The Chicken β-Globin Gene Promoter Forms a Novel "Cinched" Tetrahelical Structure

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We have previously shown that the G-rich sequence G\textsubscript{12}CG(GGT)\textsubscript{2}GG in the promoter region of the chicken β-globin gene poses a formidable barrier to DNA synthesis in vitro (Woodford et al., 1994, J. Biol. Chem. 269, 27020–27035). The K\textsuperscript{+} requirement, template-strand specificity, template concentration independence, and involvement of Hoogsteen bonding suggested that the underlying basis of this new type of DNA synthesis arrest site might be an intrastrand tetrahelical structure. However, the arrest site lacks the four G-rich repeats that are a hallmark of previously described intramolecular tetraplexes and contains a number of noncanonical bases that would be expected to greatly destabilize such a structure. Here we report evidence for an unusual K\textsuperscript{+}-dependent intrastrand “cinched” tetraplex. This structure has several unique features including the incorporation of bases other than guanine into the stem of the tetraplex, interaction between loop bases and bases in the flanking region, and base pairing between bases 3’ and 5’ of the tetrahelix-forming region to form a molecular “cinch.” This finding extends the range of sequences capable of tetraplex formation as well as our appreciation of the conformational complexity of the chicken β-globin promoter.

Sequences that cause arrest of DNA synthesis have been identified in plasmids, viruses, and chromosomes. Some of these arrest sites signal the point of replication termination in plasmids and chromosomes (1). Others are associated with phenomena such as the amplification of genomic sequences (2), strand switching during replication (3–6), or mutational hotspots (7). Some of these sequences act by binding specific proteins that then block progression of the polymerase (8–10), whether they block DNA synthesis by forming DNA structures that are a hallmark of previously described tetraplexes and contains a number of noncanonical bases that would be expected to greatly destabilize such a structure. Here we report evidence for an unusual K\textsuperscript{+}-dependent intrastrand “cinched” tetraplex. This structure has several unique features including the incorporation of bases other than guanine into the stem of the tetraplex, interaction between loop bases and bases in the flanking region, and base pairing between bases 3’ and 5’ of the tetrahelix-forming region to form a molecular “cinch.” This finding extends the range of sequences capable of tetraplex formation as well as our appreciation of the conformational complexity of the chicken β-globin promoter.

In Fig. 1A, the arrest site is composed of three independent blocks to DNA synthesis (K1, K2, and K3), suggesting three different structural blocks to DNA synthesis. The first block is the strongest, and under some conditions no chain extension is seen beyond this site (Fig. 1B). The characteristics of this region are not consistent with any previously defined category of DNA synthesis arrest site.

We have previously shown that the underlying physical basis of this block to DNA synthesis is the formation of a series of intrastrand DNA structures that involve Hoogsteen base interactions between guanines (18). It is known that some G-rich sequences associate into higher order structures via guanine tetrad formation. Four DNA strands containing sequences with a single G-rich motif can associate to form an intermolecular tetraplex referred to as G4 DNA (19). Sequences containing two G-rich repeats can form G-G hairpins that can then dimerize to form tetraplexes made up of two DNA strands (20), and sequences with four G-rich repeats or long G runs (21) can fold back to form an intrastrand tetraplex. An example of a generic intrastrand tetraplex is shown in Fig. 1C.

The properties of the chicken β-globin DNA arrest site are consistent with the formation of an intrastrand tetraplex, in that they are template concentration-independent, are specific to the G-rich strand, are stable at elevated temperatures, require K\textsuperscript{+}, and involve non-Watson-Crick base interactions between guanines. The K\textsuperscript{+} specificity is particularly compelling since the binding constants of alkali metal ions to the phosphate groups in DNA are known to decrease slightly with increasing metal ion radius, i.e. Li\textsuperscript{+} > Na\textsuperscript{+} > K\textsuperscript{+} > Rb\textsuperscript{+} > Cs\textsuperscript{+}, and it is therefore difficult to rationalize the K\textsuperscript{+} specificity in terms of a hairpin or other similar structure. It has been suggested that the K\textsuperscript{+} specificity for tetraplexes results from some sort of size constraint for which K\textsuperscript{+} ions are particularly well suited (20). Hydrogen bonding between four DNA strands in an intramolecular tetraplex creates an internal cavity that would exclude large ions such as Cs\textsuperscript{+}. Small ions such as Li\textsuperscript{+} can fit inside the cavity but are too small to form stable complexes with multiple ligand binding sites within the cavity. It has been claimed that the K\textsuperscript{+} ion is small enough to fit inside the cavity and large enough to be able to bridge multiple binding sites within the cavity, thus forming octahedral coordinate complexes with O-6 atoms in adjacent tetrad, thereby stabilizing the tetraplex (20). However, the chicken β-globin promoter arrest site sequence G\textsubscript{12}CG(GGT)\textsubscript{2}GG lacks the repeated motif normally associated with tetraplexes and contains a number of non-guanine bases that might be expected to reduce the stability of the tetraplex.

