One left-handed strand in DNA-oligonucleotide complexes?

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A single strand of oligonucleotide can bind to double helical DNA under certain conditions. This must involve some unwinding of the original double helix in a process leading to the formation of a three-stranded region. The free energy for such an entropically unlikely reaction may come from a change in the degree of supercoiling of the original DNA. The conformation of the triple strand is investigated here using computer graphics and molecular mechanics calculations. It is suggested that on binding the oligonucleotide (strand 3) to two paired strands (1 and 2) in a supercoiled DNA molecule, strand 2 might adopt a left-handed conformation whilst strand 1 and strand 3 pair in the normal Watson-Crick B-configuration.

DNA, supercoiled; DNA, left-handed; Oligodeoxynucleotide; Molecular modelling

1. INTRODUCTION

In the course of semiconservative replication, it is essential for the parental strands of DNA to unwind from each other if they are to separate and be used as templates [1]. The separation exposes the bases of each strand to the action of enzymes. In the case of closed circular duplexes, the polynucleotide strands cannot rotate freely about the helix axis, and unwinding of the double helix is accomplished by one or more topoisomerase molecules. These enzymes transiently break and rejoin one or two of the DNA strands [2] in an endergonic reaction that leads to supercoiling. The algebraic sum of the number of supercoils and the number of double helical turns is the linking number [3] that, being a topological property, can be altered only if one of the backbones of the double helix is broken. Thus, one negative superhelical turn is introduced in the molecule for every

Watson-Crick turn removed from the parental strand, i.e. for every 10.4 base pairs (bp). A ring in which the ratio of base pairs to linking number is 10.4 is said to be relaxed. The supercoiled configuration is strained with respect to the relaxed state and the excess free energy is found to be closely proportional to the square of the difference in linking number between the two [3].

As a result of the free energy associated with its formation [2], supercoiled DNA can participate in a variety of important and complex biological reactions that are not possible for the relaxed circular from [4]. Among them it its capacity to take up and form stable complexes with homologous single-stranded DNA fragments in a reaction that is accelerated by heating and the addition of salt [5]. Such a mechanism has been suggested for the initiation of genetic recombination [5] and could also be relevant to the initiation of transcription.

We have attempted to create a computer model that would enable us to visualize the geometry of the three strands in a complex of this type. A better understanding of the stereochemistry of this interaction can be used in the design of new tools in molecular biology and possibly for the development of new chemotherapeutic approaches [6].

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2. MATERIALS AND METHODS

Both the duplex (assumed to be a short fragment of a super-coiled DNA molecule) and the oligonucleotide were constructed in the B-form from idealized regular coordinates [7]. The double helix consisted of 24 bp with the repeating random sequence 5'-(A-G-C-T)$_6$-3' while the homologous decamer had the sequence 5'-T-A-G-C-T-A-G-C-T-A-3'. This base composition was chosen so as to be complementary to the central part of the duplex while leaving 7 bp flanking both ends of the oligo-nucleotide in the complex. If it is sufficiently long, the conformation of minimum energy for a relaxed circular DNA molecule will depart only slightly from the B configuration found for linear DNA molecules in solution [8]. Since circular DNA molecules with a reduced linking number (i.e. underwound) minimize the deformation energy by writhing, giving rise to negative supercoiling, we have assumed that they will also adopt the B form.

Molecular modelling was performed on a Silicon Graphics IRIS 3120 workstation using the interactive molecular graphics program HYDRA [9]. It is obvious that different types of model could be built, but we have concentrated on that one that seems to use more plausible on energetic grounds. In this model all the bases in the oligonucleotide are forming Watson-Crick hydrogen bonds with the paired bases on the original strand of the supercoiled DNA molecule, as suggested by the experimental findings [5]. Since the vital stabilizing force in DNA is the stacking interaction [10], it seemed reasonable to us that the unstacking had to be kept to a minimum. We find this can be done while keeping all bond distances and angles (including dihedral angles) stereochemically acceptable. The molecular mechanics suite of programs AMBER [11] was used to minimize the energy of the resulting complex and also to evaluate the conformational energy change upon binding of the oligonucleotide (table 1).

