Analysis of the possible helical structures of nucleic acids and polynucleotides. Application of (n-h) plots.

N. Yathindra and M. Sundaralingam

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, WI 53706, USA

Received 28 January 1976

ABSTRACT

The two helical parameters n and h where n is the number of nucleotide residues per turn and h is the height per nucleotide residue have been evaluated for single stranded helical polynucleotide chains comprising C(3') endo and C(2') endo class of nucleotides. The helical parameters are found to be especially sensitive to the C(3')-C(3') (sugar pucker) and the C(4')-C(5') torsions. The (n-h) plots display only one important helix forming domain for each class of nucleotides characterized by the sugar pucker and the C(4')-C(5') torsion. A correlation between the (n-h) plots and the known RNA (A,A') and DNA (A,B,C) helical forms has been established. It is found that all forms of helices except the C-DNA possess a favorable combination of P-0 torsions. The analysis of the (n-h) plots suggests that C-DNA can have a conformation very similar to B-DNA. Although the (n-h) plots predict the stereochemical possibility of both right-handed and left-handed helices, nucleic acids apparently prefer right-handed conformation because of the energetics associated with the sugar-phosphate backbone and the base.

INTRODUCTION

Helices represent the single most important secondary structural form of nucleic acids. It is therefore important to examine the possible types of helices that could be generated from the monomer nucleotide unit. Although there have been attempts,\(^1\)\(^2\) no detailed analysis is reported so far on the possible types of helical and double helical nucleic acid conformations presumably because of the complexity arising from the presence of six rotatable single bonds in the monomeric unit. This has been simplified to a considerable degree by the suggestion\(^3\)\(^-\)\(^5\) that nucleic acids and polynucleotides may be regarded as being composed of nucleotide units possessing either the preferred C(3') endo or C(2') endo type of conformations (Fig. 1) and that the primary flexibility in the polynucleotide backbone conformation involves the torsions around the phosphodiester bonds linking the successive nucleotide units. Using this idea we report here the evaluation of the helical parameters, viz., the number of nucleotide residues per turn (n) and the unit translation (h) for single stranded polynucleotides as a function of the internucleotide phosphodiester
torsions P-0(3') (ω') and P-0(5') (ω). These results in addition to furnishing valuable information on the possible single stranded helical conformations of nucleic acids, such as those in certain viruses, also provide a basis for examining the possible double helical and multistranded nucleic acid and polynucleotide structures.

METHODS

NUCLEOTIDE GEOMETRY

The calculation of helical parameters depends only on the backbone torsions, and is independent of the glycosyl torsions although they have important influence in deciding the stereochemical feasibility and stability of the resulting helical structures. We have shown[3,4] that the torsions around the six backbone bonds of the monomer nucleotide are restricted. For the most preferred conformation of the nucleotide, the torsion ψ' around the backbone C(4')-C(3') bond of the furanose ring is centered around gauche⁺ (76°) or trans (150°) domain corresponding to the C(3') endo and C(2') endo pucker of the sugar respectively. The other backbone torsions around C(5')-0(5') (ψ), C(3')-0(3') (ψ') and C(4')-C(5') (ψ) of the nucleotide are centered around the values 180° (trans), 210° (trans) and 60° (gauche⁺) respectively (see Fig. 1). Although the gauche⁺ is the most predominant conformation around the C(4')-C(5') bond in nucleotides as well as nucleotides of double helices[3,4,6] and is favored energetically,[5] calculations have been performed for the trans and gauche⁻ conformations also to examine the nature and types of possible helical structures that these nucleotides generate. Standard bond angles and bond distances[7] have been used for the sugar-phosphate backbone and all the calculations are for O(3')-P-0(5') bond angle of 103°.

