ghiggino also reported fluorescence quantum yields for NFW of 0.82 in water and 0.95 in ethanol, respectively.²¹

Fig. 1. Fluorescence spectra of 0.2 μM NFW recorded in water (circles) and 10 mM aqueous β -cyclodextrin (squares) at room temperature. Excitation at 350 nm.



3.2. NFW complexation by β -CD

To obtain insight into the complexation between NFW and β -CD cavities, a series of CD spectroscopic titration experiments were performed using both fluorescence and absorbance data. In each case, changes in spectroscopic characteristics were observed at a constant NFW concentration with changing β -CD concentrations. The changes in the absorbance values were too small to provide a reliable measure of complexation between NFW and β -CD.

The variation of NFW fluorescence intensity in aqueous solution is sensitive to added β -CD as shown in Fig. 2. The observed increase in intensity, while modest, is consistent with the formation of an inclusion complex between NFW and β -CD. When the integrated fluorescence intensity values are plotted as a function of $[\beta\text{-CD}]$, a typical binding isotherm^{22,23} is observed with essentially all the curvature taking place over a narrow range of β -CD concentration, suggesting a relatively strong association.

The most common form of the CD-guest complex is the so-called 1:1 complex, in which one CD molecule hosts one guest molecule within its cavity.^{24,25} Assuming a 1:1 β -CD-NFW complex, represented here as $\beta\text{-CD:NFW}$, complexation will be controlled by the equilibrium process:



The equilibrium constant for this process, K_1 , is defined as follows:

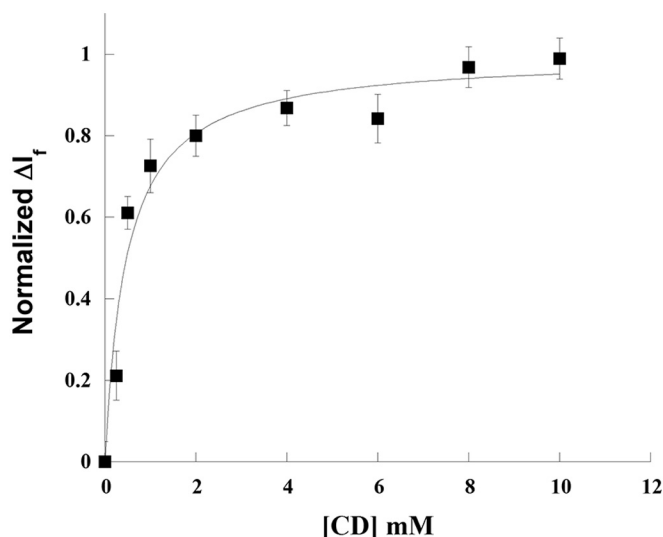
$$(2) \quad K_1 = \frac{[\beta\text{-CD:NFW}]}{[\text{NFW}][\beta\text{-CD}]_0}$$

A value for K_1 can be obtained from non-linear regression analysis of the binding isotherm. The binding isotherm is defined as^{26–28}

$$(3) \quad \Delta I = K_1 \left(\frac{\Delta i[\text{NFW}]_0 [\beta\text{-CD}]_0}{1 + K_1 [\beta\text{-CD}]_0} \right)$$

Here, ΔI refers to the difference between the fluorescence intensity at total cyclodextrin concentration, $[\beta\text{-CD}]_0$, and that

Fig. 2. NFW + β -cyclodextrin binding isotherm in water. The solid line corresponds to a fit to a 1:1 binding model (eq. 3). $[\text{NFW}] = 0.2 \mu\text{M}$.



observed in the absence of β -CD, $[\text{NFW}]_0$ is the total NFW concentration, and Δi reflects the maximum value of ΔI . Note that $[\beta\text{-CD}]_0$, rather than $[\beta\text{-CD}]$, is used in these equations as it is always true in our systems that $[\beta\text{-CD}] \gg [\text{NFW}]$, so that under all conditions, $[\beta\text{-CD}]$ is essentially equal to $[\beta\text{-CD}]_0$. From these calculations, binding constant values at different concentrations of NFW (0.1 and 0.2 μM) were derived. In each case, the fit of the data to the 1:1 model is good, and the overall binding is rather strong. The value of K_1 was found to be independent of NFW concentration for tests done at 0.1 and 0.2 μM NFW, a fact that is consistent with the formation of a 1:1 complex. At higher $[\text{NFW}]$ (i.e., 100 μM), significant precipitation was observed from this system, so it was not possible to determine K_1 under these conditions. At the lower values of $[\text{NFW}]$, there is no precipitation and the average K_1 value determined (four replicates) was $2540 \pm 380 \text{ M}^{-1}$.

