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Visualisation of inter loop tertiary base pairing and stacking interactions in an unusual slipped loop DNA structure†

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Abstract. The conformation of an unusual slipped loop DNA structure exhibited by the sequence d(GAATTCCCGAATTC)$_2$ is determined using a combination of geometrical and molecular mechanics methods. This sequence is known to form a B-DNA-like duplex with the central non-complementary cytosines extruded into single stranded loop regions. The unusual feature is that the interior guanine does not pair with the cytosine across, instead, it pairs with the cytosine upstream by skipping two cytosines, leading to a slipped loop DNA structure with the loops staggered by two base pairs. The two loops, despite being very small, can fold across minor or major groove symmetrically or asymmetrically disposed, with one of the loop bases partially blocking the major or minor groove. Most interestingly, for certain conformations, the loop bases approach one another at close proximity so as to engage even in base pairing as well as base stacking interactions across the major groove. While such pairing and stacking are common in the tertiary folds of RNA, this is the first time that such an interaction is visualized in a DNA. This observation demonstrates that a W-C pair can readily be accomplished in a typical slipped loop structure postulated for DNA. Such tertiary loop interaction may prevent access to regulatory proteins across the major groove of the duplex DNA, thus providing a structure-function relation for the occurrence of slipped loop structure in DNA.

Keywords. Slipped loop DNA structure; unusual DNA structure; inter loop base pair in DNA; inter loop base stacking in DNA; staggered loop DNA structure; regulatory protein inhibition.

1. Introduction

Gene sequences comprising characteristic sequence elements promoting unusual structures in solution are being now and then identified from enzymatic and other solution experiments. Structures, such as cruciforms by inverted repeat sequences, triple helices by long stretches of homopurine-homopyrimidine sequences and quartets by repeating guanine stretches, are a few of the well known non-canonical polymorphs of DNA. Similarly, results from S1 nuclease studies on DNA containing direct repeat sequences have led to the postulation (Hentschel *et al* 1982; Mace *et al* 1983; McKeon *et al* 1984) of a slipped loop DNA structure. Direct repeat sequences having the potential to give rise to slipped loop DNA structure, occur near regulatory regions of both prokaryotic and eukaryotic DNA. Such sequences have also been shown to be conserved during evolution (McKeon *et al* 1984). For instance, slipped structures

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are postulated for promoter sequences of chicken beta globin, sea urchin histone gene (Hentschel *et al* 1982), α2(I) collagen gene of mouse and collagen gene of chicken (McKeon *et al* 1984). The fact that sequences forming slipped DNA structure are conserved in evolutionarily widely separated species such as mouse and chicken suggest that they might play a crucial role in regulation of these genes. Moreover, locally unpaired residues forming the loop in slipped DNA structures can serve as

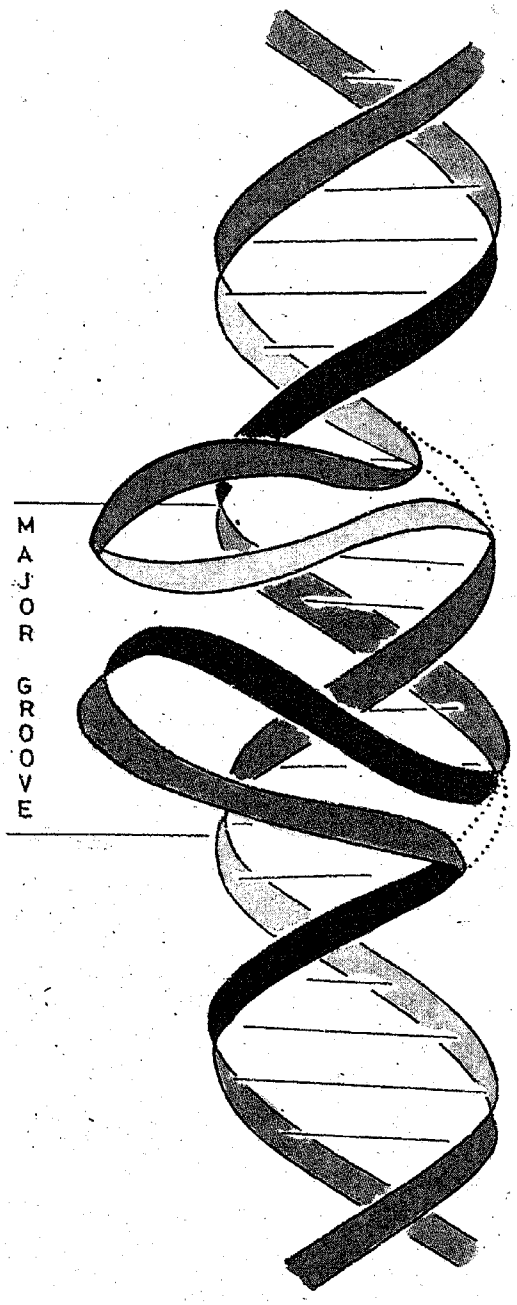

Figure 1. Visualisation of a slipped loop DNA structure. The two loops emanating from the two strands of the DNA are staggered by the "spacer" base pairs. The loops are spatially located at the major groove and show a tendency to interact with one another leading to tertiary interactions.