3. RESULTS AND DISCUSSION

The three major sources of flexibility in DNA are the sugar pucker, the rotation about the phospho-diester linkages (P-O bonds) and the glycosyl torsion angles [12]. Small changes in the values of the P-O5' (α) and O3'-P (ζ) torsional angles (to label the conformational angles, the IUB/IUPAC [13] recommended nomenclature has been followed:

$$O3' - P \overset{\alpha}{-} O5' \overset{\beta}{-} C5' \overset{\gamma}{-} C4' \overset{\delta}{-} C3' \overset{\epsilon}{-} O3' \overset{\zeta}{-} P - O5'$$
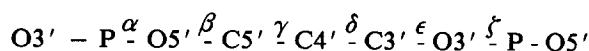
where χ is the angle about the glycosidic bond)

have been shown to alter the sense of the helix [14]. In the model presented in this paper, a 10-bp region in one of the strands of the supercoiled DNA adopts a left-handed conformation that makes it possible for a right-handed single-stranded decamer to form Watson-Crick hydrogen bonds

with the complementary bases of the other strand (fig.1) with the minimum of unstacking. Since a twist of the double helix is subtracted in the closed circular molecule (bringing about a positive super-coil), the total number of negative superhelical turns is effectively reduced by one.

The nature of the base pairs in the uncoiled DNA region is immaterial to the model. We saw no reason why the model should be restricted to alternating purines and pyrimidines. The energy required to break the hydrogen bonds in the super-coiled DNA will depend to some extent on the nature of the base pairs involved. But, since in this state the double helix is under stress, the right-hand to left-hand transition we propose for the 10-bp segment in one of the strands will release part of this stress and is likely to be independent of base composition. This torsional deformation minimizes the total conformation free energy involved in the unwinding process.

An assessment of the stability of the different conformations is of interest and can be obtained from the energy calculations. The positive value found for the difference in energy between the sum of the individual molecules and the complex (table 1) accounts for the fact that the relaxed form of DNA cannot take up oligonucleotides [5]. The total interaction energy in the complex is 55 $kcal \cdot mol^{-1}$ less than that in the supercoiled molecule. The distortion energy of the separated strand amounts to the same value. These two factors, together with the small conformational energy change in the oligonucleotide and the slight repulsion calculated for the interaction between the oligonucleotide and the separated strand, make it clear why the stress on the supercoiled structure must provide the extra free energy necessary for the uptake process. We observe a distinct repulsion between one of the oxygens of the first phosphate group in the oligonucleotide and the O_2 in the thymidine residue close to the right-left junction in the double helix. At the other junction, however, the two oxygens of a phosphate group form two very good hydrogen bonds with the N_1 and N_2 atoms of a guanidine residue from the separated strand which would presumably stabilize the structure. Thus the repulsion term for the interaction between the oligonucleotide and the second strand of the original double helix is bound to depend to some extent on base composition.
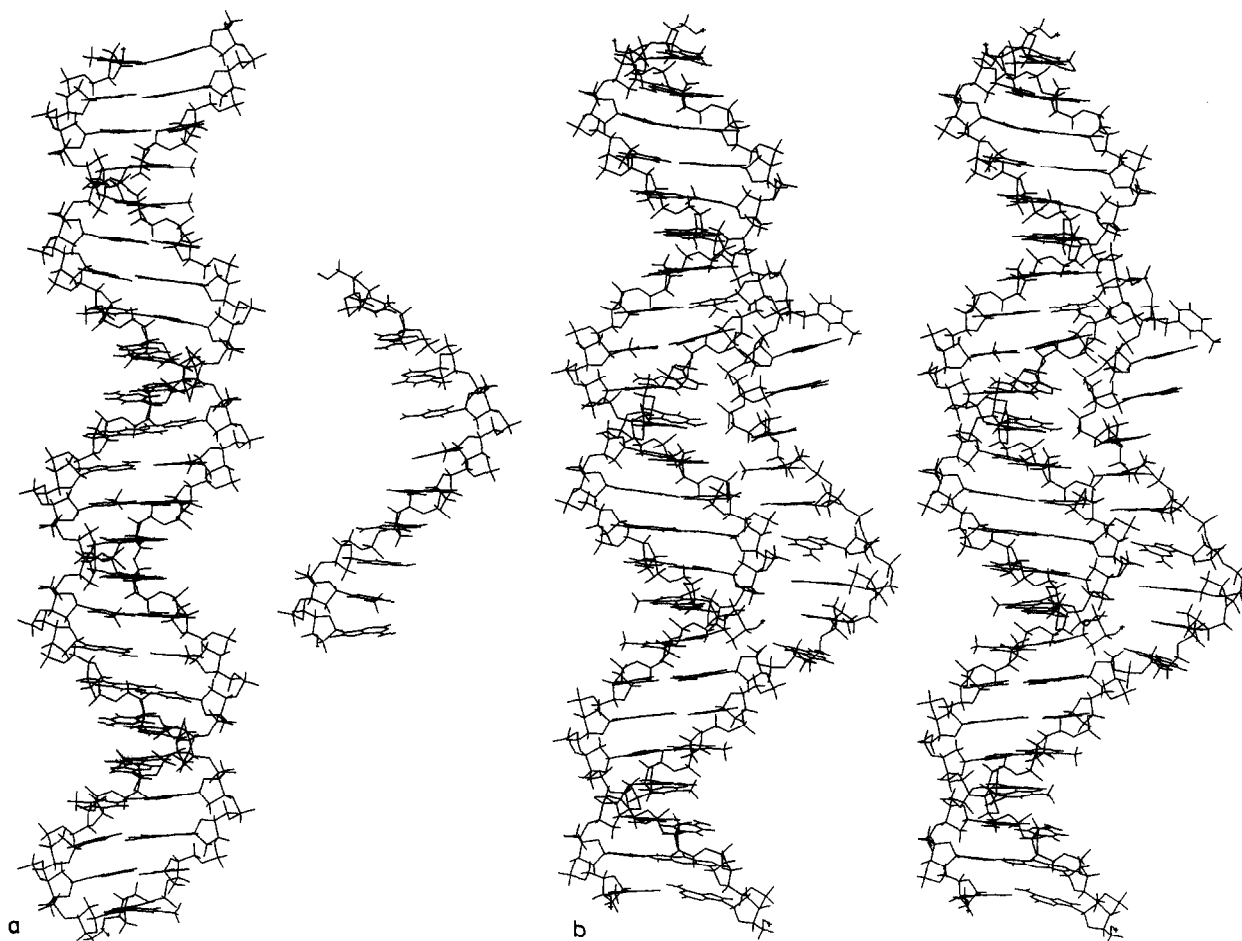
Fig.1. (a) Conformation of the DNA duplex (assumed to be a short fragment of a supercoiled DNA molecule) and the oligonucleotide after minimizing the energy of the structures obtained from idealized regular coordinates for B-DNA. (b) Stereo view of the complex after uptake of the oligonucleotide by the double helix. No significant changes were observed in this structure when 'hydrated' counterions [22] were included in the simulation.

