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Studies of charge transfer interaction of nucleosides with proflavine

Molina Choudhury[1], Rama Basu*

Department of Chemistry, University College of Science, 92, Acharya Prafulla Chandra Road, Calcutta-700009, India

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Abstract

The interaction of several nucleosides with proflavine hemisulphate was investigated by absorption and fluorescence spectroscopy in solvents with different dielectric constants. In each case, the ground state charge transfer complex was formed (observed from the difference absorption spectra) and the formation constants were calculated using the Benesi–Hildebrand equation (H.A. Benesi and J.H. Hildebrand, *J. Am. Chem. Soc., 71* (1949) 2703). Fluorescence spectroscopy ruled out the possibility of exciplex formation as no new band was observed. However, static quenching occurred within a limited range of acceptor to donor concentration ratio. The formation constants calculated were found to be higher than those observed by absorption spectroscopy.

Keywords: Charge transfer interaction; Nucleosides; Proflavine

1. Introduction

The interaction of acridine derivatives with DNA has been the subject of considerable research over the last two decades due to the antibacterial and mutagenic properties of these compounds. The nature of the binding of acridine derivatives with DNA has been explained by Peacocke and Skerret [1] and Ramstein et al. [2]. Georghiou [3] found that proflavine (PF) formed molecular complexes with nucleotides in aqueous solutions and the optical properties of these complexes were studied. The absorption spectrum of the dye with DNA was studied by Karmakar and Basu [4] in various solvents. Complex formation between PF and nucleosides was indicated by the absorption and fluorescence properties of the dye [5,6].

In this paper, PF complexes with five nucleosides in different solvents are investigated using absorption and fluorescence spectroscopy. The formation constants of the complexes are calculated and compared, an aspect which, to our knowledge, has not been reported previously.

2. Experimental details

2.1. Materials

The nucleosides adenosine, guanosine, thymidine, uridine and cytidine (Sigma Chemical Company, USA) were used as received. Proflavine hemisulphate (Allied Chemical, NY) was used without further purification. The solvents ethylene glycol (dielectric constant, 37) and propylene glycol (dielectric constant, 32) (S.D. Chemicals, Bombay) were thoroughly dried and freshly distilled before use. Conductivity water (3–4 Ω^{-1}) (dielectric constant, 80) was used for aqueous solutions.

2.2. Spectrophotometry

Spectrophotometric measurements were performed using a Cary 2390 spectrophotometer with a matched pair of stoppered fused silica cells (path length, 1 cm). The PF to nucleoside concentration ratio was around 1:1000. A temperature of 23 ± 1 °C was used.

2.3. Fluorometric studies

These were performed using a Perkin–Elmer MPF 44B fluorescence spectrophotometer. The concentration of both proflavine hemisulphate and nucleosides was

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*Corresponding author.

[1] Permanent address: Bethune College, 181 Bidhan Sarani, Calcutta-700006, India.

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10^{-5} M. A temperature of 23±1 °C was used. All binding studies were carried out at pH 5 in acetate buffer.

3. Results

Fig. 1 shows the absorption spectra of mixed solutions of the dye and adenosine in water (at a fixed concentration of the dye and various concentrations of the nucleoside). Acceptor absorption in the solvent was nullified by subtracting the absorption of the acceptor at the same concentration as in the complex. Fig. 2 shows the Benesi–Hildebrand plot [7] of $[A_0]L/A$ against $1/[D_0]$ from the data obtained from Fig. 1. The formation constant is calculated from Fig. 2. The experiments were repeated for the other four nucleosides and in the other two solvents (ethylene glycol and propylene glycol). The same types of curve were obtained. The ionization potentials of the nucleosides are not available and hence could not be plotted against $h\nu_{CT}$. Linear regression analysis was carried out in the final stages of computation of K in each case. All the data are summarized in Table 1.

Fig. 3 shows the quenching of the fluorescence of PF on addition of various volumes of adenosine of the same concentration as PF in water. Similar graphs were also obtained for the other nucleosides. The formation constants calculated are given in Table 1.

