Available online at www.sciencedirect.com
SCIENCE DIRECT
Spectrochimica Acta Part A 62 (2005) 736–739
SPECTROCHIMICA ACTA PART A
www.elsevier.com/locate/saa

Interaction of phenazine with water and DNA bases

Sharmistha Dutta Choudhury, Samita Basu *

Chemical Sciences Division, Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Kolkata 700 064, India

Received 14 December 2004; accepted 24 February 2005

Abstract

The fluorescence spectrum of aqueous phenazine (PZ), an *N*-heterocyclic compound, shows some interesting features that indicate the formation of PZ–water complex in the excited state. Two types of complexes are postulated; Type I, formed by the association of water molecule with one of the nitrogen of PZ and Type II, formed by the association of water molecules with both the nitrogen of PZ. In addition, PZ also interacts with the DNA bases, adenine and thymine and the corresponding nucleosides, adenosine and thymidine. Fluorescence and laser flash photolysis studies indicate that the mode of interaction may be photoinduced electron transfer.

Keywords: Phenazine; DNA base; Photoinduced electron transfer; Fluorescence; Transient absorption

1. Introduction

Phenazine (PZ) is an interesting, symmetric, *N*-heterocycle and its spectral properties have been extensively studied [1–8]. It has importance both in Chemistry and Biology. It undergoes efficient intersystem crossing and most of the earlier work was focused on the triplet state of PZ [4–8]. This molecule has been used as a potential sensitizer in organic photochemistry [9]. We have earlier reported for the first time exciplex formation of PZ with some aromatic amines in the singlet state and the different nature of complexation of PZ with *N*,*N*-dimethylaniline and 4,4′-bis(dimethylamino)diphenylmethane [10,11]. The biological interest in phenazines results primarily from their staining properties. PZ derivatives are used as drugs for the treatment of tuberculosis [12]. They are also active against many other mycobacterial infections, particularly those caused by *Mycobacterium leprae* [13]. Recently, XR11576 and XR5944, novel phenazines, were developed as promising new anti-tumor agents [14,15]. These biological properties of phenazines instigated us to look at the molecule from a different perspective. In this work we have studied the interaction of PZ with water and DNA bases.

Water is so ubiquitous that we consider it as an ordinary liquid solvent. However, due to its exceptionally high density of hydrogen bonds, it displays unique physical, chemical and biological properties. The fluorescence spectrum of aqueous PZ reminds us that the solvent is not a silent spectator and we cannot always ignore the molecular details of solvent–solute interactions. This is even more relevant in biochemistry where water plays a profound role in influencing molecular interactions [16,17].

Considering the use of various PZ derivatives as drugs, the study of the interaction of the bare PZ molecule with the DNA bases also presents an interesting problem. We have chosen the purine base adenine (A), the pyrimidine base thymine (T) and also the corresponding nucleosides, adenosine (AD) and thymidine (TD). The nucleosides were chosen to see role of the sugar unit if any. Our studies indicate the occurrence of photoinduced electron transfer (PET).

2. Experimental

PZ was purchased from Aldrich and was used after repeated re-crystallisation from ethanol. A, T, AD, TD (Aldrich) were used as such without further purification. Deuterated water (D_2O) was obtained from Merck and sodium lauryl sulphate (SDS) was obtained from Sigma. UV

* Corresponding author. Tel.: +91 33 23375345; fax: +91 33 23374637.
E-mail address: samita@nuc.saha.ernet.in (S. Basu).