The left-handed segment is not in the Z-DNA conformation. For the alternating C-G sequences in solution, the Z-form has a different helical periodicity from that of B-DNA (11.6 ± 0.3 vs 10.5 ± 0.1 bp per turn, respectively) and the calculated free energy difference between the two is + 0.33 kcal/mol per base pair [15]. Left-handed models other than Z-DNA have been explored and characterized [13] and it has been shown how they can combine with right-handed ones in the same molecule to give right-left (RL) models [12]. The separated strand in our model was constructed in the left-handed B-form [12], taking the mononucleotide as the repeating unit. Thus, the

sugar pucker is C2'-endo (130° ≤ δ ≤ 160°) and the orientation of the bases *anti* for all the nucleotides in both conformations before energy refinement. This means that in the central region of the second strand of the double helix the phosphates have rotated away from the minor groove and the ten bases have flipped over. The greatest change in the backbone torsion angles is observed at the junctions of both conformations. At the 5'-end α and ϵ are the ones altered most, whereas at the 3'-end of this region it is α and β which experience the largest shift.

The possibility of finding left-handed DNA in the midst of a right-handed genome was initially

Table 1

Energy decomposition (kcal · mol^{-1}) for the individual molecules (a) and the complex (b)

	Double helix strand 1 · strand 2		Oligonucleotide strand 3	Interaction energy			Total energy
				1-2	1-3	2-3	
(a)	− 682.3	− 682.3	− 292.4	− 272.2	−	−	− 1929.2
(b)	− 691.3	− 626.9	− 266.2	− 136.9	− 104.6	25.3	− 1800.6
ΔE	− 9.0	55.4	26.2	135.3	− 104.6	25.3	128.6

For each term, ΔE expresses the difference in energy between the two states

considered diminished by the instability of a left-right interface [16]. It is now known, however, that certain sequences can from left-handed Z-DNA in negatively supercoiled plasmids [17], including some which are not composed of alternating purines and pyrimidines [18], and also in physiological ionic conditions [18,19]. In our model it is much simpler to arrive at that interface since it involves only one of the strands. Furthermore, there is now crystallographic evidence [20,21] that in DNA duplexes with one extra base inserted in each strand the double helix appears nearly intact in the B-conformation with the unpaired bases looped out from the rest of the structure.

