Microcalorimetry of interaction of dihydro-imidazo-phenanthridinium (DIP)-based compounds with duplex DNA

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Abstract

Isothermal titration (ITC) and differential scanning calorimetry (DSC) have been used to screen the binding thermodynamics of a family of DNA intercalators based on the dihydro-imidazo-phenanthridinium (DIP) framework. All members of this DIP-based ligand family bind to both genomic (calf thymus and/or salmon testes) and a synthetic dodecamer d(CGCGAATTCGCG) duplex DNA with broadly similar affinities regardless of side chain size or functionality. Viscosity measurements confirm that binding satisfies standard criteria for intercalation. Binding is exothermic but with an additional favourable positive entropy contribution in most cases at 25 °C, although a significant negative heat capacity effect (ΔC_p) means that both $\Delta H°$ and $\Delta S°$ decrease with increasing temperature. DIP-ligand binding to DNA also shows significant entropy–enthalpy compensation effects that are now almost standard in such situations, probably reflecting the conformational flexibility of macromolecular systems involving a multiplicity of weak non-covalent interactions. This ability to vary side chain functionality without compromising DNA binding suggests that the DIP framework should be a promising basis for more adventurous chemistry at the DNA level. © 2006 Elsevier B.V. All rights reserved.

Keywords: Isothermal titration calorimetry (ITC); Differential scanning calorimetry (DSC); Viscosity; Intercalation

1. Introduction

Julian Sturtevant pioneered the use of calorimetric methods to probe the thermodynamics of stability and interactions of DNA almost 50 years ago [1]. What was then a technically demanding exercise has now become a routine laboratory method for the non-invasive analysis of nucleic acid interactions and for the exploration of the still poorly understood fundamentals of biomolecular interaction thermodynamics in solution, not least for its importance in the rational design and potential applications of chemotherapeutic agents, DNA probes and antibiotic/antiviral compounds [2–6]. Here, we describe the use of calorimetric titration (ITC) and differential scanning (DSC) methods, together with classical viscometry measurements, to characterize the binding (intercalation) to DNA of a range of new compounds based on the dihydro-imidazo-phenanthridinium (DIP) framework [7]. This expands the database for DNA-intercalation interactions for which thermodynamic data are currently relatively scarce [5,8].

The versatility of the DIP framework (Figs. 1 and 2) and the associated synthetic methods [7,9] allows the design of a wide range of potential DNA-binders through the facile incorporation of various features, which can be tailored to affect minor/major groove binding and inter-chain interactions in addition to the intercalation effects that might be anticipated for planar aromatic molecules of this kind. This has already been demonstrated to yield promising cytotoxic compounds with inherent stability in a biological environment as suitable candidates for therapeutic purposes [9]. Work is now focused on the elaboration of different substituents on the underlying DIP framework to give enhanced binding, selectivity, solubility and other physicochemical features relevant to practical drug design. The thermodynamics of such interactions and the way in which they are affected by different substituents forms a major component of this programme. In this initial thermodynamic screen we compare data for a series of 18 compounds for binding to genomic DNA (salmon testes, calf thymus) and a synthetic DNA duplex.

2. Experimental

DIP-based ligands and related compounds **1**–**18** were synthesized, purified and characterized following our established techniques [7]. Other reagents, purchased from Sigma-Aldrich and used without further purification, were as follows: salmon testes DNA ("ST-DNA", D1626), calf thymus DNA ("CT-DNA", D1501), ethidium bromide ("EtBr", E8751) and netropsin hydrochloride (N9653). HPLC-purified B-DNA (Dickerson et al. [10,11]) dodecamer ("12-mer"), d(CGCGAATTCGCG), was obtained from MWG-Biotech AG (Germany). All solutions were prepared in pH 7.0 phosphate buffered saline (12 mM Na_2HPO_4, 4 mM NaH_2PO_4, 1 mM EDTA, 0.2 M NaCl) unless otherwise indicated. Concentrations were determined by weight (for DIP ligands) or from UV absorbance (for DNA, expressed per mole of base pairs) using the following extinction coefficients: ε_{260} (DNA)=12824 Mbp^{-1} cm^{-1}, ε_{480}(EtBr)=5600 M^{-1} cm^{-1}, ε_{296} (netropsin)=21,500 M^{-1} cm^{-1}. B-DNA dodecamer solutions were annealed prior to use by repeated heating to 95 °C and slow cooling overnight to room temperature, and predominant duplex formation confirmed by monitoring the pronounced hypsochromic shift at 260 nm.
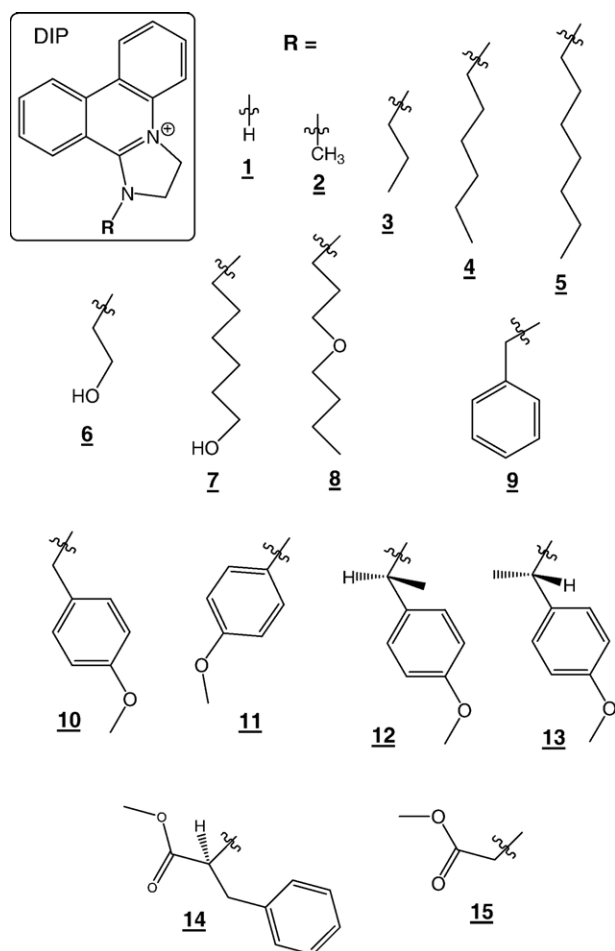

