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CORRELATION OF THE BASE SPECIFICITY OF DNA – INTERCALATING LIGANDS WITH THEIR PHYSICO-CHEMICAL PROPERTIES

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1. Introduction

Several heterocyclic compounds are known to show a preferential binding to one of the base pairs of DNA, the most widely studied of these specific interactions is the preference of the actinomycins for the G. C. base pair [1]. In an investigation into the basis of this type of preferential binding Müller and co-workers [2,3] have recently studied the base specificity of a series of 36 intercalating heterocycles and have shown that preference for the G.C. base pair increases as the absorbance maximum of the ligand chromophore shifts to longer wavelength presumably due to increased polarisation of the molecule [2]. To determine the nature of this specificity in binding we have examined eight of the compounds (fig.1) used by Müller and Crothers [2] and have correlated the quoted values for relative binding to G.C. and A.T. rich DNA with physicochemical properties using the methods of multiparameter regression analysis pioneered by Hansch [4]. The chromatographic parameter (R_m) and the relative charge transfer affinity parameter were selected to mirror the hydrophobic and electronic properties of the ligands respectively as these were the properties which would be expected to be important in directing binding to G.C. in preference A.T. The R_m value has been shown to be linearly related to the hydrophobic substituent parameter π [5] and the relative charge transfer affinity linearly related to the Hammett substituent parameter δ [6]. Regression analysis shows that there is an excellent correlation between charge transfer affinity of the ligand and the preference for G.C. base pairs in DNA.

2. Materials and methods

The ligands used in this work were obtained as follows: phenosafranine perchlorate and thionine perchlorate were gifts from Professor W. Müller, proflavine hemisulphate, methylene blue, acridine orange, pyronine G, neutral red and toluidine blue were obtained from normal commercial sources. All ligands were shown to be chromatographically pure by T.L.C. on silica gel using chloroform–methanol–acetic acid (8 : 1 : 1) as solvent. Guanosine-5′-phosphate, sodium salt (GMP) 100% by spectral analysis was purchased from P.L. Biochemicals Inc.

2.1. *Determination of charge transfer affinities*

The relative charge transfer affinities of the ligands were determined by measuring their relative abilities to accept electrons from a suitable donor molecule. The donor molecule chosen was GMP as this was thought to most closely mirror the likely in vivo donor, guanine. In order to ascertain whether a charge transfer interaction was actually taking place between GMP and the ligands, the presence of a bathochromic shift and an isosbestic point was sought. The presence of a bathochromic shift in the absorbance of the ligand is good evidence that a charge transfer interaction is occurring [7] and an isosbestic point would indicate that the interaction is between free ligand and a single type of complexed ligand [8]. Suitable concentrations of the ligands in Sorensens phosphate buffer, pH 7.0, were prepared and the u.v. absorption spectra determined using a Cecil CE 505 spectrophotometer (fig.1). The spectra were rerun at the same ligand

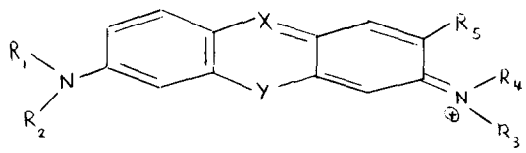

Fig.1. Heterocyclic ligands.

	X	Y	R_1	R_2	R_3	R_4	R_5	λ_{max} (nm)	Molar concn.
1. Proflavine	CH_2,	N,	H,	H,	H,	H,	H,	445	2.0×10^{-5}
2. Acridine orange	CH_2,	N,	CH_3,	CH_3,	CH_3,	CH_3,	H	492	1.0×10^{-5}
3. Pyronine G	CH_2,	O,	CH_3,	CH_3,	CH_3,	CH_3,	H	550	5.0×10^{-5}
4. Thionine	N,	S,	H,	H,	H,	H,	H	600	2.0×10^{-5}
5. Methylene blue	N,	S,	CH_3,	CH_3,	CH_3,	CH_3,	H	660	0.5×10^{-5}
6. Toluidine blue	N,	S,	CH_3,	CH_3,	H,	H,	CH_3	600	2.5×10^{-5}
7. Neutral red	N,	N-H,	CH_3,	CH_3,	H,	H,	CH_3	525	2.5×10^{-5}
8. Phenosafranine	N,	N-Ph,	H,	H,	H,	H,	H,	525	1.25×10^{-5}

concentration, in the presence of increasing concentrations of GMP. A bathochromic shift and an isosbestic point were found in every case. Crystal violet on the other hand, a ligand which Müller found to exhibit no base specificity, we also found to exhibit neither a bathochromic shift nor an isosbestic point on the addition of GMP.

The relative constants for this charge transfer interaction (K_{GMP}) were determined by spectrophotometric titration using a Pye-Unicam SP 3000 spectrophotometer. Solutions of the various ligands were prepared as above. An aliquot (3 ml) of the ligand solution was then titrated as its λ_{max} by the addition of increasing amounts of GMP. (1.5×10^{-2} M) using a microlitre syringe until no further drop in the molar extinction coefficient (ϵ) of the dye occurred after making allowance for concentration changes. Usually about 500 μl of GMP (in 10 μl steps) was required. The concentration of free and complexed ligand were calculated from the molar extinction values determined by spectrophotometric titration using the method of Double and Brown [9]. From these values the relative affinity constants (K_{GMP}) were determined by the Scatchard method [10]. Originally these determinations were carried out at pH 7.0, at which pH, 7 of the ligands are fully ionised (table 1). Neutral red however, (pK_a 6.8) is only 40% ionised at this pH. The K_{GMP} value for neutral red was therefore redetermined at pH 5.0, at which pH neutral red is fully ionised.