potential foci (Mace *et al* 1983) during recombinational events. Slipped loop structure is a kind of a stem-loop structure formed when two complementary strands of DNA base pair to form a duplex stem with a few nucleotides extruded as loop from either strand staggered by a few spacer base pairs (figure 1). A thorough understanding of their biological implications requires a complete knowledge of their three dimensional structures. Stereochemical features of such an unusual structure will also contribute towards elucidation of the ability of duplex DNA to accommodate single stranded segments as well as their tertiary folding characteristics. There is evidence from nmr (Hosur *et al* 1986) and chemical modification studies (Gorgoshidze *et al* 1992) for occurrence of slipped DNA structures in tailor-made sequences as well. We report here a computer simulation of a slipped loop structure in the oligodeoxynucleotide $d(GAATTCCCGAATTC)_2$ which comprises a direct repeat sequence GAATTC and show that such a structure can exhibit a variety of tertiary foldings facilitating even base pairing and base stacking interactions. An NMR study (Hosur *et al* 1986) on this oligomer, has infact shown that the central two mismatched cytosines loop out from both the strands of B-DNA helix formed by the rest of the sequence in a manner typical of a slipped loop structure, wherein the loops are staggered as shown in figure 1. The oligonucleotide structure, however, differs from the canonical slipped structures that are proposed for typical gene sequences, in that, the two loops are staggered by a short spacer of only two base pairs and contain less number of (two) nucleotides, besides the absence of complementary bases in them. Notwithstanding this, the present study is expected to serve as a prototype and elucidate the stereochemical principles that would enable modelling or simulation of typical slipped loop structure.

2. Method

A 12-mer B-DNA duplex with the sequence d(GAATTCGAATTC) is generated using fibre coordinates (Arnott *et al* 1974). A two nucleotide fragment corresponding to the two looped cytosines C6 and C7 is grafted to the loop priming point L_S (shown in figure 2) at the 5th thymine residue, leaving the free end dangling. The dangling end is then directed towards the loop closure point L_T (*a*, in figure 2) of the 8th cytosine residue through a novel loop closure approach. Alternatively, the two nucleotide fragment can be grafted to the O5′ atom of the C8 residue with O3′ of the T5 residue as the desired loop closure point (*b*, in figure 2). This method selects the best bond along a chain fragment with a given conformation, such that appropriate rotation effected around this bond enables movement of the free end of the chain fragment L_N towards the desired loop closure point L_T (figure 3). It can be shown that the best bond corresponds to the one which lies perpendicular to the vector $\mathbf{r}_{nt}$ joining the free end of the chain fragment L_N and the loop closure point L_T (figure 3). In practice, it is difficult to find such a bond and hence, a bond that lies very close to the plane which bisects the vector $\mathbf{r}_{nt}$, and at the same time makes a large angle with the vector $\mathbf{r}_{nt}$ as shown in figure 3, is chosen as the best bond. Mathematically, such a bond is identified from the condition that

$$(S_1^2 - S_2^2) = (S_1 + S_2) \times (S_1 - S_2) \quad \text{be small,}$$

where S_1, S_2 are the distances of the two atoms (J_1, J_2) forming the best bond with

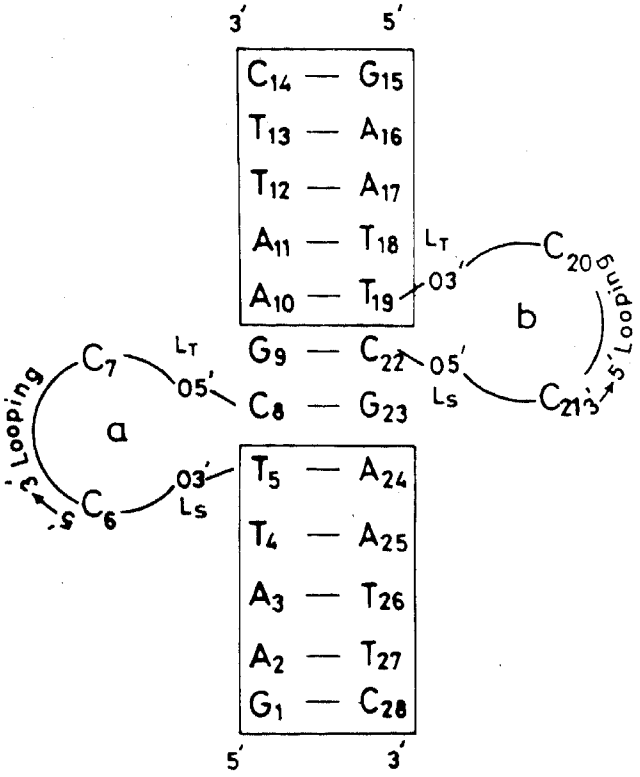

Figure 2. Schematic representation of the slipped loop structure in the oligodeoxy nucleotide $(GAATTCCCGAATTC)_2$. Two different ways (a) and (b) of loop closure possible (see text) are depicted with loop starting (L_S) and loop closing (L_T) points. The direct repeat sequence GAATT is boxed. Note that the two loops here are staggered by a two base pair spacer.

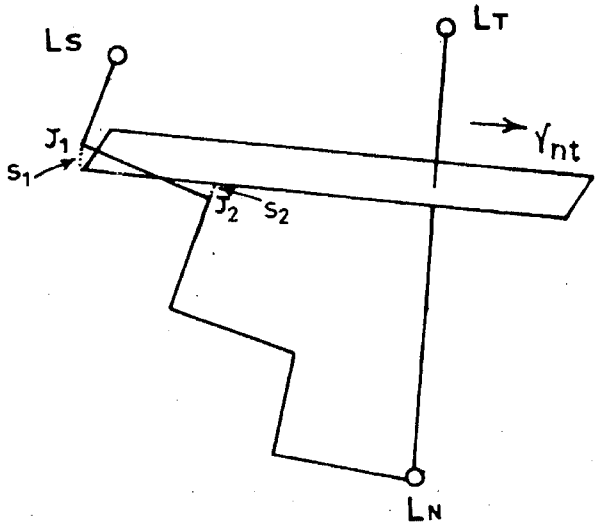

Figure 3. Schematic representation of the principle of looping procedure. The best bond $J_1 - J_2$ shown lies perpendicular to the vector $\mathbf{r}_{nt}$ connecting the end point (L_N) of the loop fragment and the desired point (L_T). S_1 and S_2 are the distances made by the best bond $J_1 - J_2$ chosen with the plane perpendicular to the vector $\mathbf{r}_{nt}$.

the perpendicular plane, as shown in figure 3. The dihedral angle between planes J_1, J_2, L_N and J_1, J_2, L_T formed by the best bond selected with the free end point L_N, and the loop closure point L_T, is the angle through which rotation around the selected bond is made to achieve loop closure. This process is repeated iteratively until the free end of the chain fragment L_N coincides with the desired point L_T. In reality the free end of the chain can only come very close to the loop closure point instead of exact coincidence. In the present case about 10 cycles are needed on an average to obtain the required loop closure. This quick procedure can be used effectively before applying the tweaking method of optimisation (Fine *et al* 1987) since the latter is valid and effective only for small rotations.