Fig. 1. Structures of DIP-based ligands **1**–**15**. The parent dihydro-imidazo-phenanthridinium (DIP) framework is shown framed, with appropriate substituent (**R**) groups numbered. All compounds synthesized as the Br salt.
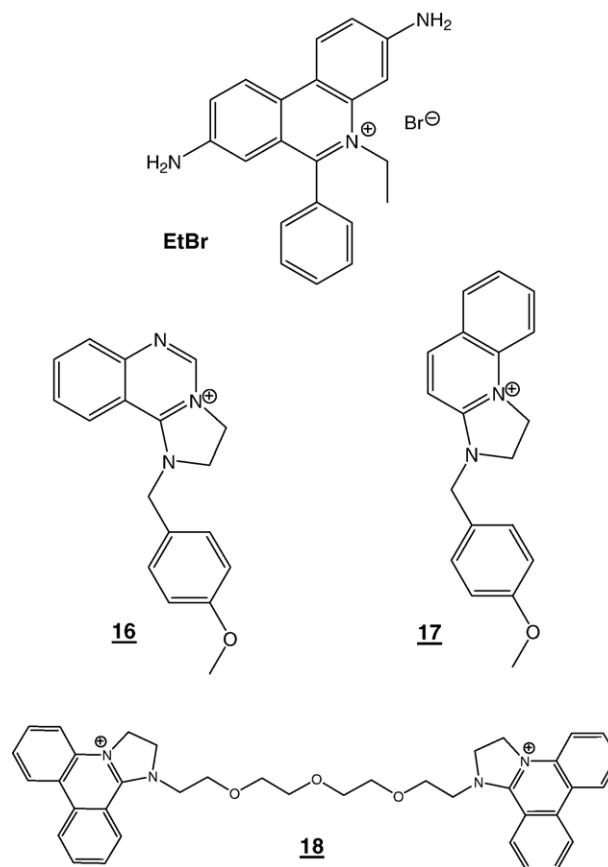

Fig. 2. Structures of ethidium bromide (EtBr), non-DIP ligands **16**–**17** and the "double-headed" DIP–ligand **18**.

2.1. Isothermal titration calorimetry

DNA–ligand complexation thermodynamics in solution were measured by isothermal titration calorimetry (MicroCal VP-ITC) in the 10–40 °C temperature range following standard instrumental procedures [12,13]. A typical experiment involved an initial 1 μl pre-injection followed by 25–30 sequential 10 μl injections of ligand solution (ca. 1 mM) into the ITC cell containing DNA (ca. 0.3 mM base pairs, 1.4 ml working volume, 320 rpm stirring). Control experiments involved identical injections into buffer alone for ligand dilution heats. Titration data were corrected for dilution heats and analyzed using a single-set-of-sites equilibrium binding model (MicroCal Origin™) to give the apparent binding stoichiometry (N), association/dissociation constants (K_A=1/K_D) and enthalpy of binding (ΔH^0). Other thermodynamic quantities were calculated using standard expressions: $\Delta G° = -RT \ln(K_A) = \Delta H^0 - T\Delta S°$, $\Delta C_p = d\Delta H^0/dT$, 1 cal=4.184 J.

2.2. Differential scanning calorimetry

DSC experiments on DNA and ligand complexes were conducted on a VP-DSC calorimeter and thermal transitions were analyzed using MicroCal Origin 5.0™ software following subtraction of the instrumental buffer–buffer baseline. The sample cell in each experiment contained a pre-mixed solution of 0.3 mM bp salmon testes DNA and 1 mM ligand prepared in

phosphate buffer solution, with identical buffer in the reference cell. Samples were briefly degassed before loading and scanned over a 20–120 °C temperature range at a scan rate of 60°/h.