1386-1425/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.saa.2005.02.042

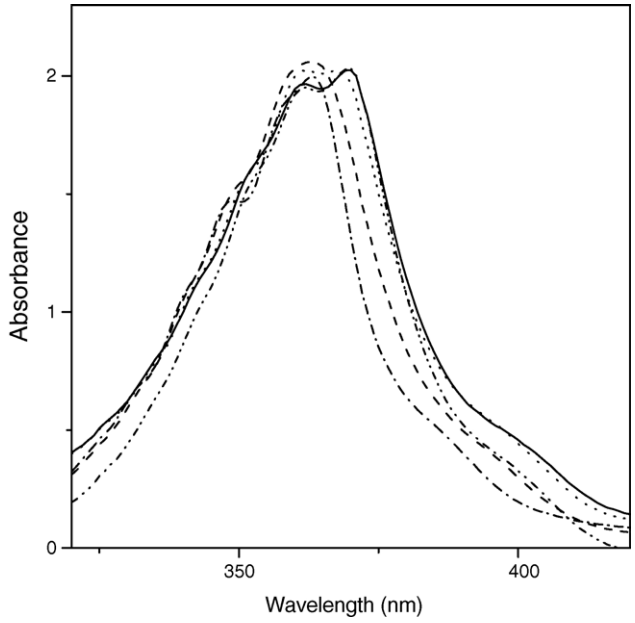


Fig. 1. Absorption spectra of PZ (1×10^{-4} M) in ACN (–·–), EtOH (– – –), water (—), D_2O (— - -) and 10% SDS (. . .).

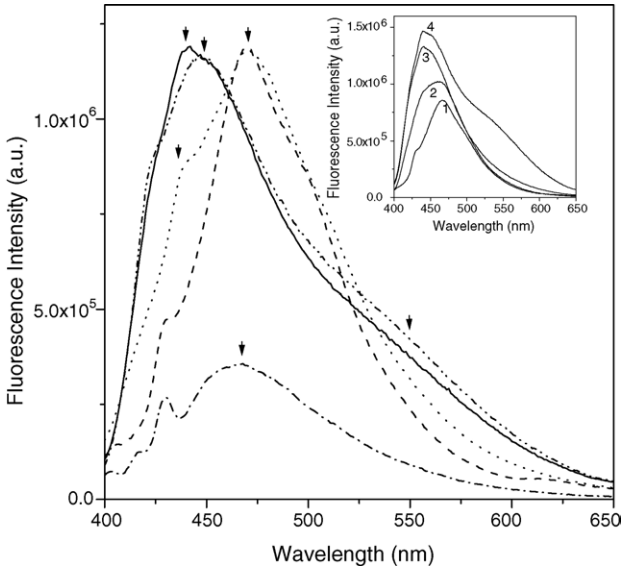


Fig. 2. Fluorescence spectra of PZ (1×10^{-4} M) in ACN (– – –), EtOH (– – –), water (—), D_2O (– ·· –) and 10% SDS (. . .). Peaks around 440 nm for water and SDS; 450 nm for D_2O; 470 nm for ACN, EtOH and SDS and the hump around 550 nm for water and D_2O are marked by arrows. Inset shows fluorescence spectrum of PZ (1×10^{-4} M) in (1) pure EtOH, (2) water (25% by volume)-EtOH, (3) water (75% by volume)-EtOH and (4) pure water.

spectroscopy grade solvents (Spectrochem), ethanol (EtOH) and acetonitrile (ACN) were used without further purification. Water was triply distilled.

Absorption spectra were recorded using UNICAM UV 500 absorption spectrophotometer. Fluorescence spectra were recorded using a Spex Fluoromax-3 spectrofluorimeter. Transient absorption spectra were measured using nanosecond flash photolysis set-up (Applied Photophysics) containing an Nd:YAG laser (DCR-II, Spectra Physics). The sample was excited by 355 nm laser light (FWHM = 8 ns). Transients were monitored through absorption of light from a pulsed Xe lamp (250 W). The photomultiplier (IP28) output was fed into a combiscope (Fluke PM3394B, 200 MHz) and the data was analysed using Fluke View Combiscope software (SW33W).

All solutions were de-aerated by purging pure argon for 20 min prior to the laser flash photolysis experiments. All aqueous and micellar solutions were prepared by sonication. There was no degradation of the samples during experiment.

