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A Small Unstructured Nucleic Acid Disrupts a Trinucleotide Repeat Hairpin

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Abstract

A variety of neurodegenerative disorders are associated with the expansion of trinucleotide repeat (TNR) sequences. These repetitive sequences are prone to adopting non-canonical structures, such as intrastrand stem-loop hairpins. Indeed, the formation and persistence of these hairpins during DNA replication and/or repair have been proposed as factors that facilitate TNR expansion. Given this proposed contribution of TNR hairpins to the expansion mechanism, disruption of such structures via strand invasion offers a potential means to negate the disease-initiating expansion. In this work, we investigated the strand invading abilities of a (CTG)₃ unstructured nucleic acid on a (CAG)₁₀ TNR hairpin. Using fluorescence, optical, and electrophoretic methods, instantaneous disruption of the (CAG)₁₀ hairpin by (CTG)₃ was observed at low temperatures. Additionally, we have identified three distinct duplex-like species that form between (CAG)₁₀ and (CTG)₃; these include 1, 2, or 3 (CTG)₃ sequences hybridized to (CAG)₁₀. The results presented here showcase (CTG)₃ as an invader of a TNR hairpin and suggest that unstructured nucleic acids could serve as a scaffold to design agents to prevent TNR expansion.

Keywords

Trinucleotide repeat expansion; Strand invasion; Molecular beacon; Polyglutamine disorders

1. Introduction

Several neurodegenerative disorders are known to be caused by the expansion of a trinucleotide repeat (TNR) DNA sequence [1,2]. In many cases, disease onset and severity is dictated by the length of these TNR regions [3]. Furthermore, these repetitive sequences are capable of folding into discrete non-canonical DNA structures [4,5,6]. For example, the TNR associated with polyglutamine disorders, (CAG)_n, has been shown to fold into a stem-loop hairpin *in vitro* [7] as well as *in vivo* [8]. It has been proposed that formation and persistence of stem-loop hairpins interferes with DNA replication and/or repair and causes TNR expansion [1,9,10]. Therefore, the stability of these non-canonical structures has become an intriguing and active area of investigation. In particular, ability of TNR stem-loop hairpins to persist in the presence of the complementary DNA, as opposed to forming

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canonical duplex, is an important factor when considering the potential contributions of these non-canonical structures to the expansion mechanism.

Studies addressing the stability of TNR sequences have typically been limited to calorimetric and/or optical methods. These techniques, although informative, are not sufficient to describe how a TNR sequence behaves in presence of the complementary sequence; this is because all of the DNA in the sample contributes to the calorimetric or optical signal. To address this limitation we have employed a fluorescence-based assay that allows for selective monitoring of the structure adopted by a TNR sequence in presence of the complementary DNA. Through the use of this strategy, we have shown previously that the TNR sequences (CAG)₁₀ and (CTG)₁₀ form kinetically-trapped hairpins that irreversibly convert to duplex at near physiological temperatures [11]. We have also shown that the conversion to duplex is strongly dependent on hairpin-hairpin interactions and whether the TNR sequence contains an even or an odd number of repeats [12]. Given these properties, TNR hairpins are attractive targets for strand invasion, a process in which a structured nucleic acid, such as a hairpin, is disrupted by another sequence (e.g. a complementary nucleic acid) through hybridization [13,14,15,16].

In this work we explore the use of unstructured TNR repeats as strand invading sequences and, specifically, their ability to disrupt the (CAG)₁₀ hairpin. Using our fluorescence-based assay, in conjunction with native gel electrophoresis and optical measurements, we found that an unstructured TNR sequence, (CTG)₃, invades the (CAG)₁₀ hairpin and forms a series of duplex-like species. The ability of this unstructured sequence to efficiently disrupt a TNR hairpin could provide the foundation for the development of strand invasion agents that could reduce the lifetime of TNR hairpins formed during DNA replication and/or repair, and thus limit the likelihood of expansion.

2. Materials and Methods

2.1. Synthesis and Purification of Oligonucleotides

Oligonucleotides were synthesized using standard phosphoramidite chemistry [17]. All phosphoramidites, including 5' fluorescein (FL) and 3'-(*E*)-*N*-(3-(dihydroxymethoxy)propyl)-4-((4-(dimethylamino)phenyl)diazonyl) benzamide (DCL) were purchased from Glen Research. Oligonucleotides were purified using a Dynamax Microsorb C18 reverse phase HPLC column (10 × 250 mm) as described previously [11]. Quantification was performed at 90 °C using ϵ_{260} values estimated for single-stranded DNA [18].