2.3. Viscometry

The relative viscosities ($\eta = (t - t_0)/t_0$) of DNA solutions in the presence and absence of ligands were determined by capillary viscometry at 25 °C using a Cannon-Fenske viscometer with flow time for buffer (t_0) of ca. 60 s. Salmon testes DNA (0.4 mM base pairs) and ligand solutions (up to 1 mM) were prepared in phosphate buffer and quantified by UV absorbance. For each experiment 10 ml of DNA (for η_0) or DNA/ligand solution (η) was added to the flow tube and left to equilibrate in the viscometer bath for 30 min prior to flow time measurements. Controls using ligand solutions in the absence of DNA were performed to confirm that any changes in viscosity were solely attributable to ligand-induced effects on DNA conformation. Each experiment was repeated four times.

3. Results

Typical experimental ITC data for the binding of DIP ligands to DNA are illustrated in Fig. 3 and the resulting binding
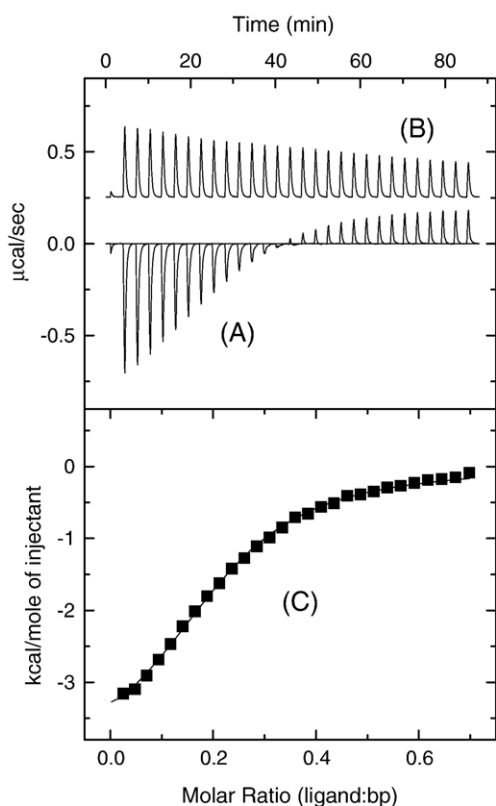

Fig. 3. Illustrative ITC data for binding of ligand **4** to salmon testes DNA at 25 °C. The upper panel shows the raw data (sequential heat pulses) for both ligand–DNA binding (A) and ligand dilution control (B) (offset for clarity). The lower panel (C) shows the integrated heat data after subtraction of the (endothermic) dilution heats, fit to a single-set-of-sites binding model with parameters given in Table 1.

parameters tabulated in Table 1. Data for binding of ethidium bromide are in close agreement with previously published studies under similar conditions [6,14]. All the DIP ligands examined so far give ITC thermograms consistent with exothermic binding to DNA. In addition, ligand dilution control experiments in all cases show relatively large endothermic heats of mixing consistent with dissociation of non-covalent intermolecular homo-dimers or larger oligomers, as is commonly seen for planar hydrophobic compounds such as these in aqueous solution. After taking into account such heats of dilution, the differential titration curves arising from the integrated heat pulse data in most cases fit reasonably well to a simple non-interacting identical site model that gives empirical estimates for the apparent stoichiometry N (ligands per mole base pairs), affinity K_A and enthalpy of binding. ITC experiments at different temperatures show that the enthalpies of binding of DIP ligands to ST-DNA decrease linearly with temperature, giving rise to the negative ΔC_p values reported in Table 1. (Similar experiments with the 12-mer were less informative because of the broad thermal melting profile for such small oligomeric DNA duplexes.)

The DIP framework seems to be obligatory for binding in this series, since compounds lacking this feature (e.g. **16** and **17**) show no heat effects upon mixing with DNA. This pattern is confirmed by DSC experiments (Fig. 4) on the effects of added ligands on the thermal stability of calf thymus DNA. In the absence of ligands, DSC thermograms show a sharp melting transition with a T_m of 88 °C, as anticipated for DNA duplex of this base composition, and this is unaffected by addition of the non-binding compounds (e.g. **17**). By contrast, in the presence of excess EtBr or DIP ligands the stability of the DNA is significantly enhanced, with T_m's increasing to around 100 °C or higher. The largest effect ($T_m = 109$ °C, $\Delta T_m = 21$ °C) arises with the DIP-dimer compound **18**, suggesting that possible cooperativity or inter-strand linkage with this double-ended intercalator is further stabilizing the duplex DNA in this case.