3. Results and discussion

Fig. 1 shows the absorption spectra of PZ in the solvents, ACN, EtOH, water, D_2O and in 10% SDS. It is known that the peak of PZ around 365 nm is due to $\pi \leftrightarrow \pi^*$ transition [18]. Absorption bands in water, D_2O and 10% SDS are similar. The absorption bands are broader in protic than in aprotic solvents. Also there is a red shift of these bands with increasing solvent polarity. In general the electronic spectra of *N*-heterocycles are very sensitive to solvents. The formation of hydrogen bonds in protic media may completely alter the photophysical properties of *N*-heterocycles by changing the relative energies of the $n\pi^*$ and $\pi\pi^*$ states [19]. Again, on addition of A, T, AD or TD there is no change in the absorption spectrum of PZ indicating that there is no interaction of PZ with the DNA bases in the ground state.

Fig. 2 shows the fluorescence spectra of PZ in ACN, EtOH, water, D_2O and 10% SDS. The fluorescence spectrum in water is strikingly different from that in EtOH or in the non-protic solvent ACN. Fluorescence spectra of PZ in the latter two solvents are similar. The emission peak that is observed at around 470 nm for EtOH and ACN is blue shifted to around 440 nm in water. In addition a new hump appears at around 550 nm. In the presence of a strong acid, a hump appears around 530 nm; therefore the 550 nm hump is not for the protonated molecule. To understand this intriguing behaviour of PZ in water, fluorescence spectrum of PZ was studied in D_2O. We see that in D_2O the hump on the red side of the spectrum appears as usual (∼550 nm) but the blue shift is less i.e. around 450 nm. In 10% SDS the fluorescence spectrum of PZ resembles those in other non-aqueous solvents since the molecule is now incorporated inside the micelle, however a slight hump around 440 nm is also discernible. All the above observations can be explained if we consider that PZ in the excited state forms a complex only with water/D_2O. Inset to Fig. 2 shows that on gradually increasing the water: EtOH ratio the fluorescence spectrum of PZ first undergoes a blue shift and only at a very high water concentration the 550 nm hump starts appearing. Thus two types of complex formation are possible. Type I appears at low water concentrations and is probably due to the association of one of the nitrogens of PZ molecule with a water molecule. This would lead to a distorted geometry of the molecule and rapid exchange of the

water molecule would cause further destabilization leading to a blue shift. In D_2O, on the other hand, the rapid exchange is arrested. This increases the stability of the complex and consequently the blue shift in D_2O is also less. Type II complex is formed by association of water/D_2O molecules with both the nitrogen of PZ. This complex probably leads to the hump at around 550 nm. In micellar medium, since PZ resides in the hydrophobic interior, water molecules cannot freely approach PZ and hence, PZ–water complex formation is not facilitated. However, the presence of water molecules cannot be totally ruled out and whatever little water is there in the vicinity of PZ leads to the formation of only Type I complex which gives the hump around 440 nm.

The appearance of several sub spectra due to the formation of different hydrogen bond complexes with protic solvents has been observed earlier for *p*-diazines [2,20,21]. Marzzacco has observed various hydrogen-bonded species of pyrazine in its low temperature phosphorescence and absorption spectra in EtOH water mixtures [20]. Rossetti and Brus have reported vibronically resolved excitation and emission spectra of isolated pyrazine, pyrazine(H_2O) and pyrazine(H_2O)$_2$ in solid neon host at 4.2 K [21]. Kallir et al. have used the technique of total luminescence spectroscopy in the analysis of excitation dependent phosphorescence of PZ in EtOH at 77 K and have identified three different PZ species in the protic medium: free molecules, molecules with one hydrogen bond to either EtOH or water and molecules forming two hydrogen bonds at least one of which is to water [2]. The complex formation with EtOH, which is possible at 77 K, is not observed at room temperature in our case.

To the best of our knowledge there has been no report so far for the complex formation of PZ with water at room temperature and in the singlet state. Although our interpretation is purely qualitative we feel that it is the only logical explanation for the observed results.