The two nucleotide chain fragment, comprising two cytosines C6 and C7, in different starting conformations (generated with different combinations of phosphodiester and C4′–C5′ bond rotations), is grafted to the loop priming point (L_S in figure 2) and then allowed to fold into a loop according to the procedure mentioned above. The various loop conformations thus obtained are screened for stereochemical acceptance and further subjected to energy minimization in the molecular mechanics formalism using AMBER. Initially, the loop atoms alone are allowed to vary with the rest of the duplex region kept frozen, and subsequently the entire 14-mer structure is subjected to energy minimization. Slipped loop DNA structures with identical conformation for both the loops, as well as different conformations, are considered in an effort to optimise their interactions with the duplex and to scan several possible structures. Force field parameters corresponding to all atom model and the point charges for the atoms listed in the AMBER data base are adopted. Distant dependent dielectric constant of $4r_{ij}$ is used as it is shown (Tidor *et al* 1983) to dampen the electrostatic forces due to solvent molecules. The structure is allowed to minimize until the root mean square deviation of energy gradient is less than or equal to 0·1 kcal/mol.

3. Results and discussion

Consequent to consideration of different starting conformations for the two chain fragments for loop closure, several stereochemically acceptable conformations are obtained. These differ primarily in the way the loops are disposed in relation to double helix and they interact with themselves or with the double helix. For convenience of discussion, these conformations are characterised as those folding across the major or minor groove of the duplex.

3.1 *Slipped loop structure with the loops folding into the major groove*

In one such model shown in figure 4, it is seen that the two nucleotide loop, comprising two cytosines folds by making a sharp turn towards major groove such that one of the loop cytosines (C6) projects into the major groove. The other cytosine (C7) is oriented away from the minor groove of the helical stem. As expected, the nucleotides of the loop, necessarily undergo considerable modifications from the preferred conformational states in order to negotiate the sharp loop. The phosphate group of the second nucleotide (C7) of the loop is pushed away from the helical path on to the minor groove by a *trans* conformation around the C4′–C5′ bond. A g^+g^+ phosphodiester conformation provides a sharp turn to locate the cytosine (C7) at the

