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REVERSAL OF HANDEDNESS IN DNA: A STABLE LINK BETWEEN RU AND LZ HELICES

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Summary

Two types of left-handed zig-zag (LZ) helices were obtained following stereochemical guideline. They are referred to as LZ1 and LZ2 helices. LZ1 helices have conformations similar to those found in the single crystals of $d(C-G)_3$ and $d(C-G)_2$[5,6]. Z-character is more prominent in LZ2 than in LZ1 helix. The conformations of a stable link between RU and LZ helical fragments are given. The link involves inverted stacking arrangement of the bases: a characteristic feature of all RL models proposed by us[9,10].

Introduction

Left-handed duplexes can be classified into two types: uniform (LU) and zig-zag (LZ) helices[1]. LU helices can either have mononucleotide (or dinucleotide) as the repeating unit such that successive phosphate groups are related by identical (or nearly identical) helical twist and axial rise. LZ helices, on the contrary, would invariably have dinucleotide as the repeating unit so that alternate phosphate groups are helically related but not the successive ones. For the first time, we have demonstrated that LU helices are possible for various polymorphous forms of DNA[2,3]. In some special cases, for example polynucleotide duplexes with alternating purine-pyrimidine sequence (in short PAPP), we have shown the possibility of LZ helices[4]. But it is only after the observation of LZ-fragments in $d(C-G)_3$ and $d(C-G)_2$[5,6]

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that left-handed helices for DNA are being taken more seriously than they used to be. However, judgement on the handedness in retrospect of the single crystal structure of DNA fragments makes it impossible to find an answer to the question as to why DNA exists in either handedness. Quest of such an answer calls for an independent approach which we took. We first identified conformational flexibility of the polynucleotide backbone as the root cause of DNA being present in either handedness. We then formulated a stereochemical guideline to exploit this flexibility. This led to both right- and left-handed DNA duplexes. Our systematic analyses revealed that LZ helices are only one type of left-handed duplexes and there can be LU type present as well in DNA[2,3,4]. In this paper, we deal only with the structural features of LZ-helices. We also discuss how a stable link could be made between LZ and RU helices.

LZ-Helices: Two Types

As stated above, LZ-helices have dinucleotide as the repeating unit. For this purpose PAPP happens to be a model system in which purine nucleotide has a conformation different from the pyrimidine one. It was earlier shown[1] that the conformation of a nucleotide unit can either be in a helical or a non-helical domain in the ($\beta - \gamma$) space. It turns out that, LZ-helices can either have a dinucleotide repeat in which one nucleotide is in helical domain and the other in non-helical domain or both the nucleotides in non-helical domains. The two types are designated as LZ1 and LZ2 respectively. In this paper, we consider one example in each type. Example of the LZ1 type is the one in which the dinucleotide unit has purine nucleotide in (C3'-endo, g^-t) conformation (a helical domain) connected to the pyrimidine

nucleotide in (C2'-endo, g^+g^+) conformation (a non-helical domain). The example of LZ2 type involves a dinucleotide unit with purine nucleotide in (C3'-endo, g^+g^+) conformation and pyrimidine nucleotide in (C2'-endo, g^+g^+) conformation (both being in non-helical domain). In both the types of LZ helices, the following are the common conformational features: (i) all C3'-endo sugars have gt conformation around C4'-C5' bond while C2'-endo sugars have gg conformation around the same bond; (ii) purines are all attached to C3'-endo sugars and have syn conformation while pyrimidines are connected to C2'-endo sugars and have anti conformation.

Using the two conformations discussed above, molecular models were generated for B(n = 5, h = 6.80 Å) and Z(n = 6, h = 7.50 Å) forms of DNA. The LZ-helices in Z-form are reminiscent of the single crystal structures of $d(C\text{-}G)_3$ and $d(C\text{-}G)_2$[5,6]. The possibility of LZ-helices in B-form suggests that the conformational flexibility of the dinucleotide unit allows compression of the helix from a pitch of 45 Å (in Z-form) to that of 34 Å (in B-form). The conformational parameters of the two types of LZ helices in B- and Z-forms are given in Table 1. It is seen that the torsion angles in the two cases are only slightly different. Therefore, to discuss various structural features of LZ1 and LZ2 helices we have restricted ourselves to B-form as an example.

