H-DNA: DNA TRIPLEX FORMATION WITHIN TOPOLOGICALLY CLOSED PLASMIDS

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I. INTRODUCTION

Nucleic acid triplex structures were discovered in 1956 by Davies and Rich who observed an apparent increase in solution viscosity upon interacting equimolar mixtures of the synthetic ribo-homopolymers poly(A) and poly(U) (Rich and Davies, 1956). Although originally interpreted as poly(A-U) duplex formation, stoichiometric analysis later indicated that in the presence of moderate concentrations of Mg\(^{2+}\) (or other cations) the predominant structure formed from this mixture was actually a three-stranded complex (Felsenfeld et al., 1957). Since that time a wealth of information regarding the structure, stability, thermodynamics, etc. of triple helices has emerged (for example see recent review by Cheng and Pettitt, 1992). In addition, the potential biological significance of triplex DNA structures is a topic that has also received considerable recent attention (e.g. Helene, 1991; Durland et al., 1991, etc.). Although there still exists many uncertainties regarding the detailed molecular properties of these structures it is possible to summarize some of their general principles. Typically, nucleic acid triplex structures can form along tracts of all-purine (or purine-rich) duplex helical regions. This is a consequence of the triplex H-bonding scheme which requires residues of the third-strand to form “Hoogsteen-type” hydrogen bonds with N-6 and N-7 positions along runs of duplex purine residues. These purine positions project into the major groove of the duplex and are available for triplex hydrogen bonding with residues on the third strand bound within the major groove. This arrangement provides for considerable sequence specificity in triplex formation. Although details of these specificities are still emerging, it generally appears that either A or T residues can form triplex opposite A residues on a duplex, and either G or C\(^+\) can form triplex opposite G (the C residues require protonation at the N-3 position in order to form triplet hydrogen bonds).
Although unique sequences which meet these specificity requirements are clearly able to form triplex, it also appears that third-strand pyrimidine residues adopt different geometries than purines in the third strand, and therefore most stable triplexes contain mostly either all-purines or all-pyrimidines in the third strand.

The stability and various melting transitions of triplexes have been extensively investigated. In general, because of the large anionic charge density due to the phosphate groups along the triplex backbone, these structures are highly sensitive to cation concentration. Third strands containing C residues are additionally sensitive to pH because of the requirement for these residues to protonate in order to form triplex. Manipulation of temperature along with these conditions has enabled the study of different association and disassociation (melting) transitions. One particularly interesting transition involves the $2 \rightarrow 3$ disproportionation of double helix into triplex. In this transition one half of the duplex structures dissociate into single strands, and half of the single strands then associate with the remaining duplex to form triplex. For example:

$$2 \text{poly}(A \cdot T) \rightarrow \text{poly}(T \cdot A \cdot T) + \text{poly}(A).$$

This transition has been extensively investigated; it is generally favored at moderate to high ionic strength and intermediate temperatures (sufficiently high temperatures will eventually favor the complete dissociation into all single strands). The thermodynamics of this transition has also been studied and it is believed that the increased entropy of the dissociated poly(A) single strands is the principle component favoring disproportionation (Stevens and Felsenfeld, 1964).

1. **H-DNA Formation**

This very interesting and unusual structure is formed through a unimolecular intrastrand disproportionation along purine-rich tracts of DNA which are topologically constrained within closed circular plasmids. In this transition one half of the tract dissociates into single strands, and one of these single strands (usually the pyrimidine-rich strand) swivels (or "hinges") its backbone parallel to the remaining purine residues to form Hoogsteen-type triplex hydrogen bonds. The resulting structure is comprised of a three-stranded helix along one half of the purine-rich insert, plus a region of unpaired single-stranded residues along its remaining half (see Fig. 1). This structure was originally observed in negative supercoiled circular plasmids containing inserts with alternating G-A and other all-purine "mirror repeat" sequences (later termed "H-palindromes") (see Fig. 2). It was detected from the change in topological supercoil density which accompanies this transition (discerned from 2-D gel electrophoresis) (Lyamichev et al., 1985), and from increased sensitivity to S1 nuclease (Pulleyblank et al., 1985; Lyamichev et al., 1986; Mirkin et al., 1987; Vojtiskova et al., 1988). Originally, because of the requirement for C residues in the third strand to protonate in order to form C$^+ \cdot G \cdot C$ triplets the structure was termed H-DNA. Later it was also noted that this term is additionally appropriate because of the requirement for "hinging" of the denatured single-stranded region into a parallel orientation along the intact purine duplexes (Htun and Dahlberg, 1989). Investigations using site-specific DNA chemical modification reagents (such as dimethylsulfate, diethyl pyrocarbonate, OsO$_4$, etc.) followed by Maxim–Gilbert-type cleavage provided strong direct evidence for the unimolecular nature of this transition, as well as additional details about its structure (Voloshin et al., 1988). Other studies have also provided insights into the stability, structure, topology, and sequence effects of this structure. In the following sections some of these findings will be summarized and discussed.

