J. Chem. Soc., Faraday Trans. 1, 1981, **77**, 1405–1410

Study of Charge-transfer Interaction in Biomolecules

BY JYOTIRMAY LAHIRI AND RAMA BASU*

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

Received 24*th June*, 1980

The interaction of the antibiotic drugs tetracycline and oxytetracycline with purines, pyrimidines and amino acids were studied spectrophotometrically in water, ethylene glycol and propylene glycol. In each case a new absorption band was located and denoted as a charge-transfer band. The formation constants for the charge-transfer complexes were determined using Benesi–Hildebrand's method followed by an iterative procedure. Different linear plots for $h\nu$ against the highest filled π-molecular orbital of the donors (purines, pyrimidines and amino acids) were obtained indicating different stabilization energies for each system.

Spectroscopic observations have shown that many drugs form charge-transfer complexes with chloranil, 1,4-dinitrobenzene, 1,3,5-trinitrobenzene[1] and other well known organic acceptors in a solvent of low dielectric constant. On the other hand, various workers have shown that purines, pyrimidines and amino acids undergo charge-transfer complexation with various electron-accepting and electron-donating species.[2-8]

However, no systematic investigation has yet been undertaken with any specific drug and cellular receptors such as proteins and nucleic acids. The object of the present investigation is to determine whether charge-transfer complexation occurs between purines, pyrimidines and different amino acids with drugs like tetracycline and oxytetracycline. Moreover, most of the workers in this field have worked with solvents of low dielectric constant although most biochemical reactions occur in aqueous medium, which has a high dielectric constant. We have carried out spectrophotometric measurements in aqueous medium and in ethylene glycol and propylene glycol, which have dielectric constants of 37.0 and 32.0, respectively.

The formation constants of the charge-transfer complexes formed between the tetracycline/oxytetracycline and the purines, pyrimidines and amino acids were measured.

EXPERIMENTAL

All the purines, pyrimidines and amino acids used were from E. Merck (A.G.-Darmstadt, Germany; Bombay, India), B.D.H. (England) or Loba Chemicals (Wien-Fischmend, Austria) and were used without further purifications. The solvents used were water, ethylene glycol and propylene glycol. The water had a conductivity of 3-4 Ω^{-1} and the other two solvents were carefully dried and freshly distilled. Tetracycline belongs to a family of important and low toxicity antibiotic drugs which contain the hydronaphthacene skeleton as a structural unit. Tetracycline and oxytetracycline were from Sigma (St Louis, U.S.A.) and were used directly without further purification. The absorption spectra were measured using a Beckman Quartz spectrophotometer model DU with matched pair of stoppered silica cells. Measurements with strongly absorbing systems were made in 1 cm cells whereas 5 cm cells were used when the intensity of the absorption was low. The temperature was 23 ± 0.1 °C.

In locating the position of a characteristic absorption maximum, a solution of the donor and the acceptor (tetracycline or oxytetracycline) in the respective solvent was balanced against

a solution of tetracycline or oxytetracycline of the same strength as the purines, pyrimidines and amino acids do not absorb in the region being studied. The concentration ratio of the donor to acceptor was nearly 500:1 for the purines and pyrimidines whereas for the amino acids the ratio was nearly 1000:1. A ratio of 500:1 for the amino acids did not give a measurable intensity for the complex. The donors were used at a concentration of 10^{-2} mol dm^{-3} and the drugs at a concentration of 10^{-5} mol dm^{-3}.

RESULTS

Fig. 1 shows the absorption spectra of tetracycline and tetracycline with adenine in water. Tetracycline shows a peak at 360 nm while the adenine + tetracycline mixture shows two peaks at 360 and 375 nm. Adenine itself has no absorption band in this region. As the adenine concentration is increased at a fixed tetracycline concentration, the optical density at 375 nm increases and that at 360 nm diminishes. Evidently this