3.3. Quenching study and the influence of ionic strength

It is known that host-guest binding can be either increased or decreased by salts, depending on the nature of the host (charged vs. uncharged CD), the guest (charged or uncharged, size compared with CD cavity), and the salt (salting in or salting out species, tendency to bind to the CD cavity).^{29–35} Inorganic salts such as NaI may also quench the fluorescence of fluorophores such as, for example, naphthalene and 2-naphthol. Thus, these quenchers are convenient tools to probe the relative importance of various complex species that may form in the presence of β -CD in an aqueous medium.^{27,28,36,37} We carried out a quenching study on NFW both in the presence and in the absence of 10 mM β -CD. The NFW concentration was approximately 0.2 μM and the quencher concentration ranged up to 50 mM in most cases. We used NaI, KNO_3 , NaCl, CsBr, EDTA, succinamide, and MV as salts or quenchers.

The quenchers employed quenched NFW fluorescence in water to a greater or lesser extent. In most cases, Stern-Volmer plots (eq. 4) were linear at all quencher concentrations up to ~ 50 mM. However, this was not the case with MV (vide infra) in the absence of β -CD. The Stern-Volmer slope (K_{SV}) values, tabulated in Table 2, were determined at quencher concentrations not exceeding 50 mM. Under these conditions, the Stern-Volmer plots were all linear, again with the exception of MV in the absence of β -CD. NaCl and KNO_3 had no influence on the observed fluorescence intensity of NFW at concentrations as high as 1 M.

Table 2. Quenching of NFW fluorescence by various substances in water at 25 °C.

Quencher ^a	[CD]	K_{SV} (M ⁻¹) ^b	k_q (M ⁻¹ s ⁻¹) ^c
NaI	0	8.7 ± 0.7	7.4 × 10 ⁹
NaI	10 mM β-CD	6.8 ± 0.6	5.7 × 10 ⁹
CsBr	0	2.4 ± 0.3	2.0 × 10 ⁹
CsBr	10 mM β-CD	2.5 ± 0.1	2.1 × 10 ⁹
EDTA	0	20.2 ± 1.1	17 × 10 ⁹
EDTA	10 mM β-CD	14.0 ± 1.2	12 × 10 ⁹
Succinimide	0	11.6 ± 1.3	9.8 × 10 ⁹
Succinimide	10 mM β-CD	11.8 ± 1.3	10 × 10 ⁹
Methyl viologen	0	~5.0 × 10 ^{3d}	~4.2 × 10 ³
Methyl viologen	10 mM β-CD	105 ± 12	89 × 10 ⁹
Methyl viologen	0 + 0.5 M NaCl	106 ± 9	90 × 10 ⁹

^aThe maximum quencher concentration was 50 mM in each case except methyl viologen where it was 6 mM. [NFW] = 0.2 μM in each case.

^bPlots were linear ($r^2 > 0.98$) over the entire concentration range with the exception of methyl viologen (see footnote d). NaCl and KNO₃ had no effect on the observed value of I_F up to 1 M salt.

^cAssuming purely dynamic quenching, $k_q = K_{SV}/\tau$, where τ is the unquenched NFW fluorescence lifetime. $\tau = 1.18$ ns in water.²¹ Note that based on the experimental error in K_{SV} , the k_q values calculated from them are expected to have uncertainties of at least 10%.