### II. CONDITIONS INFLUENCING FORMATION AND STABILITY

#### 1. **pH and Topological Stress**

This structure has typically been formed within closed plasmids by increasing negative supercoil stress at constant temperature and (reduced) pH. In this transition (which can be considered analogous to unimolecular B$\rightarrow$Z transitions within closed plasmids also induced
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Fig. 1. Two representations of H-DNA structure. Regions with solid dots (●) depict duplex structure, regions with open dots (○) and (+) depict triplex (adopted from Lyamichev et al., 1986 (a), and Hanvey et al., 1988a (b)).

Fig. 2. Duplex tract showing a typical mirror repeat “H-palindrome”. The boxed area represents the region of symmetry axis where the single-stranded “hinge” region of the H structure is formed.

by topological stress) the torsional stress provides the energy required to promote the B→H transition.

The magnitude of supercoil stress required to induce this transition is strongly dependent on pH. For example, in an early investigation 2-D gel electrophoresis was used to monitor the transition within closed plasmids containing a repeating tract of (G−A)$_{16}$ alternating
purines (Lyamichev et al., 1985). Various topoisomers of this plasmid were analyzed for the ability to undergo the B→H transition at varying pH (at room temperature and moderate ionic strength, 0.1 M NaCl). The relationship between pH and the linking number required to promote the transition clearly shows that this disproportionation can be induced at lesser supercoil densities as pH decreases (Fig. 3). In this particular insert it was observed that negative supercoil densities of −0.08 were necessary to induce the transition at pH 7, whereas at pH 6 and pH 4.3 the transition was induced at biological supercoil densities of −0.055 and −0.01, respectively. Other approaches utilized reactivity to chemical modification followed by analysis of site-specific termination of in-vitro RNA transcription as a more sensitive structural probe of this transition (Htun and Dahlberg, 1988). Plasmids containing (A→G)18 inserts also showed increased stabilization of this structure at decreased pH and increased supercoil densities, but also showed (by these analyses) some very low levels of H-DNA structure present at pH 7 and 8 at biological supercoil densities (approximately −0.05). These studies also suggested the possibility of H structures within topologically relaxed plasmids at pH = 4.0.

2. Effects of Base Sequence and Length

There have been several studies investigating the influence of sequence and length of the purine-rich insert on the formation and stability of this structure. One of the earliest observations of this transition occurred along a run of (G→A)16 alternating purines (Lyamichev et al., 1985, 1986). Subsequently, it was demonstrated that the requisite sequence feature was a region of all-purine residues containing a mirror repeat (termed an “H-palindrome”) along a two-fold symmetry axis (Mirkin et al., 1987) (Fig. 2). This requirement is, in fact, a predictable outgrowth of the structure of the transition product and the binding specificities of third-strand residues which predict that pyrimidine residues can form triplex only opposite purines with which they can also form Watson–Crick base-pairs. In addition, it was also observed that the nucleotide region containing the axis of symmetry of this palindrome can include an additional region (of variable sequence and length) which itself, does not have to contain all-purine sequences or repeat symmetry. This region, containing the symmetry axis within the middle of the repeat, comprises the “hinge” region of the H-DNA disproportionation structure and forms a single-stranded loop which is the only region of this structure containing both the 5′→3′ and the 3′→5′ strands in a denatured, single-stranded conformation (a property which has been successfully exploited in various structural probe investigations).

