Interaction of 1,8-acridinedione dye with urea dimer in methanol

Chellappan Selvaraju a, Viruthachalam Thiagarajan a, Perumal Ramamurthy a,b,*

a Department of Inorganic Chemistry, University of Madras, Guindy campus, Chennai 600 025, India
b National Centre for Ultrafast Processes, University of Madras, Taramani Campus, Chennai 600 113, India

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Abstract

The hydrogen-bonding interaction between acridinedione dye (ADD) and urea dimer in methanol is reported by using steady state and time resolved fluorescence techniques.

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1. Introduction

The behaviour of urea in solution is a very important topic in biological science because of its involvement as a waste product. Due to the complicated hydrogen bonding and the acid–base properties associated with both the carbonyl group and amines in the urea molecule, it has distinct chemical and physical properties in solutions such as demicellization [1], denaturation of proteins [2], dissolution and interaction of hydrophobic species in aqueous solution [3], etc. Various models such as hydrogen bond breaking [4], replacement of solvent molecule from the solvation layer [5], interaction with the hydrophobic moieties of the molecules and association of urea molecules [6] have been proposed to explain the anomalous behaviour of urea in solution.

1,8-Acridinedione (ADD) dye has biological importance due to its structural similarity with coenzyme NADH [7–9]. These dyes form inclusion complexes with cyclodextrin [10,11]. In this Letter, we report the interaction of urea molecules with the following ADD dye in methanol by using the steady state and time resolved fluorescence techniques.

2. Experimental

The ADD dye was prepared by following the procedure reported in the literature [8]. Urea (molecular biology grade) was obtained from SRL and used as received. Methanol used in this
investigation was of HPLC grade purchased from Qualigens India Ltd. Absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. Fluorescence spectra were recorded using a Perkin–Elmer LS5B luminescence spectrometer.

Fluorescence decays were recorded using TCSPC method using the following setup. A diode pumped millena CW laser (Spectra Physics) 532 nm was used to pump the Ti:sapphire rod in Tsunami picosecond mode locked laser system (Spectra Physics). The 750 nm (80 MHz) was taken from the Ti:sapphire laser and passed through pulse picker (Spectra Physics, 3980 2S) to generate 4 MHz pulses. The second harmonic output (375 nm) was generated by a flexible harmonic generator (Spectra Physics, GWU 23PS). The vertically polarized 375 nm laser was used to excite sample. The fluorescence emission at magic angle (54.7°) was dispersed in a monochromator (f/3 aperture), counted by a MCP PMT (Hamamatsu R 3809) and processed through CFD, TAC and MCA. The instrument response function for this system is ~52 ps, the fluorescence decay was analyzed by using the software provided by IBH (DAS-6) and PTI global analysis software.

3. Results and discussion

The absorption spectrum of ADD dye was recorded with and without urea and the dye shows absorption maximum at 382 nm. No change is observed in the shape and position of ADD absorption band on addition of urea. ADD dye has emission maximum at 437 nm on excitation at 382 nm. The fluorescence intensity of ADD dye increases with increase in urea concentration. The fluorescence emission spectra of ADD at different urea concentration are shown in Fig. 1.

The fluorescence enhancement of ADD may be due to the one of the following reason: (i) prevention of aggregation of ADD dye by urea or (ii) interaction of ADD dye with urea molecules. With increasing concentration of ADD, there is no new peak in the absorption and emission spectra indicate the absence of aggregation of this dye and which rules out the first possibility. The fluorescence enhancement of ADD in the presence of urea is assigned to the direct interaction between urea and ADD molecule. The fluorescence behaviour of ADD–urea system could be analyzed using the Benesi–Hildebrand equations [12,13] for 1:1(1) and 1:2(2) complex:

Fig. 1. Fluorescence spectra of ADD at different concentration of urea in methanol: (1) 0 M, (2) 0.20 M, (3) 0.40 M, (4) 0.60 M, (5) 0.81 M, (6) 1.00 M, (7) 1.20 M, (8) 1.40 M and (9) 1.60 M urea. Inset shows the Benesi–Hildebrand plot for the 1:1 inclusion complex between ADD and urea dimer.
where $K_1$, $I_0$, $I$ and $I'$ are the equilibrium constant, the observed fluorescence intensity in the absence of urea, the observed fluorescence intensity in the presence of urea and the fluorescence intensity of the ADD–urea complex, respectively. The plots of $1/I_0 - I$ vs $[U]^{-1}$ and $[U]^{-2}$ are not linear, which indicates the absence of 1:1 and 1:2 ADD–urea interaction.

Solid urea inclusion complexes are known [14–16] in the literature, in which urea molecules form a hydrogen bonded host structure that contains linear and parallel tunnels. The tunnel structure of urea is stable only in the presence of guest molecules. Hamilton and Stockes [17] have reported the formation of urea dimers and other higher order aggregates in methanol. Than Htun et al. [18] studied the excited state proton transfer from 4-hydroxy-1-naphthalenesulphonate to urea in methanol and reported the involvement of urea dimer in proton transfer reactions. The following equilibrium is expected to occur between urea monomer and urea dimer:

$$2U \xrightleftharpoons[K]{} U_2,$$

where $U$ and $U_2$ are urea monomer and urea dimer, respectively. The equilibrium constant for the formation of urea dimer was obtained [18] from the apparent molar volume data of urea in methanol and the reported $K$ value is 0.0238 M$^{-1}$. From the equilibrium constant and the total urea concentration, urea dimer concentration can be calculated.