Fig. 3 shows the fluorescence spectrum of PZ in water with increasing concentration of the purine base, A. We see that on adding A, the fluorescence of PZ is gradually quenched. T, AD and TD also show similar behaviour. Quenching by energy transfer can be ruled out because the absorption of the DNA bases are at much lower wavelength with respect to the emission band of PZ [22]. The quenching process obeys the Stern–Volmer relationship:

$$\frac{I_0}{I} = 1 + k_{SV}[Q] = 1 + k_q\tau_0[Q] \quad (1)$$

Here I_0 and I are the intensities of PZ in the absence and presence of quencher, Q, respectively, k_{SV} the Stern–Volmer constant, k_q the bimolecular quenching constant and τ_0 the lifetime of PZ in the absence of Q. The calculated values of k_{SV} are 107.9 M^{-1}, 38.0 M^{-1}, 74.8 M^{-1} and 27.3 M^{-1} for A, T, AD and TD, respectively. PZ is a very weakly fluorescent compound with a very short lifetime. Therefore, we were unable to measure τ_0 with our available nanosecond set-up and could not calculate the k_q values. It is seen that the purine bases (A, AD) have higher k_{SV} values and hence, are far better

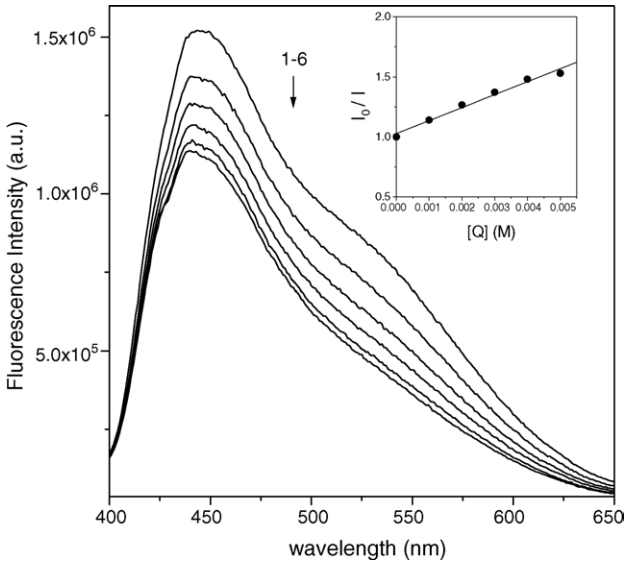
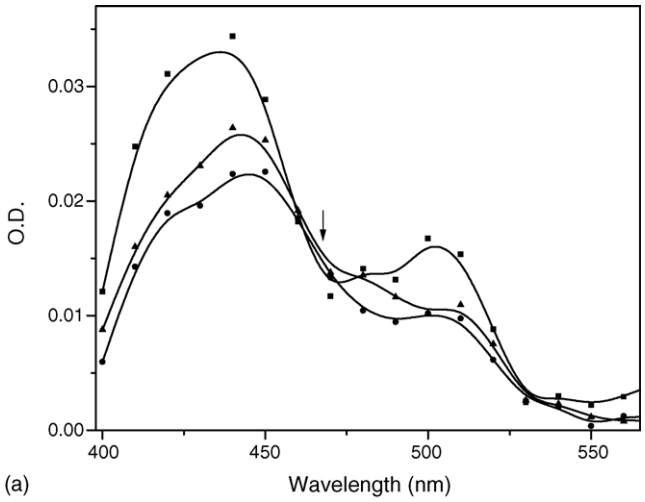


Fig. 3. Fluorescence quenching of PZ (1×10^{-4} M) with increasing concentration of A: (1) 0.0 M, (2) 0.001 M, (3) 0.002 M, (4) 0.003 M, (5) 0.004 M, and (6) 0.006 M in water. Excitation wavelength was 380 nm. Inset shows the corresponding Stern–Volmer plot.

quenchers compared to the pyrimidine bases (T, TD). Now, the oxidation potentials for A and T are known to be 1.6 eV and 2.26 eV, respectively [23]. This is in accordance with the calculated k_{SV} values of A and T i.e. k_{SV} values decrease with increasing oxidation potential and this indicates that the quenching is due to electron transfer from the DNA bases to PZ [24].