Data presented here indicate that the chicken β-globin promoter DNA synthesis arrest site does indeed form an intrastrand tetrahelical structure in the presence of K\textsuperscript{+}. However, this structure differs from conventional tetraplexes in a number of important respects. In addition to the incorporation of a number of non-guanine bases into the stem of the tetraplex, the structure is stabilized by interactions between a loop guanine

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and a guanine in the flanking region and hydrogen bonding between bases in the 5′- and 3′-flanking regions to form a “cinch” that holds one end of the tetraplex together. We suggest that the stabilizing effect is due to duplex formation by the G-rich flanking sequence that effectively closes off the “open” end of the tetraplex. We also demonstrate that in the absence of K⁺, part of this region is able to form a hairpin containing a mixture of G-G and G-C base pairs. Our findings, together with those that describe the triple-forming ability of this same sequence, demonstrate the structural complexity of the chicken β-globin promoter. This conformational complexity may have implications for the transcriptional regulation of this gene. Our data also indicate that since the absence of four perfect G-motifs does not preclude tetraplex formation, the number of potential tetrahelix-forming sequences is much broader than previously thought. Our observations demonstrate a clear link between K⁺-dependent DNA synthesis arrest sites and tetra- helix formation, suggesting that the K⁺-dependent blocks to DNA synthesis might be a general feature and useful diagnostic property of this class of structures.

MATERIALS AND METHODS

Oligomer Synthesis and Purification—Oligonucleotides were synthesized using an Applied Biosystems 381A synthesizer according to standard procedures and resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 (TE). Oligonucleotides used for sequencing and polymerase chain reaction amplification were used without further purification. Oligonucleotides used for chemical modification experiments were purified by electrophoresis on 20% denaturing polyacrylamide gels. Bands containing full-length synthesis products were excised and eluted with 100 mM Tris-HCl, pH 8.0, 5 mM EDTA, and 0.3 M NaCl overnight at 55°C. Eluates were filtered through a Durapore filter and precipitated with ethanol. Oligonucleotides were labeled with [γ-32P]ATP using 6 units of T4 polynucleotide kinase (U.S. Biochemical Corp.) in 10 mM Tris-HCl, 2.5 mM MgCl₂, or TE at a concentration of approximately 13 nM, 1 μl of DMS was added to 1 μl with distilled H₂O and mixed per reaction. The positions of the previously described DNA synthesis arrest sites are also marked, K1-K3, respectively, and the relative strength of each arrest site is indicated by the number of filled arrowheads at that position. B, arrest of DNA synthesis by the templates G13(GGT)5G (chicken β-globin promoter) and G6B in the presence (+) and absence (−) of 40 mM K⁺. DNA synthesis arrest assays were performed as described previously (18). C, diagram of generic intrastrand tetraplex.

Modification of labeled oligonucleotides with dimethyl sulfate (DMS) was performed using reagents from a Maxam-Gilbert sequencing kit (Epicentre Technologies) and end-labeled primers Zseq (5′-AGT GCC ACC TGA CGT TCT GAC TTG AGC GTC-3′), supFR1(18) as described previously (18) to create pT(G₁), and pCStem. The pM clones 1–3 were constructed in a similar manner from fragments amplified using pBG6 (18) as template and M1, M2, or M3 (M1 is 5′-GTA CGA CTG GAG ACC ACC CG-3′, M2 is 5′-GTA CGA CTG GAG ACC ACC CGC CCC CCC CCC CCA GTG AYT ACA TCT T-3′, or M3 is 5′-GTA CGA CTG GAG ACC ACC ACC CGC CCC CCC CCC CCG GAG CAA CTG AYT ACT T-3′) as 5′ primers and supFR1 as the 3′ primer.

DNA Synthesis Arrest Assay—DNA synthesis arrest assays were performed with the SequiTHERM DNA sequencing kit (Epicentre Technologies) and end-labeled primers Zseq (5′-AGT GCC ACC TGA CTT GGC ACC CG-3′, or supFR4 (5′-ATG CTT TTG GGC ACC ACC CG-3′) as described previously (18).