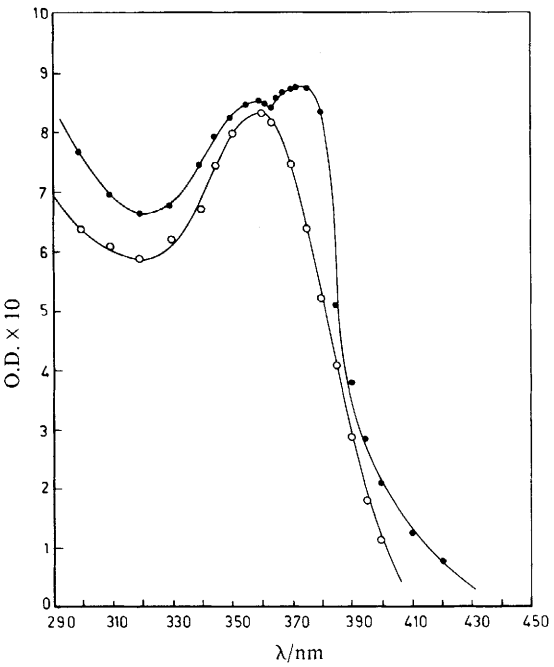

FIG. 1.—Absorption spectra of tetracycline (○) and complex of tetracycline with adenine in water (●).

red-shifted band at 375 nm arises from a molecular complex between tetracycline and adenine. When balanced against the acceptor at the same concentration the band appeared at 380 nm. Note that all the band positions of the complexes were recorded by balancing against the same concentration of the acceptors.

In order to establish if the complex formation arises from a proton donor–acceptor type of interaction between the constituents, the absorption spectra of tetracycline in water was measured in the presence of an acid [pH = 4.64 and μ = 0.0006083 (acetic acid)] and a base [pH = 11.85 and μ = 0.01 (NaOH)]. Apart from a change in the intensity of the 360 nm band, neither the acid nor the base altered the band shape of tetracycline in water. From this it appears that the complex is an electron donor–acceptor complex or, in other words, a charge-transfer complex. A Benesi–Hildebrand[9] plot for a tetracycline + adenine mixture in water gave a perfect straight

TABLE 1.—RESULTS WITH WATER AS SOLVENT

donor	$\bar{\nu}/10^3$ cm^{-1}		K (iterative)/dm^3 mol^{-1}		ε_c (iterative)/dm^3 mol^{-1} cm^{-1}		HFMO/β
	tetra-cycline	oxytetra-cycline	tetra-cycline	oxytetra-cycline	tetra-cycline	oxytetra-cycline	
adenine	26.32	26.18	72.04±0.54	90±0.40	11 494±108	13 333±125	0.486
guanine	26.04	25.97	48.40±0.40	54	18 182±130	6 667±65	0.307
xanthine	25.71	26.11	33.60±0.60	49±3	4 762±60	4 082±95	0.397
hypoxanthine	26.18	26.04	52±0.50	65.30±0.80	21 739±588	9 804±76	0.402
thymine	27.62	27.03	67±0.65	70.50±0.50	14 295±645	12 500±900	0.510
uracil	27.17	26.74	58	78±1.50	17 241±709	15 385±275	0.597
phenylalanine	26.11	26.32	75±0.20	102±2	13 333±305	10 000±940	0.908
tyrosine	25.64	25.91	49±1.00	68±0.85	28 571±90	20 000±850	0.792
histidine	25.51	25.77	60±0.25	77±1.20	20 000±506	18 182±90	0.660
tryptophan	25.38	25.64	65	84±1.00	15 385±78	16 667±75	0.534

TABLE 2.—RESULTS WITH ETHYLENE GLYCOL AS SOLVENT

donor	$\bar{\nu}/10^3$ cm^{-1}		K (iterative)/dm^3 mol^{-1}		ε_c (iterative)/dm^3 mol^{-1} cm^{-1}		HFMO/β
	tetra-cycline	oxytetra-cycline	tetra-cycline	oxytetra-cycline	tetra-cycline	oxytetra-cycline	
adenine	28.17	28.57	70±3.5	162±2	28 571	25 000	0.486
guanine	28.01	28.17	49±1	125±3	30 303±560	40 000±95	0.307
xanthine	28.17	28.33	45	90±5	5 000	2 222±50	0.397
hypoxanthine	27.78	28.25	40±0.5	105±7	40 000±650	28 571±350	0.402
thymine	28.74	28.90	60	70±1	25 000±450	20 000	0.510
uracil	28.57	28.65	66±0.82	81±0.5	12 500±575	22 222±200	0.597
phenylalanine	28.65	28.90	90±1	204±8	33 333±950	25 000±980	0.908
tyrosine	27.62	27.93	56±5	176±7	35 714±310	28 409±412	0.792
histidine	27.70	27.78	65±2	79.2±0.85	40 000	30 303±960	0.660
tryptophan	27.55	27.62	30	115.2±1.5	66 666	31 250±50	0.534