2.2. Fluorescence Measurements

The FL-(CAG)₁₀-DCL molecular beacon was prepared in 20 mM sodium phosphate, 10 mM NaCl, pH 7.0 at a concentration of 3 μ M with all sample manipulations conducted in the dark. In order to obtain the thermodynamically-favored DNA structures, FL-(CAG)₁₀-DCL was incubated for 5 min at 90 °C and slowly cooled to room temperature. The (CTG)₃ oligonucleotide was prepared in the same fashion but at a 3.6-fold excess (10.8 μ M). The FL-(CAG)₁₀-DCL and (CTG)_n oligonucleotides were chilled on ice for 1 h prior to mixing in a 1:1 volumetric ratio for a final sample (10 μ L) that contained 1.5 μ M FL-(CAG)₁₀-DCL and 5.4 μ M (CTG)_n in 20 mM sodium phosphate, 10 mM NaCl, pH 7.0. After mixing, the samples were incubated on ice for 20 min and subjected to the following temperature cycle on a MX3005P QPCR (Stratagene): 25 °C for 30 s, 25 °C to 90 °C at 1 °C/min, 5 min at 90 °C, and 90 °C to 25 °C at 1 °C/min. Over the course of this temperature cycle the samples were excited at 492 nm and the emission was monitored at 520 nm.

2.3. Optical Melting Analysis

Optical melting profiles for the structures adopted by each oligonucleotide were obtained using a Beckman Coulter DU800 UV-visible spectrophotometer equipped with a Peltier thermoelectric device. (CAG)₁₀ and (CTG)₃ were prepared at concentrations of 3 μ M and 9 μ M, respectively, in 20 mM sodium phosphate, 10 mM NaCl, pH 7.0. Prior to optical analysis samples were incubated for 5 min at 90 °C and slowly cooled to room temperature. The samples were then incubated at 10 °C prior to mixing in a 1:1 volumetric ratio, for a total sample volume of 325 μ L, and final DNA concentrations of 1.5 μ M (CAG)₁₀ and 4.5 μ M (CTG)₃. The sample was heated at a rate of 1 °C/min from 10 to 90 °C while monitoring absorbance at 260 nm, held at 90 °C for 5 min, and returned to the starting temperature at a rate of 1 °C/min.

2.4. Native Gel Electrophoresis

The (CAG)₁₀ strand was 5'-[³²P] end-labeled. Radiolabeled DNA was supplemented with unlabeled DNA to obtain the desired concentration of 3 μ M (CAG)₁₀. The (CAG)₁₀ hairpin was obtained by incubating the oligonucleotide at 90 °C for 5 min, followed by slow cooling to room temperature. A solution of 10.8 μ M (CTG)₃ was prepared in the same fashion. Prior to mixing, each solution was incubated at 10 °C for 30 min; 5 μ L of each of the (CTG)₃ and (CAG)₁₀ samples were combined to reach a concentration of 1.5 μ M (CAG)₁₀ and 5.4 μ M (CTG)₃. After mixing, samples were incubated at 10 °C for 20 min. 5 μ L of ice-cold non-denaturing loading buffer (15% Ficoll, 0.25% xylene cyanol, and 0.25% bromophenol blue) were added and samples were immediately loaded onto a 12% non-denaturing polyacrylamide gel and electrophoresed at 80 V for 7 h at 4 °C. A second experiment was performed in which (CAG)₁₀ and (CTG)₃ were at final concentrations of 1.5 μ M and 3 μ M, respectively, and following mixing the non-denaturing loading buffer was immediately added and the samples were loaded onto a non-denaturing gel.