The zig-zag progression of the phosphate groups along the helix-axis is shown in Fig.1. For LZ1 type, two neighbouring phosphate groups either have a twist angle, $t = 23^\circ$ and axial rise h = 5.3 Å or $t = 49^\circ$ and h = 1.5 Å. LZ2 type has two phosphate groups either with $t = 0^\circ$ and h = 6.8 Å or with $t = 72^\circ$ and

Table 1. Conformational Parameters of LZ-helices in B- and Z-forms*.

Type of the LZ-helix	Torsion Angles (in deg)	B-Form		Z-Form	
		Pyr-Pur Sequence	Pur-Pyr Sequence	Pyr-Pur Sequence	Pur-Pyr Sequence
LZ1	α (C3'-03')	259	237	262	242
	β (03'-P)	105	325	96	320
	γ (P-05')	87	210	90	212
	δ (05'-C5')	190	178	194	182
	ε (C5'-C4')	160	69	161	68
	ζ (C4'-C3')	95	120	85	120
	χ (C1'-N9)	240	-	220	-
	χ (C1'-N1)	-	15	-	10
LZ2	α (C3'-03')	235	192	275	183
	β (03'-P)	94	98	85	102
	γ (P-05')	66	108	67	99
	δ (05'-C5')	184	148	168	146
	ε (C5'-C4')	154	85	150	75
	ζ (C4'-C3')	86	156	96	153
	χ (C1'-N9)	210	-	220	-
	χ (C1'-N1)	-	20	-	28

* The alphabetical nomenclature for the torsion angles were adopted from Seeman et al[11].

h = 0 Å. Thus in LZ2 type Z-character is more prominent than in LZ1 (compare Fig.1a and 1b).

The stacking patterns in the two types, as shown in Fig.2 have the same essential features. In both the types, in purine-pyrimidine sequence one finds intrastrand stacking whereas inter-strand stacking occurs between two pyrimidine bases in pyrimi-dine-purine sequence.

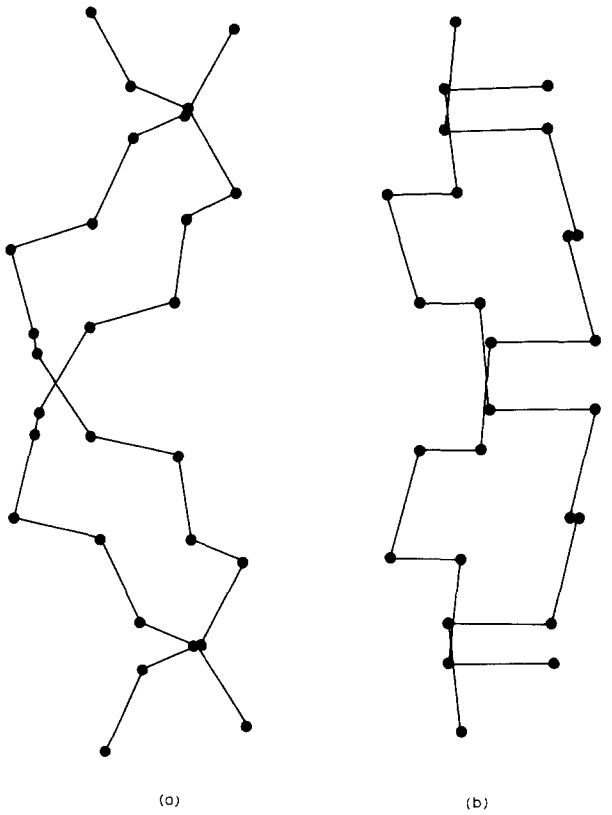

Fig. 1: Schematic representation of the LZ-helices in B-form. Zig-zag progression of the phosphate groups is shown along the helix axis. (a) For LZ1 type, two neighbouring phosphate groups either have t = 23° and h = 5.4 Å or t = 49° and h = 1.4 Å. (b) For LZ2 type, two neighbouring phosphate groups either have t = 0° and h = 6.8 Å or t = 72° and h = 0 Å. Hence, Z-character is more prominent in LZ2 than in LZ1 type.

DNA fragments in $d(C\text{-}G)_3$ and $d(C\text{-}G)_2$[5,6] show conformations similar to that of LZ1 helices. However, same kind of stacking arrangement as found in the single crystals can be brought about by the backbone conformation of LZ2 helices[7].