To further investigate the process of charge transfer we have performed laser flash photolysis experiments. The triplet–triplet absorption of PZ in water with a maximum around 440 nm is similar to that in ACN and no special feature of PZ water interaction in the triplet state is apparent [10]. Fig. 4a and b show that on adding A, T and AD, TD, the absorption at 440 nm is quenched. A new hump appears around 460 nm and this is more prominent for the nucleosides AD and TD. This indicates that the sugar unit plays some role in the interaction with PZ. Considering PET to take place from the DNA bases to PZ, we expect to observe transient absorption spectra corresponding to PZ radical anion ($PZ^{\bullet -}$) and base radical cation ((DNA base)$^{\bullet +}$), but these could not be directly observed. It is known that (DNA base)$^{\bullet +}$ are very unstable and they readily undergo de-protonation or hydration giving rise to neutral radicals [22]. In an aqueous medium hydration would be more favoured [25]. The $TOH^{\bullet}$ radical is known to absorb in the 450 nm range [26]. The N^6,N^6-dimethyladenosine$OH^{\bullet}$ radical also absorbs in this region [27]. Hence, the hump around 460 nm can be assigned to the species formed upon hydration of (DNA base)$^{\bullet +}$.

Otaga et al. have observed a peak at 550 nm corresponding to $PZ^{\bullet -}$ in the PET between PZ and triethylamine (0.17 M) in ACN medium [9]. We have seen that at lower concentrations of triethylamine, the triplet absorption of PZ is quenched but the 550 nm hump due to $PZ^{\bullet -}$ is not observed. Now, the DNA



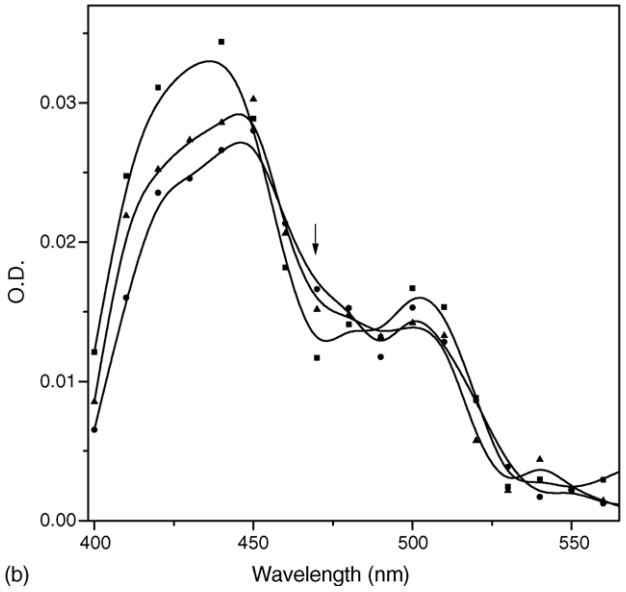


Fig. 4. Transient absorption spectra of PZ (1×10^{-4} M) (■) and PZ (1×10^{-4} M) with (a) A (1×10^{-2} M) (●), T (1×10^{-2} M) (▲); (b) AD (1×10^{-2} M) (●), TD (1×10^{-2} M) (▲) in water at 0.8 μs time delay after the laser flash.

bases are not highly soluble in water and ACN and because of this limitation we could not increase their concentration in solution. Probably our working concentration is insufficient for observing the transient absorption by $PZ^{\bullet -}$. Further $PZ^{\bullet -}$ can also form the $PZH^{\bullet}$ radical that absorbs around 440 nm, by protonation [9]. So the results suggest that PET may be the initial mode of interaction, but it is dominated by the subsequent hydration and protonation reactions.

4. Conclusion

The intriguing behaviour of aqueous PZ can be explained as complex formation of PZ with water. Two types of complex formation are possible; one in which only one of the nitrogens of PZ molecule is associated with water molecule and the other in which both the nitrogens of PZ are associated with water molecules. Studies with the DNA bases, A, T, AD and TD, reveal that PET may be the mechanism of interaction with PZ.

