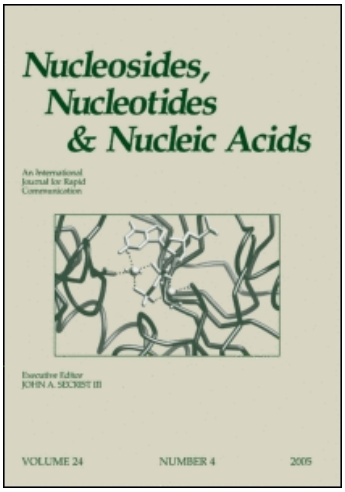

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

^{1}H NMR Thermodynamical Analysis of the Interactions of Proflavine with Self-Complementary Deoxytetranucleotides of Different Base Sequence

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To cite this Article Davies, D. B. , Djimant, L. N. and Veselkov, A. N.(1994) '^{1}H NMR Thermodynamical Analysis of the Interactions of Proflavine with Self-Complementary Deoxytetranucleotides of Different Base Sequence', Nucleosides, Nucleotides and Nucleic Acids, 13: 1, 657 — 671
To link to this Article: DOI: 10.1080/15257779408013270
URL: http://dx.doi.org/10.1080/15257779408013270

NUCLEOSIDES & NUCLEOTIDES, 13(1-3), 657-671 (1994)

^{1}H NMR THERMODYNAMICAL ANALYSIS OF THE INTERACTIONS OF PROFLAVINE WITH SELF-COMPLEMENTARY DEOXYTETRANUCLEOTIDES OF DIFFERENT BASE SEQUENCE†

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ABSTRACT

Enthalpies and entropies of complex formation (1:1, 2:1, 1:2, and 2:2 complexes) between proflavine and tetranucleotides of different base sequence have been determined by 500 MHz proton NMR chemical shifts, enabling the contributions to be differentiated for the formation of different types of complexes in solution. Comparison of the calculated thermodynamical parameters has lead to an understanding of the nature of the intermolecular interactions responsible for the formation of dye complexes with the different tetranucleotides.

INTRODUCTION

It is known that the bacteriostatic and mutagenic properties of acridine dyes are due to their ability to bind to the DNA helix by intercalation[1]. The majority of papers published on the investigation of dye-DNA interactions have been directed towards determining the binding equilibria involved, the structures of the intercalated complexes and any alterations in conformations of nucleic acids resulting from intercalation[2-6]. Less attention has been given to determining the thermodynamics of intercalation reactions which is necessary for a thorough understanding of the nature of the intermolecular interactions involved in the

† This paper is dedicated to the memory of Roland K Robins and his many contributions to the chemistry of nucleosides and nucleotides.

intercalation process. The development of high-sensitivity methods of microcalorimetry has recently enabled enthalpies of the reactions of ligand binding to DNA or its fragments to be determined at low concentrations in order to exclude the effect of molecular aggregation[7].

For short oligonucleotides, as a rule, there is a rather complex dynamic equilibrium of the interacting molecules, which includes different types of molecular association and complex formation[6]. The contributions of different reactions to the total thermal effect of complex formation of molecules in solution cannot be differentiated by calorimetric studies; also this problem cannot be solved by spectrophotometric and optical methods. NMR spectroscopy has certain advantages compared with calorimetric and optical investigations of molecular complex formation, because it can be used to determine both the equilibrium and structural details of multicomponent complex formations in solution[8]. In addition, investigation of NMR parameters as a function of temperature makes it possible to determine the thermodynamic characteristics of formation of different complexes in solution.

Complex formation between the acridine dye proflavine and self-complementary deoxytetraribonucleoside triphosphates in aqueous solution at constant temperature has been studied previously by one-dimensional and two-dimensional NMR-spectroscopy[6,9]. Using experimental concentration dependences of proton chemical shifts of the interacting molecules and 2D-NOE spectra, the possible types of complexes and their structures have been determined, and analysis of the relative content of different complexes (1:1, 2:1, 1:2, 2:2) has been made[6]. In this work the thermodynamic characteristics of complex formation have been derived from the observed temperature dependences of proton chemical shifts of proflavine and four tetranucleoside triphosphates, 5'-d(CGCG), 5'-d(GCGC), 5'-d(ACGT) and 5'-d(AGCT). The tetramers have different numbers and positions of the preferential pyrimidine-purine binding sites for proflavine, and that binding site is flanked by various nucleoside residuals. Thus, the first of the tetramers contains two CG-sites of preferential binding of proflavine to the duplex, the second and the third tetramer have in the centre one such CG site, flanked at the 5'- and 3'-ends by different nucleosides, whereas the remaining tetranucleotide has no site with pyrimidine-purine base sequence, but contains a GC-site which potentially can be a binding site of the dye to the tetranucleotide both in the monomer and duplex forms[6].

MATERIALS AND METHODS

500 MHz ^{1}H-NMR spectra were recorded on a JEOL GSX-500 NMR spectrometer. Chemical shifts of non-exchangeable protons were measured relative to an internal

reference TMA (tetramethylammonium bromide) and then re-calculated with respect to DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate), i.e. $\delta_{DSS} = \delta_{TMA} + 3.178$ ppm. The residual water peak was saturated during relaxation. The method of preparation of solutions and conditions of the NMR experiments were described earlier[9,10]. The sample temperature during measurements was regulated using the JEOL NM-GVT3-unit. The experimental uncertainty in the temperature measurements was estimated as $\pm$ 0.2°C.