Of course, the calorimetric data do not give any direct information about the way in which these DIP ligands or other compounds interact with the DNA, whether as intercalators, major/minor-groove binders or in some other more complex fashion. For non-fluorescent compounds such as these, changes in solution viscosity are most informative, in particular the increase in viscosity arising from increased lengthening and stiffening of the DNA chain by insertion of planar ligands between adjacent base pairs is a strong indicator of intercalation [15]. Fig. 5 illustrates how the viscosities of ST-DNA solutions are increased in the presence of a classic intercalator (ethidium bromide) or DIP-based ligands **1** and **11**, but not by a known minor-groove binder (netropsin) nor by compounds such as **16** for which ITC and DSC data indicate no binding to DNA. For the DIP-based ligand **1**, this increase in viscosity depends on the relative concentrations (Fig. 5) and saturates at a ligand/bp molar ratio of around 0.4, similar to the apparent N values obtained by calorimetric titrations.

The possible polyelectrolyte contribution to the free energy of ligand binding was examined by repeating the ITC titra-

Table 1
Thermodynamic parameters for the binding of DIP-based and related ligands to DNA in phosphate buffer (12 mM Na_2HPO_4, 4 mM NaH_2PO_4, 1 mM EDTA, 0.2 M NaCl, pH 7.0) at 25 °C, as determined by isothermal titration calorimetry (ITC)[a]

Ligand	DNA	N	K_A (10^4 M^{-1})	ΔG^0 (kcal mol^{-1})	ΔH^0 (kcal mol^{-1})	ΔS^0 (cal mol^{-1} K^{-1})	ΔC_p (cal mol^{-1} K^{-1})
EtBr	12-mer	0.38	7.13	−6.6	−5.0	5.5	
	ST	0.32	12.9	−7.0	−8.9	−6.5	−133.5
	CT	0.32	11.7	−6.9	−7.3	−1.4	(lit. −139±30) [14]
1	12-mer	0.31	1.5	−5.7	−3.6	7.0	
	ST	0.30	2.6	−6.0	−3.6	8.0	nd
	CT	0.30	2.0	−5.9	−4.8	3.5	
2	12-mer	0.39	1.7	−5.8	−4.0	5.9	
	ST	0.19	4.6	−6.4	−5.9	1.5	−132
3	12-mer	0.39	1.7	−5.8	−3.3	8.2	
	ST	0.17	3.5	−6.2	−5.0	4.0	nd
	CT	0.17	4.1	−6.3	−5.2	3.8	
4	12-mer	0.42	1.7	−5.8	−1.65	13.9	
	ST	0.21	4.9	−6.4	−4.0	7.9	−130
	CT	0.21	4.3	−6.3	−4.1	7.5	
5	12-mer	0.37	1.7	−5.8	−2.6	10.8	
	ST	0.20	5.4	−6.5	−4.1	7.9	nd
	CT	0.19	4.3	−6.3	−4.2	7.1	
6	12-mer	0.32	1.6	−5.7	−3.9	6.1	
	ST	0.16	2.0	−5.9	−5.5	1.2	nd
	CT	0.15	2.1	−5.9	−6.1	−0.7	
7	12-mer	0.45	2.3	−5.9	−2.2	12.5	
	ST	0.36	7.0	−6.6	−4.3	7.8	−207
8	12-mer	0.61	0.94	−5.4	−1.6	12.7	
	ST	0.19	2.2	−5.9	−4.7	4.2	−127
9	12-mer	0.37	1.1	−5.5	−2.9	8.7	
	ST	0.15	4.3	−6.3	−4.7	5.3	nd
10	12-mer	0.40	1.1	−5.5	−4.3	4.1	
	ST	0.26	2.9	−6.1	−4.4	5.8	nd
	CT	0.22	2.0	−5.9	−5.3	1.9	
11	12-mer	0.29	2.1	−5.9	−2.6	11.2	
	ST	0.18	4.2	−6.3	−5.8	1.9	−183
	CT	0.19	3.1	−6.1	−5.7	1.4	
12	12-mer	0.40	1.9	−5.8	−3.0	9.5	
	ST	0.21	5.2	−6.4	−4.7	5.8	nd
	CT	0.25	4.1	−6.3	−5.0	4.3	
13	12-mer	0.42	1.2	−5.6	−2.1	11.6	
	ST	0.26	2.8	−6.1	−4.1	6.6	nd
	CT	0.26	2.6	−6.0	−4.0	6.8	
14	All	nd	nd	See note 1	Exothermic	nd	See note 1
15	ST	0.18	1.6	−5.7	−6.2	−1.5	nd
16	All	–	–	–	No binding	–	–
17	All	–	–	–	No binding	–	–
18	12-mer	0.45	29.3	−7.5	−1.2	20.9	
	ST(1)	0.18	150	−8.4	−6.5	6.5	nd
	−(2)	0.47	80	−8.1	+1.1	31	See note 2