EVALUATION OF HELICAL PARAMETERS

When the torsions along the successive nucleotide backbone bonds and the phosphodiester linkages are kept constant, the polynucleotide backbone chain folds into a helical structure. In such a situation, each nucleotide residue can be generated from the previous one by rotating through an angle τ (=360°/n, where n is the number of residues per turn) and advancing it through a distance h, the unit height along the helix axis. The evaluation of the helical parameters n and h from the dimensions of the monomer nucleotide unit reduces to obtaining the transformation matrix which transforms a vector from the coordinate system associated with the (i + 1)th unit or virtual bond to the coordinate system of the ith unit.
Fig. 1: The two energetically most favored conformations for the nucleotide building blocks (a) C(3') endo nucleotide and (b) C(2') endo nucleotide. The notations defining the bond rotations are indicated. Note that the main difference between the two nucleotide units is in the sugar pucker. The torsions around the C(4')-C(5') (\(\phi\)), C(5')-O(5') (\(\psi\)) and C(3')-O(3') (\(\phi'\)) bonds have values 60°, 180° and 210° respectively in both types.

or virtual bond. The mathematical formulation of this matrix is based on the rigid body transformation similar to that described by Ramachandran et al. The helical parameters \(n\) and \(h\) are determined from this matrix on the same lines as detailed by Ramakrishnan for polypeptides and Rao et al. for polysaccharides. The helical parameters are computed for each class of nucleotides as a function of internucleotide P-O(3') (\(\omega'\)) and P-O(5') (\(\omega\)) torsions at intervals of 10°. The possible values of \(n\) and \(h\) are depicted as contours of constant values of \(n\)'s and \(h\)'s on a \(\omega', \omega\) plot. The dotted lines correspond to curves of constant \(n\) and the full lines correspond to curves of constant \(h\). Positive values of \(h\) represent a right-handed helix while negative values of \(h\) denote a left-handed helix.
HELICAL PARAMETERS FOR THE C(3') ENDO (3E) NUCLEOTIDES

The possible values of \( n \) and \( h \) computed as a function of the inter-nucleotide P-O torsions for a C(3') endo nucleotide unit is shown in Fig. 2 in the form of a \((n-h)\) plot. The points of intersection of the \( h \)-curves with the \( n \)-curves are interpreted in terms of single stranded helical structures \( (n_h) \) characterized by specific values of \( n \) and \( h \). Thus the \((n-h)\) plot provides at a glance the possibilities of various helical structures. Values of \( n \) greater than 12 and less than 3 are not shown in Fig. 2 for clarity. The maximum permissible value of \( n = 28.2 \) occurs at \((\omega', \omega) = (260^\circ, 310^\circ)\). The maximum value of \( h \) corresponds to the length \((\approx 5.9 \text{ A})\) of the nucleotide unit and values of \( h \) greater than this are obviously not

![Fig. 2: Curves of iso-n (---) and iso-h(-----) for single stranded C(3') endo polynucleotide helices (\( \psi = \varphi' \)). The two possible positions of occurrence of right-handed A-RNA (\(^\text{112A}\)) and A'-RNA (\(^\text{123A}\)) are shown.](image_url)
It is noticed that curves of constant n from 3-12 occur in the broad region of the (\(\omega',\omega\)) map encompassing the \(\omega'\) torsions from -210 to +20° and \(\omega\) from 190 to 360°. This region corresponds to the \(\text{g}^-\text{g}^-\) (300°,300°), \(\text{tg}^-\) (180°,300°) and upper portions of \(\text{g}^-\text{t}\) (300°,180°) conformational domains of the (\(\omega',\omega\)) map. Similarly curves of constant h ranging from -5 to +5 also occur in the same region intersecting the n-curves indicating the possibility of a variety of helical polynucleotide structures. Another region where the n and h curves intersect corresponds to the \(\text{g}^+\text{g}^+\) (180°,60°) conformation domain which generates helices comprising 3 and 4 nucleotide residues per turn. The n values between 2 and 3 occur in the \(\text{g}^+\text{g}^+\) (60°,60°), \(\text{g}^+\text{t}\) (60°,180°), \(\text{g}^-\text{g}^-\) (60,300°), \(\text{g}^+\text{g}^-\) (300°,60°) and lower portions of \(\text{g}^-\text{t}\) (300°,180°) domains. Some of these correspond to higher energy conformations and are therefore unlikely to form stable helices. For instance, it is impossible to generate a stereochemically acceptable polynucleotide chain with successive phosphodiesters in the \(\text{g}^+\text{g}^+\) or \(\text{g}^-\text{t}\) conformations. Similarly, the \(\text{g}^-\text{g}^-\) region has also been shown to be energetically unfavorable. Both in the skewed \(\text{g}^-\text{g}^-\) as well as the \(\text{tg}^+\) conformations, the adjacent bases lie on opposite side of the central phosphodiester and therefore cannot generate the energetically favored stacked helical structure. These conformations have therefore been suggested as loop phosphodiesters. Thus the broad region comprising the \(\text{tg}^-\) and \(\text{g}^-\text{g}^-\) conformational domains for which the adjacent bases are on the same side of the phosphodiester represent the single most important secondary structural region for nucleic acids.

