Importance of Single-Strand DNA Conformational Changes in the Determination of DNA Duplex Stability

The thermodynamics of oligonucleotide interactions at ambient temperatures is normally determined from extrapolation of the thermodynamics of oligonucleotide dissociation at high temperature. For example, the thermodynamic stability of a DNA duplex at ambient temperatures is normally described by the temperature where half of the duplex is dissociated into single DNA strands or by its "melting temperature". However, isothermal titration calorimetry measurements of the interaction of DNA sequences with their complementary DNA sequences have shown that the thermodynamics of DNA duplex formation at ambient temperatures can be significantly different from the thermodynamics of DNA duplex dissociation at high temperature extrapolated to ambient temperatures.

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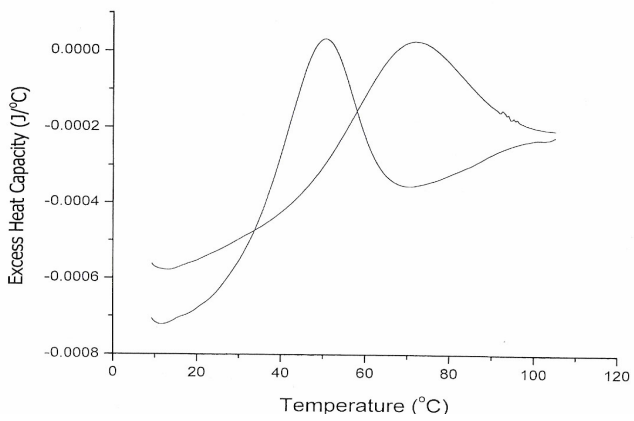
The thermodynamic stability of a DNA duplex is of practical importance because the design of high-performance DNA microarrays employed for DNA identification and for gene expression measurements is based on the ability to accurately predict the interaction thermodynamics of DNA with its complementary DNA and RNA sequences. Since only four different bases make up DNA, various estimation schemes have been developed to predict the interaction thermodynamics of a DNA strand from its sequence at all temperatures. The estimation schemes are based on the dissociation thermodynamics of the duplex at the melting temperature and do not take into account the thermodynamic contributions from temperature-dependent conformational changes in single-stranded DNA. A quantitative knowledge of these contributions will improve the predictive schemes and, ultimately, DNA microarray performance.

Early measurements on increases in the UV optical density of single-stranded DNA with increase in temperature implied that single-stranded DNA undergoes a transition from a "stacking" nucleotide base conformation at ambient temperatures to an unstacked random coil conformation at high temperatures. Recent modeling of the small-angle neutron scattering measurements on a ten-base single- stranded DNA sequence performed at 10 ^{o}C increments from 25 ^{o}C up to 85 ^{o}C demonstrate that the DNA sequence does indeed undergo a stacking to unstacking transition over this temperature range. In addition, the results are in agreement with differential scanning calorimetry (DSC) measurements that show the heat capacity changes which accompany these transitions over the same temperature range. It was found that this transition is dependent on the sequence and that the transition temperature increases with increase in the salt concentration. The thermodynamic contribution from the conformational change of the single-stranded 10 mer DNA was shown to be responsible for the discrepancy between the thermodynamics of the DNA interacting with its complementary sequence at 25 ^{o}C and the thermodynamics of the DNA duplex dissociation extrapolated from the melting temperature.

The thermodynamics of single-strand DNA transitions are being determined from DSC measurements in order to more accurately predict the thermodynamics of DNA interactions at all temperatures. This would improve predictive specificities of DNA interactions on microarrays and allow for the more accurate determination of how well DNA microarrays distinguish different DNA or/and RNA sequences.



DSC results for the excess heat capacity as a function of temperature for two different 10 base-pair DNA strands. The DSC peaks are the stacking ⇔ unstacking transitions

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