Note 1: Although DIP-ligand **14** (and its enantiomer, not shown) gave strong exothermic effects with DNA, the resulting ITC thermogram did not fit well to a one-set-of-sites model, indicating a more complex mode of binding in this case. Note 2: ITC data for CT-DNA: **18** approximate fit to two separate sites model.
[a] Estimated errors (based on repeat measurements and systematic errors arising from accuracy limitations of concentrations, etc.): $N\pm0.1$, $K\pm10\%$, $\Delta H^0\pm0.1$ kcal mol^{-1}, $\Delta C_p\pm10$ cal mol^{-1} K^{-1}. nd=not determined.

tions of DIP-ligand **4** with ST-DNA in phosphate buffer at 25 °C over a range of salt concentrations up to 1.2 M NaCl. Increased salt concentration destabilizes the DNA–ligand interaction and the behaviour is very similar to that seen here with ethidium bromide under the same conditions. A plot of $\ln(K_A)$ versus $\ln[NaCl]$ (Fig. 6) is linear with slope −0.81, which compares to the range −0.88 to −1.24 reported elsewhere [5,6,16] for the binding of other monovalent cations to DNA.

4. Discussion

We have shown here that a broad family of ligands involving simple derivatives of the dihydro-imidazo-phenanthridinium (DIP) framework can bind to duplex DNA from various sources. Viscosity measurements confirm expectation that these compounds bind predominantly by intercalation between adjacent base pairs in the DNA double helical structure, and the thermodynamic parameters–particularly ΔC_p
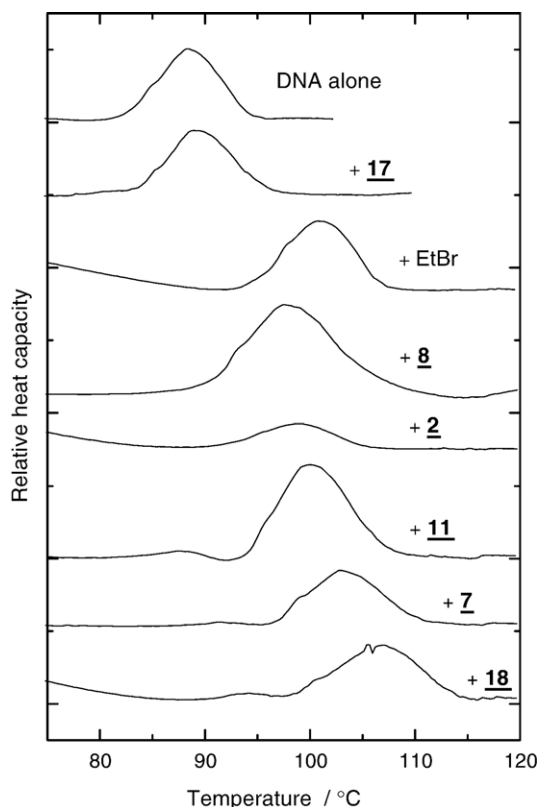
Fig. 4. DSC thermograms for ST-DNA in the presence and absence of various putative ligands.
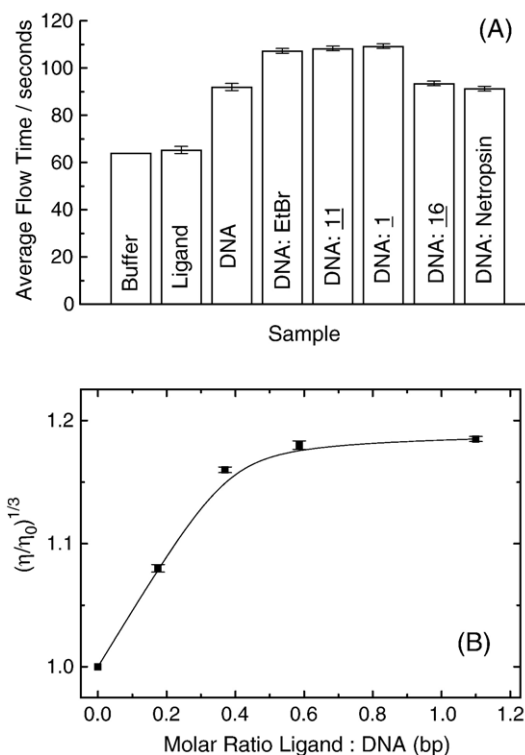

Fig. 5. The effect of added ligand on the relative viscosities of DNA solutions at 25 °C. (A) Comparison of the average flow times for ST-DNA (0.4 mM bp) and DIP-ligand (1 mM) mixtures, together with an intercalator (EtBr) and (minor) groove-binder (netropsin) as controls. (B) Increase in apparent DNA length $(\eta/\eta_o)^{1/3}$, with increasing concentration of DIP-ligand **1**.

and salt effects–are similar to what has been observed previously for other known intercalators. Presumably, this mode of binding primarily involves intercalation of the planar DIP moiety, leaving the pendant substituent R-groups free to interact (or not, as the case may be) with the phosphate backbone, major/minor groove or other regions of the DNA duplex.