(a) Variations in loop size

Several studies have specifically investigated the effects of varying the size of this “hinge” region. Studies on perfect mirror repeats (GAA)9 within circular plasmids generated
structure-specific chemical modification profiles consistent with the formation of single-stranded hinge regions that were four nucleotides in length (Hanvey et al., 1988a,b). Because these perfect mirror repeats (containing no variable sequence region in the center of the symmetry axis) could support the formation of any sized single-stranded hinge-loop structure this result was interpreted as indicating that four nucleotides is the energetically ideal loop size in these structures. Other investigations utilized chemical probes to characterize the B→H transition in different plasmids, each containing two regions of (GAA), separated by varying lengths of different "hinge" sequences (ranging from 3 to 10 nucleotides long) (Shimizu et al., 1989). Thermal stability studies (at uniform pH 5, and biological supercoil densities) demonstrated that all of these structures were able to undergo the B→H transition, but it was more favored in plasmids with smaller hinge loop regions. Structural analysis by site-specific chemical modification patterns confirmed that the energetically preferred loop size contained 4 nucleotides.

(b) Sequence composition effects

The effects of sequence of the mirror repeat region have also been investigated. Studies utilizing site-specific chemical modification patterns (Hanvey et al., 1989) and structure-specific enzymatic digestion (Hanvey et al., 1988b) of plasmids containing different sequence mirror repeats (of approximately equal length ≈20–24 nucleotides) investigated the thermal stability of these structures (at constant pH and native supercoil density), and the dependence of their B→H transitions on different levels of torsional stress (at constant pH 4.0). The trend from these results shows a clear relationship between increased %GC composition (within the repeat) and increased stabilization of the H-DNA structure (Fig. 4). In addition, there appears to be a minimum requirement for at least 20% GC composition within the entire repeat in order for this topologically-induced transition to proceed; inserts containing less than 20% GC (including tracts of pure oligo(A·T)) consistently showed no detectable evidence of this transition across any range of ionic strength, supercoil density, and pH (including neutrality). This latter observation appears surprising because of the established ability for poly(dA·dT) to undergo intermolecular disproportionation (Blake et al., 1967), and because of the increased energetic cost of having to protonate more C residues as %GC composition increases. The rational for this may reside in the increased stacking properties of G-rich single-stranded purine strands, or in some (as yet poorly understood), sequence-dependent topological properties of these structures. It is noteworthy that this relationship between GC composition and increased H-DNA stability was observed at decreased pH (4.5–5.0), and it is not clear to what extent this dependence would hold up at higher ranges of pH where the increased energetic cost of protonating C residues might play a role.

An additional investigation demonstrated that inserts containing 100% oligo(G·C) seemed to undergo two different, structurally-distinct transitions depending on the presence or absence of Mg2+ (Kohwi and Kohwi-Shigematsu, 1988). Investigations (utilizing structure-dependent enzymatic digestion, and chemical modification probes) studying oligo(dG·dC)30 inserts within natively supercoiled plasmids revealed significantly different cleavage patterns in the presence or absence of 2 mM MgCl2. These results indicated that in the absence of Mg2+, and at mildly decreased pH (< 5.5), native levels of topological stress induced the previously observed B→H structure with C+ · G· C triplex structures along one half of the insert, plus single-stranded oligo(G) residues. However, in the presence of 2 mM Mg2+ the cleavage pattern clearly indicated the exclusive formation of an alternative disproportionation product containing G· G· C triple helices, plus oligo(C) single-stranded regions. This latter disproportionation product was the only one observed across a wide range of pH (5.0–8.0) when Mg2+ was present. This is an interesting finding which may suggest that in the presence of Mg2+ the G· G· C triplet appears more stable than C+ · G· C (even under mildly acidic conditions, pH 5.0).

(c) Mirror repeat sequence length variations

There is limited information concerning the stability of this structure within inserts
containing similar sequence but varying length. However, some observations may be summarized from the present literature. A tendency appears to be increased stabilization of this structure as length of the repeat increases. In one investigation different repeat lengths of oligo(A–G) (ranging from 10–60 nucleotides long) were cloned into circular plasmids which were induced (with negative supercoil stress) to undergo the B→H disproportionation (pH 5.0) (Htun and Dahlberg, 1989). These results indicated that smaller repeat lengths required increased levels of negative supercoil stress in order to induce the transition. Smaller repeat lengths of (A–G)$_{18–26}$ (containing insert lengths of 18–26 nucleotides) required larger levels of torsional stress to induce the transition, and (A–G)$_{10}$ (containing a 10 nucleotide insert) failed entirely to undergo the transition under any of these experimental conditions. This trend implies that longer repeat lengths stabilize this structure and is probably accounted for by the recognition that longer repeat lengths relax greater levels of supercoiled writhe upon transition (see Section III.2).

