

A Prototype System for Measuring Thermodynamic Energy to Predict the Affinity of DNA Probes Used For Genetic Testing

Scientists at NIST are employing calorimetry to determine the energy of binding between complementary strands of DNA/DNA and DNA/RNA in order to predict the tightness of the interaction. The ability to measure the thermodynamic properties of binding is critical to designing DNA probes that work properly in genetic tests and biological assays. The model system being developed will enable innovation of new products and methods in the forensic sciences, clinical diagnostic and pharmaceutical industries.

B. Lang and F. P. Schwarz (Div. 831)

The conditions which stabilize DNA-DNA and DNA-RNA double-strands in solution are important considerations for applications such as the design of PCR and gene chip array measurements, as well as gaining a fundamental understanding of these important reactions. The stabilization of these DNA-DNA and DNA-RNA duplexes is measured in terms of thermodynamic quantities, the standard molar enthalpy changes $\Delta_r H^\circ$, standard molar Gibbs free changes $\Delta_r G^\circ$, and dissociation temperatures T_m . Although there are models which can be used to predict thermodynamic quantities for DNA-DNA and DNA-RNA duplexes, the models have not been validated over a wide range of conditions. In fact, most predictive models rely on UV melting data extrapolated to 37 °C. Consequently, we have performed a series of detailed measurements on a model system, namely an oligomer (10 bases on each strand) of single-stranded DNA binding to its complementary DNA and RNA strands.

New methods of measuring the binding energy of DNA/DNA and DNA/RNA interactions will enable the development of more accurate tests for human identification, disease diagnosis and prediction and new drug development

We used isothermal titration calorimetry (ITC) to measure equilibrium constants and molar enthalpy changes for the DNA hybridization reactions over a wide range of temperature, pH, and salt (sodium chloride) concentration in order to obtain an understanding of how the thermodynamic quantities depend on changes in these parameters.

Differential scanning calorimetry (DSC) was also used to determine the thermodynamic quantities ($\Delta_r H^\circ$, $\Delta_r G^\circ$, T_m) for the reverse reaction (melting of double-stranded DNA). DSC results on the melting of DNA duplexes gave results consistent with existing predictive models (based on UV melting). However, we did find an inconsistency with the results obtained from the ITC measurements. This inconsistency was explained by the presence of a transition between two different conformational states in the single-stranded oligonucleotides, which occurs between the temperatures of the ITC and DSC measurements.

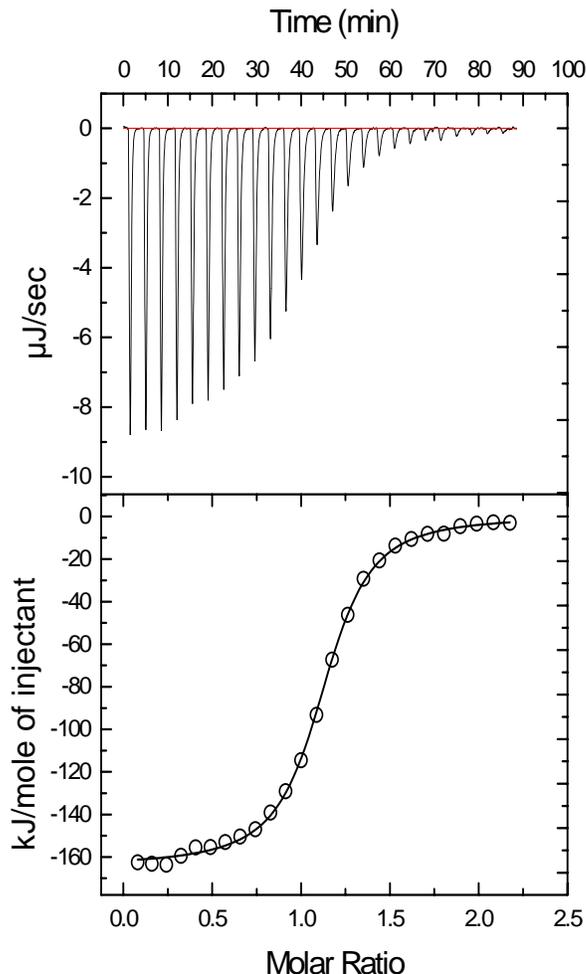


Figure Description: (top) the ITC scan of 10 μl aliquots of 177 μM DNA(TG) into 15.8 μM DNA'(CA) in pH = 7.0 PBS buffer at 298.15 K. (bottom) The binding isotherm for this titration.

This study has shown that the standard molar Gibbs free energy change, $\Delta_r G^\circ$, for the binding reaction is temperature dependent and contrary to the assumptions used in existing predictive models. Also, it was found that the dependence of $\Delta_r G^\circ$ on salt concentration between 0.1 M and 1.0 M is both non-linear and less than what the current models would predict. Additionally, both $\Delta_r G^\circ$ and $\Delta_r H^\circ$ for the binding reaction exhibit only a slight dependence on pH from pH = 6.0 to pH = 8.0. Thus, these results allow for the prediction of the thermodynamic behavior of this prototypical model system over a wide range of temperature, pH, and salt concentration and also provide insight into the behavior of many similar reactions.

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