An interesting feature of the thermodynamic data (Table 1) is that, despite the relatively wide variety in shape, size and functionality of the R-groups explored here, the variations observed in apparent binding free energies are relatively small. This would suggest that binding free energy is dominated by the DIP group and that the substituent groups can be varied almost at will without compromising binding. In some respects, this might be somewhat disappointing, since it suggests that the R-groups have relatively little interaction with the DNA, thereby reducing any possibility of using such groups to engineer-in any additional features such as enhanced binding or base-pair/ sequence specificity for instance (and certainly we see little evidence of such specificity here). However, this does leave us free to contemplate incorporation of more useful chemical functionalities without too much fear of impeding binding. This might allow us, for example, to have greater control over ligand solubility and membrane permeability, or to incorporate photo-cleavable or other chemo-active groups suitable for DNA-targeted reactions.

Although the free energies of binding of the DIP ligands show relatively little variation, either between different ligands

or with different DNAs, the enthalpies and entropies show much greater spread. It is interesting to plot the apparent correlation between binding enthalpies (ΔH^0) and entropies (ΔS^0) for all the species considered here (Fig. 7). This apparently strong linear correlation, even involving different chemical species, is in reality just another example of the enthalpy–entropy compensation frequently seen in such circumstances [17,18]. Such effects may simply be artifacts arising from experimental limitations. Alternatively, they may reflect the underlying linkage between enthalpy and entropy, especially in relatively flexible macromolecular systems involving a multiplicity of
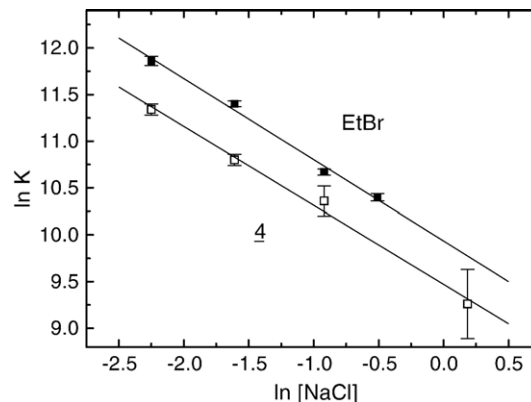

Fig. 6. Comparison of the effect of salt concentration, [NaCl], on the apparent binding affinity of ethidium bromide and DIP ligand **4** with ST-DNA at 25 °C.
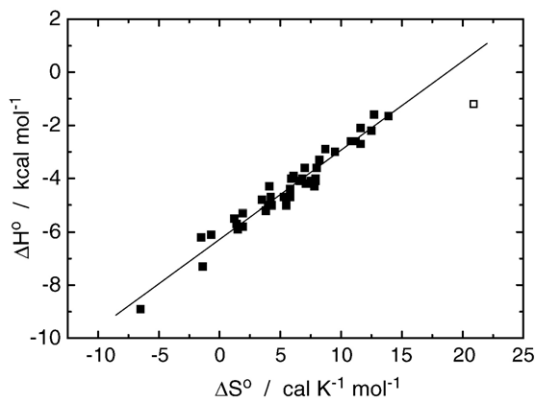
Fig. 7. Entropy–enthalpy plot for DNA-intercalating ligands at 25 °C using data from Table 1. Note that each point corresponds to a different ligand with a different DNA. The outlying point (open symbol) is for ligand **18** binding to the DNA dodecamer (12-mer).

cooperative weak interactions [17,19]. Interestingly, the one $\Delta H^0/\Delta S^0$ point that does not follow this trend is for the relatively strong interaction of the DIP-dimer (ligand **18**) with DNA dodecamer (12-mer) for which the mode of binding is likely to be more complex than for other systems due to the possible dual insertion of the DIP moieties into the relatively small DNA molecule.

The apparent stoichiometry (N) values estimated here lie in the range 0.15–0.4 for most ligands binding to DNA. This is consistent with what might be expected from neighbour exclusion models and compares well to values obtained for other intercalators [20]. The reciprocal ($1/N \approx 2.5$–7) might be viewed roughly as the number of base pairs spanned by each ligand, so again the numbers are not unreasonable. However, the absolute magnitudes of such estimates should be treated with some caution for a number of reasons. Firstly, N values obtained by the ITC methods used here rely on the accuracy of the ligand and DNA concentration estimates. For DNA concentrations determined by UV absorbance, this is particularly troublesome, since measured and calculated extinction coefficients can differ by as much as 20% depending on conditions [21]. In addition, measurements for ST/CT DNA may be subject to further error due to the unknown fraction of single-stranded DNA possibly present in the commercial materials and, despite careful annealing, the assumption that synthetic dodecamer samples are entirely self-complementary duplex may be compromised by the presence (hopefully small) of single-strand hairpin conformers. Moreover, although the data do not usually merit more complex analysis, the minimal identical-sites binding model used here for empirical fitting of the ITC differential binding curves is clearly too simple to express the inevitable heterogeneity of intercalation sites and neighbour interactions in the DNA. Nor do we know whether the mode of insertion of these DIP intercalators involves approach from the major groove, minor groove or both. Despite all these caveats, the N values obtained here are physically reasonable and, although the absolute values may be subject to error, any trends or variations within a particular ligand series may be informative.