RESULTS

We have previously shown that the chicken β-globin gene promoter contains a G-rich sequence, G₁₃(GGT)₅G, that forms a strong DNA synthesis arrest site in the presence of K⁺ (18). The location of this arrest site in the promoter is shown in Fig. 1A. The individual bases in the arrest site are labeled 1–26 with base 1 being the 5′-most guanine in the arrest site (G₁). In fact, this arrest site consists of a series of three successive blocks to DNA synthesis, since under some conditions

1. The abbreviations used are DMS, dimethyl sulfate; BAA, bromoacetaldehyde.
three steps are seen opposite successive T residues in the template (see arrows in Fig. 1A, labeled K1, K2, and K3). Polymerase arrest is significantly more efficient at K1 than at K2 and K3, and under some conditions almost no read-through is seen past K1 (Fig. 1B). The amount of DNA synthesis arrest by the chicken β-globin promoter is similar to that seen for a run of uninterrupted guanines of the same length (Fig. 1B). These blocks to DNA synthesis are eliminated if some of the guanines in this sequence are blocked at the N-7 position, suggesting that formation of a series of structures involving non-Watson-Crick base interactions is responsible for DNA synthesis arrest. Arrest of DNA synthesis is independent of the anion present and is not seen in the presence of other cations such as Li\(^+\), NH\(_4\)\(^+\), Rb\(^+\), or Cs\(^+\) (18). This K\(^+\)-specific effect is thus not simply a general ion-screening effect. We have also previously shown that a hairpin-forming sequence, G\(_{13}\)C\(_{13}\), of the same length as the β-globin arrest site does not form a K\(^+\)-specific block to DNA synthesis, suggesting that the K\(^+\)-specific effect seen in the chicken β-globin promoter is not due to hairpin formation. Neither the pattern of DNA synthesis arrest nor the ion specificity are consistent with the formation of triplexes (2).

Previous data showed that the arrest site is found only when the G-rich strand served as template and was independent of template concentration, with the arrest of DNA synthesis being observed even when only femtomoles of template were present (18). These findings suggested that unusual intrastrand tetraplex-like structures might form the basis of the blocks to DNA synthesis. The intrastrand nature of these structures was confirmed by the observation that even at very low oligonucleotide concentrations, corresponding to template concentrations at which the blocks to DNA synthesis are still clearly visible, no intermolecular associations of an oligonucleotide containing the arrest site were observed by nondenaturing polyacrylamide gel electrophoresis (data not shown). However, in gels containing KCl, this oligonucleotide migrates slightly faster than an oligonucleotide containing the complement of the arrest site, suggesting that it can form a more compact intrastrand structure. While the difference in mobility is small, it is reproducible and is consistent with mobility differences that we have found for known tetraplex-forming sequences (26).

Evidence for Two Classes of Structures That Can Form on the Template Strand—To examine the K\(^+\)-dependent structures at single-nucleotide resolution we probed the arrest site oligonucleotide with DMS, OsO\(_4\), and BAA. These results are shown in Figs. 2 and 3.

Chemical modification reactions carried out under the same conditions as the assay of DNA synthesis arrest produce a result that reflects the sum total of the chemical modification of all the structures in the mixture. From a comparison of the amount of prematurely terminated polynucleotide chains relative to full-length products it is clear that the major molecular species present in the reaction represent those that cause DNA synthesis arrest at K1, with minor contributions from the structures that cause arrest at K2 and K3 (see Fig. 1), and that for all intents and purposes the chemical modification data will reflect the K1 structure.

To examine if the cytosine residue at position 17 was base-paired we first reacted the oligonucleotide with BAA and then treated it with formic acid, followed by piperidine. BAA reacts with the N-3 and the N-4 position of unpaired C residues. Treatment of a BAA-modified cytosine with formic acid enhances β-elimination by piperidine. Fig. 2 shows the results obtained for the BAA/formic acid modification of the arrest site oligomer. In the presence of 40 mM KCl, a strong band corresponding to the C\(_{17}\) residue is seen on a sequencing gel. This strong band translates into a tall peak for this residue on densitometric analysis. However, in the absence of KCl the density of this band is much reduced. These data suggest that C\(_{17}\) is modified by BAA in the presence of KCl, i.e. it is unpaired, while in the absence of KCl, it is resistant to BAA modification and is thus involved in a hydrogen-bonding interaction. As expected, the C\(_{17}\) residue was not reactive in the presence or absence of K\(^+\) when treated only with formic acid (data not shown).