3. Results and Discussion

3.1 (CTG)₃ disrupts the (CAG)₁₀ stem-loop hairpin

The (CAG)₁₀ molecular beacon, FL-(CAG)₁₀-DCL, has a fluorescein (FL) functionality covalently attached at the 5' end and a dabcyI quencher (DCL) at the 3' end. When the molecular beacon adopts a hairpin conformation the fluorophore and the quencher are in close proximity, and no fluorescence is observed [11]. However, if the molecular beacon adopts an unstructured conformation, or forms a duplex with complementary DNA, fluorescence is observed. This molecular beacon approach enables specific observation of the labeled sequence and how its structure changes in response to a changing environment, namely, when exposed to other nucleic acids. We previously used the (CAG)₁₀ molecular beacon to explore how the structure of this TNR hairpin is modulated by complementary (CTG)_n hairpins [11]. Moreover, we have reported structural differences between even and odd-repeat (CTG)_n hairpins when n ranges from 6 to 14, namely, odd-repeat sequences have repeat overhangs [12]. Presence or absence of these overhangs caused a significant decrease in the lifetime of a (CAG)₁₀ hairpin and produced equally noticeable changes in the fluorescence melting profile for this molecular beacon. When expanding our studies to include (CTG)_n sequences with fewer repeats (n = 2–5), we discovered that the fluorescence profile of the molecular beacon changed dramatically in the presence of (CTG)₃ when compared to the previously studied (CTG)_n series, regardless of whether the sequences had an even or odd number of repeats (Supplementary Data). Furthermore, while the fluorescence intensity at room temperature of the molecular beacon is low when in the presence of (CTG)_n when n = 2, 4, or 5, indicative of the molecular beacon adopting a hairpin, the fluorescence intensity is high in the presence of (CTG)₃, suggesting that the (CAG)₁₀ hairpin has been disrupted in the presence of this TNR sequence. A very small

increase in fluorescence intensity was seen with (CTG)₂, however, we chose to pursue experiments with (CTG)₃ since the melting profile of the molecular beacon was dramatically altered in the presence of this sequence.

Analysis of the CTG sequences by optical melting revealed that (CTG)₃ does not form a stem-loop hairpin and is unstructured (Supplementary Data). While (CTG)₄ and (CTG)₅ show a slight but noticeable increase in absorbance with temperature, and (CTG)₆ displays a clear sigmoidal melting curve consistent with hairpin dissociation, (CTG)₃ shows no significant change in absorbance at 260 nm upon heating, consistent with an unstructured sequence. It is likely that the stem of a (CTG)₃ hairpin would not contain sufficient base pairing and stacking to form a stable intramolecular structure. While at higher concentrations, (CTG)₃ is capable of forming an intermolecular homoduplex, as shown by NMR [19,20], under our conditions we did not detect homoduplex formation. Notably, by optical melting analysis we also observed that (CTG)₂, with one fewer repeat than (CTG)₃, is also unstructured.

Given its unique effect on the (CAG)₁₀ molecular beacon, our further studies focused on characterizing (CTG)₃ as a strand invading sequence. Shown in Fig. 1A is the fluorescence melting profile of the (CAG)₁₀ molecular beacon in the absence and in the presence of a 3.6-fold molar excess of (CTG)₃. In the absence of other DNA, a single transition at 47.1 ± 1.2 °C is observed (Fig. 1A, open circles), which is consistent with melting of the hairpin conformation of the molecular beacon [11]. In the presence of (CTG)₃, the fluorescence melting profile of the molecular beacon is dramatically different (Fig. 1A, closed circles). A high level of fluorescence is observed at temperatures below ~ 35 °C (indicated by I in Figure 1A). As the temperature increases from 35 to 45 °C (II) there is a decrease in fluorescence intensity with a transition temperature of 41.5 ± 0.6 °C. Additionally, an increase in fluorescence that is centered at 48.8 ± 0.1 °C is observed, and this final transition overlays with the melting transition observed for the molecular beacon in the absence of (CTG)₃. Lastly, both in the presence or absence of (CTG)₃, a plateau in the fluorescence intensity is observed at temperatures above 60 °C and is indicative of the unstructured molecular beacon (III).

We have shown previously that (CTG)₁₀, which forms a stem-loop hairpin, disrupts the hairpin form of the (CAG)₁₀ molecular beacon [11]. Specifically, (CTG)₁₀ lowered the melting temperature of the hairpin form of the molecular beacon by 10 °C. Based on this previous observation with (CTG)₁₀, we postulated that the unstructured (CTG)₃ is a more effective disruptor the (CAG)₁₀ hairpin than (CTG)₁₀ and disrupts the hairpin by forming a duplex comprised of one (CAG)₁₀ sequence and three (CTG)₃ sequences; importantly, this duplex would be a fluorescent species and would account for the high fluorescence intensity observed below 35 °C.