2.2. *Determination of relative hydrophobicites*

The relative hydrophobicites of the ligands were characterised by the R_m values which were determined by reverse phase chromatography [11]. Sheets of Whatman No. 1 chromatography paper were soaked in a 5% v/v solution of redistilled *n*-octanol in ether. The ether was allowed to evaporate leaving the sheets evenly coated with octanol. Solutions of the ligands were spotted on to the sheets and the chromatograms développed by descending chromatography using Sorensens phosphate buffer pH 7.4. The mean R_f values from 4 measurements were determined and the R_m values calculated correcting for the degree of ionisation at pH 7.4 (table 1).

3. Results

All 8 ligands gave bathochromic shifts of about 5 nm with an isosbestic point in the presence of GMP indicating the occurrence of a charge transfer interaction. Values for relative charge transfer affinity and relative hydrophobicity for 8 heterocyclic ligands (fig.1) were determined (table 1). Using multiparameter regression analysis these values were correlated with the values for DNA affinity and G. C. specificity determined by Müller and co-workers [2,3]. The large increase in K_{GMP} for neutral red on changing the pH of the buffer from pH 7.0 to pH 5.0 (i.e. going from

Table 1
Physicochemical parameters for heterocyclic ligands

Ligand	K_{DNA}[a]	$\log K_{DNA}$	α[a]	K_{GMP}[b]	$\log K_{GMP}$	pK_a	R_f	Fraction ionised (f) at pH 7.4	$R_m = \log \frac{(1/R_f - 1)}{f}$
1	8.8×10^4	4.9444	1.26	9.3×10^5	5.9668	9.21	0.116	0.998	0.8890
2	2.0×10^5	5.3011	1.57	14.0×10^5	6.1458	10.1	0.025	0.984	1.5919
3	1.3×10^4	4.1139	1.70	18.7×10^5	6.2710	11.3	0.181	0.999	0.6556
4	3.7×10^4	4.5682	1.52	12.7×10^5	6.1027	11.76	0.099	0.999	0.9591
5	1.2×10^4	4.0792	1.84	22.8×10^5	6.3586	12.0	0.165	0.999	0.7043
6	2.4×10^4	4.3802	1.90	32.6×10^5	6.5126	11.6	0.092	0.999	0.9943
7	6.6×10^4	3.8195	1.91	3.1×10^5 34.1×10^5[c]	5.5665 6.5327	6.8	0.017	0.2	2.4218
8	8.1×10^3	3.9085	1.61	18.0×10^5	6.2545	12.0	0.233	0.999	0.5175

[a] K_{DNA} is the affinity of binding to calf thymus DNA, α is the ratio of the affinity for *M. luteus* DNA (72.5% G.C.) to the affinity for *B. subtilis* DNA (44.5% G.C.) and are taken from reference [2].
[b] K_{GMP} is the relative affinity constant for charge transfer interaction at pH 7.0.
[c] K_{GMP} is the relative affinity constant for charge transfer interaction at pH 5.0.

partially ionised neutral red to fully ionised neutral red), indicates that it is the ionic form of the ligand which is responsible for the interaction with GMP and the value for K_{GMP} at pH 5.0 is the one which has been used in the regression analysis. The following equations were generated:

$$\log K_{DNA} = -1.78 \log K_{GMP} + 15.57 \quad (1)$$
$$r = 0.67, s = 0.42, F = 4.99, n = 8$$
$$\log K_{DNA} = 0.03 R_m + 4.36 \quad (2)$$
$$r = 0.035, s = 0.56, F = 0.007, n = 8$$
$$\log K_{DNA} = 0.27 \log K_{GMP} - 2.1 R_m + 17.3 \quad (3)$$
$$r = 0.74, s = 0.41, F = 3.03, n = 8$$
$$\alpha = 1.1 \log K_{GMP} - 5.2 \quad (4)$$
$$r = 0.97, s = 0.06, F = 97.9, n = 8$$
$$\alpha = 0.11 R_m + 1.54 \quad (5)$$
$$r = 0.31, s = 0.23, F = 0.65, n = 8$$
$$\alpha = 1.12 \log K_{GMP} - 0.02 R_m - 5.31 \quad (6)$$
$$r = 0.972, s = 0.06, F = 42.8, n = 8$$

r = regression coefficient, s = standard error, F = variance ratio test, n = number of results.

4. Discussion

From analysis of eqs. 1–6 a number of conclusions may be drawn concerning the affinity and specificity of heterocyclic ligands for DNA. The relative hydrophobicities of the ligands correlate neither with the affinity for DNA nor with the base specificity (eqs. 2 and 5). At physiological pH the ligands are present almost exclusively in the ionised form in which form they interact with DNA. One would therefore expect that electronic parameters would be more important in determining binding. Eq. 1 shows some correlation between affinity for DNA and charge transfer interaction. This correlation is improved slightly by adding the hydrophobicity parameter (eq. 3). This suggests that binding affinity for DNA depends on a number of different parameters, electronic, hydrophobic and also possibly steric. Base specificity on the other hand appears to depend almost exclusively on electronic factors. This is shown by the very good linear correlation between Müller's α value and the relative charge transfer affinity constant K_{GMP} (eq. 4) which is not significantly altered by the addition of the hydrophobicity parameter (eq. 6).

Thus we have shown that base specificity in these ligands is related to their electronic characteristics. These are in turn related to chemical structure and *N*-methylation increases both base specificity and relative charge transfer affinity. The precise effect of slight variations of chemical structure on the electronic characteristics of the molecule and also the importance of steric parameters in binding affinity and base specificity needs to be further investigated.

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