To examine the thymidine residues in the arrest site, the arrest site oligonucleotide was modified with OsO\(_4\) in the presence of pyridine. Under these conditions, addition to the C-5 and C-6 double bonds of thymidine residues promotes formation of osmium esters that are susceptible to deavage with hot piperidine. OsO\(_4\) is significantly more reactive with unpaired residues and has been used successfully as a probe for DNA conformation junctions and for identifying loop regions in cruciforms (Ref. 27 and references therein). Our results are shown in Fig. 3. All thymidine residues in the sequence were reactive in the presence and absence of K\(^+\), but the intensity of modification of the T residues in the G tract was markedly increased in the presence of 40 mM K\(^+\), with T\(_{27}\) being particularly susceptible to OsO\(_4\) modification in comparison with T\(_{21}\) and T\(_{24}\).

DMS treatment of DNA results in the methylation of G residues at the N-7 position. This modification makes the residue susceptible to deavage by piperidine. In the absence of KCl, deavage with piperidine is significantly above background at all positions. G\(_{13}\) is most reactive, followed by G\(_{22}\), G\(_{26}\), G\(^+\)–G\(^5\), and the guanines outside of the arrest site (Fig. 3). Under these conditions, BAA modification indicated some protection of the C\(_{17}\) site, suggesting that it is base-paired. The pattern of slight protection from DMS by bases in the middle of

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\(^2\) M. N. Weitzmann, K. J. Woodford, and K. Usdin, manuscript in preparation.
the arrest site, combined with the hyperreactivity of G13, is consistent with the formation of a stem-loop structure with the G13 being in the loop. In such a hairpin, the N-7 of each G in a G-G base pair would be available for DMS modification about 50% of the time. The base that constitutes the hairpin loop, G13, would be the only base that was consistently available for DMS modification and would therefore appear hyperreactive. Since no arrest of DNA synthesis is seen under these conditions, it seems that this hairpin structure does not block DNA synthesis. This is consistent with our observation that a G-C hairpin of the same length also does not block DNA synthesis under these conditions.

In contrast, almost complete protection from DMS modification of some residues was seen in the presence of 40 mM KCl (Fig. 3). In the presence of K\textsuperscript{+}, DMS modification at G1 was similar to guanines outside the arrest site, and intermediate reactivity was observed at G2, G4, G7, and G11. DMS hyperreactivity was observed at position G3. The reactivity of the remaining G residues was reduced to close to background levels. Protection of the N-7 position of guanine residues is diagnostic of structures containing G-G Hoogsteen base interactions. The apparent complete protection of some of the guanine residues from DMS modification indicates that they are involved in hydrogen bonding interactions in which they act as N-7 donors almost all of the time. The DMS reactivity pattern observed in 40 mM NaCl was identical to that observed without potassium, illustrating that the DMS protection observed in the presence of K\textsuperscript{+} is not simply a general cation effect (data not shown).

The Effect of Interruption of Guanine Runs in a Tetraplex Stem—If the structure formed by the wild-type chicken \( \beta \)-globin promoter were indeed a tetrahelical structure of some sort, it would suggest that a number of non-guanine bases could be accommodated in the tetrahelix. Our chemical modification experiments with BAA indicated that the cytosine in the structure is unpaired. We addressed the question of the effect of a C residue in a tetraplex stem on structural stability in two other sets of experiments. First, a plasmid bearing the sequence \((T_2G_5)T_2G_2CG_2(T_2G_5)\) (pCstem) was constructed and tested for its ability to arrest DNA synthesis in vitro. For comparison a plasmid bearing the known tetraplex-forming sequence \((T_2G_5)_4\) that was constructed in our lab and shown to block DNA synthesis in the presence of K\textsuperscript{+} was employed. The Cstem sequence has a single cytosine in place of a guanine in the central guanine-tract that comprises one strand of the stem of the tetraplex. The Cstem sequence has a single cytosine in place of a guanine in the central guanine-tract that comprises one strand of the stem of the tetraplex. The Cstem sequence still formed a block to DNA synthesis at the same position as that observed for the \((T_2G_5)_4\) sequence (Fig. 4), although the arrest site was significantly weaker than that observed with the \((T_2G_5)_4\) sequence. This is consistent with our observation of strong protection from DMS modification for the \((T_2G_5)_4\) sequence, and much weaker protection for the Cstem sequence (data not shown).