One way we examined whether the high fluorescence intensity observed below 35 °C is due to disruption of the (CAG)₁₀ hairpin by (CTG)₃, was by performing an optical melting experiment. Different from the fluorescence-based experiments with the molecular beacon, where contributions to fluorescence are derived solely from (CAG)₁₀ and not from other DNA present in the sample, in the optical-based assay all DNA in the sample contributes to the absorbance. The optical melting data for the (CAG)₁₀ hairpin alone, and the (CAG)₁₀ hairpin in presence of (CTG)₃ are shown in Fig. 1B. (CAG)₁₀ in absence of any other DNA has a single, broad transition at 51.3 °C, which is consistent with hairpin melting. This melting temperature is ~ 4 degrees higher than the melting temperature obtained by fluorescence melting. We proposed previously that this difference is derived from the two distinct phenomena measured by fluorescence and optical methods [11]. The melting temperature obtained by fluorescence reflects the spatial separation of the fluorophore and

quencher while optical melting analysis relies on the unstacking of the base chromophores that occurs during hairpin melting.

In contrast to the data obtained for (CAG)₁₀ alone, the presence of (CTG)₃ results in an additional transition by optical melting, an increase in absorbance at $37.3 \pm 1.1^\circ\text{C}$ (II). Moreover, in the presence of (CTG)₃, a broad transition at $54.2 \pm 0.1^\circ\text{C}$ is observed which corresponds with the transition observed for melting of the (CAG)₁₀ hairpin alone. The melting curve reaches a maximum absorbance which corresponds to all of the DNA in the sample being unstructured (III).

Both the fluorescence and the optical melting profiles for (CAG)₁₀ in presence of the unstructured (CTG)₃ sequence are suggestive of the formation of a duplex species at temperatures below 35°C . As the temperature is raised above 35°C , the duplex melts as the (CTG)₃ sequences dissociate from (CAG)₁₀. Notably, both the fluorescence and optical melting data indicate that the (CAG)₁₀ hairpin exists at temperatures above the melting temperature of the duplex, but below $\sim 50^\circ\text{C}$, which is the melting temperature of the (CAG)₁₀ hairpin. Therefore, we propose that (CTG)₃ operates as an efficient disruptor of the (CAG)₁₀ hairpin by formation of a duplex species. To determine the nature of the duplex species being formed by (CTG)₃ and (CAG)₁₀ we performed native gel electrophoresis.

3.2. Characterization of (CAG)₁₀/(CTG)₃ duplex species by native gel electrophoresis

Native gel electrophoresis was performed using 5'-³²P-labeled (CAG)₁₀ in the presence of a 3.6-fold excess of (CTG)₃ following a 20 min incubation period (Fig. 1C, Lane 1). One species, which migrates slower than the (CAG)₁₀ hairpin and (CTG)₃ unstructured controls (Lanes H and U3, respectively) was observed. The same reaction was performed using 5'-³²P-labeled (CTG)₃, instead of having (CAG)₁₀ radiolabeled, and as expected the major product co-migrates with the product obtained when (CAG)₁₀ is radiolabeled (Lane 2). A minor product, which co-migrates with the 5'-³²P-labeled (CTG)₃ control is visible, consistent with the slight excess of (CTG)₃. The migration of the major species observed in Lanes 1 and 2 is consistent with formation of a duplex-like species, since we have observed that on a native gel TNR duplexes migrate slower than the corresponding hairpins [11]. Given the excess of the unstructured (CTG)₃ sequence used for this experiment and the presence of a single major product, we hypothesized that three (CTG)₃ sequences hybridize with a single (CAG)₁₀ sequence.

To determine if duplex-like species with fewer than three (CTG)₃ sequences are possible we performed a separate experiment in which there was no incubation period and the excess of (CTG)₃ was reduced to 2-fold over (CAG)₁₀. Under these conditions, three duplex-like species were observed, in addition to the (CAG)₁₀ hairpin (Fig. 1D, Lane 3). The slowest migrating species co-migrates with a (CAG)₁₀/3(CTG)₃ control (Lane D3), which in turn migrates faster than a fully complementary (CAG)₁₀/(CTG)₁₀ duplex (Lane D10). These results suggests that the three duplex-like species observed in Lane 3 correspond to 1, 2, and 3 (CTG)₃ sequences hybridized to one (CAG)₁₀.