RESULTS AND DISCUSSION

Signal assignments of the spectra of mixed solutions of proflavine with tetranucleotides were carried out earlier using two-dimensional homonuclear COSY and NOESY measurements[9]. Low field ^{1}H-NMR spectra of the solution of the tetranucleotide 5'-d(GCGC) and proflavine at different temperatures (Fig. 1) show resonances of the proflavine aromatic protons, purine and pyrimidine base ring protons, and H1' of the deoxyribose rings protons of the nucleosides. The largest chemical shift changes with temperature are observed for all non-exchangeable dye protons, and the smallest chemical shift changes with temperature are observed for signals of H8(G1) and H8(G3) protons. Considerable changes in the spectra also occur for the H5 and H6 proton resonances of the cytosine residues. It is noteworthy that the fine structure of the spectrum is well-defined at high temperatures, but that substantial broadening of spectral lines takes place at low temperatures. By analysing the concentration dependence of proton chemical shifts of the molecules and 2D-NOE spectra it was shown that there is an equilibrium between different modes of binding of the dye with the tetramer, as well as self-association reactions of the dye and tetramer molecules in solution[6]. Thus, in solutions of proflavine with the tetranucleotides 5'-d(CGCG) and 5'-d(GCGC) at relatively low initial sample concentrations (*ca* 0.5 10^{-3} M) it is found that the most likely molecular associations are dimerisation of the molecules and formation of 1:1, 2:1, 1:2 and 2:2 complexes of the dye and tetramer molecules. In the case of the interaction of proflavine with the tetranucleotides 5'-d(ACGT) and 5'-d(AGCT) formation of the 1:2 complexes is possible in two different ways, by direct binding of the dye with the tetranucleotide duplex and by formation of these complexes by interaction of the tetranucleotide monomer with the 1:1 complex, in which proflavine acts as a 'nucleation centre'. Such 1:2 complexes formed through a 'nucleation centre' are prevalent for the tetranucleotides 5'-d(ACGT) and 5'-d(AGCT) with relatively low melting temperatures[10] whereas it has been shown by us previously that 2:2 complex formation of the dye with these tetramers may be neglected in the general equilibrium in solution[9].