Acknowledgement

We thank Prof. Nihar Ranjan Ray of Plasma Physics Division, for kindly providing the Fluke Combiscope and the Fluke View Combiscope software. We are grateful to Prof. Dipak Dasgupta for providing D_2O. We also thank Mrs. Chitra Raha for technical assistance and Mr. Tamal Sengupta for helpful discussions.

References

[1] R.M. Hochstrasser, J. Chem. Phys. 36 (1962) 1808.
[2] A.J. Kallir, G.W. Suter, U.P. Wild, J. Phys. Chem. 89 (1985) 1996.
[3] J.J. Aaron, M. Maafi, C. Párkányi, C. Boniface, Spectrochim. Acta 51A (1995) 603.
[4] Y. Hirata, I. Tanaka, Chem. Phys. Lett. 43 (1976) 568.
[5] A. Grabowska, Chem. Phys. Lett. 1 (1967) 1113.
[6] T.G. Pavlopoulos, Spectrochim. Acta 43A (1987) 715.
[7] J.I. DelBarrio, J.R. Rebato, F.M.G. Tablas, J. Phys. Chem. 93 (1989) 6836.
[8] V.A. Kuz'min, P.P. Levin, Bull. Acad. Sci. USSR Div. Chem. Sci. 37 (1988) 1098.
[9] T. Ogata, Y. Yamamoto, Y. Wada, K. Murakoshi, M. Kusaba, N. Nakashima, A. Ishida, S. Takamuku, S. Yanagida, J. Phys. Chem. 99 (1995) 11916.
[10] S. Dutta Choudhury, S. Basu, Chem. Phys. Lett. 373 (2003) 67.
[11] S. Dutta Choudhury, S. Basu, Chem. Phys. Lett. 383 (2004) 533.
[12] V.M. Reddy, G. Nadadhur, D. Daneluzzi, J.F. O' Sullivan, P.R. Gangadharam, Antimicrob. Agents Chemother. 40 (1996) 633.
[13] V.M. Reddy, J.F. O' Sullivan, P.R. Gangadharam, J. Antimicrob. Chemother. 44 (1999) 615.
[14] P. Mistry, A.J. Stewart, W. Dangerfield, M. Baker, C. Liddle, D. Bootle, B. Kofler, D. Laurie, W.A. Denny, B. Baguley, P.A. Charlton, Anticancer Drugs 13 (2002) 15.
[15] A.J. Stewart, P. Mistry, W. Dangerfield, D. Bootle, M. Baker, B. Kofler, S. Okiji, B. Baguley, W.A. Denny, P.A. Charlton, Anticancer Drugs 12 (2001) 359.
[16] L. Stryer, Biochemistry, 4th ed., W.H. Freeman and Company, New York, 2000.
[17] G.A. Jeffrey, J. Mol. Struct. 322 (1994) 21.
[18] G.A. Davis, J.D. Gresser, P.A. Carapellucci, J. Am. Chem. Soc. 93 (1971) 2179.
[19] E.C. Lim, J.M.H. Yu, J. Chem. Phys. 47 (1967) 3270.
[20] C. Marzzacco, J. Am. Chem. Soc. 95 (1973) 1774.
[21] R. Rossetti, L.E. Brus, J. Chem. Phys. 70 (1979) 4730.
[22] H. Morrison, (Ed.), Bioorganic Photochemistry: Photochemistry and the Nucleic acids, vol. 1, Wiley, New York 1990.
[23] K. Kasama, A. Takematsu, S. Yamamoto, S. Arai, J. Phys. Chem. 88 (1984) 4918.
[24] J.R. Bolton, M.D. Archer, in: J.R. Bolton, N. Mataga, G. McLendon (Eds.), Electron Transfer in Inorganic Organic and Biological Systems, American Chemical Society, Washington, DC, 1991.
[25] J.R. Wagner, J.E. Van Lier, J.L. Johnston, Photochem. Photobiol. 52 (1990) 333.
[26] G.J. Fischer, E.J. Land, Photochem. Photobiol. 37 (1983) 27.
[27] A.J.S.C. Vieira, S. Steenken, J. Am. Chem. Soc. 109 (1987) 7441.