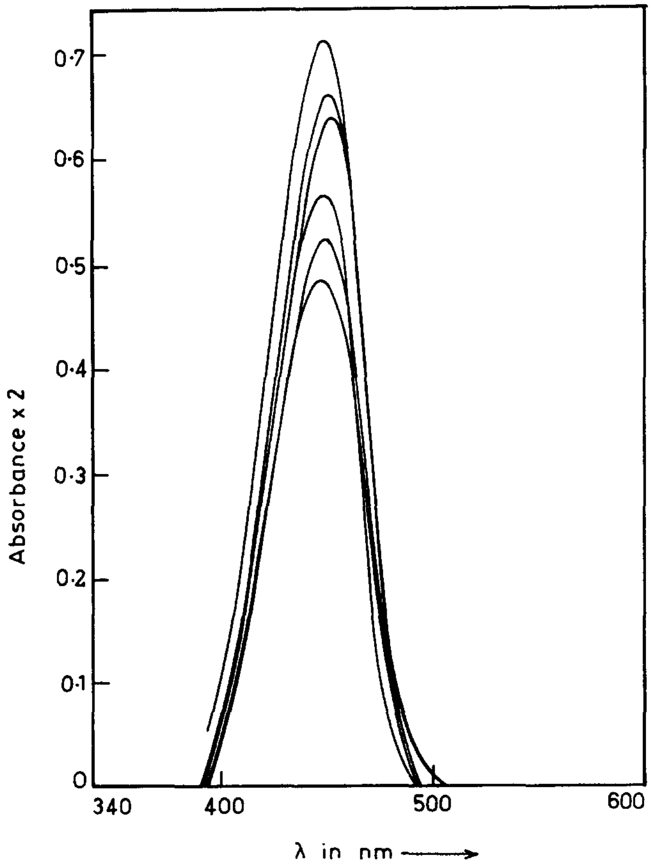

Fig. 1. Absorption spectra of mixed solutions of the dye and adenosine in water.

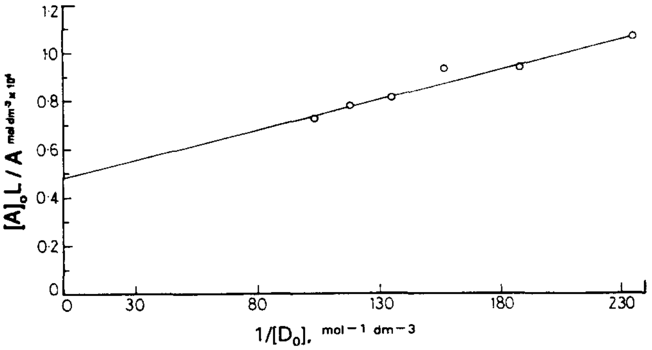

Fig. 2. Plot of $[A_0]L/A$ vs. $1/[D_0]$ for PF–adenosine complex in water (Benesi–Hildebrand plot). Data obtained from Fig. 1. $[A_0] = 2.614 \times 10^{-5}$ mol dm^{-3}; $[D_0] = (0.4252–0.9566) \times 10^{-2}$ mol dm^{-3}; $L = 1$ cm.

Table 1

Formation constants K (dm^3 M^{-1}) of charge transfer complexes in different solvents from absorption and fluorescence studies

Nucleoside	Solvent		
	Water	Ethylene glycol	Propylene glycol
Adenosine	448[a]	460	460
	225.63 (±7%)[b]	89.8 (±11%)	120.23 (±8%)
	6900 (±10%)[c]		
Guanosine[d]	444	460	464
	313.39 (±7%)	252.12 (±6%)	342.75 (±2%)
	11310 (±10%)	356	356
		431.44 (±6%)	223.71 (±4%)
Cytidine[e]	460		460
	113.77 (±5%)		112.59 (±2%)
	5250 (±12%)		
Thymidine	460	460	464
	571.94 (±8%)	468.39 (±9%)	151.99 (±8%)
	2500 (±15%)		
Uridine	450	460	460
	23.46 (±2%)	102.49 (±3%)	226.49 (±5%)
	4700 (±15%)		

[a] Absorption maximum of the complex.

[b] Formation constant from absorption spectral data (Benesi–Hildebrand equation).

[c] Formation constant from fluorescence data in water solvent only (Stern–Volmer equation).

[d] Two bands were observed in guanosine complexes in solvents of lower dielectric constant.

[e] No complex formation was detected in ethylene glycol.