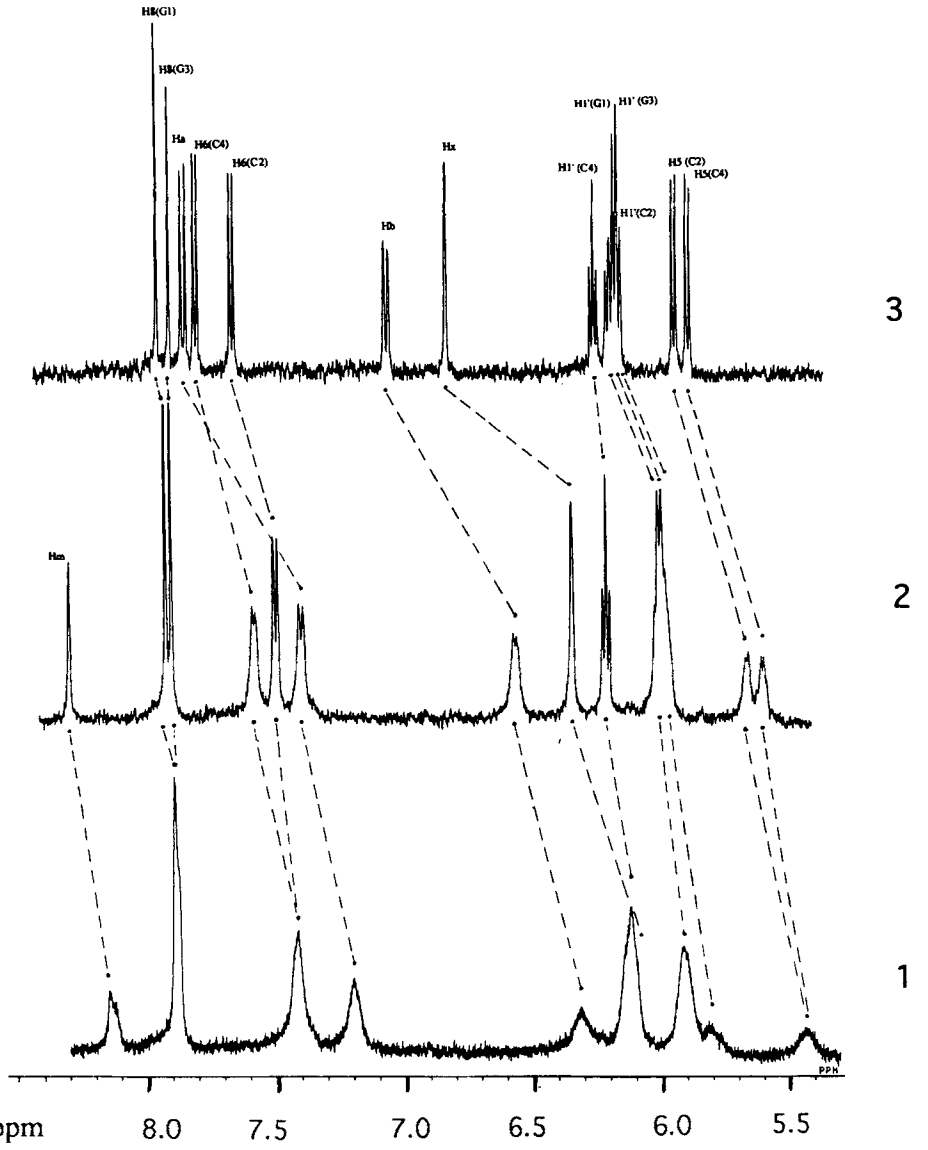

FIG. 1: 500 MHz ^{1}H NMR spectra of aqueous solutions of proflavine and the tetranucleotide 5'-d(GpCpGpC) (P_O = 0.546 mM, N_O = 1.084 mM) at different temperatures: 1-25°C; 2-45°C; 3-70°C. Chemical shifts are quoted relative to internal DSS.

In order to estimate quantitatively the thermodynamical parameters of formation of each type of complex, it is necessary to determine the dependence of their molar fractions on temperature. This can be accomplished by analysis of the experimental dependences of proton chemical shifts of the interacting molecules, taking into account the additive contributions of all associated forms in solution. It is most expedient to make such calculations using the experimental data for proflavine protons, as these resonances experience the largest displacements with variations of temperature. As an example, the experimental results for changes with temperature of the non-exchangeable protons of the dye in solution with 5'-d(GCGC) are presented in Fig. 2. The observed chemical shift δ_i of the *i*-th proflavine proton at a given temperature T can be written in the form

$$\delta_i(T) = f_m(T)\delta_{mi} + f_d(T)\delta_{di} + \sum_{k=1}^{k=4} f_k(T)\delta_{ki} \quad (1)$$

where δ_{mi}, δ_{di}, δ_{1i}-δ_{4i} and $f_m(T)$, $f_d(T)$, $f_1(T)$-$f_4(T)$ are the values of chemical shifts of the *i*-th proflavine proton and equilibrium molar fractions of proflavine at temperature T in the monomer and dimer forms, and in the above-mentioned complexes with tetranucleotides, respectively. It is assumed in relation (1) that the values of δ_{mi}, δ_{di}, δ_{1i}-δ_{4i} do not depend on temperature in the temperature range studied. Such an assumption was shown to be valid for the NMR investigation of proflavine interacting with dinucleotides[8]; specifically the experimental values of proton chemical shifts of proflavine in solution with tetranucleotides at high temperature (*ca* 373K) coincide with the corresponding values of the dye monomer determined using concentration dependences of chemical shifts at 294K. It is significant that the temperature curves of observed chemical shifts δ for all proflavine protons in solutions of the dye with tetranucleotides at high temperatures approach the same numerical values of δ as in pure dye solutions (Fig.2). It shows that at such temperatures practically all types of complexes are completely disassociated and proflavine molecules are released into solution.

In equation (1) the influence of temperature on the observed values of δ is manifested through changes in the molar fractions f_m, f_d, f_1-f_4, which are a function of temperature and simply related to the equilibrium constants of complex formation K_1-K_4. Determination of temperature dependences of equilibrium constants enables the thermodynamical parameters of complex formation ΔH and ΔS to be estimated for different types of complexes. The values of ΔH and ΔS have been calculated in this work by two different methods.

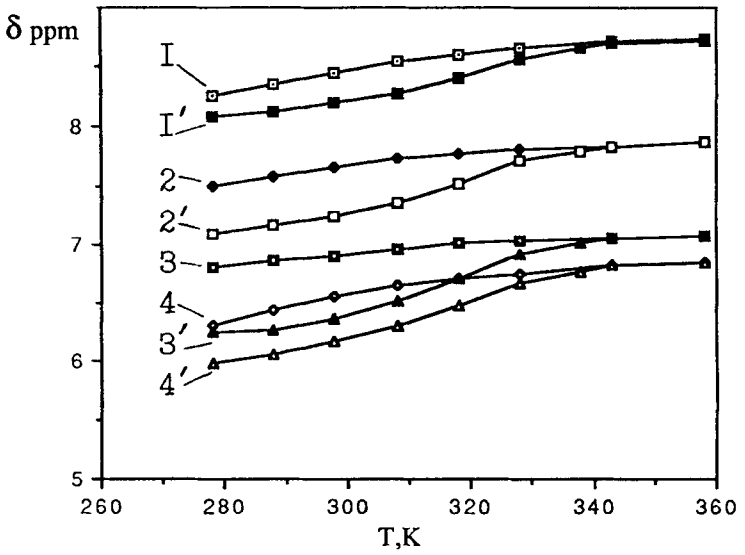

FIG. 2: Experimental temperature dependences of proflavine proton chemical shifts in solution of the dye (curves 1 - 4) and in solution of the dye with the tetranucleotide 5'-d(GCGC) (curves 1' - 4'): 1,1' - H_M; 2,2' - H_A; 3,3' - H_B; 4,4' - H_X.