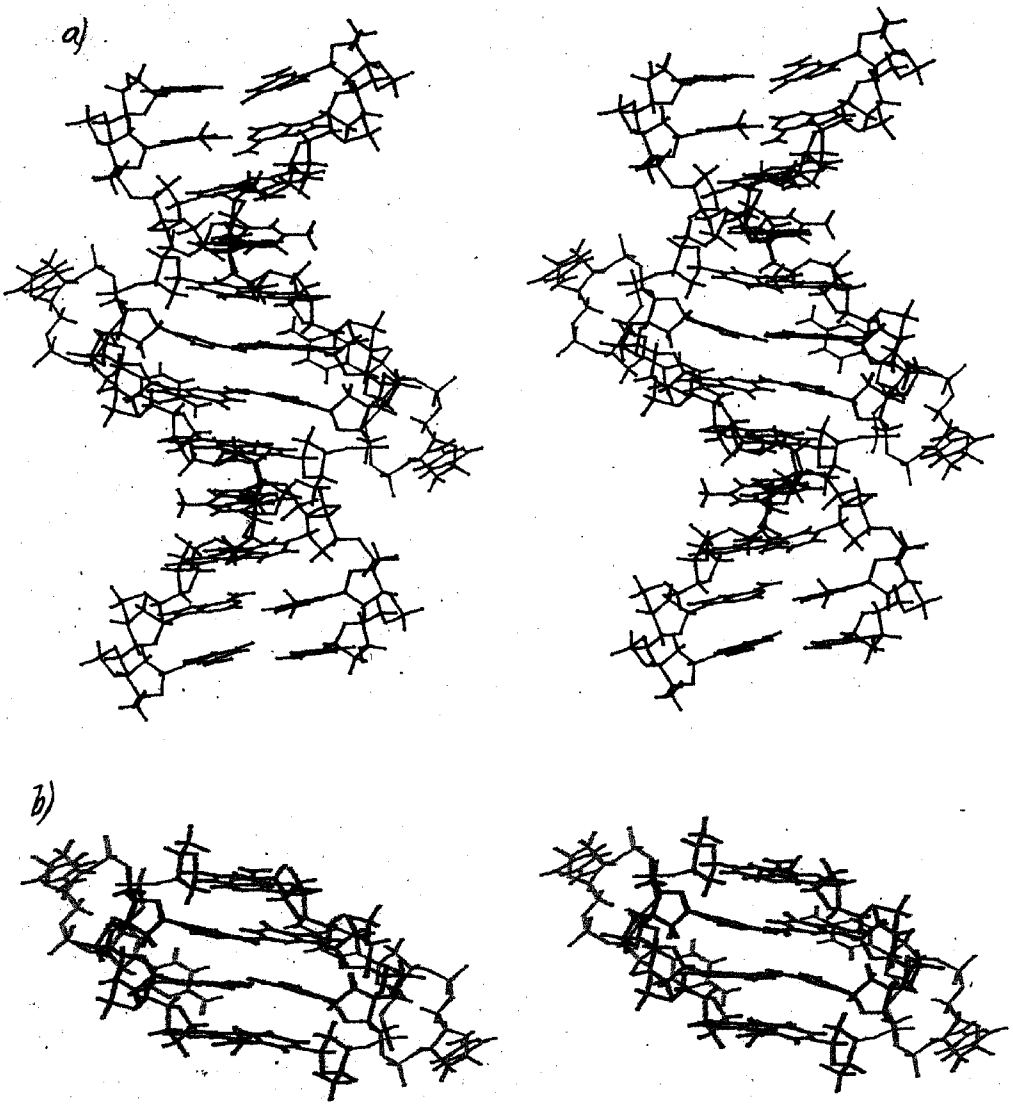

Figure 4. A stereo plot of the slipped loop structure of the sequence d(GAATTCCCGAATTC)$_2$, (**a**) major groove view of the structure. The two loop conformations are similar and fold alongside the major groove. Note that the duplex region is unaffected by the loop extrusion from either strand. (**b**) Part of the structure with only the two loop regions and the spacer base pairs.

helix surface and orient in the direction of the major groove. An extended tg^- conformation for the phosphodiester linking the two loop cytosines takes the cytosine C6 towards the major groove. This cytosine base (C6) seems to interact favourably through partial stacking (4 Å) with the C8 of the C8-G23 pair of the helix. There is also a possibility of a hydrogen bond ($d = 3{\cdot}2$ Å) between the loop cytosine C6 (N4) and guanine G9 (O6) of helical stem. Finally the sugar–phosphate chain joins the helical backbone through a g^-t phosphodiester facilitated by a near *gauche* conformation around the C5′–05′ and the C3′–03′ bonds. The two loops are nearly

identical and are stabilised by favourable phosphate...base interactions involving P6...C7 (4·8 Å) and P7...C7 (4·6 Å) and P20...C21 (4·8 Å) and P21...C21 (4·6 Å) (figure 2). The closest P...P separation in the loop is about 4·8 Å. Such tightly folded polynucleotide conformations entail short P...P separations necessitating the neutralization of electrostatic repulsions through water or metal ions. All the bases exhibit *anti* conformations although one of the loop cytosines C7 adopts a high *anti* conformation to facilitate base...phosphate stabilising interactions mentioned above. The sugars favour phase angle of pseudorotation (P) in the range 130°–150° corresponding to C1′ *exo* and C2′ *endo* type of puckers. The sugar of C7 takes up the unusual C2′ *exo* pucker while the sugars of T4 and C8 residues assume O4′ *endo* conformation. This is probably due to constraints imposed by the loop conformation. Different conformation for the sugar puckers are also suggested by NMR studies (Hosur *et al* 1986) although there are some differences between the NMR assignments and this model. It is interesting to note that the quasi-helical stack is continuous despite the break in the sugar–phosphate chain and there is no kink or bend at the loop junctions. This shows that the double helix can accommodate the staggered loops of two nucleotide in length without any major distortion. This is reminiscent of the nearly continuous nature of the long helical stem formed between the amino acid and pseudouridine helical stems of tRNAs, despite the break in one of the strands. The major stabilization of the structure comes from van der waals interactions and this overcomes additional repulsive electrostatic interactions arising due to proximity of phosphates from loop folding. The total energy of the structure is around − 119 kcal/mol. It is possible to obtain other structures having similar broad features, but slightly different conformations. An example of this is shown in figure 5.

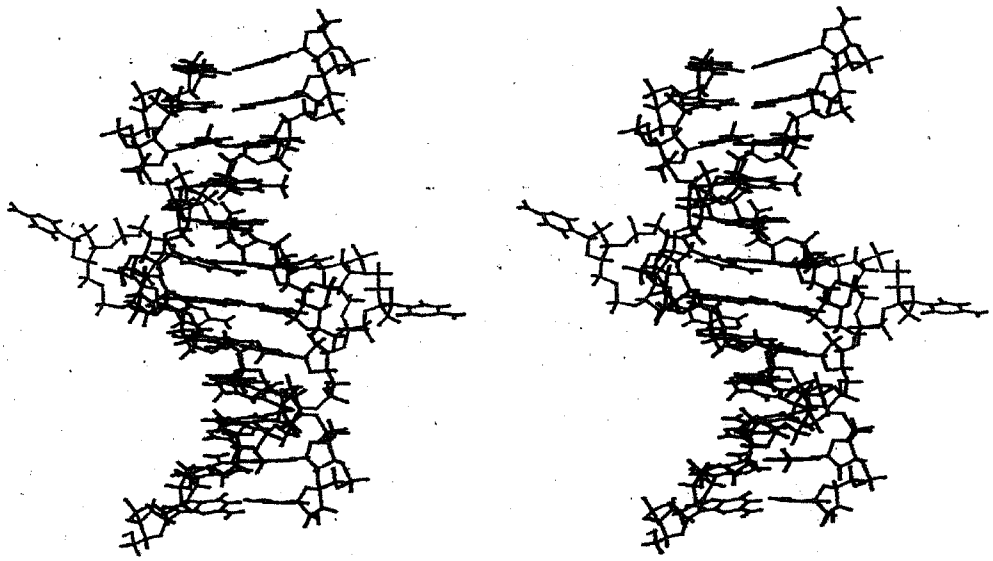

Figure 5. Stereo plot of a conformationally different slipped loop structure with loops folded alongside the major groove.