(d) Sequence symmetry imperfections

Studies investigating the necessity for perfect mirror repeat symmetry substituted one nucleotide deviations into otherwise perfect H-palindromes, and then used the relationship between pH and required levels of topological stress to evaluate the effects of these symmetry deviations on the formation and stability of the H-DNA structure (Mirkin et al., 1987). These results demonstrated that inserts containing one nucleotide deviations from perfect mirror repeat symmetry either failed to undergo detectable levels of B→H disproportionation, or did so only at increased levels of negative supercoil stress and/or highly reduced pH. Later
investigations (Hanvey et al., 1988a, 1989) utilized sensitive site-specific chemical modification probes to investigate the effects of specific types of symmetry deviations. The results indicate that the observed outcome depends on the number, type, and location of these sequence symmetry imperfections. A one nucleotide sequence interruption typically seems to allow triplex structures to form with the inclusion of a triplet mismatch loop structure (readily detected by chemical modification studies). This imperfect H-DNA triplex is thermally destabilized by 5–7°C, and requires increased levels of negative supercoil energy to form (Fig. 5). However, if the one nucleotide interruption is positioned near the end of the triplex stem, then the structure is observed to simply form a smaller triplex region in order to avoid formation of a mismatched loop structure. Other inserts containing a tandem three nucleotide sequence deviation positioned within a large region of otherwise perfect mirror repeats were also induced to undergo this transition at native supercoil densities, pH 5.0. In this instance, site-specific chemical modification probes clearly indicated that a long H-DNA structure did not form (i.e. a three nucleotide long triplet mismatch loop structure was not tolerated). Instead, the results showed that shorter triplex structures formed only along one side of the sequence imperfection in order to avoid formation of a large unstructured loop.

3. Ionic Strength Effects

Although the influence of ionic strength on interstrand disproportionation has been well studied there is currently limited information regarding ionic strength effects on the B→H transition. In general, because triplex anionic charge density exceeds the charge density of duplexes, increasing ionic strength is observed to favor interstrand disproportionations. However, this trend is not observed from the current literature on B→H transitions. Most
investigations into this structure were conducted at invariant concentrations of moderate ionic strength (e.g. 0.1–0.2 M Na+) (Lyamichev et al., 1985, 1989; Mirkin et al., 1987). In some studies (Htun and Dahlberg, 1988, 1989) low levels of Mg2+ (e.g. 0.1 mM) were used, but concentrations were typically not varied so these effects could not be assessed.

There have been some investigations in which ionic strength was varied which may provide some preliminary observations. In one series of experiments enzymatic probes were used to monitor the B→H transition across a range of pH and ionic strength (0–0.2 M NaCl) (Hanvey et al., 1988b). At decreased pH (4.6) no difference in sensitivity to enzymatic structural probes was observed across this range of ionic strength. However, at increased pH (7.5) an insert containing alternating (A–G)15 showed increased stabilization of the H structure as ionic strength increased from 0–0.2 M NaCl. (It should be noted that these results could not be quantified with the same degree of sensitivity as, for example, site-specific chemical modification experiments, so these results should must be considered preliminary.) This trend may be related to observations on the interstrand disproportionation of poly(GC)→poly(C + · G · C)+poly(G) which is stabilized by increased ionic strength at decreased pH (Lee et al., 1979). It is possible that the decreased overall stability of the H structure at neutral pH renders it more sensitive to stabilization by increased ionic strength.

4. Kinetic Considerations

There is limited information regarding the kinetics of this transition due, in large part, to the inherent inability of the procedures typically used to monitor the transition (e.g. chemical modification studies, 2-D PAGE, etc.) to quantitatively resolve information within very short time frames. However, in one investigation (Hanvey et al., 1989) a (G–A–A)8 mirror repeat sequence (separated with a three nucleotide TTC hinge sequence in the center) was rapidly dropped from pH 8.0 to pH 5.2 under conditions of moderate (native) supercoil density. P1 structure-specific nuclease analysis (which was shown to be an effective probe of the transition across this pH range) was immediately performed after decreasing pH, and H-DNA formation was detected within 20 sec (the shortest time interval that the assay permitted). Triplex formation was observed to increase over subsequent time points, and the transition was shown to be complete within 1.5 min.