Method 1

It is expected that application of parametric regression equations would be a suitable computational method for description of the temperature dependences of molar fractions. Utilisation of regression equations of the second order with respect to temperature, as accomplished previously for complex formation of proflavine with mono- and dinucleotides,[8] turned out to be inadequate for the sigmoid-shaped experimental curves (Fig.2). Instead, equations were used that took into account the cooperative nature of the temperature transitions in the PN_2 and P_2N_2 complexes of proflavine binding to 5'-d(CGCG) and 5'-d(GCGC), as well as for PN_2 complexes formed through 'nucleation centres' in solutions with 5'-d(ACGT) and 5'-d(AGCT). The specific type of regression equation was made using the known values of molar fractions at T_1=294K, which were determined from investigations of concentration dependences of proton chemical shifts of the interacting molecules[6]. The experimental fact that at high temperatures (T_O *ca* 373K) practically all the dye in solution is in the monomer form was also taken into consideration, *i.e.* $f_m(T_O)$=1. As a result the temperature dependences of the chemical shifts of the *i*-th proton of proflavine is given by:

$$\delta_i(T) = f_m(T)\delta_{mi} + [f_d(T_1) + a_d(T_o - T)(T_1 - T) +$$

$$+ f_d(T_1)\frac{T}{T_0}\frac{(T_1 - T)}{(T_0 - T_1)}]\delta_{di} + \sum_{n=1,2}[f_n(T_1) + a_n(T_0 - T)(T_1 - T)^2 + \quad (2)$$

$$+ f_n(T_1)\frac{T}{T_0}\frac{(T_1 - T)}{(T_0 - T_1)}]\delta_{ni} + \sum_{k=3,4} f_k(T_1)\frac{1 + S_k(T_1)}{1 + S_k(T)}\delta_{ki}$$

where a_d, a_n are the parameters of the regression equations for molar fractions of dye dimers and complexes 1:1 (PN) or 2:1 (P_2N) of proflavine with monomers of tetranucleotides; $S_k(T) = (\frac{T}{\theta_k})^{b_k}$, θ_k and b_k are the parameters of the regression equations for molar fractions of 1:2 and 2:2 complexes of proflavine with the duplex. Equations for $f_3(T)$ and $f_4(T)$ are presented in the form usually used for descriptions of cooperative 'helix-coil' transitions. In addition the boundary condition at $T=T_O$ and the known values of molar fractions at $T=T_1$ were taken into account. It should be noted that constants θ_k of these regression equations have a definite physical meaning. The value of θ_k corresponds to the melting temperature of the complexes, *i.e.* the temperature when the molar fraction of a given type of complex decreases to half the content compared with its value at low temperatures (*ca* 273K).

The value of $f_m(T)$, included in equation (2), was found from the following relation

$$f_m(T) + f_d(T) + \sum_{k=1}^{k=4} f_k(T) = 1 \quad (3)$$

Unknown parameters of the regression equations were determined from measured values of $\delta_i(T)$ using discrepancy functions derived from variational principles[11]. The numerical procedure of minimization of the discrepancy function was carried out by the simplex method of Nelder-Mead[12] as modified by Gusnin *et al*[13], which is an effective, direct method of minimization. The calculated values of parameters lead to temperature dependences $\delta_i(T)$ which give a good fit to the experimental data. A more accurate approximation of the experimental curves was achieved by minimization of the discrepancy function using not only the parameters indicated in (1) but also the variations of molar fractions f_d, f_1-f_4 at temperature T_1, which were fixed in equation (2). In this case the relative magnitude of the discrepancy between the experimental and calculated values of δ did not exceed 0.5%.

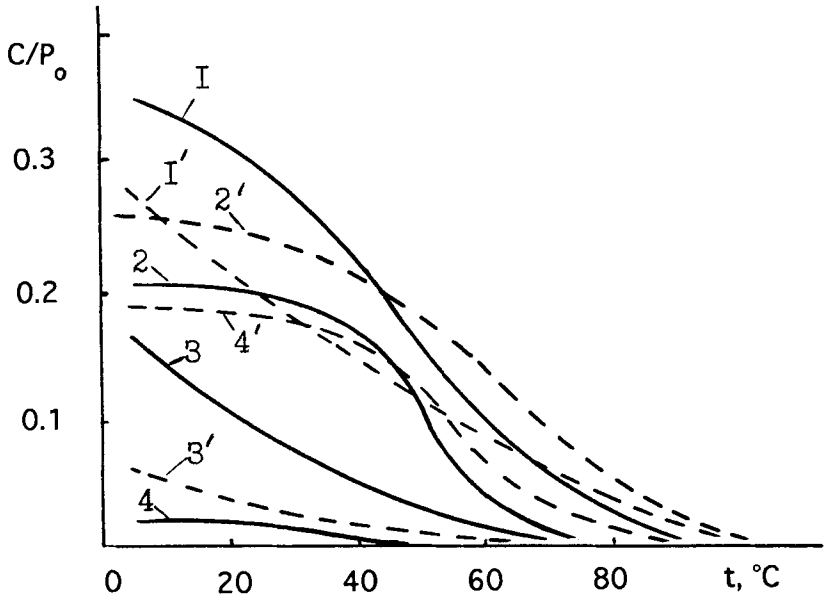

FIG. 3: Calculated temperature dependences of molar fractions of the complexes of proflavine with the tetranucleotides 5'-d(GCGC)(solid lines): and 5'-d(CGCG)(dotted lines): 1,1'-PN complexes; 2,2'-PN_2 complexes; 3,3'-P_2N complexes; 4,4'-P_2N_2 complexes.