Such concerns arising from uncertainties in exact DNA concentrations are less pronounced for the enthalpies (ΔH) and binding affinities (K_A), since, with the ITC protocols used here, these empirical parameters depend mainly on the accuracies of the ligand concentrations and are relatively insensitive to variations in estimated DNA content. However, particularly for comparison of K_A values, there is another factor that must be taken into account, at least in principle. We have seen from the ITC ligand dilution controls that, as anticipated for planar hydrophobic ligands such as these, dilution of DIP ligands shows endothermic heat effects consistent with dissociation of molecular dimers or higher aggregates. Consequently we must expect that equilibrium mixtures in solution will contain not only free ligand and DNA-ligand complexes, but also varying fractions of ligand–ligand dimers and possibly higher oligomers. As a result, the true equilibrium expression is rather more complex than assumed by simple (monomeric) ligand/DNA binding, and the empirical K_A values will therefore depend not only on the absolute affinity of ligand for DNA binding, but also on the self-association affinities of the unbound ligands. To the extent that different ligands might have different self-association properties, this might also affect rational interpretation of any differences in binding properties of the different DIP ligands.

Against this background of cautionary qualifications, it would be unwise to attempt any fundamental interpretation of the binding thermodynamics measured here. However, there are a number of general features that emerge that are of potential empirical interest in the design and application of DNA-intercalators for practical purposes. In general, and in contrast to ethidium bromide and the majority of other intercalators studied so far [6], standard entropies of binding (ΔS^0) of DIP ligands are favourably positive (at 25 °C), and this compensates for the otherwise relatively small enthalpic (ΔH^0) contributions to enhance the standard free energies of binding (ΔG^0). This is consistent with a traditional view of an interaction predominantly involving hydrophobic interactions, though it is probably unhelpful to speculate further in view of the multiplicity of factors that need to be taken into account, especially when important details regarding the inevitable conformational changes related to intercalation are yet to be determined [6]. Other than this, it is hard to discern any significant patterns in the empirical binding parameters for these ligands. The nature of the side chain appears to have little effect in most cases. Increasing the bulkiness of the R-groups does possibly reduce the apparent N values with the longer chain DNAs, possibly due to increase steric hindrance between nearest neighbour intercalators, but the pattern is not altogether consistent. In the absence of detailed structures for each of the complexes, we cannot yet determine to what extent the different substituent groups might occupy major/minor grooves and thereby impede possible neighbouring ligands, or project freely into the bulk solvent.

The relatively forgiving nature of the DIP-based binding means that all of the DIP ligands examined so far do seem to bind to DNA and, in the majority of cases, the ITC data can be fit by a relatively simple binding model. However, there are a couple of exceptions that require special mention. Firstly, although binding of DIP-ligand **14** (and its enantiomer, not

shown) is clearly exothermic, it was not possible to fit the ITC data to any simple binding model. The reasons for this are not entirely clear, but it seems possible that in this instance, although the DIP group does facilitate intercalation, the close proximity of the bulky chiral substituent might cause more disruption to the DNA chain structure than is normally the case for simple intercalators. Longer-range disruption of the duplex structure might mean that sequential ligand binding events are no longer independent of each other, and titrations involving such ligands inevitably would not conform to simple binding models.

Another example of potential long-range effects arises with the double-headed DIP-ligand **18**, which was deliberately designed to explore the possibility of enhanced binding by dual intercalation of DIP groups separated by a flexible linker, exploiting the "chelate" or "effective concentration" effect that has been much discussed elsewhere (see [22] for example). Indeed, the binding of **18** to the DNA 12-mer is significantly enhanced by about a factor 10 (in K_A) compared to other DIP ligands (Table 1). This enhanced binding does involve a more favourable entropy contribution, as might be expected from the chelate effect, though at the expense of a somewhat less favourable ΔH^0, possibly due to the conformational strains involved in the contortions required to achieve dual intercalation in the small oligomeric DNA structure. Calorimetric titrations of **18** with longer genomic DNA (salmon testes) give a more complex binding pattern, exothermic at low concentrations but becoming endothermic with further additions of ligand. This cannot be fit by a simple model, but rough estimates using a two-sets-of-sites model suggest similarly enhanced binding affinities. We ascribe the complex behaviour in this case to the interplay of inter- and intra-chain crosslinking by the double-headed DIP ligand.

Obviously, calorimetric techniques are incapable of probing structural details to any great extent, but the current work demonstrates the utility of these techniques, especially ITC, as a convenient general-purpose analytical tool for the empirical characterization of biomolecular interactions. The relatively straightforward screening of small compound libraries, as illustrated here for the DIP family of intercalators, is proving to be helpful in guiding the synthetic efforts of medicinal chemists.