However, the arrest site in the chicken \( \beta \)-globin locus seems to contain at least three non-G interruptions. The fact that this region still forms such a strong block to DNA synthesis is indicative of the fact that some additional stabilizing factors must be present that compensate in some way for these interruptions.

\textsuperscript{3}M. N. Weitzmann, K. J. Woodford, and K. Usdin, unpublished results.
Defining Arrest Site Requirements Using Arrest Site Sequence Variants—To define those bases important for the structure adopted by the G_{15}CG(GGT)_2GG sequence, we constructed a series of plasmids with slight sequence variations in the arrest site. The pattern of DNA synthesis arrest in these variants is shown in Fig. 5. Stopping at K1 was restored only in those variants pM2 and pM3, but the strength of the arrest sites was not duplicated in any of the constructed mutants. Replacement of G1–G4 with TCGA, TGGA, TCGG, and TCCC abolished stopping at K2 (BG6, pM1, pM2, and pM3), indicating that the structure forming the underlying block to synthesis at K2 requires at least the sequence G_{15}CG(TG)_2. The stop at K3 was not negatively affected by any of these mutations, indicating that the sequence necessary and sufficient for the structure that causes the stop at K3 is G_{19}CG_{19}. This observation is interesting since it demonstrates that even relatively short interrupted G runs can still form blocks to DNA synthesis. With respect to K1, replacing G1–G2 with TC (pM2) reduced the extent of stopping but did not abolish it, suggesting that these residues are important for stability but are not essential in order to get a block at this point. However, replacement of G4 by an A was sufficient to eliminate the stop (BG6), and this effect could be partially alleviated by substitution of a C for the A (pM3). Elimination of T24 changed the pattern of polymerase arrest, illustrating that this residue is not looped out of the structure but is an integral feature of the arrest site.

**DISCUSSION**

We have previously shown that the chicken β-globin promoter contains a strong composite arrest site for DNA synthesis in vitro (18). That DNA synthesis arrest is template concentration-independent and is seen only on one strand suggested that the underlying physical basis was the formation of a series of intrastrand structures. The G-richness of the arrest site (the sequence 5′-G_{15}CG(GGT)_2GG-3′ is necessary and sufficient to cause synthesis arrest) suggested that the arrest site might involve G-G base interactions. The K1 specificity suggested that despite its relatively short length, its lack of four clearly identifiable G-repeats, and the presence of a number of non-canonical bases, arrest was due to a series of intrastrand tetrahedral structures of some kind.

These conclusions are supported by experiments shown here. In gel electrophoresis of oligonucleotides containing the arrest site in the presence of K+ a high mobility species was observed consistent with intrastrand folding. The fact that a hairpin-forming sequence (G_{19}C_{19}) of the same length as the arrest site produces no K+ dependent block to DNA synthesis suggested that arrest of DNA synthesis by the chicken promoter is not due to hairpin formation.

Our chemical modification data are consistent with the major DNA synthesis arrest site being due to the formation of a novel intramolecular tetrahedral structure in the presence of K+. The complete protection of bases G^{12–26} from DMS modification indicates that guanine tetrads are involved. The hyperreactivity of G2 suggests that it might be located at the junction between the tetraplex and bases 5′ of the tetraplex, and the OsO4 hyperreactivity of the T just 3′ of the arrest site defines the 3′ limit of bases involved in the structure. A tetraplex of the length defined by the distance between these two bases i.e. 23 would have three loops spaced approximately an equal number of bases apart at around G7–G9, G13–G15, and G19–T21. The DMS reactivity seen for bases G3–G26 is confined to bases G9, G7, and G11. It is hard to fit all of these reactive bases into the loops of the tetraplex, and it seems likely that the loop bases are not in fact DMS-reactive and that reactivity at G9, G7, and G11 is the result of some other structural feature. The lack of reactivity of loop bases may be due to stacking interactions, transient base pairing in or between loops, or binding to K+. The reactivity of G4, G9, and G11 can perhaps be accounted for by placing them adjacent to some of the non-G bases. The reactivity of C17 with BAA and the OsO4 hyperreactivity of T21 and T24 suggest that these bases are all unpaired. The DMS protection of G residues that would be in the same plane as G4, G9, and G11 are consistent with G-G-G base triplets, with the DMS-reactive base acting as an N-7 acceptor but not an N-7 donor. The non-G base adjacent to the reactive G presumably fills the space that would normally be occupied by the fourth G in the tetrad but does not participate in hydrogen bonding. One possible structure that accounts for this pattern of reactivity is shown in Fig. 6B. In this structure G14 is shown as being in a loop on the same side of the structure as the base G9 and G25. Interaction among G14, G15, and G25 in a G-G-G triplet in which G14 and G25 act as N-7 donors would explain the DMS protection of G14 and G25.