The temperature dependences of the molar fractions of different types of complexes are shown in Figs. 3 and 4. It is seen that at low temperatures practically all the dye is in the bound state. The relative amounts of all the complexes gradually decrease with increasing temperature. The fraction of the 1:1 complex remains significant up to high temperatures, around 75-80°C for solutions of the dye with 5'-d(CGCG) and 5'-d(GCGC) and around 55-60°C when proflavine binds to 5'-d(ACGT) and 5'-d(AGCT). The temperature dependences of the molar fractions of 1:2 complexes of the dye with tetranucleotide duplex (curves 2, 2', Figs. 3 and 4) are typical for 'melting' curves of double-helical oligonucleotides[14]. The transition temperature T_m (melting temperature) for the duplex with the intercalated dye molecule is approximately 12-15K greater than the corresponding melting temperature for tetranucleotides without the ligand.

It should be noted that the highest melting temperature (T_m, *ca* 333K) is determined for the 1:2 complex of the dye with 5'-d(CGCG) whereas the equivalent complex with 5'-d(AGCT) is the least thermally stable (T_m *ca* 311K). Complexes of tetranucleotides both in

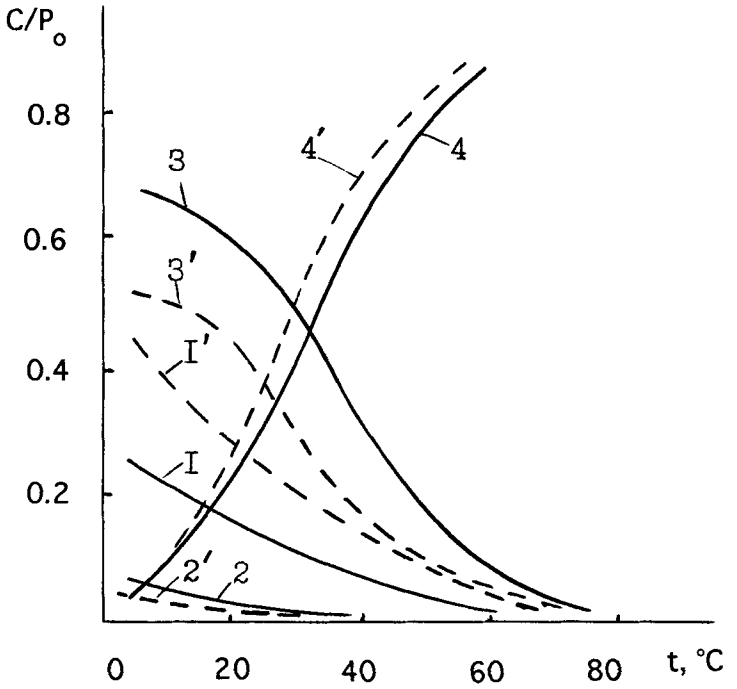

FIG. 4: Calculated temperature dependences of molar fractions of proflavine and complexes of the dye with the tetranucleotides 5'-d(ACGT)(solid lines) and 5'-d(AGCT)(dotted lines). 1,1'-PN complexes; 2,2'-P_2N complexes; 3,3'-PN_2 complexes forming through the "nucleation centre"; 4,4'-proflavine monomers.

the monomer and duplex states with two proflavine molecules are much less stable than the appropriate complexes with one dye molecule; this conclusion is also supported by the calculated values of thermodynamical parameters of reactions of complex formation. The value of T_m determined directly from the experimental dependences of chemical shifts (Fig.2) is somewhat lower than the calculated melting temperature of the 1:2 complex, which is the most stable complex in solution for all the tetranucleotides studied. It must be emphasised that the experimentally-observed melting curves for such multicomponent systems are averages in character and, without the appropriate quantitative analysis, as in this work, it is impossible to make definite conclusions about the temperature stability of one complex or another in solution as the contribution of each type of complex needs to be differentiated. It was shown earlier, that the relative amounts of different types of complexes depend substantially not only on the temperature of the solution but also on the relation of the initial concentrations of dye and tetranucleotide and on the nucleotide sequence in the tetramer[6].