HELICAL PARAMETERS FOR THE C(2'-) ENDO (\(2E\)) NUCLEOTIDES

The primary difference between the C(3'-) endo and C(2'-) endo nucleotides lies in the backbone \(\text{C}(\h')\text{-C}(\h')\) torsion which is nearly gauche \(\text{+} (76°)\) in the former and trans \(\text{+} (150°)\) in the latter. It is apparent from a comparison of the (n-h) plots given in Fig. 2 and Fig. 3 that the change in the backbone \(\text{C}(\h')\text{-C}(\h')\) torsion from gauche \(\text{+} (\text{C}(3'-\text{endo pucker})\) to trans \(\text{+} (\text{C}(2'-\text{endo pucker})\) produces considerable shift towards lower P-O(3') (\(\omega'\)) torsions. The contours which occur at \((\omega',\omega) = (270°,310°)\) (\(\text{g}^-\text{g}^-\)) for the C(3'-) endo nucleotide now occur at \((\omega',\omega) = (200°,280°)\) (\(\text{tg}^-\)) for the C(2') endo nucleotide causing a shift of nearly 70° along \(\omega'\) and 30° along \(\omega\). Nevertheless the other general features in the (n-h) plot are very similar to those obtained for the C(3'-) endo nucleotide (Fig. 2). The maximum permissible value of n = 8.9 occurs at \((\omega',\omega) = (200°,270°)\) and is much higher than the value (28.2) obtained for C(3') endo nucleotides indicating that C(2') endo
nucleotides can generate wider helices than the C(3') endo nucleotides. As previously n-values greater than 12 are not shown in Fig. 3 for clarity. The broad $tg$ domain represents the single most important helix forming domain in the entire $(\omega', \omega)$ surface. Other features in the $(n-h)$ plot are similar to Fig. 2.

Fig. 3: $(n-h)$ plot for C(2') endo polynucleotide helices with C(U')-C(5') in the preferred gauche conformation. The two possible positions of occurrence of B-DNA and C-DNA are shown (see also text).

$(n-h)$ PLOT FOR C(3') EXO NUCLEOTIDES

Since C(3') exo, a form of C(2') endo pucker, is frequently observed for 2'-deoxyribose sugars$^b,12$ and is also favored in B-DNA,$^{13}$ it is of interest to compute the $(n-h)$ plot with the C(3') exo nucleotide dimensions. Fig. 4 shows the $(n-h)$ plot obtained using C(3') exo nucleotide torsions, $\psi = 214^\circ$, $\phi' = 155^\circ$, $\psi = 36^\circ$, $\phi' = 155^\circ$ as found in the B-DNA model.$^{13}$ These torsions differ from their preferred values and are used in this calculation for the purpose of correlating the current B-DNA model with the $(n-h)$ plot. Noticeable shifts towards higher values of $\omega$ and $\omega'$ are
Fig. 4: (n-h) plot for C(3') exo polynucleotide helices with C(h')-C(5') in the preferred gauche domain. The two possible positions of occurrence of B-DNA and C-DNA are shown.

observed in the (n-h) plots compared to Fig. 3. These shifts are due to the changes in the nucleotide backbone torsions ($\psi$ and $\psi'$) rather than the change in the sugar pucker from C(2') endo to C(3') exo. Calculation shows that the (n-h) plots for C(3') exo nucleotide with usual backbone torsions ($\psi = 60^\circ$, $\psi' = 210^\circ$) is virtually identical to that obtained for C(2') endo nucleotide (Fig. 3).