Taking the fluorescence, optical melting, and native gel results into consideration, a clearer picture of the disruption of the (CAG)₁₀ hairpin by the (CTG)₃ unstructured DNA emerges. High fluorescence occurs at low temperatures due to the formation of duplex species composed of a single (CAG)₁₀ sequence and multiple (CTG)₃ sequences (Fig. 2, panel I). When sufficient (CTG)₃ is present, as is the case when there is a 3.6-fold excess of (CTG)₃ to (CAG)₁₀, three (CTG)₃ sequences will hybridize to (CAG)₁₀. However, when a lower amount of (CTG)₃ is present it is also possible to form species in which only one or two (CTG)₃ sequences hybridize to (CAG)₁₀. For clarity, in panel I of Fig. 2 we have provided a representation of only three of the potential duplex-like species. For instance, when one

(CTG)₃ is hybridized to (CAG)₁₀, it could be hybridized in several positions along the (CAG)₁₀ sequence. Additionally, the seven repeats overhanging the +1 duplex species could form a hairpin. In the temperature range of 35–45 °C, the duplex-like species revert back to (CAG)₁₀ hairpin and (CTG)₃ unstructured nucleic acid (Fig. 2, panel II). As the temperature is increased further, the increase in fluorescence, as well as increase in absorbance, can be attributed to melting of the (CAG)₁₀ hairpin; thus, at 90 °C, only the unstructured (CAG)₁₀ and (CTG)₃ sequences are present (Fig. 2, panel III). It is noteworthy that the effectiveness of (CTG)₃ as a strand invading nucleic acid is best showcased at low temperatures; it converts to duplex-like species with (CAG)₁₀ instantaneously. In contrast, conversion of the fully complementary (CAG)₁₀ and (CTG)₁₀ hairpins to duplex is extremely slow at low temperatures [11].

Structured strand-invasion targets, such as a TNR DNA hairpin, incur a thermodynamic penalty for hybridization, which in turn reduces the favorability of the hybridization reaction [21]. Therefore, it is significant that a stable target such as the (CAG)₁₀ hairpin is so efficiently disrupted by the (CTG)₃ sequence; this result suggests that hybridization with the (CTG)₃ sequence overcomes the free energy barrier. As described by Kushon and coworkers in their exploration of the ability of peptide nucleic acids (PNA) to invade DNA hairpins, instantaneous duplex formation at low temperatures via strand invasion occurs when the duplex product has a lower free energy than the hairpin [22]. This behavior is a direct consequence of the enthalpic favorability resulting from the gain in the number of base pairs achieved by formation of duplex versus the number of base pairs present in the hairpin target and the invading strand. Furthermore, it is the lack of structure in (CTG)₃, and therefore the lack of intramolecular base pairing, that makes this sequence such an effective disruptor of the (CAG)₁₀ hairpin; if this sequence were structured there would be a smaller gain in thermodynamic stability upon formation of duplex with (CAG)₁₀.

Interestingly, the hybridization process does not pose a requirement for a particular (CAG)₁₀ to (CTG)₃ stoichiometry. Although formation of a duplex species composed of one (CAG)₁₀ sequence and three (CTG)₃ sequences is thermodynamically more stable, formation and persistence of +2 and +1 (CTG)₃ species is possible as we demonstrated by native gel electrophoresis, showcasing that minimal amounts of (CTG)₃ are sufficient to disrupt the (CAG)₁₀ hairpin.

3.3. Unstructured nucleic acids and their implications in TNR expansion

As reviewed by Armitage [21], TNR DNA is an attractive target for the use of strand invasion and/or interference. However, towards this end, studies taking advantage of strand invasion have been limited to isolating transcriptionally active TNR DNA *in vitro*, determining in-cell localization of the TNR target, and allele specific RNA silencing of TNR RNA hairpins [23, 24]. In the work described here a TNR DNA hairpin is the intended strand invasion target. A disruptor DNA such as (CTG)₃ could prove to be useful to minimize expansion *in vivo*. As expansion has been proposed to be dependent upon formation of (CAG)_n hairpins during DNA replication and/or repair [1, 6, 9], destabilization of these hairpins could be achieved by the use of (CTG)₃ or a derivative with improved intracellular stability. Furthermore, it is known that the propensity for TNR expansion increases with the length of the repeat sequence and it has been proposed that as the length of the repeat sequence increases, complex non-canonical structures may form, possibly comprised of several smaller hairpins rather than a single stem-loop hairpin [25]. Consequently, a small unstructured nucleic acid could be effective as a disruptor should smaller TNR hairpins, such as the one used in this work, form within the TNR tracts.