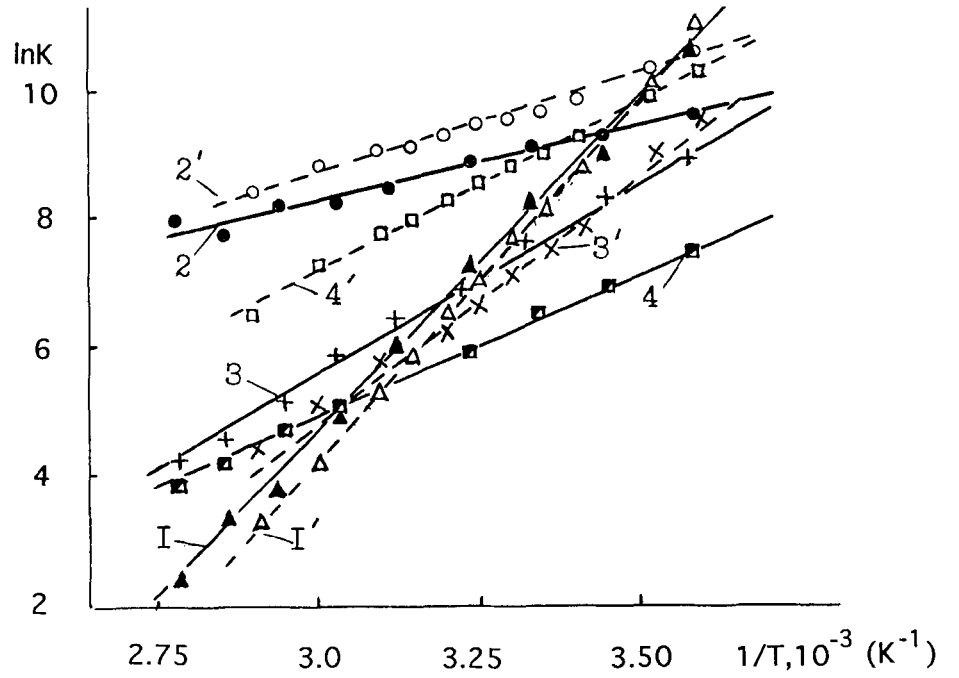

FIG. 5: Plots of ln K vs 1/T for the reactions of complex formation of proflavine with the tetranucleotides 5'-d(GCGC) (solid lines) and 5'-d(CGCG)(dotted lines): 1,1'-PN complexes; 2,2'-P_2N complexes; 3,3'-PN_2 complexes; 4,4'-P_2N_2 complexes.

Equilibrium constants of complex formation K_1-K_4 at different temperatures were determined from calculated values of molar fractions using the mass law equations and the mass conservation law for each reaction. Van't-Hoff's plots, lnK = f(1/T), used to estimate the enthalpy and entropy of complex formation between proflavine and the tetranucleotides in solution are shown in Figs 5 and 6. The data are fitted well by straight lines confirming the negligibly small influence of heat-capacity change in the temperature range studied. Enthalpies of the reactions were evaluated from the slopes of approximately straight lines in conformity with Van't-Hoff's relation

$$\frac{d(\ln K)}{d(1/T)} = -\frac{\Delta H}{R} \qquad (4)$$

Entropy was estimated from Gibbs' free energy, $\Delta G = -RT\ln K$, and enthalpy

i.e., $\Delta S = -(\Delta G - \Delta H)/T$ (5)

The derived values of enthalpy and entropy of the reactions of proflavine with each tetranucleotide in solution are summarised in the Table.

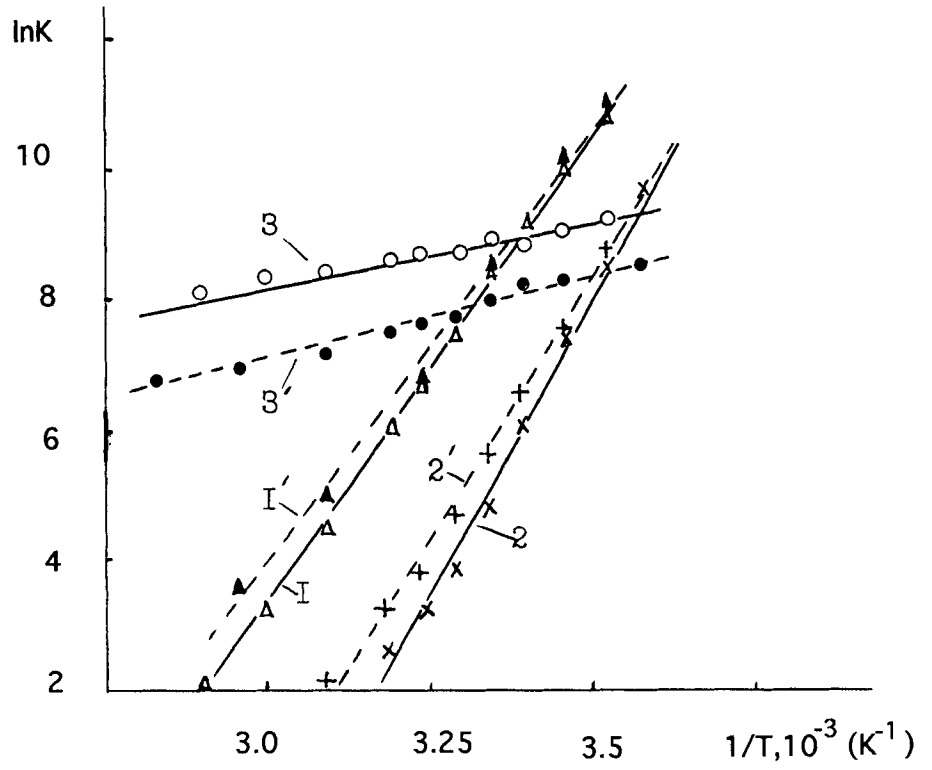

FIG. 6: Plots of ln K vs 1/T for the reactions of complex formation of proflavine with the tetranucleotides 5'-d(ACGT)(solid lines) and 5'-d(AGCT)(dotted lines): 1,1'-PN complexes; 2,2'-P_2N complexes; 3,3'-PN_2 complexes formed through the 'nucleation centre'.