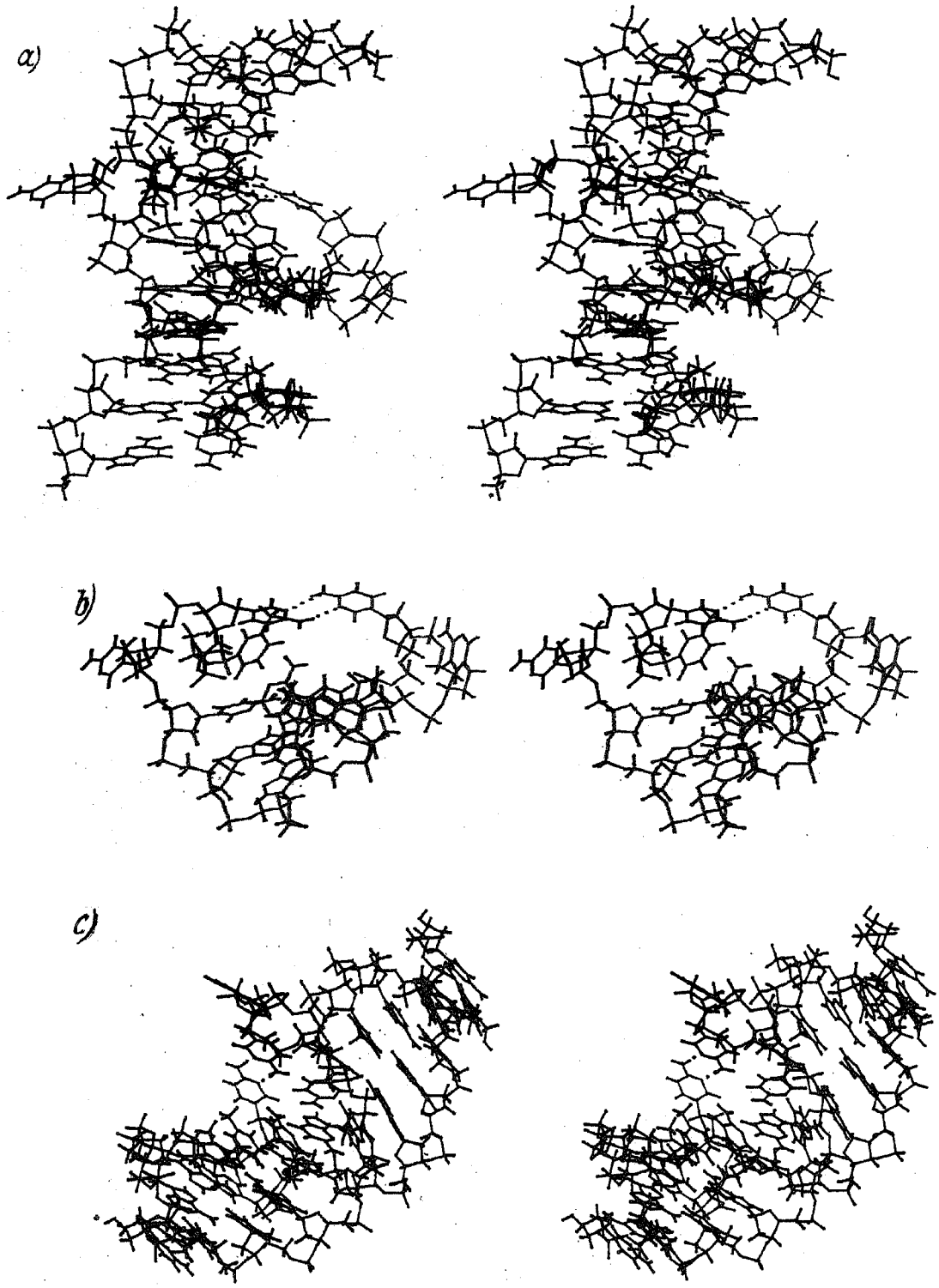

Figure 6. Stereo plot of the slipped loop structure with the loop cytosines paired. (**a**) Viewed perpendicular to the helix axis. The C...C pair partially blocks the bases exposed in the major groove and prevents their possible interaction with regulatory proteins. (**b**) Part of the structure viewed down the major groove. (**c**) View of the structure in another direction.

3.2 *Slipped loop DNA structure with base pairing*

While a stereochemically viable slipped loop structure is readily accomplished, it is extremely important to probe into the possibility of base pairing between looped out cytosines since it is an essential feature of a typical slipped loop DNA structure. Although the present sequence does not permit complementary Watson–Crick pairing, a possibility of pairing between the looped out cytosines is conceived and a viable model is simulated. The two cytosines involve in symmetric pairing interactions across the major groove as depicted in figure 6. The staggered loops fold along the major groove of the duplex with one of the loop cytosines (C6) located deep in the major groove of the duplex, while the other cytosine (C7) is positioned at the mouth of the major groove. In the loop (a) (figure 2), a g^+g^- phosphodiester conformation concomitant with a *gauche*$^-$ conformation about the C4′–C5′ bond facilitate the loop extrusion away from the helical stack and directs its base C6 into the major groove. The backbone of the next nucleotide takes a gradual turn and orients its base (C7) in the same plane as the previous loop base but in the opposite direction outside of the major groove. Such a gradual turn is rendered by a *gauche*$^-$ conformation about the C3′–O3′ bond of the preceding loop nucleotide (C6), a g^+g^- phosphodiester conformation and a *trans* conformation about the C4′–C5′ bond. The tg^+ conformation around the loop closing phosphodiester (P8) and a *trans* conformation about the C4′–C5′ bond folds the loop back onto the helical stem. Similarly, in the loop (b) (figure 2), the first loop nucleotide (C6) produces the loop expulsion in the major groove direction and disposes the base (C6) in the centre of the major groove (figure 6). This is facilitated by a g^+g^+ conformation around the phosphodiester bonds accompanied by a *trans* conformation about the C4′–C5′ bond. The second loop nucleotide (C7) brings about a sharp turn and orients the loop backbone in the opposite direction again via g^+g^+ phosphodiester and a *trans* conformation about the C4′–C5′ bond. The torsion about the C5′–O5′ bond assumes a value about 90°. The favoured g^-g^- phosphodiester (P22) concommitant with a *gauche*$^+$ conformation around the C4′–C5′ bond for the following cytosine nucleotide (C22) facilitates a gradual loop closure. The two loop cytosines disposed into the centre of the major groove interact with one another via symmetrical hydrogen bonds involving the amino group (N4) and N3 sites. The nearly linear hydrogen bonds are slightly longer (3·2 Å) in length.