To assess the kinetics of the reverse, H→B transition, supercoiled plasmids with the same insert (at pH 5.2) were relaxed with topoisomerase, and the loss of triplex was monitored by sensitivity to P1 nuclease. These results indicate that ≈ 50% of triplex had dissociated within 2 min, and the structure was completely undetected within 3 min. These time frames correspond to previously observed rates of supercoil relaxation of circular plasmids, so it is likely that triplex dissociation (and the H→B transition) instantaneously followed topological relaxation.

III. STRUCTURAL AND TOPOLOGICAL CONSIDERATIONS

1. H-DNA Structure

A structural observation that was made early on, and repeatedly confirmed (Htun and Dahlberg, 1988; Hanvey et al., 1988a,b) was that H-DNA consistently assumes only one out of two possible structural conformations. Figure 6 shows that this structure can form either by the 5′ end of the pyrimidine region swiveling around to form triplex with the 5′ end of the purine duplex (termed the H-Y5 conformation), or alternatively, the 3′ end of the denatured pyrimidine sequences swiveling to associate with the 3′ end of intact double helical purines (termed H-Y3). Despite the apparent energetic equivalence of these two possible conformations, essentially every structural investigation into the B→H disproportionation has conclusively demonstrated that the latter (H-Y3) conformer is usually the exclusive disproportionation product observed from topologically-induced B→H transitions. One exception was detected from chemical modification analysis of an (A–G)18 insert induced to undergo the B→H transition in the absence of topological stress (Htun and Dahlberg, 1989). This finding implied that there were topological differences between these two conformations which were influencing the structural outcome (see next section).
H-DNA: DNA triplex formation

Fig. 6. The two possible structural conformation of H-DNA. Experimental data indicates that structures topologically induced to form consistently adopt the H-Y3 conformation (adopted from Wells et al., 1988).

Fig. 7. Negative supercoil relaxation as a function of repeat length. The maximum number of negative supercoils relaxed by H-DNA formation was determined for TC<AG> repeats of various lengths by two-dimensional gel electrophoresis. The slope of the line between 15–30 repeat units shows that one supercoil is relaxed for every 11 base-pair of repeat (adopted from Htun and Dahlberg, 1989).

2. Topological Considerations

The mechanism by which topological stress is able to induce this B→H transition is an interesting topic that has been investigated. In one very elegant work, investigators used 2-D gel electrophoresis to quantitate the number of supercoils relaxed (at pH 5.0) as alternating (A–G) inserts of varying length were induced to undergo the B→H transition (Htun and Dahlberg, 1989). These results (summarized in Fig. 7) show the relationship between number of relaxed supercoils and mirror repeat length. The slope of this plot indicates that one supercoil is relaxed for every eleven base-pairs undergoing this transition. Although the eleven base-pairs correspond to approximately one region of helical “twist” (which might be expected to unwind for every relaxed supercoil), it is noteworthy that for every region of mirror repeat that undergoes this transition only one half of the repeat residues actually denature into single strands. This implied that there was an additional source of reduced helical twist accompanying this transition, and led to the formation of the “corkscrew” model of H-DNA formation. This model predicts that as one (“donor”) region of mirror repeat denatures into single strands the remaining (“acceptor”) region rotates in the direction of negative twist in order to form triplex by winding its major groove around the single strand from the “donor” region. This model predicts that one full turn of negative helical twist
Table 1. Thermostability and Transition Free Energy of Formation of Intramolecular DNA Triplexes

<table>
<thead>
<tr>
<th>Loop size</th>
<th>Loop sequence</th>
<th>GC content (%)</th>
<th>T_{50%} °C</th>
<th>(\sigma_{50%})</th>
<th>(-\langle x - x\rangle_{1/2})</th>
<th>(\Delta T_w)</th>
<th>(\Delta G_{tr}) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>CGAA</td>
<td>50</td>
<td>55.0</td>
<td>0.028</td>
<td>7.2</td>
<td>3.7</td>
<td>12.8</td>
</tr>
<tr>
<td>6</td>
<td>CGCGAA</td>
<td>67</td>
<td>52.9</td>
<td>0.036</td>
<td>7.6</td>
<td>4.1</td>
<td>14.8</td>
</tr>
<tr>
<td>8</td>
<td>CGAATTAA</td>
<td>25</td>
<td>53.6</td>
<td>0.034</td>
<td>7.1</td>
<td>4.3</td>
<td>13.7</td>
</tr>
<tr>
<td>10</td>
<td>CGCGAATTAA</td>
<td>40</td>
<td>51.7</td>
<td>0.043</td>
<td>8.4</td>
<td>4.4</td>
<td>17.6</td>
</tr>
</tbody>
</table>