4. Discussion

PF in dilute solution (less than 10^{-5} M) shows two bands in the visible absorption spectrum: one at 452 nm and the other as a shoulder at 440 nm. At con-

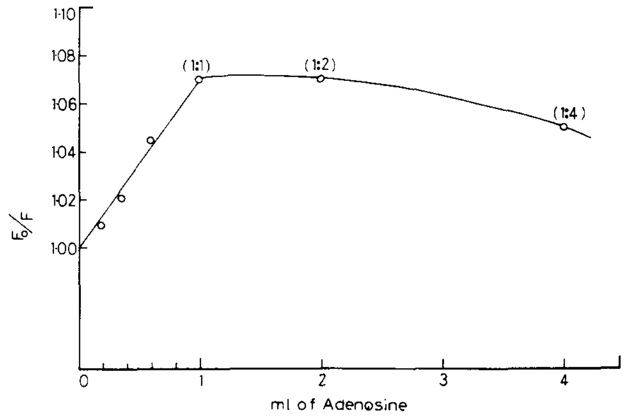

Fig. 3. Quenching of fluorescence emission of PF (510 nm) on addition of adenosine at pH 5 in acetate buffer. The desired amount of 10^{-4} M adenosine was added to 1 ml of 10^{-4} M PF and made up to 10 ml with buffer.

centrations above 10^{-5} M only one band appears at 440 nm. As shown in Fig. 1, all the complexes exhibit a red-shifted band with respect to the donor or acceptor, thus suggesting a π–π^* transition. The Benesi–Hildebrand plots (Fig. 2) for all the complexes are linear, suggesting the formation of a 1:1 charge transfer complex.

The charge transfer complex proposed by Mulliken [8] suggests that the ground state wavefunctions for the donor–acceptor complex may be represented by

$$\Psi_G = a\phi_0(D, A) + b\phi_1(D^+ A^-) \quad (1)$$

where ϕ_0 represents a "no bond" structure and ϕ_1 represents a "dative" structure in which transfer of an electron from donor D to acceptor A has taken place. A possible experimental approach for investigating the relative importance of charge transfer and electrostatic contributions to the ground state wavefunction is to measure the formation constants in solvents of varying dielectric constant. If electrostatic interaction holds the molecules together, the formation constant will decrease as the dielectric constant increases. However, from Table 1 we can see that there is very little change in K in the three solvents ethylene glycol, propylene glycol and water, indicating zero ionic contributions.

Ground state charge transfer complex formation occurs in all the dye–nucleoside mixtures, but on excitation of the complex at λ_{max} no new, broad, structureless emission band, with mirror symmetry to the corresponding charge transfer absorption spectrum, is observed. Thus the possibility of exciplex formation can be ruled out. The fluorescence spectrum of free PF solution (concentration, 10^{-5} M), excited at 452 nm, shows a peak at 510 nm which does not shift on addition of nucleosides, but shows moderate quenching up to a PF to donor concentration ratio of 1:1 in all cases. For higher concentrations of donor, the fluorescence intensity increases.

The quenching data were analysed according to the following scheme [9]

$$\begin{tikzcd}
\text{Dynamic quenching } A^* + Q \arrow[r, shift left, "K_e"] & AQ^* \arrow[l, shift left] \arrow[d, "\text{non-fluorescent}"] \\
\text{Static quenching } A + Q \arrow[u, "h\nu"] \arrow[r, shift left, "K_g"] & AQ \arrow[l, shift left]
\end{tikzcd}$$

where A is the fluorophore, Q is the quencher, AQ is the ground state complex, AQ* is the excited state complex and K_g and K_e are the corresponding association constants. When the complex AQ* is non-fluorescent or very weakly fluorescent, static quenching is more prevalent and the fraction of fluorescence F/F_0 which remains is given by [9]

$$F/F_0 = \frac{1}{1+K_g[Q]} \quad (2)$$

or

$$F_0/F = 1 + K_g[Q] \quad (3)$$

Eq. (3) is identical with the Stern–Volmer equation for dynamic quenching except that the quenching constant is now the formation constant.

For a PF to nucleoside ratio up to 1:1, the fluorescence decreases markedly. It then remains constant (Fig. 3) up to a certain value and increases thereafter. This is consistent with earlier observations on similar systems [10], and suggests complex binding between the dye and donor at high concentration. The calculated values of K_g are higher than those obtained from absorption spectral data (Table 1). This comparison is anomalous as absorption studies were performed at very high donor concentrations without buffer, whereas quenching occurs only at very low donor concentrations in buffer solution. The effect of viscosity on quenching was studied by changing the solvent: no viscosity dependence was observed thus eliminating dynamic quenching. Thus the participation of a diffusion-controlled dynamic process in the non-radiative degradation of the excited state is unlikely.

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