The folding of both the loops to achieve inter-strand hydrogen bonding between bases brings about noticeable deviations in the duplex structure, especially in the spacer base pairs, near the loop region. For instance, the phosphodiester (P5) exhibits a tg^+ conformation and a *gauche*$^-$ conformation about the C4′–C5′ bond. This is necessitated by the need for the deep penetration of the base into the centre of the major groove leading to hydrogen bonding interactions with the other cytosine base (C20) in the major groove. In spite of the presence of a non-traditional phosphodiester conformation, the stacking with adjacent pairs is maintained due to the correlated rotations around the sugar–phosphate backbone bonds. The large positive propeller twisting (19°) of the T5–A24 base pair, concomitant with a large buckle lays the seed for the distortion in the continuity of the helical stack. This distortion is further augmented by the high negative propeller twisting of the central spacer base pairs which hold the loop regions between continuous helical stems. Actually, the spacer base pairs, C8–G23 and G9–C22, exhibit higher propeller twist of about $-53°$ and

$-27°$ respectively. The adjoining base pair A10–T19 on the other side exhibits a propeller twist of about $-20°$. Rest of the base pairs on either side of the loop exhibit normal values of propeller twist usually found in DNA oligonucleotide structures. Similarly, the helical repeat angles between base pairs show considerable variation especially at the spacer base pair region and at the loop extrusion region. The helical twist angle at the spacer base step (C8/G9) is 40°. The adjacent base steps C8/T5 and C22/T19 on either side of the spacer step (C8/G9) are distinctive in the sense, the base pairs constituting the step are continuous on one side and are interrupted on the other side by loop expulsion. The helical twist angle at these pseudo base steps C22/T19 and C8/T5 is 38·3° and 36° respectively. Twist angle at the AT base steps (A17/T18 and A3/T4) is around 30°, a few degrees lower than its neighbouring A/A (33°) (A2/A3, A10/A11) base steps. Such a low value for the AT base step is observed in the previous model also. Twist angles at rest of the base steps fall in the usual range of 36°. The average width of the major (P12...P18, P11...P19, P10...P19) and minor groove (P10...P25, P10...P26, P6...P27, P5...P28) of the two helical stems is around 18 Å and 10 Å respectively. Major groove seems to expand while the minor groove contracts from their initial value of 17·5 Å and 11·5 Å found in the fibre model (Arnott *et al* 1974).

The sugar puckers of most of the nucleotides fall in the broad range (125°–175°) of pseudorotation phase angle (P). The sugar rings of the base paired loop cytosines (C6 and C20) from either strand exhibit C2′ *exo* and C3′ *exo* conformation respectively. Other nucleotide residue C21 of the loop (b) (figure 6), exhibits a C3′ *endo* conformation while the sugars of thymine residues (T4 and T18) penultimate to the looped out cytosines (C6 and C20) favour O4′ *endo* conformation. The total energy of the structure is -131 kcal/mol, indicating the preference of the base paired loop structure.

It is clear from above that loops with even two nucleotides can exhibit base pairing interaction. Formation of W–C base pairs between such looped out bases appears, therefore, natural when the loops contain complementary bases. A prototype sequence such as $d(GAATTATCCGAATTC)_2$, capable of forming a slipped loop structure with three nucleotides in the loop and two spacer base pairs, can give rise to a W–C base pair between the looped out bases. A logical extension of this argument, to the case of a canonical slipped loop structure, with a large number of complementary nucleotides in the loops, is the presupposition of inter loop helix formed by stacked adjacent bases of one loop interacting through W–C pairing with the complementary bases in the other strand loop. Another salient feature of the above shown slipped loop structure with inter loop pairing lies in the fact that it gives a prelude to the site or location of extruded helical stem and its possible interaction with duplex DNA. The major groove of DNA seems the most plausible and stereochemically favoured site for such an extra helical stem. Occurrence of a base-paired stem across the major groove might stereochemically hinder the access of bases of the DNA for the regulatory proteins thus providing a neat stereochemical rationale for the preference of direct repeat sequences at promoter regions.

3.3 *Slipped loop DNA structure with bases stacked*

Apart from base pairing, the other major stabilizing interaction giving rise to characteristic secondary or tertiary structures in nucleic acids is the stacking interaction between bases. Possibility of such an interaction between looped out bases