Method 2

By taking into account the mass law equations and the mass conservation law, the equation for determining the observed chemical shift of i-th proton of proflavine can be written in the form[6]

(i) when the dye interacts with 5'- d(CGCG) and 5'-d(GCGC)

$$\delta_i = \frac{P}{P_O}(\delta_m + 2K_P P\delta_d + K_1 N\delta_1 + 2K_1K_2PN\delta_2 + K_1K_3N^2\delta_3 + 2K_TK_3K_4PN^2\delta_4) \quad (6)$$

(ii) when the dye interacts with 5'-d(AGCT) and 5'-d(ACGT)

$$\delta_i = \frac{P}{P_o}(\delta_m + 2K_P P\delta_d + K_1 N\delta_1 + 2K_1K_2PN\delta_2 + K_1K_3N^2\delta_3 + K_TK_4N^2\delta_4) \quad (7)$$

Here P_0 and N_0 are the initial molar concentrations of dye and tetranucleotide. In relations (6) and (7) the influence of temperature on the values of molar fractions f_m, f_d, f_1-f_4 is determined by the dependence on temperature of the equilibrium constants K_P, K_T, K_1-K_4 and molar concentrations of proflavine (P) and tetranucleotide (N) monomers. Constants of the appropriate reactions, in turn, can be expressed with the help of thermodynamical parameters ΔH^o and ΔS^o

$$K(T) = \exp\left(\frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT}\right) \qquad (8)$$

assuming that values of ΔH^o and ΔS^o do not depend substantially on temperature in the range studied. The thermodynamical parameters of proflavine (ΔH_P^o, ΔS_P^o) and tetranucleotide (ΔH_T^o, ΔS_T^o) self-association were determined previously[10,11] from investigations of the temperature dependences of proton chemical shifts of the molecules at the same conditions of the experiment. Thus, taking into account the known values of δ_m, δ_d, δ_1-δ_4[6,11] the observed chemical shift δ in relations (6) and (7) is a function of 8 unknown parameters ΔH_i^o, ΔS_i^o (i = 1-4). Minimisation of the discrepancy function between experimental and calculated values of δ enables the optimum values of the thermodynamical parameters of complex formation to be determined in solution. The calculations were carried out using experimental chemical shift data for all the non-exchangeable protons of proflavine. The mean values of ΔH^o and ΔS^o are summarised in the Table. It is significant that, within error limits, the thermodynamical parameters obtained by the two different methods are in good agreement with each other.

It is seen from the Table that the enthalpies of complex formation are negative in all cases, but their absolute values differ significantly. It is known that exothermic reactions are typical for aggregation processes including stacking interactions of molecules with unpaired π-electrons such as purine bases, acridine dyes and so on[15-17]. In general the thermodynamical characteristics of dye interactions with oligonucleotides can be determined by the following factors[18]: i) molecular interactions (hydrogen bonding, hydrophobic, van der Waals and electrostatic interactions); ii) conformational alterations in the tetranucleotide and dye molecules on dye binding; iii) changes in hydration, release of the solvent counterion or proton in the interaction of the molecules. Unfortunately, there is no consensus as to the magnitude of the contributions of each of the above factors to the measured thermodynamical parameters. It is known that dispersive van der Waals forces are characterised both by negative enthalpy and negative entropy[19]. Hydrogen bonding in complex formation also leads to negative values of ΔH and ΔS[20,21]. At the same time some processes of complex formation give positive contributions to the overall changes of

TABLE: Thermodynamic parameters of complex formation between proflavine and deoxytetranucleotides in aqueous solution