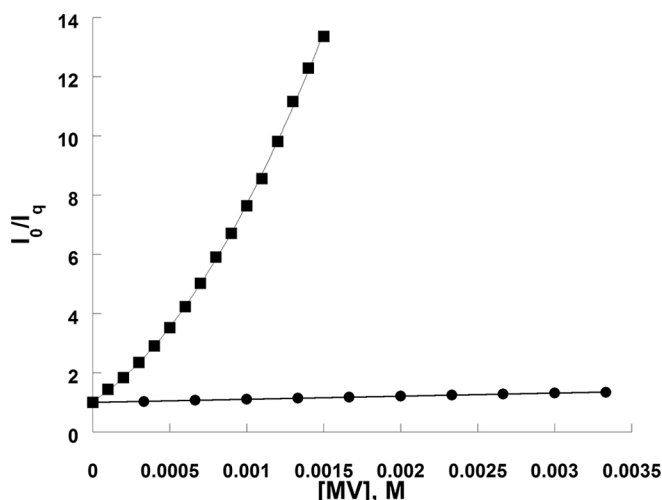
^dNominal value based on the low [methyl viologen] region of the Stern–Volmer plot. See text for further explanation.

$$(4) \quad \frac{I_0}{I_q} = 1 + K_{SV}[Q]$$

where I_0 is the observed fluorescence intensity of NFW in the absence of quencher and I_q is the observed fluorescence intensity of NFW in the presence of quencher at concentration $[Q]$.

For dynamic quenching, the value of the Stern–Volmer slope, K_{SV} , is the product of the bimolecular quenching rate constant, k_q , and the unquenched lifetime, τ , of the fluorescent state. The only reported lifetime value for NFW in water is 1.18 ns.²¹ Using this value in conjunction with the measured K_{SV} values, estimates of k_q can be obtained. These are also presented in Table 2. In each case, with the exception of MV and the non-quenching salts NaCl and KNO₃, the value of k_q is consistent with a dynamic quenching process occurring close to the diffusion-controlled limit. We note that the k_q values obtained for EDTA and succinimide are both slightly larger than one would anticipate. By contrast, the value calculated for MV as quencher dramatically exceeds the diffusion-controlled limit in water.

In most cases, the quenching behaviour is unremarkable. Quenching proceeds at or near the diffusion-controlled limit and β-CD provides a modest protective effect. However, MV behaves very differently. Of the quenchers examined, MV was by far the most potent. This is not surprising in that MV, an excellent electron acceptor, is a di-cationic quencher and NFW is a di-anionic fluorophore. Figure 3 shows Stern–Volmer plots for the MV quenching of NFW with and without 10 mM β-CD present over a range of quencher concentrations. In the absence of β-CD, the Stern–Volmer plot shows the strong upward curvature usually associated with a contribution from static quenching. When β-CD is present, the plot is linear and the quenching response is weaker by at least a factor of 10. If the first few points from the measurements under β-CD free conditions are plotted, a roughly linear fit is obtained, yielding a nominal K_{SV} of about $\sim 5.0 \times 10^3$ M⁻¹. We report this nominal K_{SV} value to only two significant figures due to the extensive curvature observed in the quenching plot in water alone. Combining this nominal value with the published lifetime of 1.18 ns for the unquenched NFW excited state yields a k_q value on the order of 4×10^{12} M⁻¹ s⁻¹; a value that exceeds the diffusion-controlled limit in

Fig. 3. Stern–Volmer plots for methyl viologen quenching of 0.2 μM NFW fluorescence in the absence (squares) and presence (circles) of 10 mM β-cyclodextrin in water at 25 °C. Ionic strength is not held constant.

water by several orders of magnitude. When β-CD is present, the estimated k_q value is near the diffusion-controlled limit and there is no evidence of static quenching.

The literature reports that CD cavities are effective in protecting fluorophores from the quenching effect of MV in water, acting, in essence, to “insulate” the chromophore from the water-based quencher.³⁸ For example, β-CD threading on poly(4,4'-diphenylenevinylene) rotaxanes reduces the observed Stern–Volmer constant for MV quenching by a factor of nearly 1800 compared with the unthreaded poly-chromophore.³⁸ In the current context of MV as a quencher in an aqueous β-CD system, we should point out that this CD does not form complexes with the fully oxidized (dicationic) form of MV in water. This has been established by several groups over the years using techniques as varied as cyclic voltammetry and ¹H NMR.^{39–41} Therefore, in our system, MV acts as an exclusively water-based quencher in much the same way the smaller ionic quenchers do.

Static quenching is normally observed because of (i) formation of a ground-state complex between the quencher and fluorophore or (ii) the implication of a “sphere of action”, which suggests the fluorophore will be quenched any time there are one or more quencher molecules within an active volume surrounding the excited state. The latter situation tends to be important only at high quencher concentrations.

