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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.

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.

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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.

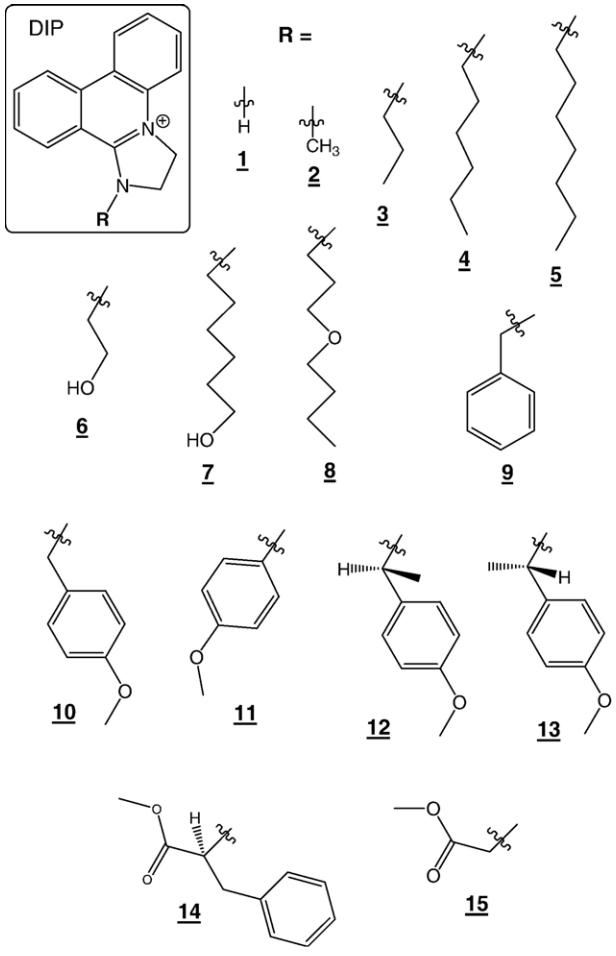

Fig. 1. Structures of DIP-based ligands **1**–**15**. The parent dihydro-imidazophenanthridinium (DIP) framework is shown framed, with appropriate substituent (**R**) groups numbered. All compounds synthesized as the Br salt.

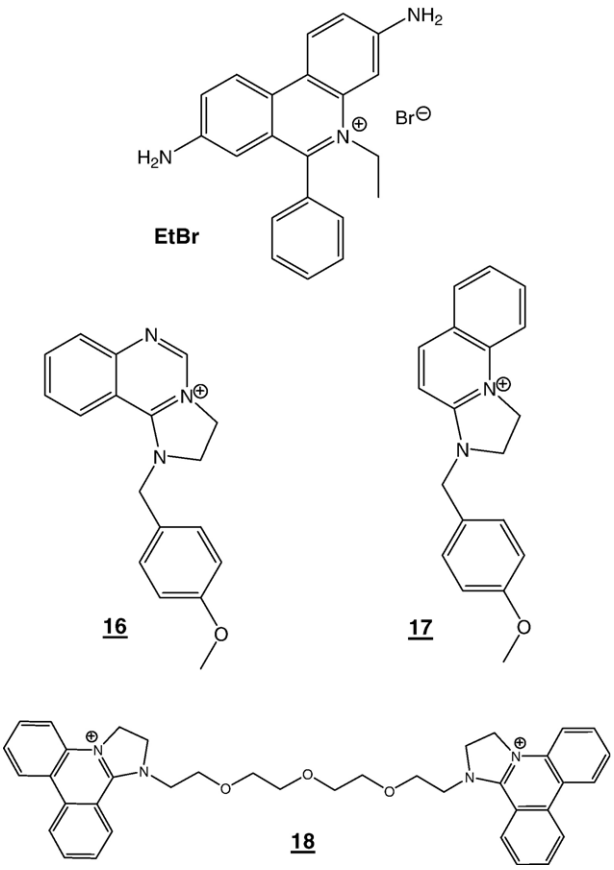

Fig. 2. Structures of ethidium bromide (EtBr), non-DIP ligands **16**–**17** and the "double-headed" DIP–ligand **18**.

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