TABLE 3.—RESULTS WITH PROPYLENE GLYCOL AS SOLVENT

donor	$\bar{\nu}/10^3$ cm^{-1}		K (iterative)/dm^3 mol^{-1}		ε_c (iterative)/dm^3 mol^{-1} cm^{-1}		HFMO/β
	tetra-cycline	oxytetra-cycline	tetra-cycline	oxytetra-cycline	tetra-cycline	oxytetra-cycline	
adenine	27.78	27.40	180 ± 5.0	198 ± 3.0	20 000	22 222 ± 334	0.486
guanine	27.47	27.32	112 ± 2.0	140 ± 7.0	28 571 ± 975	28 500 ± 91	0.307
xanthine	27.62	27.47	175 ± 6.5	210	8 000 ± 350	2 667	0.397
hypoxanthine	27.32	27.03	80	152 ± 2.0	25 000 ± 637	24 000 ± 67	0.402
thymine	28.41	27.78	126 ± 1.0	138 ± 3.0	14 285 ± 676	20 000 ± 58	0.510
uracil	28.65	27.86	150 ± 0.5	160 ± 5.0	16 666 ± 351	23 810 ± 312	0.597
phenylalanine	28.82	28.90	187 ± 4.0	240 ± 0.56	18 182 ± 256	33 333	0.908
tyrosine	27.62	27.93	156 ± 1.0	226 ± 1.0	19 231 ± 50	31 250 ± 79	0.792
histidine	27.47	27.86	81 ± 2.2	130	22 222	15 385 ± 995	0.660
tryptophan	27.32	27.78	136 ± 6.0	184.5 ± 3.75	25 000 ± 756	30 303 ± 196	0.534

line indicating the 1:1 nature of the molecular complex. The first molar extinction coefficients and formation constants used in the iterative procedure were calculated from the slope and intercept of the curves using the relation

$$[A]l/(O.D.)_{complex} = \frac{1}{K\varepsilon_c}\left(\frac{1}{[D]}\right)+\frac{1}{\varepsilon_c}$$

as given by Benesi and Hildebrand where l is the optical path length and [A] and [D] are the initial concentrations of the acceptor and the real donor, respectively.

Similar measurements were made with other purines, pyrimidines and amino acids and new bands were detected in all cases. Oxytetracycline behaved in a similar fashion.

Tables 1, 2 and 3 summarize the results giving the wavenumber of the charge-transfer band (ν/cm^{-1}), the formation constant (K/dm^3 mol^{-1}), the molar extinction coefficient (ε_c/dm^3 mol^{-1} cm^{-1}) and the highest filled π-molecular orbital (HFMO/β), measured by an iterative procedure proposed by de Maine.[10] The iterative procedure was carried out using the IBM 1130 of the Regional Computer Centre of Calcutta University.

DISCUSSION AND CONCLUSIONS

It may be observed that the complex absorption in water shows a maximum which is shifted to longer wavelengths with respect to the tetracycline band while in ethylene and propylene glycol there is a definite blue-shift in the respective maxima for all the donors with respect to the tetracycline band. Oxytetracycline behaves in a similar fashion. Oxytetracycline was always found to give stronger complexes than tetracycline.

It remains to be established which of the two partners in the molecular complex is acting as donor and which as acceptor.