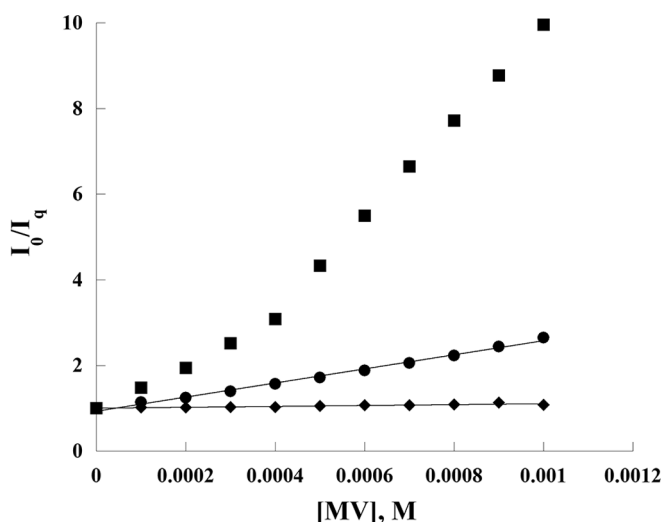
3.4. Formation of ground-state complex

We propose that the strong component of static quenching observed in the present system in the absence of β-CD is due to formation of a ground-state complex between MV and NFW that arises due to the electrostatic attraction between the two species. There are several pieces of evidence to support this proposal.

3.4.1. Changes in absorption spectrum

When increasing concentrations of MV (0 to 6 mM) are added to a 0.2 μM aqueous solution of NFW in water, a minor red shift (349 to 355 nm) is observed in the position of the NFW λ_{max} value. In a second experiment, using a split absorption cell, the absorption spectra of NFW and MV were measured unmixed (i.e., the two components in their separate cell chambers) and mixed (i.e., the two components combined). With the split cell experiment, any observed difference between the unmixed and mixed measurements suggests an interaction in the ground state. We observed a

Fig. 4. Effect of ionic strength on NFW quenching by methyl viologen in water at 25 °C. Stern–Volmer plots of methyl viologen quenching of 0.2 mM NFW fluorescence in the absence (squares) and presence (50 mM, circles; 500 mM, diamonds) of NaCl. Ionic strength is not held constant.



small but significant difference of $\Delta A \sim 0.012$ (A, sample absorbance) between the unmixed and mixed situations, as measured at 260 nm.

3.4.2. Observation of a coloured precipitate

At elevated concentrations of NFW (i.e., $\sim 100 \mu\text{M}$) in water, an orange coloured precipitate was observed upon addition of MV. We did not characterize this substance, but it is consistent with an interaction between the ground states of NFW and MV.

3.4.3. Effect of salt concentration on quenching behaviour

A non-covalent ground-state complex between NFW and MV would be expected to be held together by electrostatic attraction. Adding a large excess of a non-quenching salt such as NaCl would lower the attractive forces between the complexation partners. This would lead to less effective quenching of the NFW fluorescence by MV. Figure 4 shows the effect of increasing NaCl concentration on the quenching behaviour. The slope of the curve with 0.5 M NaCl present is $106 \pm 9 \text{ M}^{-1}$ (Table 2). The elevated salt content strongly suppresses the quenching and eliminates the upward curvature observed in the absence of salt.

3.4.4. Effect of increased temperature on quenching behaviour

Stern–Volmer plots for NFW quenching by MV were determined at 25, 45, and 70 °C in the absence of salt and the absence and presence of 10 mM β -CD. In addition, Stern–Volmer plots were obtained at 25 and 50 °C in the presence of 50 mM NaCl (without β -CD). In the absence of β -CD, quenching was suppressed at higher temperatures. For example, at 25 °C without salt or β -CD, the nominal slope (i.e., the initial part of the curve) was $\sim 4.2 \times 10^3 \text{ M}^{-1}$, whereas at 70 °C, it was reduced to $\sim 1.4 \times 10^3 \text{ M}^{-1}$. In the presence of 50 mM NaCl, the slopes were $1.4 \times 10^3 \text{ M}^{-1}$ and $8.9 \times 10^2 \text{ M}^{-1}$, respectively. This is the trend one would expect if a ground-state complex formed via an exergonic process such as electrostatic attraction.