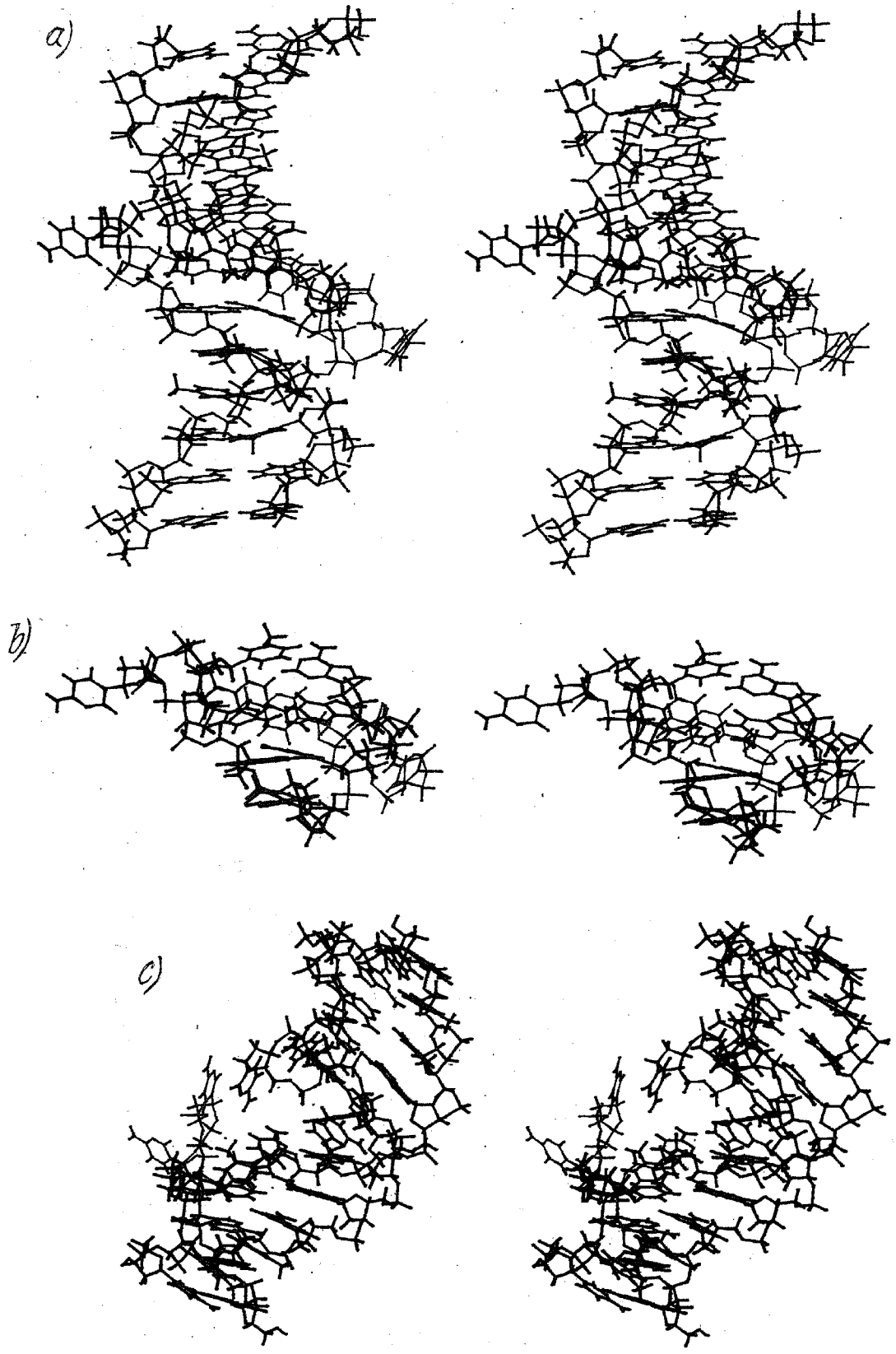

Figure 7. Stereo plot of the slipped loop structure with the loop cytosines stacked one over the other across the major groove. (**a**) View along the major groove. (**b**) Portion of the same structure. (**c**) The structure viewed in another direction. High propeller twisting of the "spacer" base pairs intervening the two loops are clearly visible.

in the oligonucleotide under study is also examined. Figure 7 shows a model of a slipped loop structure in which the looped out cytosines of cross strands exhibit stacking interaction. The two loops fold again across the major groove side of the duplex to facilitate such an energetically favourable interaction. The amino group of one cytosine overlaps (3·7 Å) with the centre of the cytosine base and vice versa.