Research Highlights

Unstructured nucleic acids as trinucleotide repeat hairpin invaders
(CTG)₃ invades a (CAG)₁₀ repeat hairpin
Three duplex-like invasion products are possible
(CTG)₃ can serve as a foundation to design disruptors that prevent TNR expansion

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Mirkin SM. Expandable DNA repeats and human disease. *Nature*. 2007; 447:932–940. [PubMed: 17581576]
2. Castel AL, Cleary JD, Pearson CE. Repeat instability as the basis for human diseases and as a potential target for therapy. *Nat. Rev. Mol. Cell Biol.* 2010; 11:165–170. [PubMed: 20177394]
3. Orr HT, Zoghbi HY. Trinucleotide repeat disorders. *Annu. Rev. Neurosci.* 2007; 30:575–621. [PubMed: 17417937]
4. Mirkin SM. DNA structures, repeat expansions and human hereditary disorders. *Curr. Opin. Struc. Biol.* 2006; 16:351–358.
5. Wells RD. Non-B DNA conformations, mutagenesis and disease. *Trends Biochem. Sci.* 2007; 32:271–278. [PubMed: 17493823]
6. Vasquez KM, Zhao JH, Bacolla A, Wang GL. Non-B DNA structure-induced genetic instability and evolution. *Cell. Mol. Life Sci.* 2010; 67:43–62. [PubMed: 19727556]
7. Gacy AM, Goellner G, Juranic N, Macura S, McMurray CT. Trinucleotide Repeats That Expand in Human-Disease Form Hairpin Structures in-Vitro. *Cell.* 1995; 81:533–540. [PubMed: 7758107]
8. Liu G, Chen X, Bissler JJ, Sinden RR, Leffak M. Replication-dependent instability at (CTG) x (CAG) repeat hairpins in human cells. *Nat. Chem. Biol.* 2010; 6:652–659. [PubMed: 20676085]
9. Kovtun IV, McMurray CT. Crosstalk of DNA glycosylases with pathways other than base excision repair. *DNA Repair.* 2007; 6:517–529. [PubMed: 17129768]
10. McMurray CT. Mechanisms of trinucleotide repeat instability during human development. *Nat. Rev. Genet.* 2010; 11:786–799. [PubMed: 20953213]
11. Ávila Figueroa A, Delaney S. Mechanistic Studies of Hairpin to Duplex Conversion for Trinucleotide Repeat Sequences. *J. Biol. Chem.* 2010; 285:14648–14657. [PubMed: 20228068]
12. Ávila Figueroa A, Cattie D, Delaney S. Structure of even/odd trinucleotide repeat sequences modulates persistence of non-B conformations and conversion to duplex. *Biochemistry.* 2011; 50:4441–4450. [PubMed: 21526744]
13. Peffer NJ, Hanvey JC, Bisi JE, Thomson SA, Hassman CF, Noble SA, Babiss LE. Strand-invasion of duplex DNA by peptide nucleic acid oligomers. *Proc. Natl. Acad. Sci.* 1993; 90:10648–10652. [PubMed: 8248156]
14. Egholm M, Buchardt O, Christensen L, Behrens C, Freier SM, Driver DA, Berg RH, Kim SK, Norden B, Nielsen PE. PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen-bonding rules. *Nature.* 1993; 365:566–568. [PubMed: 7692304]
15. Nielsen PE, Egholm M, Berg RH, Buchardt O. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science.* 1991; 254:1497–1500. [PubMed: 1962210]

