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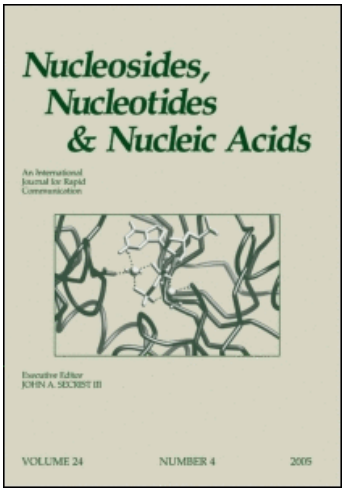

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Structural Studies on DNA Triple Helix Formed by Intronic GAA Triplet Repeat Expansion in Friedreich's Ataxia

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INTRODUCTION

It is well established that GAA/TTC base triplet expansion is the cause of degenerative disorder in Freidreich's Ataxia. It is also known that these repeat sequences fold back to form the unusual intramolecular triple helix structures in DNA of the type Pyrimidine *Purine•Pyrimidine or Purine *Purine•Pyrimidine. In this paper we report on the stability of Purine *Purine•Pyrimidine intermolecular triple helix DNA containing GAA/TTC repeats under physiological conditions. Using the oligonucleotides 5′ TCGC GAA GAA GAA GAA GAA CGCT 3′, 5′-AGCG TTC TTC TTC TTC TTC GCGA-3′ for duplex and 5′- AAG AAG AAG AAG AAG -3′ as triplex forming strand (TFO), we have established the formation of triplex by UV–melting temperature (Tm), and circular dichroic spectra. This was

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further confirmed by gel-retardation assay. The thermodynamic parameters ΔG, ΔH and ΔS for both duplex and triplex formation were determined at different salt concentrations. The results suggest the formation of a stable intermolecular, antiparallel triplex in GAA/TTC repeat sequences where each repeat unit contains two A*A•T and one G*G•C triplet. The therapeutic agents and TFOs, which competitively inhibit the in-vivo intra-molecular triplex by formation of a more stable inter-molecular triplex, could be used to reverse the transcription deficit in GAA/TTC expansions in Frataxin gene.

MATERIAL AND METHODS

The PAGE purified oligonucleotides 5′-TCGC $(GAA)_5$CGCT-3′, (23 R); 5′-AGCG $(CTT)_5$GCGA-3′, were used to form the duplex (23Y). The (TFO) 5′-$(AAG)_5$-3′, (15R), was added to the duplex to generate the triplex (23R:15R). Otherwise indicated all spectroscopic measurements were done in 10 mM sodium-Cacodyalte, 150 mM NaCl and 10 mM $MgCl_2$ at pH 7.4 and 20°C. The thermodynamic parameters were evaluated by the shape analysis two state model of the UV melting curves at different salt concentration as explained earlier.[1]

RESULTS AND DISCUSSION

The normalized melting curves of duplex; 23RY alone and in presence of TFO; 15R and their first derivatives curves respectively were measured at 260 nm. The melting profile of the duplex alone shows a monophasic sharp melting at 73.10°C but the 1:1 mixture of 23R:15R shows biphasic melting with distinctly separate meting temperatures at 52.60°C and 73.10°C. The biphasic melting indicate that the present structure forms the triplex. The CD spectra also recorded for the duplex, 23RY alone and 1:1 ratio of 23RY:15R in SC buffer at pH 7.4 and 20°C. The calculated CD spectrum of the complex (weighted sum total of 23 RY and 15R) is significantly different from that of the experimentally measured triplex. However, the CD spectrum obtained on the experimental addition of 15R to the 23RY duplex showed strong changes; the positive band at 220 nm has disappeared while an intense negative band appeared at 210 nm. The negative band ~210 nm is characteristic of the triplex and generally considered as a ''hall mark'' for triplex formation in oligonucleotides contains GA or GT or CT repeats.[2–4] Hence the intense distinct negative band around 210 nm indicates the formation of triplex. The differential mobilities of duplex and triplex on gel retardation assay further proved the Triplex formation. The dependence of first melting of triplex to duplex, Tm_1 and second melting of duplex to single strands, Tm_2 on increasing the Na^+ concentration was done. The triplex to duplex melting shows a stronger dependence on salt (slope = 12.44 deg/mole) than that of duplex-to-open strands (slope = 8.64 deg/mole). The enthalpy, entropy and free energy changes associated with the transitions of triplex-to- duplex and duplex- to- open strands as described earlier.[1] The enthalpy changes ΔH of triplex dissociation is much small for example, at 150 mM salt it is 48 Kcal. mol^{-1} as compared to the duplex dissociation about 70 Kcal. mol^{-1}.

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CONCLUSIONS

The interstrand triplex formed by GAA repeats is anti-parallel, quite stable under physiological conditions. The knowledge from the present study can provide an insight into the role of GAA/TTC repeats in the triplex formation. The results can be useful in developing TFOs as potential therapeutic agents which will inhibit the in-vivo triplex formation in frataxin gene so that the transcriptional inefficiency can be inhibited in FRDA.

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