Benesi–Hildebrand plot can be used to elucidate the stoichiometric relationships between host (urea dimer) and guest molecules as well as the strength of the arrangement of association. Consider the 1:1 complex between ADD and urea dimer

$$\text{ADD} + U_2 \xrightleftharpoons[K_0]{} \text{[ADD–U}_2\text{]},$$

where ADD–U$_2$ is the association complex between ADD and urea dimer. The fluorescence enhancement of the ADD–U$_2$ system could be analyzed by the Benesi–Hildebrand equation (1) for 1:1 complex formation. The dependence of $(1/I_0 - I)$ on the reciprocal concentration of urea dimer is found to be linear, indicating the 1:1 complex formation between ADD and urea dimer. The equilibrium constant ($K_1$) is determined from the slope and intercept of this plot and the value of $K_1$ is around 75 M$^{-1}$.

The complexation between urea dimer and ADD has also been investigated by the time resolved fluorescence technique. The fluorescence decay of ADD at different concentration of urea in methanol is shown in Fig. 2. The fluorescence decay of ADD is single exponential with lifetime of 666 ± 15 ps in neat methanol, whereas in the presence of urea, the fluorescence decay of ADD is biexponential. In presence of urea, the fluorescence decays were first analyzed by unlinked free biexponential analysis and the two lifetimes obtained are found to be in the range of 450–650 and 800–1000 ps. To get further insight, the fluorescence decay of ADD in presence of urea was analyzed by global analysis, which gives two lifetimes $\tau_1 = 675$ and $\tau_2 = 995$ ps. The short lifetime ($\tau_1$), which is similar to the lifetime of ADD in methanol, is assigned to the unbound ADD and the longer one ($\tau_2$) is attributed to the interaction of ADD with urea dimer. The lifetime ($\tau$), relative amplitudes ($A$) and $\chi_2$ of the various decay analysis of the ADD–urea system are listed in Table 1. The single, bi and global biexponential decay analyses of ADD in 0.65 M urea are shown in Fig. 3.

The ratio of the pre-exponential factors ($P_2/P_1$) is related to the concentration of the two components by the following equation:

$$\frac{P_2}{P_1} = \frac{C_2 k_{e2} \varepsilon_2}{C_1 k_{e1} \varepsilon_1},$$

where $C$, $k_i$ and $\varepsilon$ are the concentration of ADD, the radiative rate constant and the molar absorption coefficient at the excitation wavelength, respectively. The subscripts 1 and 2 stand for the free and bound ADD, respectively. Since $k_i$ is constant and $\varepsilon_1 = \varepsilon_2$, then Eq. (3) is simplified as $P_2/P_1 \cong C_2/C_1$. In the presence of excess of [U$_2$] with respect to the dye, $P_2/P_1$ can be written as

$$\frac{P_2}{P_1} = K_1[U_2],$$

(4)
Table 1

Single, biexponential and global fluorescence analysis data of ADD in methanol at different concentration of urea

<table>
<thead>
<tr>
<th>S. No.</th>
<th>[Urea] (M)</th>
<th>Analysis</th>
<th>Lifetime (ps)</th>
<th>Amplitude</th>
<th>$\chi^2$</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>$\tau_1$</td>
<td>$\tau_2$</td>
<td>$A_1$</td>
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<tr>
<td>1</td>
<td>0</td>
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<td>Single exp.</td>
<td>720</td>
<td>–</td>
<td>100</td>
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<td></td>
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<td>733</td>
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<td>Global biexp.</td>
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<td>995</td>
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<td>3</td>
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<td>717</td>
<td>–</td>
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<td>767</td>
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<td>Global biexp.</td>
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<td>995</td>
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<td>791</td>
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<td>Biexp.</td>
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<td>836</td>
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<td></td>
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<td>Global biexp.</td>
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<td>631</td>
<td>934</td>
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<tr>
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<td>955</td>
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<td></td>
<td></td>
<td>Global biexp.</td>
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<td>995</td>
<td>27.00</td>
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Fig. 2. Fluorescence decay profiles of ADD in the absence and presence of urea in methanol, $\lambda_{ex} = 375$ nm and $\lambda_{em} = 440$ nm: (1) laser profile, (2) ADD alone, (3) 0.2 M, (4) 0.4 M, (5) 0.8 M and (6) 1.08 M urea. Inset shows the plot of $P_2/P_1$ vs [U$_2$].
The plot of $P_2/P_1$ vs $[U_2]$ is linear and from the slope, the association constant for the ADD–urea dimer complex is obtained as 65 M$^{-1}$. The association constant calculated by this method is in good agreement with that determined by steady-state method.

From the analysis of the fluorescence intensity and lifetime, it can be established that the enhancement of fluorescence intensity and lifetime is due to the interaction of excited state of ADD with urea dimer. The ADD dyes without methoxy group do not show such fluorescence enhancement which reveals that methoxy group is involved in the interaction. The most probable interaction of methoxy group with urea dimer would be hydrogen bonding. The presence of electron donating methoxy substituent reduces the fluorescence quantum yield of this dye when compared to the dye without the methoxy substituent. The hydrogen bonding of urea dimer with methoxy group prevents the electron donation, which enhances the fluorescence intensity and lifetime.

Similar experiments were also done with less hydrogen bond active compound like thiourea. Instead of fluorescence enhancement, quenching is observed in the presence of thiourea for all dyes irrespective of the substituent and this could be due to the presence of sulphur heavy atom.

This is the first report regarding the interaction of fluorophore with urea dimer in solution. From these
studies, it is established that this dye acts as a very good probe to identify the aggregation of urea.

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References