NUCLEOTIDES WITH ALTERNATIVE BACKBONE CONFORMATIONS

Although the gauche ($g'$) torsion around the C(h')-C(5') bond of a nucleotide is energetically the most favored conformation, it can also adopt the other two staggered but less favored trans (t) and gauche ($g^*$) orientations. Such conformations may be important in the single stranded loop structures and also in helical nucleic acids such as in viruses and
chromatin which are involved in complicated tertiary structures. Hence, it is important to examine the nature of helical structures that the alternative nucleotide conformations generate. These results furnish information on the effect of C(h')-C(5') torsions on the helical parameters. We have considered both the C(3') endo and C(2') endo sugar puckers as before.

**Fig. 5** shows that when the C(h')-C(5') torsion is changed from the preferred gauche (g) to the trans (t) orientation, the (n-h) curves are shifted along ω to the g-t domain (ω,ω) = (300°,180°). The maximum value of n = 60.3 occurs at (ω,ω) = (280°,180°) suggesting that the trans nucleotide conformation permits wider nucleic acid helices than the preferred standard C(3') endo or C(2') endo nucleotide conformations (see above). The n-values greater than 12 occur in the g-t domain and are not shown in Fig. 5 for clarity. The g-t represents the single most important helix forming domain for this class of nucleotide monomer. Furthermore potential energy...
calculations have shown\textsuperscript{14} that this conformation is energetically favored and model building studies show that it is the only phosphodiester domain that would provide possible stacking between the adjacent bases. Interestingly, the original Watson-Crick structure had this stereochemistry. Other details are similar to those found in Fig. 2 and are self explanatory.

\textbf{(n-h) PLOT FOR C(3') ENDO NUCLEOTIDE WITH THE GAUCHE (g') C(4')-C(5') TORSION}

Fig. 6 shows the (n-h) plot obtained with the gauche (g') torsion for the nucleotide. Compared to Fig. 2, Fig. 6 shows a shift in the (n-h) plot along P-O(5') (\(\omega\)) from the \(g'g\) domain to the \(g'g^+\) domain (\(\omega',\omega\)) (300°,60°) while the general features of the (n-h) curves are very similar, as was also noticed in Fig. 5. The maximum permissible value of \(n\) is only 13.6 and it occurs at (\(\omega',\omega\)) = (270°,60°) indicating that this nucleotide conformation would generate narrow helices compared to the gauche\textsuperscript{\*} and trans nucleotide conformations. The \(g'g^+\) is the only important helix forming phosphodiester domain. The skewed \(g'g\) is one of the sterically accessible domain (although energetically not highly favored) for the phosphodiester and is the only conformation which leads to the overlap of the adjacent bases.\textsuperscript{14}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig6}
\caption{(n-h) plot for C(3')-endo polymucleotide helices with C(4')-C(5') in the gauche conformation.}
\end{figure}
The (n-h) plots computed for the C(2') endo nucleotide with the trans and gauche orientations around the C(U')-C(5') bond showed shifts of 120° along P-0(5') (ω) very similar to those observed for C(3') endo nucleotides (Figs. 5 and 6) and hence are not described. The important helix forming domains occur at the tt and tg* phosphodiester (Table 1). These analyses however demonstrated that the position of occurrence of the helix forming domain on the (ω',ω) surface is intimately correlated to the adjacent C(4')-C(3') torsion associated with the sugar pucker. While the changes in the C(4')-C(5') torsion produce shifts mainly along P-0(5') (ω), changes in the sugar pucker (C(4')-C(3') torsion) produce shifts along P-0(3') (ω'). Hence it would be expected that lower values of C(4')-C(5') torsion (<60°) would shift the helical domain towards higher P-0(5') torsions (eg. Fig. 4). The (n-h) plots obtained with nucleotides having values other than these would be expected to exhibit properties intermediate to those discussed above.