In the presence of 10 mM β -CD, without salt, the Stern–Volmer slope was not appreciably affected by changes in the temperature range of 25–70 °C. This suggests β -CD is effective at suppressing the formation of the ground-state complex responsible for the static quenching effect. This could presumably be a result of β -CD

Table 3. Impact of ionic strength and presence of β -CD on the quenching of NFW by methyl viologen in water at 25 °C.

[NaCl] or total ionic strength (M) ^a	[β -CD] (mM)	$K_{SV} (\text{M}^{-1})$	$k_q \times 10^9 (\text{M}^{-1} \text{s}^{-1})^c$
7×10^{-3}	0	$\sim 5.0 \times 10^{3b}$	$\sim 4.2 \times 10^3$
7×10^{-3}	10	105 ± 12	89
0.1	0	$(1.97 \pm 0.18) \times 10^3$	1.67×10^3
0.1	10	124 ± 14	106
0.5	0	106 ± 9	90
0.5	10	80 ± 6	67

^a $7 \times 10^{-3} \text{ M}$ represents the highest ionic strength value achieved corresponding to 0.2 μM NFW and 3.5 mM methyl viologen. Otherwise, total ionic strength was kept constant at 0.1 or 0.5 M as indicated.

^bPlot shows upward curvature. The reported K_{SV} is an estimate based on the initial slope of the curve.

^cAssuming $\tau = 1.18 \text{ ns}$ for the unquenched NFW fluorescent state.²¹ Note that based on the experimental error in K_{SV} , the k_q values calculated from them are expected to have uncertainties of at least 10%.

interfering with the ionic interaction between NFW and MV and (or) sequestering the fluorophore.

Finally, contributions from static quenching have been reported for MV quenching of other anionic fluorophores.^{42–44} In these reports, the static quenching was also attributed to the formation of ground-state complexes between the quenching partners.

As mentioned above, complexation of charged guests with CD can be influenced by ionic strength. It is also clear from the data presented here that ionic strength alters the quenching behaviour. It is likely that this reflects changes in the ground state interaction between MV and NFW as the ionic strength increases. To control for these effects, a series of quenching experiments in the absence and presence of 10 mM β -CD were performed at elevated but constant ionic strength. The results are summarized in Table 3. In each case, the Stern–Volmer plots were linear with smaller slopes than observed at low ionic strength, and the presence of β -CD reduced the slopes further. Assuming that the lifetime of NFW is independent of the conditions used, we can estimate the k_q values. These are also presented in Table 3. In all cases without β -CD, the resulting k_q value exceeds the diffusion-controlled limit, suggesting that even at relatively high ionic strength, there is still association between the ground-state NFW and MV. To test this hypothesis, we used a modified Stern–Volmer equation to treat our data assuming a mixed static–dynamic quenching scenario, where separate values of the static (K_s) and dynamic (K_d) quenching parameters can be determined:

$$(5) \quad \frac{I_0}{I_q} = 1 + (K_s + K_d)[Q] + K_s K_d [Q]^2$$

This is reminiscent of the approach taken by Schmehl and Whitten.⁴² In their study, they identified the parameter K_s as the product of the ion-pairing equilibrium constant with the ratio of absorption coefficients for a ground-state ion-pair complex and the free chromophore (anionic porphyrins in their study) on the assumption that quenching within the ground-state complex is essentially instantaneous. Our quenching data were fit to eq. 5 using non-linear regression. This analysis was performed for the NFW–MV system at a series of [NaCl] ranging from 0 to 0.5 M (Table 4) without β -CD. The assumption for the model is that a shared contribution of static and dynamic quenching is the sole reason for the curvature. The value of K_s dropped from a high of $3.50 \times 10^3 \text{ M}^{-1}$ in the absence of salt to a constant value of approximately 260 M^{-1} between 0.2 M and 0.5 M NaCl. At low [NaCl], the K_d value fluctuates significantly around a value of approximately 50 M^{-1} . We note that this relatively high uncertainty in the value

Table 4. K_s and K_d values extracted from the fit of the quenching data to eq. 5 for the NFW, MV, and β -CD system.

[NaCl] (M)	K_d (M^{-1})	K_s (M^{-1})
0.0	23	3.50×10^3
0.02	82	1.60×10^3
0.04	32	11.1×10^3
0.06	59	897
0.2	282	282
0.4	245	282
0.5	250	249

Note: [NFW] = 0.2 μ M, [MV] ranges from 0 to 5 mM.

of K_d is not surprising, as most of the quenching observed in the low [NaCl] regime will be due to static quenching.