A Stable Link Between RU and LZ Helices

We have earlier shown[4] that a right-handed uniform (RU) helix is possible for PAPP wherein purine-nucleotides are in (C3'-endo, g^-g^-) conformation while pyrimidine-nucleotide are in

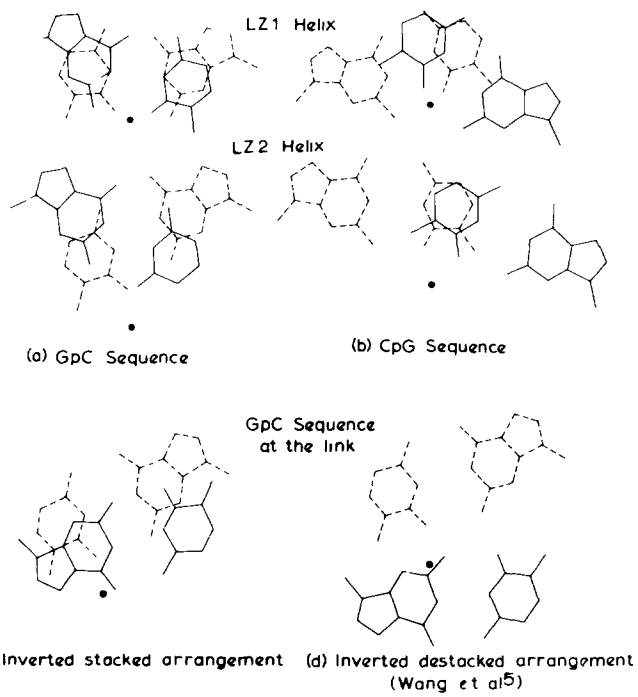

Fig. 2: a & b Stacking arrangements of (a) pur-pyr and (b) pyr-pur sequences in LZ1 and LZ2 helices (G_pC and C_pG sequences are chosen as examples). In both the types of LZ helices, G_pC sequence involves intrastrand stacking overlap where interstrand stacking overlap between two Cs occurs for C_pG sequence. In LZ2 helix intrastrand stacking overlap is less (in G_pC fragment) than that in LZ1 helix while interstrand stacking overlap (between two Cs) is more than that in LZ1 helix.

Fig. 2: c & d Stacking arrangement of the bases at the link between RU and LZ-helices. 2c shows the inverted stacking arrangement at the link as suggested. 2d gives a rough indication of inverted arrangement of the bases at the link of B- and Z-DNA proposed by Wang *et al*[5]. Note, for 2c both the base-pairs are on the same side of the helix-origin (hence stacked) while for 2d two base-pairs are on opposite sides of the helix-origin (thus largely destacked).

(C2'-*endo*, tg^-) conformation. Joining of RU to LZ helix resulting in a RL-model is possible only when a stable link can be made between the two. By 'stable', we imply that the link should involve allowed stereochemistry of the backbone, favourable stacking arrangement of the bases and Watson-Crick base-pairing. In what follows, we describe the conformational features of such a

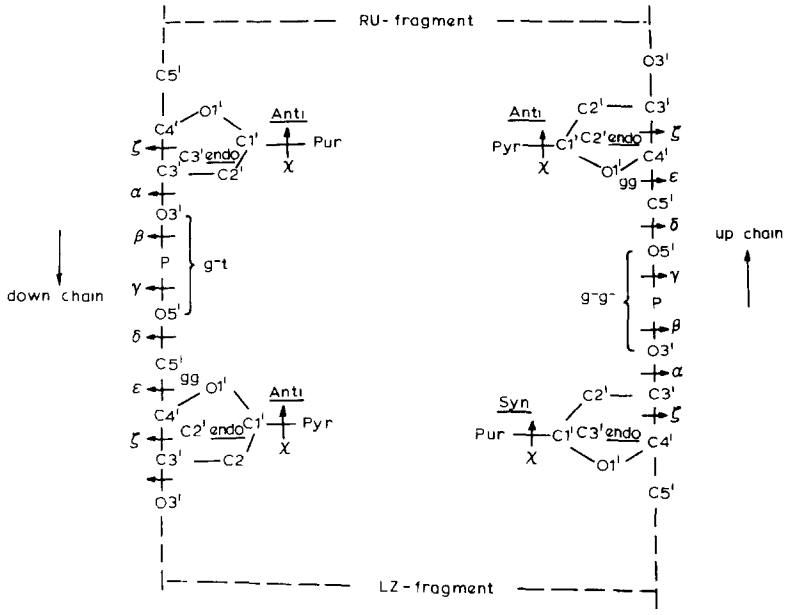

Fig. 3: Conformational features of the link between LZ-helix at the bottom and RU helix at the top. Note that the backbone conformations of the up-and down-chains are different (see the text).

stable link between RU and LZ helices.