DISCUSSION

CYCLIC STRUCTURES

It is seen from Figs. 2-6 that the value of h = 0 occur in the important helix forming domain separating the curves of positive and negative h-values. The intersection of the h = 0 curve with an n-curve is interpreted as a helix with residue height equal to zero, in other words a flat helix. Thus the points of intersections of h = 0 and different n-curves generate ring

<table>
<thead>
<tr>
<th>C(4')-C(5') torsion (ϕ)*</th>
<th>Important Helix Forming Phosphodiester Domain (ω',ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C(3') endo</td>
</tr>
<tr>
<td>1</td>
<td>$g^+$</td>
</tr>
<tr>
<td>2</td>
<td>$t$</td>
</tr>
<tr>
<td>3</td>
<td>$g^-$</td>
</tr>
</tbody>
</table>

*The C(5')-O(5') (ϕ) and C(3')-O(3') (ϕ') torsions are assigned 180° and 210° respectively.
structures comprising different number of nucleotide residues \(n\). It would be interesting to examine whether these macro-cyclic ring structures comprising integral number of residues are in fact stereochemically feasible. The ring structures develop into right-handed helices as the value of \(\omega\) torsion increases.

**RIGHT-HANDED HELICES**

**HELICES COMPRISINC C(3') ENDO NUCLEOTIDES - A,A' FORMS OF RNA**

The points of intersection of \(h\)-curves \((h>0)\) with the \(n\)-curves in the \((n-h)\) plot represent right-handed helices. It is clear from Fig. 2 that the \(g-g\) region represents the most important helix forming domain and the \(h\)-curves intersect the \(n\)-curves in this region at two different points suggesting two different types of helical conformations for a given \(n\) and \(h\). For instance \(h = +3\) curve intersects \(n = 11\) curve at \((\omega',\omega) = (290°,290°)\) and also at \((240°,320°)\). To distinguish the two models, we refer to the region near the phosphodiester \((290°,290°)\) as the type 1 and the region near the phosphodiester \((240°,320°)\) as type 2. The preference for either of these conformations is decided by energy and other geometric and structural considerations like hydrogen bonding and stacking. Although both of these phosphodiester conformations are stereochemically accessible, type 1, with the phosphodiester near \((\omega',\omega) = (290°,290°)\), has been shown to be energetically most favored for a dinucleoside triphosphate and a polynucleotide backbone. Furthermore, it is readily apparent from model studies that type 1 provides better stacking interactions between the adjacent bases, satisfying at the same time the requirements of base-pairing geometry. The type 2 model corresponding to the phosphodiester conformation \((240°,320°)\) on the other hand results in poor stacking. Consequently, the type 1 model \((\omega',\omega) = (290°,290°)\) is expected to prevail in single as well as double stranded nucleic acid and polynucleotide helices. It is noteworthy that all the observed helical nucleic acid and polynucleotide structures comprising C(3') endo nucleotides such as RNA-11, RNA-12, A-DNA, poly A and other synthetic polynucleotides indeed possess the phosphodiester in this narrow \(g-g\) domain corresponding to the type 1 model (Table 2).

It is striking that values of \(n > 6\) and \(h = 1\) to 5 occur in a narrow region around \((\omega',\omega) = (300 ± 20°, 300 ± 20°)\) indicating that relatively small variations in the internucleotide P-O(3') and P-O(5') torsions would result in a variety of helical conformations for nucleic acids. This also suggests that the possible transitions among these different but very similar structures are expected to be smooth and noncooperative in nature. The close proximity of the positions of occurrence of A-RNA \((n = 11, h =

739
Correlation between the helical parameters, the nucleotide geometry and the phosphodiester conformation. Comparison of experimental and theoretically predicted values