The pattern of DNA synthesis arrest by sequence variants confirms various details of the structure shown in Fig. 6B. Replacement of G1–G2 with the residues T-C eliminates the second arrest site (K2) and reduces the extent of arrest at K1. Replacement of these residues together with a substitution of A for G4 eliminates the first arrest site altogether. On the other hand, replacement of G4 by a C reduces but does not eliminate this arrest site. This might indicate that G4 is involved in hydrogen bonding in a context in which a C can substitute at least partially. We interpret the hydrogen-bonding contribution made by G3 in terms of a molecular cinch that holds the end of the tetraplex closed, making it more difficult for the polymerase to traverse this region. The fact that a G-to-A substitution at G4 eliminates the stop at K1 and that a C at that position partially restores the stop might be due to the fact that the C permits interaction with the top portion of the stem that, according to this model, becomes folded back, while an A at that position would hydrogen bond to the T in the same end of the stem, providing no stabilization of the fold-back structure.

The fact that the ability of all of these variants to block DNA synthesis is considerably less than that of the wild type suggests that bases G1–G2 also make a contribution to the stability of the structure, perhaps as a result of stacking interactions on
the single strand or from pairing with bases outside the tetraplex-forming region. The DMS protection of G26 is consistent with a G-G interaction between G26 and G2 in which G3 acts as the N-7 acceptor. Deletion of T24 (Tout in Fig. 5) abolished the original pattern of DNA synthesis arrest and resulted in the formation of two new arrest sites both located at bases 3' of the original arrest site. This illustrates that T24 plays an important role in the arrest site structure. The pattern of arrest in this mutant is also consistent with the structure shown in Fig. 6B, if it is assumed that formation of a G-G base pair with bases immediately flanking the tetrahelix is an important stabilizing factor. In this case G25 would move into the tetrahelix, and G26 would be available for hydrogen bonding to G2 and G14. However, in the absence of a hydrogen bonding partner for G2 3', this interaction might not be stable, resulting in the stop at G26. In spite of the paucity of complete tetrads in the wild type arrest site structure, K+ may still be able to bind to guanines in adjacent rungs of the structure since the internal dimension of the channel might still resemble a more conventional tetraplex.

Direct evidence for the ability of non-G bases to be accommodated into the stems of tetrplexes was obtained by comparing a known tetraplex-forming sequence (T2G5)4, and a sequence T2G5T2G2C(T2G5)2 that is identical except for a single C residue that disrupts one of the four G-rich repeat motifs. Both the ability to block DNA synthesis and the DMS protection of stem guanines were decreased markedly in the template containing the interrupted motif, but clear evidence for tetrplex formation was still visible. The extent of DNA synthesis arrest by the full-length arrest site is comparable for tetrahelix sequences to form a cinch, greatly extends the range of sequences that could potentially form tetrahelical structures that block DNA synthesis.

In theory the structure we have described could form in vivo any time that the duplex region containing the sequence becomes unpaired. Replication or transcription would provide such an opportunity, as would local melting of the duplex or formation of the triplex, found in this region (29). In fact a large region of the chicken $\beta$-globin gene promoter is known to be $K^+$-sensitive switch. The structure may act by chemical modification as described in the text. In the absence of $K^+$ the sequence G26CG(GGT)2GG forms a K+-dependent tetrahelix. Bases adjacent to the four-stranded tetrplex structure interact to stabilize the structure. This structure presents a formidable block to DNA synthesis in vitro. The G residues shown in outline are those modified by DMS.

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FIG. 6. Structures formed by the chicken $\beta$-globin sequence in the absence and presence of $K^+$.

Structural models were generated on the basis of chemical modification data as described in the text. In the presence of $K^+$ the sequence G26CG(GGT)2GG forms a hairpin structure (A) that does not present a block to DNA synthesis in vitro. In the presence of $K^+$ the sequence G26CG(GGT)2GG forms a cinched tetrahelix (B). Bases adjacent to the four-stranded tetrplex structure interact to stabilize the structure. This structure presents a formidable block to DNA synthesis in vitro. The G residues shown in outline are those modified by DMS.
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