Tetranu-cleotide	Type of complex	Method 1		Method 2	
	P:N	ΔH kcal mol^{-1}	ΔS(293K) cal.$mol.^{-1}K^{-1}$	ΔH^o kcal. $mol.^{-1}$	ΔS^o cal.$mol.^{-1}$ K^{-1}
5'-d(CGCG)	1:1	-22.5(±1.8)	-60(±5)	-18.4(±1.4)	-46(±4)
	2:1	-13.4(±1.1)	-30(±3)	-15.0(±1.1)	-33.3(±1.8)
	1:2	-4.6(±0.4)	+4.1(±0.4)	-4.8(±0.4)	+3.6(±0.3)
	2:2	-10.2(±0.9)	-16.4(±1.4)	-9.7(±0.8)	-16.5(±1.1)
5'-d(GCGC)	1:1	-20.5(±1.6)	-53(±4)	-17.0(±1.4)	-41.4(±3.6)
	2:1	-12.4(±1.1)	-26.0(±2.1)	-13.0(±1.2)	-27.0(±2.1)
	1:2	-4.9(±0.4)	+1.6(±0.5)	-2.3(±0.4)	+4.3(±0.7)
	2:2	-8.8(±0.8)	-16.9(±1.5)	-8.3(±0.8)	-17.7(±1.5)
5'-d(ACGT)	1:1	-29.7(±2.2)	-82(±7)	-28.5(±2.1)	-81(±5)
	2:1	-37.0(±2.4)	-114(±8)	-36.3(±2.6)	-110(±7)
	1:2	-3.7(±0.5)	+6.0(±1.5)	-2.9(±0.5)	+5.6(±0.8)
5'-d(AGCT)	1:1	-27.1(±1.8)	-74(±4)	-26.2(±2.0)	-71.5(±4.5)
	2:1	-34.0(±2.7)	-103(±9)	-33.1(±2.6)	-100(±8)
	1:2	-5.5(±0.5)	-5.3(±1.6)	-5.1(±0.5)	-4.1(±1.2)

enthalpy and entropy. Hopkins *et al* [7] suggest that enthalpy changes for dye binding with double-helical DNA is a sum of at least six components

$$\Delta H = \Delta H_{bp} + \Delta H_{bc} + \Delta H_{el} + \Delta H_{sol} + \Delta H_{conf} + \Delta H_{H\text{-}b} \quad (9)$$

where the following factors are included: diminution of the stacking interactions of the bases caused by formation of the cavity for the dye molecule to occupy (ΔH_{bp}); stacking interactions of the bases in the cavity with dye chromophore (ΔH_{bc}); electrostatic interactions between dye cations and negatively charged phosphates of nucleotides (ΔH_{el}); alteration of solvation due to complex formation (ΔH_{sol}); different arrangements of the intercalator in the complex (ΔH_{conf}); specific hydrogen bonds which dye molecules form in the complex ($\Delta H_{H\text{-}b}$). Additional components for ΔH may also be taken into account if the intercalator induces considerable conformational alterations in DNA. Some components of enthalpy change may adopt positive values such as ΔH_{bp}, ΔH_{sol} and ΔH_{conf}[7,20].

Positive entropy contributions are determined, first of all, by hydrophobic interactions due to transfer of the dye molecule from solvent to the intercalation site. Considerable hydrophobic interactions may be expected for 1:2 complexes of the dye with the tetranucleotide duplex, which is confirmed in this work by the entropy changes being more positive for the reaction of 1:2 complexes formation of proflavine with 5'-d(GCGC), 5'-d(CGCG) and 5'-d(ACGT), respectively. The entropy change for the 1:2 complex of the dye with 5'-d(AGCT) is negative. One may assume that in 1:2 complexes some contribution to the negative values of ΔS will be given by an increase of the rigidity of the double-helical structure due to intercalation of the dye molecule[22]. It should be accompanied by a decrease of entropy in view of limiting the number of accessible conformational states[21].

It is likely that the negative values of entropy observed for the 2:2 complex formation of the dye with the tetranucleotides 5'-d(CGCG) and 5'-d(GCGC) can be explained by an increase of the rigidity of the double-helical structures containing two intercalated dye molecules. However, the absolute values of ΔS for such complexes are considerably smaller than the entropy changes for complex formation between proflavine and monomers of these tetranucleotides which shows that hydrophobic interactions play an essential part in 2:2, as well as in 1:2, complex formation in solution. It should be noted that some positive contributions to enthalpy and entropy result from the increase of the length of oligonucleotide molecule due to intercalation of the dye and disruption of normal base stacking[23]; this is likely to be the explanation for the differences in ΔH and ΔS magnitudes for 1:1 and 2:1 complexes of proflavine with the tetranucleotides 5'-d(CGCG) and 5'-d(GCGC). Relatively large negative values of ΔH and ΔS for the 2:1 compared with the 1:1 complexes between proflavine and the tetranucleotides 5'-d(ACGT) and 5'-d(AGCT) indirectly confirm the assumption previously made[6] that the second dye molecule binds to the 1:1 complex from the outside by means of vertical stacking to the nucleosides at the end of the chain. Such a process of complex formation does not require bases in the tetramers to move aside and accordingly disrupt stacking interactions giving a positive contribution to ΔH and ΔS.

In this work methods have been developed for analysing the temperature dependences of NMR experimental parameters of drug-nucleic acid interactions enabling the contributions of different reactions of complex formation to be differentiated as well as separate enthalpy and entropy effects of each interaction in solution. The results for the acridine dye proflavine and four tetranucleotide duplexes can be interpreted in terms of the main types of intermolecular interactions responsible for formation of the different complexes (1:1, 1:2, 2:1, 2:2) in aqueous solution.

ACKNOWLEDGEMENTS

We thank the British Council for a Senior Travel Fellowship (to A.N.V.) and the University of London Intercollegiate Research Service for access to the 500 MHz Biomedical NMR facility (at Birkbeck College).

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Received 8/2/93
Accepted 10/26/93