16. Hanvey JC, Peffer NJ, Bisi JE, Thomson SA, Cadilla R, Josey JA, Ricca DJ, Hassman CF, Bonham MA, Au KG, et al. Antisense and antigene properties of peptide nucleic acids. *Science*. 1992; 258:1481–1485. [PubMed: 1279811]
17. Beaucage SL, Caruthers MH. Synthetic strategies and parameters involved in the synthesis of oligodeoxyribonucleotides according to the phosphoramidite method. *Curr. Protoc. Nucleic Acid Chem*. 2000;3.3.1–3.3.20.
18. Warshaw MT, Tinoco I. Optical Properties of sixteen dinucleoside phosphates. *J. Mol. Biol.* 1966; 20:29–38. [PubMed: 5970660]
19. Smith GK, Jie J, Fox GE, Gao XL. DNA CTG Triplet Repeats Involved in Dynamic Mutations of Neurologically Related Gene-Sequences Form Stable Duplexes. *Nucleic Acids Res.* 1995; 23:4303–4311. [PubMed: 7501450]
20. Zheng MX. Genetically unstable CXG repeats are structurally dynamic and have a high propensity for folding. An NMR and UV spectroscopic study. *J. Mol. Biol.* 1996; 264:323–336. [PubMed: 8951379]
21. Armitage BA. The impact of nucleic acid secondary structure on PNA hybridization, *Drug Discov. Today*. 2003; 8:222–228.
22. Armitage BA, Kushon SA, Jordan JP, Seifert JL, Nielsen H, Nielsen PE. Effect of secondary structure on the thermodynamics and kinetics of PNA hybridization to DNA hairpins. *J. Am. Chem. Soc.* 2001;10805–10813. [PubMed: 11686681]
23. de Mezer M, Wojciechowska M, Napierala M, Sobczak K, Krzyzosiak WJ. Mutant CAG repeats of Huntingtin transcript fold into hairpins, form nuclear foci and are targets for RNA interference. *Nucleic Acids Res.* 2011
24. Gagnon KT, Pendergraff HM, Deleavey GF, Swayze EE, Potier P, Randolph J, Roesch EB, Chattopadhyaya J, Damha MJ, Bennett CF, Montaillier C, Lemaitre M, Corey DR. Allele-Selective Inhibition of Mutant Huntingtin Expression with Antisense Oligonucleotides Targeting the Expanded CAG Repeat. *Biochemistry*. 2010; 49:10166–10178. [PubMed: 21028906]
25. Petruska J, Arnheim N, Goodman MF. Stability of intrastrand hairpin structures formed by the CAG/CTG class of DNA triplet repeats associated with neurological diseases. *Nucleic Acids Res.* 1996; 24:1992–1998. [PubMed: 8668527]

Fig. 1. Effect of $(CTG)_3$ unstructured nucleic acid on a $(CAG)_{10}$ hairpin

(A) Fluorescence melting profile of $(CAG)_{10}$ molecular beacon hairpin in absence (open circles) or presence (closed circles) of a 3.6-fold excess of $(CTG)_3$. (B) Optical melting profile of $(CAG)_{10}$ hairpin in absence (open circles) or presence (closed circles) of a 3-fold excess of $(CTG)_3$. The y-axis on the left corresponds to the open circles while that on the right corresponds to the closed circles. I, II and III correspond to temperatures at which proposed species form and are described in detail in the text. (C) Autoradiogram depicting the migration of the DNA species observed with 3.6:1 ratio of $(CTG)_3:(CAG)_{10}$. Lanes are as follows: H, $(CAG)_{10}$ hairpin; U3, unstructured $(CTG)_3$; 1, radiolabeled $(CAG)_{10}$ hairpin with a 3.6-fold excess of $(CTG)_3$ and; 2, $(CAG)_{10}$ hairpin with a 3.6-fold excess of radiolabeled $(CTG)_3$. All samples are in 20 mM sodium phosphate, 10 mM NaCl, pH 7.0. (D) Autoradiogram depicting migration of the DNA species observed with 2:1 ratio of $(CTG)_3:(CAG)_{10}$. Lanes are as follows: H, $(CAG)_{10}$ hairpin; 3, radiolabeled $(CAG)_{10}$ with a 2-fold excess of $(CTG)_3$; D3, $(CAG)_{10}/3(CTG)_3$ duplex and; D10, $(CAG)_{10}/(CTG)_{10}$ duplex. The D3 and D10 duplex controls are obtained by mixing the complementary sequences, heating to $90^{\circ}C$, and slowly cooling to room temperature prior to loading onto the gel. All samples are in 20 mM sodium phosphate, 10 mM NaCl, pH 7.0.

Fig. 2. Schematic representation of DNA species present at different temperatures. Left, (CAG)₁₀/n(CTG)₃ duplex-like species; center, (CAG)₁₀ hairpin and (CTG)₃ unstructured sequences; right, unstructured DNA. Roman numerals I, II, and III correspond to Fig. 1.