Acknowledgements

References

[1] J.M. Sturtevant, E.P. Geiduschek, The Heat of Denaturation of DNA, vol. 80, 1958, p. 2911.

[2] M.A. Lynch, O. Duval, A. Sukhanova, J. Devy, S.P. MacKay, R.D. Waigh, I. Nabiev, Synthesis, biological activity and comparative analysis of DNA binding affinities and human DNA topoisomerase I inhibitory activities of novel 12-alkoxy-benzo[c]phenanthridinium salts, Bioorg. Med. Chem. Lett. 11 (2001) 2643–2646.

[3] S.T. Mullins, P.G. Sammes, R.M. West, G. Yahioglu, Preparation of some new intercalating europium(III) sensitizers, J. Chem. Soc., Perkin Trans., II 1996 (1996) 75–81.

[4] L.H. Hurley, F.L. Boyd, DNA as a target for drug action, Trends Pharmacol. Sci. 9 (1988) 402–407.

[5] I. Haq, Thermodynamics of drug–DNA interactions, Arch. Biochem. Biophys. 403 (2002) 1–15.

[6] J.B. Chaires, Energetics of drug–DNA interactions, Biopolymers 44 (1997) 201–215.

[7] A.D.C. Parenty, L.V. Smith, A.L. Pickering, D.L. Long, L. Cronin, General one-pot, three-step methodology leading to an extended class of N-heterocyclic cations: spontaneous nucleophilic addition, cyclization, and hydride loss, J. Org. Chem. 69 (2004) 5934–5946.

[8] D.E. Graves, L.M. Velea, Intercalative binding of small molecules to nucleic acids, Curr. Org. Chem. 4 (2000) 915–929.

[9] A.D.C. Parenty, L.V. Smith, K.M. Guthrie, D.-L. Long, L. Cronin, J. Plumb, R. Brown, Highly stable phenanthridinium frameworks as a new class of tunable DNA binding agents with cytotoxic properties, J. Med. Chem. 48 (2005) 4504–4506.

[10] R.E. Dickerson, H.R. Drew, Structure of a B-DNA dodecamer: II. Influence of base sequence on helix structure, J. Mol. Biol. 149 (1981) 761–786.

[11] H.R. Drew, R.M. Wing, T. Takano, C. Broka, S. Tanaka, K. Itakura, R.E. Dickerson, Structure of a B-DNA dodecamer: conformation and dynamics, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 2179–2183.

[12] T. Wiseman, S. Williston, J.F. Brandts, L.N. Lin, Rapid measurement of binding constants and heats of binding using a new titration calorimeter, Anal. Biochem. 179 (1989) 131–137.

[13] A. Cooper, C.M. Johnson, Isothermal titration microcalorimetry, in: C. Jones, B. Mulloy, A.H. Thomas (Eds.), Microscopy, Optical Spectroscopy, and Macroscopic Techniques, Humana Press, Totowa, NJ, 1994, pp. 137–150.

[14] J.S. Ren, T.C. Jenkins, J.B. Chaires, Energetics of DNA intercalation reactions, Biochemistry 39 (2000) 8439–8447.

[15] D. Suh, J.B. Chaires, Criteria for the mode of binding of DNA binding agents, Bioorg. Med. Chem. 3 (1995) 723–728.

[16] J.B. Chaires, W. Priebe, D.E. Graves, T.G. Burke, Dissection of the free energy of anthracycline antibiotic binding to DNA: electrostatic contributions, J. Am. Chem. Soc. 115 (1993) 5360–5364.

[17] A. Cooper, C.M. Johnson, J.H. Lakey, M. Nollmann, Heat does not come in different colours: entropy–enthalpy compensation, free energy windows, quantum confinement, pressure perturbation calorimetry, solvation and the multiple causes of heat capacity effects in biomolecular interactions, Biophys. Chemist. 93 (2001) 215–230.

[18] K. Sharp, Entropy–enthalpy compensation: fact or artefact? Protein Sci. 10 (2001) 661–667.

[19] A. Cooper, Heat capacity effects in protein folding and ligand binding: a re-evaluation of the role of water in biomolecular thermodynamics, Biophys. Chemist. 115 (2005) 89–97.

[20] J.B. Chaires, Analysis and interpretation of ligand–DNA binding isotherms, Methods Enzymol. 340 (2001) 3–22.

[21] G. Kallansrud, B. Ward, A comparison of measured and calculated single- and double-stranded oligodeoxynucleotide extinction coefficients, Anal. Biochem. 236 (1996) 134–138.

[22] M.I. Page, W.P. Jencks, Entropic contributions to rate accelerations in enzymic and intramolecular reactions and the chelate effect, Proc. Natl. Acad. Sci. U. S. A. 68 (1971) 1678–1683.