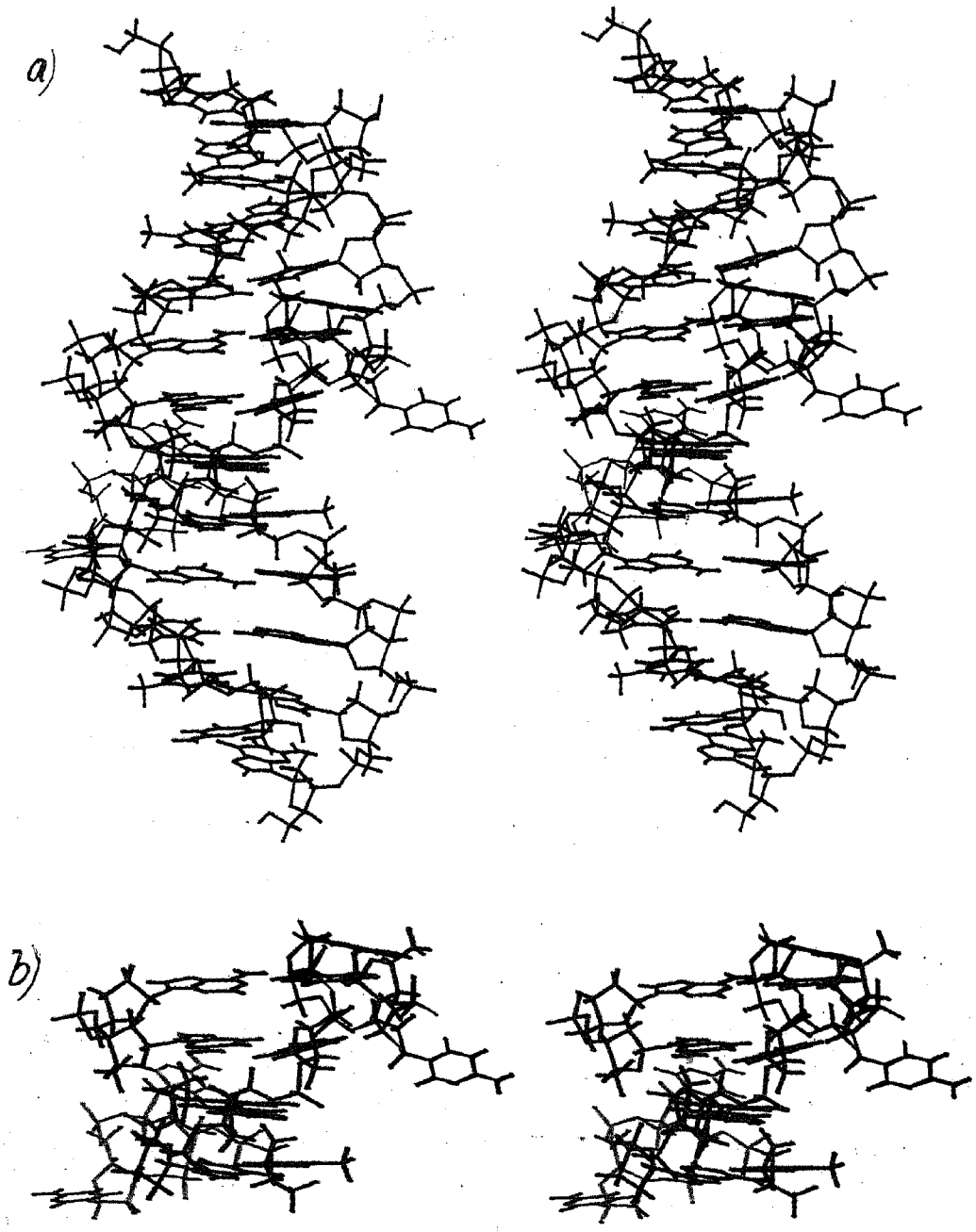

Figure 8. Stereo view of the slipped loop structure with the loops folded alongside the minor groove. (**a**) View along the minor groove. Entire sugar–phosphate backbone of the loop folds along the minor groove with one of the loop bases projecting towards the major groove. (**b**) Part of the above structure showing only the loops and the spacer base pairs.

The two loops are not identical and the nucleotides undergo considerable variation from the energetically most favoured conformations to facilitate loop closure. The base pairs extruding the loop regions and the spacer base pairs exhibit large propeller twists. One of the spacer base pairs C8–G23 exhibits a very high propeller twist of about $-60°$, while the propeller twist around the other pairs, A10–T19, G9–C22 and T5–A24 are $-32°$, $-20°$ and $-15°$ respectively. Here the spacer base pairs as well as the adjoining base pairs from which the loop extrudes show propeller twist in the same direction unlike in the base paired structure discussed above. The helical twist angle at the C8/G9 spacer step is around 40° (same as in the base paired model), whereas at the adjoining base steps on either side of the spacer is about 34·4° and 35°, respectively, at the T19/C22 and T5/C8 steps. Here again, the A/T base steps (A17/T18, A3/T4) on either side of the loop exhibit a low value of helical twist (30°). Rest of the base steps exhibit an average helical twist angle of 36°. This structure is energetically a couple of kcal (-133 kcal/mol) more favoured than the paired structure.

3.4 *Slipped loop structure with the loops folding into the minor groove*

In order to probe into the possibility of loop folding across the minor groove instead of the major groove of the duplex, slipped loop structures with loop folding into the minor groove is simulated. In one of the slipped structures shown in figure 8, the loops shown in blue and red, fold alongside the minor groove. The conformation of both the loops are similar. In this structure, the sugar–phosphate backbone of the loop is located completely in the minor groove. The loop cytosine C7 is disposed into the minor groove while the other cytosine C6 is oriented towards the major groove. Neither the loop bases nor the sugar–phosphate backbone seem to exhibit any interaction with the duplex. Because of this, the intrinsic properties of the base pairs at the loop-helix junction remain unaltered. For example, the propeller twist of the spacer base pairs and the adjoining base pairs encompassing the loop show values in the range ($-8°$ to $-13°$). The helical twist angle, at the spacer base step C8/G9 is around 33°, at the A/T base steps (A17/T18, A3/T4) on either helical strand is 33° and at the rest of the base steps forming the duplex region are around 36°, same as in the case of loop folding into the major groove described in § 3.1. This structure is energetically nearly equally favoured (-129 kcal/mol) as the other structure (figure 6) with the loops folding alongside the major groove of the duplex.

4. Conclusion

It has been possible to generate a variety of loop conformations for the slipped loop structure with minimum number of nucleotides in the loop. A noteworthy feature has been the possibility of generation of stereochemically acceptable model with the loops folded along major as well as minor groove of the uninterrupted duplex stems. All the more, inter loop pairing which remained a speculation so far, has now been visualized. Realization of such inter loop pairing and stacking interactions in an extremely short looped slipped structure corroborate the possibility of such interactions in structures with longer loops. The distortions occurring in the interloop base paired and base stacked structures is understandable, as the looped out portion

is too short to be folded without hindering the helical stack of the quasi duplex. Under the given circumstances, stabilizing interactions between looped out bases can be maximized readily by increasing the length of the loop and by having complementary nucleotide composition in the loops. This can be easily demonstrated from a prototype sequence $d(GAATTATCCGAATTC)_2$ designed, which is capable of forming a slipped loop structure, with three nucleotides in the loop and two spacer base pairs, having the potential to form W–C base pairs between the looped out bases. In addition, such tertiary pairing and stacking interaction can be optimized to achieve a tertiary inter loop helix by having spacers of suitable length. Above all, occurrence of base-pairing and base-stacking interactions in the major groove of the duplex become important from the functional aspect as they might inhibit the access of bases of DNA for the regulatory proteins. Thus, a structure–function rationale emerges for the occurrence of one other polymorph of DNA, namely, the slipped loop structure. Also, generated models adequately explain the various ways in which unpaired and mismatched nucleotides are accomodated by DNA and the conformational resilience exhibited by duplex DNA.

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