An important point is how much of a DNA supercoiled double helix can be uncoiled to let an oligonucleotide in. It is intuitively obvious from our model that if the length of the oligonucleotide is a multiple of 10.4 bases the distortion inflicted on the molecule will be minimized. When this length is sufficiently long (in principle over about 31 bases, i.e. three times 10.4), the central part of the separated strand can presumably adopt the more favourable right-handed conformation. By using this mechanism the DNA molecule can expose a small group of base pairs without too great an expenditure of energy.

DNA is a very flexible molecule and its conformation will vary depending on the environment. Although this model must be regarded as speculative, it could provide some clues for the understanding of biological processes which involve specific DNA-protein or DNA-DNA interactions that depend not only on the primary base sequence of the DNA but also on the conformational state of the double helix. If the complexes are sufficiently stabilized, unique resonance and circular dichroism signals associated with the particular configuration of the separated strand, and, or cleavage patterns by single-strand specific endonucleases could confirm the mechanism proposed. On the basis of the structural information presented, experiments such as these can be designed to test the validity of our suggested model.

REFERENCES

[1] Watson, J.D. and Crick, F.H.C. (1953) Nature 171, 964-967.
[2] Gellert, M. (1981) Annu. Rev. Biochem. 50, 879-910.
[3] Crick, F.H.C. (1976) Proc. Natl. Acad. Sci. USA 73, 2639-2643.
[4] Bauer, W.R. (1978) Annu. Rev. Biophys. Bioeng. 7, 287-313.
[5] Holloman, W.K., Wiegand, R., Hoessli, C. and Radding, C.M. (1975) Proc. Natl. Acad. Sci. USA 72, 2394-2398.
[6] Toulmé, J.J., Krizh, H.M., Loreau, N., Thuong, N.T. and Hélene, C. (1986) Proc. Natl. Acad. Sci. USA 83, 1227-1231.
[7] Arnott, S. and Hukins, D.W.L. (1972) Biochem. Biophys. Res. Commun. 47, 1504-1510.
[8] Dickerson, R.E., Drew, H.R., Conner, B.N., Wing, R.M., Fratini, A.V. and Kopka, M.L. (1982) Science 216, 475-485.
[9] Hubbard, R. (1987) HYDRA (Harvard York Drawing Program), Chemistry Department, University of York, Heslington, York YO1 5DD, England.
[10] Freier, S.M., Alkema, D., Sinclair, A., Neilson, T. and Turner, D.H. (1985) Biochemistry 24, 4533-4539.
[11] Singh, U.C., Weiner, S.J., Caldwell, J.W. and Kollman, P.A. (1986) AMBER (UCSF): Assisted Model Building with Energy Refinement, version 3.0, Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco.

[12] Gupta, B., Bansal, M. and Sasisekharan, V. (1980) Proc. Natl. Acad. Sci. USA 77, 6486–6490.

[13] IUB Biochemical Nomenclature and Related Documents, Spattiswoode Ballantyne Press, London.

[14] Gupta, G., Bansal, M. and Sasisekharan, V. (1980) Biochem. Biphys. Res. Commun. 97, 1258–1267.

[15] Peck, L.J. and Wang, J.C. (1983) Proc. Natl. Acad. Sci. USA 80, 6206–6210.

[16] Drew, H.R. and Dickerson, R.E. (1981) J. Mol. Biol. 152, 723–736.

[17] Nordheim, A. and Rich, A. (1983) Proc. Natl. Acad. Sci. USA 80, 1821–1825.

[18] Ellison, M.J., Kelleher, R.J., Wang, A.H.-J., Habener, J.F. and Rich, A. (1985) Proc. Natl. Acad. Sci. USA 82, 8320–8324.

[19] Singleton, C.K., Klysik, J., Stirdivant, S.M. and Wells, R.D. (1982) Nature 299, 312–316.

[20] Joshua-Tor, L., Rabinowich, D., Hope, H., Frolow, F., Appella, E. and Sussman, J.L. (1988) Nature 334, 82–84.

[21] Miller, M., Harrison, R.W., Wlodawer, A., Appella, E. and Sussman, J.L. (1988) Nature 334, 85–86.

[22] Singh, U.C., Weiner, S.J. and Kollman, P.A. (1985) Proc. Natl. Acad. Sci. USA 82, 755–759.