Critical Performance Characteristics of the Nano ITC SV
When Used in the Study of Complex Binding Reactions

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Introduction

Isothermal titration Calorimetry (ITC) has become one of the most powerful tools for characterizing the structure–function relationship for a wide variety of molecular binding events. The ability to generate thermodynamic data that defines the driving forces of a binding event provides signature characteristics that can be critical in fragment based drug design, biotherapeutic characterization and other process analysis steps where a subsequent toxicity or in vivo failure can be quite expensive.

In most cases of analyzing a ligand-target interaction, ITC can provide the required sensitivity to fully characterize even the most complex binding reactions that may not be evident in other in-vitro binding assay techniques, such as Surface Plasmon Resonance (SPR). Two examples of previously published ligands, distamycin and netropsin, that bind to AT-rich DNA sequences are characterized by the Nano ITC. These two ligands have very different binding modes and show distinguishable thermodynamic profiles for their target DNA sequences.

Materials and Methods

The instruments used in this study were the Nano ITC Standard Volume (SV) and the VP ITC. The test KHCO₃–HCl reagents were provided by the TA Instruments (P/N 601116.901). The reagents used in the netropsin and distamycin titrations were provided by the laboratory of Professor W. David Wilson at Georgia State University. All titrations on both instruments were performed by the same personnel in Dr. Wilson’s laboratory. The titrations of netropsin and distamycin were performed with cacodylic acid buffer containing 100 mM NaCl, 10 mM cacodylic acid, 1 mM EDTA adjusted to pH 6.25 with NaOH.

Results

1. KHCO₃–HCl Titrations

Standardized reagent titrations with KHCO₃–HCl have been carried out on both instruments. 10 μL aliquots of 1.0 mM HCl was titrated into 0.155 mM KHCO₃ in the 942 μL Nano ITC SV cell, and into 0.178 mM KHCO₃ in the 1414 μL VP ITC cell. Both data were then fitted to a one-site independent model. The binding affinity ($K_a$) obtained from the Nano ITC SV is $1.8 \pm 0.3 \times 10^6$ M⁻¹,
enthalpy (\(\Delta H\)) is \(-2.15 \pm 0.02\) kJ/mol and the binding stoichiometry (n) is \(0.986 \pm 0.005\). The profile obtained from the VP ITC was similar \((K_a=1.5 \pm 0.1 \times 10^6 \text{ M}^{-1}, \Delta H=-2.15 \pm 0.01\) kJ/mol, n= 1.02 \pm 0.002\). The results from both instruments are in agreement with one another, as well as the published literature [1].

2. Distamycin–AAATTT Titrations

Distamycin is highly specific for binding to DNA sites containing four or more AT base pairs, and it can bind to the AAATTT DNA minor groove as a dimer with negative cooperativity [2]. Distamycin 0.2 mM was titrated into 0.011 mM of AAATTT on both the Nano ITC and the VP ITC. The binding is quite strong and the second molecule to bind does so with a significantly more negative enthalpy than the first molecule (Figure 1). Both calorimeters show a break at around a 1:1 molar ratio for the first binding and a final equivalence at a 2:1 ratio for the dimer. Because of the strong binding of the first molecule and the negative cooperativity, the titration transition of enthalpy differences is clearly seen. The profiles obtained from the Nano ITC using two-site model \((K_1=7.0 \times 10^8 \text{ M}^{-1}, K_2=4.3 \times 10^6 \text{ M}^{-1}, \Delta H_1=-6.3\) kcal/mol, \(\Delta H_2=-13.8\) kcal/mol) and the VP ITC \((K_1=3.2 \times 10^8 \text{ M}^{-1}, K_2=1.9 \times 10^6 \text{ M}^{-1}, \Delta H_1=-6.4\) kcal/mol, \(\Delta H_2=-15.9\) kcal/mol) are comparable.

Figure 1. Distamycin–AAATTT Titrations.
3. Netropsin–AATT Titrations

Netropsin is similar in structure to distamycin and it binds to the hairpin duplex AATT site in a 1:1 complex (Figure 2). It can bind in two orientations on the duplex site but because the site is symmetric, the orientations have the same thermodynamic parameters (the hairpin loop has a negligible effect) [3]. The titrations of 0.3 mM netropsin into 0.03 mM AATT were performed on the Nano ITC (Figure 2), and the biphasic results are surprising for a 1:1 complex. Both the biphasic nature of the titration in Figure 2 and the thermodynamic results are reproduced in a very similar manner as the results obtained by the VP ITC [3]. One explanation for this behavior is that netropsin binds to the AATT site both with and without a strongly bound water molecule to couple it to the DNA site, and the two complexes sum to 1:1 but have quite different thermodynamics of binding.

![Figure 2. Netropsin–AATT Titration, compound structures and DNA sequences.](image)

**Summary & Discussion**

Heterocyclic cations that bind to the DNA minor groove have demonstrated effectiveness as therapeutic agents and probes for use to extend our understanding of the basis of DNA molecular
Their thermodynamic profile including binding affinity, enthalpy and stoichiometry can be accurately determined by ultrasensitive ITC instruments such as the Nano ITC. In this work, two complex DNA-minor groove binder interactions have been investigated using a Nano ITC from TA Instruments: monomeric binding of netropsin to AATT and dimeric binding distamycin to AAATTT in the DNA minor groove. The monomer binding of netropsin with AATT has been verified by several techniques, however the two distinct binding modes of the complex have not been found until the study by ITC [3]. The complexes in two modes have very different enthalpies and show a dip in the ITC titrations. Distamycin targets AAATTT as a dimer with a negative cooperativity and shows a very distinguishable titration curve from other binding modes. All the results obtained in this study are very comparable with previously published results [2, 3]. The experiments presented here demonstrate the critical role of ITC in the understanding of DNA-minor groove binder interactions, and show that the Nano ITC from TA Instruments can provide the required sensitivity to fully characterize the most complex binding reactions. Moreover, standardized reagents of KHCO$_3$–HCl titrations have been run on both the Nano ITC and the VP ITC and the results from both instruments are in good agreement.

References