It was found that the linking must be done at the phosphate groups in purine-pyrimidine sequence. The conformational features of the link are shown in Fig.3. It is seen that in the up-chain the phosphodiester conformations are g^-g^- while in the down-chain they are g^-t. The sugars attached to purines are in C3'-endo conformation while those attached to pyrimidines are in C2'-endo conformation. Thus even at the link the alternation of sugar puckering is maintained. The lower base pair marks the end of the LZ-fragment and hence it has purine in syn and pyrimidine in anti conformation. The upper base-pair being the beginning of the RU fragment has both the bases in anti conformation.

The conformational parameters of the link between LZ2 and RU helices are given in Table 2. The lower base-pair is fix-

Table 2. Conformational Parameters of the Links between RU and LZ-helices*.

Torsion Angles (in deg)	Up-Chain	Down-Chain
(5'-end)χ	240	50
(5'-end)ζ	85	86
α	189	203
β	305	325
γ	320	154
δ	140	218
ε	68	43
(3'-end)ζ	156	153
(3'-end)χ	75	65

* The torsion angles are as indicated in Fig.3.

ed in the frame of reference of LZ-helix and the upper base-pair is connected by sugar phosphate backbone constrained to the requirements of a stable link mentioned earlier. The stacking arrangement at the link is given in Fig.2c. The stacking pattern is of inverted type[8]: a characteristic feature of all RL-models. The physical overlap of the bases is almost identical to the stacked arrangement of purine-pyrimidine sequence in LZ-helices (see Fig.2a and Fig.2b). It may be recalled that Wang et al.[5] suggested a link between Z- and B-DNA. They also arrived at inverted arrangement of the bases at the link, but bases are largely destacked (see Fig.2d). This was perhaps the reason why they felt that the link they suggested was different from the one in RL model proposed by us[9,10]. However, stacking interaction being an important stabilizing factor in DNA, completely

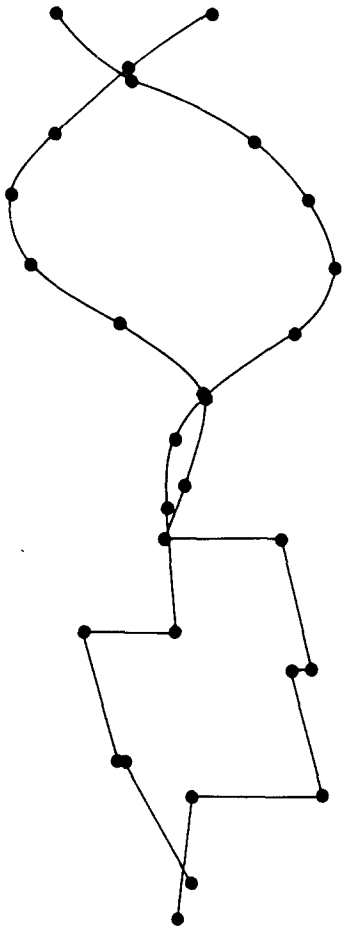

Fig. 4: A schematic drawing of RU and LZ helical fragments with a stable link in between. The phosphate groups (dark circles) are shown.

destacked arrangement of the bases would be a rather weak link between two DNA fragments. It would, therefore, be surprising if such a link occurs at all in the middle of a structure. In Fig.4, the phosphate groups in the RL model obtained by joining LZ end RU helices are shown. It is seen that the gross feature of a cylinder is still retained.

Conclusion

Thus, following a stereochemical guideline it was possible to obtain two distinct types of LZ-helices, of which one is

conformationally similar to the Z-DNA fragments in the single crystals. Both the types of LZ helices can be joined to RU helices by a stable link. The resulting RL model involves inverted stacking arrangement at the link.

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