It is known that the energy of charge-transfer transition, $h\nu_{c.t.}$, is a linear function of the ionization energy of the donor, when using the same acceptor. Since the experimental ionization energies of the purines, pyrimidines and amino acids are not available, the energies of the highest occupied π-molecular orbital (which is related to its ionization energy) as calculated by Pullman and Pullman[11] were used in plotting $h\nu_{c.t.}$ against HFMO curves. Fig. 2 shows that the plot is fairly linear for purines,

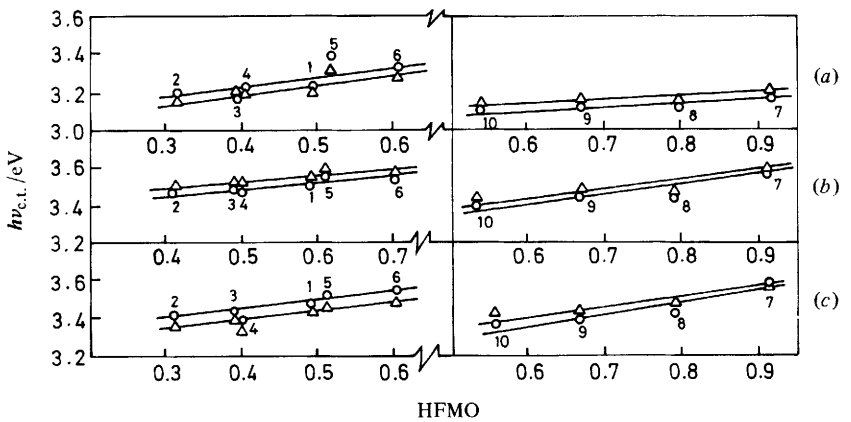

FIG. 2.—Linear $h\nu_{c.t.}$ against HFMO plots. Acceptors: ○, tetracycline; Δ, oxytetracycline. Donors: 1, adenine; 2, guanine, 3, xanthine; 4, hypoxanthine; 5, thymine; 6, uracil; 7, phenylalanine; 8, tyrosine; 9, histidine; 10, tryptophan. Solvents: (*a*) water, (*b*) ethylene glycol, (*c*) propylene glycol.

pyrimidines and amino acids + tetracycline, although two different plots are obtained. The reason for the two different plots is quite obvious. The energy of a charge-transfer transition is given as

$$h\nu_{c.t.} = I_D - E_A - \Delta$$

where I_D is the ionization energy of the donor, E_A is the electron affinity of the acceptor and Δ is the stabilization energy of the donor–acceptor pair. The linear $h\nu_{c.t.}$ against HFMO plot indicates a constant value for Δ. As purines, pyrimidines and amino acids are dissimilar chemical compounds, the stabilization energies may be different for the two series. The same is true for the oxytetracycline system (fig. 2).

We may conclude that linear $h\nu_{c.t.}$ against HFMO plots (fig. 2) indicate that the purines, pyrimidines and amino acids are acting as donors and tetracycline and oxytetracycline as acceptors in the molecular complexes in the three solvents.

Preliminary observation shows that while tetracycline can be reduced at the dropping mercury electrode surface adenine cannot be reduced. This indicates that tetracycline has a greater electron affinity than adenine (purine). Thus we may conclude that tetracycline is acting here as an electron acceptor while adenine is the electron donor. However, a thorough investigation along these lines is necessary with all the purines, pyrimidines and amino acids before a definite conclusion can be drawn. This is in progress in this Laboratory.

We thank U.G.C. of India for a teacher fellowship to one of us (J.L.).

[1] W. A. Strickland Jr and L. Robertson, *J. Pharm. Sci.*, 1965, **54**, 452.
[2] G. Cilento and P. Tedeschi, *J. Biol. Chem.*, 1961, **236**, 907.
[3] R. Foster and C. A. Fyfe, *J. Chem. Soc. B*, 1966, 926.
[4] R. Foster and P. Hanson, *Trans. Faraday Soc.*, 1964, **60**, 2189.
[5] P. Machmer and J. Duchesne, *Nature (London)*, 1965, **206**, 618.
[6] G. Cilento and P. Guisti, *J. Am. Chem. Soc.*, 1959, **81**, 3801.
[7] D. Agin, *Nature (London)*, 1965, **205**, 805.
[8] R. Foster and C. A. Fyfe, *Biochim. Biophys. Acta*, 1966, **112**, 490.
[9] H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703.
[10] P. A. D. de Maine and R. D. Sea Wright, *Digital Computer Programs for Physical Chemistry* [Macmillan, New York, 1963 (vol. 1) and 1965 (vol. 2)].
[11] B. Pullman and A. Pullman, *Rev. Mod. Phys.*, 1960, **32**, 428.

(PAPER 0/980)