To test the reliability of the fitting, we selected a K_d value close to what would be expected for a purely diffusion-controlled process and refit using this as a fixed K_d value. The value we used was $K_d = 10 M^{-1}$, which is based on a value of 1.18 ns for the fluorescence lifetime and a value of $k_{diff} \sim 8 \times 10^9 M^{-1} s^{-1}$ in water. The values and trend in K_s remained reproducible even when we forced the fit to this fixed value of K_d . In fact, the K_s values obtained were rather insensitive to the value of K_d when even substantially different values of K_d used to achieve the fit. This seems reasonable as these parameters reflect two quenching processes that are largely independent of one another apart from the amount of MV available for each process.

This behaviour supports the existence of two quenching regimes: a static effect and a dynamic effect. The static effect dominates at lower ionic strengths but becomes less important as the concentration of NaCl is raised. This is exactly the trend one would expect if the static quenching is facilitated by a ground-state ion-pair association between a cationic quencher and an anionic fluorophore. We conclude that the static value, K_s , is an apparent equilibrium constant for the association that results in static quenching (most likely a ground-state electrostatic complex). The behaviour and meaning of K_d are less clear. If the K_d values at low (<0.2 M) NaCl are combined with the fluorescence lifetime of 1.18 ns, we obtain k_q values that range from $19 \times 10^9 M^{-1} s^{-1}$ to $70 \times 10^9 M^{-1} s^{-1}$, which are, again, on the high side if K_d is truly a simple dynamic parameter.

There are several possible reasons why the k_q value obtained this way is larger than expected. These include the following: (i) the reported τ value is not invariant across conditions as we have assumed here; (ii) an electron transfer is taking place in the NFW–MV system; (iii) even at high [NaCl], there is still an important contribution of static quenching not captured by the simple model employed here (eq. 5).

The first possibility is unlikely, as Smit and Ghiggino²¹ have reported that the lifetime in ethanol is 0.92 ns, very close to the water value of 1.18 ns. Although the second option is possible in light of MV's well-known behaviour as an electron acceptor,⁴⁵ the MV radical cation resulting from a full electron transfer results in a very distinctive absorption spectrum and a strong blue colour in solution.⁴⁶ We observed neither of these in our study.

We therefore favour the third explanation. Based on the model calculation, K_s is significantly greater than zero even at 0.5 M NaCl, implying that both dynamic and static quenching are contributing to the observed quenching behaviour. At higher ionic strengths, specific ion pairing between sodium cations and NFW and (or) chloride anions and MV may become significant enough to perturb the values of K_s and K_d derived from the quenching model of eq. 5, which considers only MV as quencher Q and only NFW as the fluorophore. In this case, the assumption that the

value we are calling K_d is simply equal to $k_q\tau$ will be inaccurate under conditions of high ionic strength.

When 10 mM β -CD is present, the MV quenching rate constant becomes diffusion controlled and is comparable across conditions. This suggests that complexation of NFW by β -CD becomes the dominant factor in controlling quenching when the host molecule is present. The fact that the MV k_q values in the presence of β -CD are all comparable (same order of magnitude) with or without NaCl suggests that changing ionic strength does not have a large impact on the association of NFW with this particular host. It is interesting to note that the value of K_1 (the 1:1 complexation constant for NFW with β -CD) is comparable in size with the value of K_s observed in the absence of salt. That is, β -CD complexation of NFW is competitive with formation of the NFW ground-state complex with MV under these conditions.

4. Conclusion

The commercial fluorescent whitening agent NFW forms rather stable 1:1 complexes with β -CD in water. NFW fluorescence is quenched at or near the diffusion-controlled limit by several neutral and anionic quenchers. Quenching of NFW by the di-cationic electron acceptor MV is very different. This highly efficient process is a combination of static and dynamic contributions, and there is evidence for formation of a ground-state complex between NFW and MV, the formation of which can be controlled by ionic strength and the presence of β -CD.

Acknowledgements

The authors thank Ryerson University and the Natural Sciences and Engineering Research Council of Canada (NSERC) for their financial support. JvS thanks Karlstad University for a travel grant. We also thank Ciba-Geigy Canada for their generous gift of Uvitex NFW liquid solution.

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