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A Molecular Beacon Strategy for Thermodynamic Characterization of Triplex DNA: Triplex Formation at the Promoter Region of Cyclin D1

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The formation of triplex DNA in the purine-pyrimidine-rich promoter site sequence of cyclin D1, located at -116 to -99 from the transcription initiation site, was studied with a molecular beacon comprised of a G-rich 18-mer triplex forming oligodeoxyribonucleotide. Formation of triplex DNA was monitored by enhanced fluorescence of the beacon, due to the weakening of fluorescence energy transfer, upon its binding to the target duplex.. In low salt buffer (10 mM Na^+), triplex DNA formation was not observed in the absence of a ligand such as spermine. At room temperature (22℃), the equilibrium association constant (Ka) calculated in the presence of 1 μM spermine and 10 mM Na^+ was 3.2×10^8 M^{-1}. The Ka value was 1.0×10^9 M^{-1} in the presence of 150 mM Na^+, and it increased by 10-fold by the addition of 1 mM spermine. Structurally related polyamines exerted different degrees of triplex DNA stabilization, as determined by binding constant measurements. Comparison of spermine versus hexamine showed a 17-fold increase in the equilibrium association constant, whereas bis(ethyl) derivatization lead to a 4-fold decrease of this value. In the absence of added duplex and polyamines, the molecular beacon dissociated with a melting temperature of 67℃. These results demonstrate that molecular beacons can be used for the direct determination of the equilibrium association constants and thermodynamic parameters of triplex DNA formation in the presence of ligands such as polyamines.