GC content is the base composition of the sequence of the loop regions. \(T_{50\%}\) is the temperature at 50% initial triplex remaining as determined by OsO_4 modification. \(\sigma_{50\%}\) is the average supercoiling density at 50% OsO_4 modification. \(-\langle x - x\rangle_{1/2}\) is the critical linking difference, the linking difference at which the B and non-B states possess equal free energy. \(\Delta T_w\) is the amount of relaxation observed. \(\Delta G_{tr}\) is the change of free energy of supercoiling (kcal/mol) for the B to triplex transition calculated from:

\[
\Delta G_{tr} = \frac{1100RT}{N} \left[ \langle \langle -\langle x - x\rangle_{1/2} \rangle \rangle - \langle \langle -\langle x - x\rangle_{1/2} \rangle \rangle - \langle \Delta T_w \rangle^2 \right]
\]

where \(R\) is the gas constant, \(T\) the absolute temperature, and \(N\) the plasmid size (from Shimizu et al., 1989).

(Anchored one negative supercoil) is generated from each eleven base-pair turn of insert undergoing this transition.

This model was also used to explain the observation of only H-Y3 conformational structures generated when the transition is topologically induced. Model analysis of this mechanism predicted that there was increased negative twist associated with the formation of H-Y3 structures versus H-Y5 structures. Furthermore, it was shown that the H-Y5 structure is in fact, the favored conformer when the transition is induced under conditions of very low or no topological stress (explained by predicted increased stacking energy in the H-Y5 conformer), whereas high topological stress generated only the H-Y3 conformer (which relaxes more supercoil writhe).

IV. THERMODYNAMIC CONSIDERATIONS

Various analyses of interstrand disproportionation transitions have demonstrated that the energetic drive promoting this transition essentially comes from increases in entropy accompanying the transition at increased temperature (Stevens and Felsenfeld, 1964). In contrast, the B→H intrastrand disproportionation is typically induced isothermally within closed circular plasmids with the energy stored in the plasmid as topological writhe. Previous analyses of stored supercoil energy predicts that free energy of supercoiling is expressed as:

\[
\Delta G = \frac{1100RT}{N} \langle \Delta L \rangle
\]

where \(N\) is total number of base-pairs in the plasmid, \(R\) is the gas constant, and \(\Delta L\) is the total change in linking number (Peck and Wang, 1983). By experimentally quantitating the number of negative supercoils relaxed for a particular B→H transition, calculations have been made for the accompanying free energy change. By using these approaches investigations into the effects of hinge loop size showed that free energies associated with this transition range from 13–18 kcal/mol (Shimizu et al., 1989). It was also observed that structures containing a ten nucleotide hinge loop region required \(\approx 5\) kcal/mol additional torsional free energy to form triplex than a structure containing the more favorable loop size of four nucleotides (Table 1).

Another investigation experimentally monitored the change in linking number accompanying these transitions within various sized repeat regions, and at different pH (Lyamichev et al., 1989). The extent of transition was experimentally evaluated at different supercoil densities and the 50% transition point was assumed to correspond to the equilibrium position where \(-\Delta G\) generated from supercoil relaxation equals \(\Delta G\) for the B→H transition. These results suggest that this transition contains a "length-independent" unfavorable "energy of nucleation" equal to \(\approx 18\) kcal/mol (which is also independent of pH), plus a favorable free energy of triplex formation ranging from \(-0.23~0.39\) kcal/mol, which is linearly dependent on pH. The energy necessary to overcome the unfavorable free energy component was consistent with that provided by the \(\Delta G\) generated from the relaxation of negative supercoils.
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REFERENCES


Lee, J. S., Johnson, D. A. and Morgan, A. R. (1979) Complexes formed by (pyrimidine)n • (purine)n DNAs on lowering the pH are three-stranded. Nucl. Acids Res. 6(9), 3073–3091.