<table>
<thead>
<tr>
<th>Polynucleotide Helices</th>
<th>Internucleotide phosphodiester ((\omega', \omega))</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>(h)</td>
</tr>
<tr>
<td><strong>C(3')-endo</strong> Helices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>poly AH(^+), poly AH(^+)</td>
<td>8</td>
<td>3.8</td>
</tr>
<tr>
<td>poly A</td>
<td>9</td>
<td>2.82</td>
</tr>
<tr>
<td>RNA-10</td>
<td>10</td>
<td>2.9</td>
</tr>
<tr>
<td>A-RNA</td>
<td>11</td>
<td>2.81</td>
</tr>
<tr>
<td>A'-RNA</td>
<td>12</td>
<td>3.0</td>
</tr>
<tr>
<td>A-DNA</td>
<td>11</td>
<td>2.56</td>
</tr>
<tr>
<td><strong>C(2')-endo (C(_3)-exo)</strong> Helices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>poly (dA-dT).poly (dA-dT)</td>
<td>8</td>
<td>3.03</td>
</tr>
<tr>
<td>C-DNA</td>
<td>9.3</td>
<td>3.32</td>
</tr>
<tr>
<td>C-DNA</td>
<td>9.3</td>
<td>3.32</td>
</tr>
<tr>
<td>B-DNA</td>
<td>10</td>
<td>3.38</td>
</tr>
<tr>
<td>B-DNA</td>
<td>10</td>
<td>3.38</td>
</tr>
</tbody>
</table>

2.81) and A'-RNA \((n = 12, h = 3)\) in the \((n-h)\) plot (Fig. 2) indicate that the possible A-A' transition in polynucleotide structures is noncooperative, in agreement with the conclusions from circular dichroism studies.\(^{15}\)

**CONFORMATIONS OF DNA HELICES - A, B, C FORMS**

DNA is known to exist in several polymorphic modifications. In addition to A- and B- forms, DNA has been found to exhibit the C- form in the presence of lithium\(^{16}\) as well as sodium\(^{17}\) salts. A- form is well characterized and has essentially the same structure as RNA-11.\(^{6}\) The position of occurrence of A-DNA in the \((n-h)\) plot lies therefore very close to A-RNA (Fig. 2). Both B-DNA and C-DNA have been implicated to possess the C(2') endo class of nucleotides.\(^{13,18}\) It is of considerable importance to examine their positions of occurrence in the \((n-h)\) plot since this would reveal information on the possible similarity or dissimilarity in the conformational structures of the two forms. B-DNA\(^{13,18}\) has the helical parameters \(n = 10\) and \(h = 3.37\) and C-DNA,\(^{16}\) \(n = 9\) and \(h = 3.32\). It is clear from Fig. 3 that the phospho-
diesters corresponding to these structures lie in the broad $t_g$~ domain. Interestingly, there are two possible phosphodiester conformations for a single set of helical parameters since the $h$-curves intersect the $n$-curves at two points, a feature also noticed in Fig. 2. To distinguish the two regions which generate two different structures we refer to the phosphodiester $(\omega',\omega) = (240^\circ,270^\circ)$ as type 1 and the phosphodiester $(\omega',\omega) = (180^\circ,300^\circ)$ as type 2. It is striking that the points corresponding to B-DNA and C-DNA in both type 1 and type 2 regions occur very close to each other in the $(n-h)$ plot (Figs. 3 and 4). This strongly suggests that the conformational structures of B-DNA and C-DNA should be very similar. However, in previous models the type 1 model was favored for B-DNA and type 2 for C-DNA. It has therefore been regarded that the conformation of C-DNA is considerably different from B-DNA. We agree that the type 1 model is favored for B-DNA, probably because of favorable stacking interactions and the base pairing geometry, and it is now clear from the $(n-h)$ plot that the type 1 model for C-DNA is also possible. We argue that C-DNA would also favor the type 1 model (skewed $g^-g^-$ phosphodiester) rather than the type 2 model (extended $t_g$ phosphodiester) and that the conformational difference between the B-DNA and C-DNA structures is one of degree rather than of kind. It is also clear from the $(n-h)$ plot obtained for C(3') exo nucleotide (Fig. 4) that the type 2 model is also energetically unfavorable because of eclipsed torsion around the P-O(5') bond further indicating the preference of the B- and C-DNA forms for the type 1 model near the $g^-g^-$ domain. The phosphodiester in the $g^-g^-$ domain tends to provide a better overlap between the adjacent bases while the $t_g$ domain results in an extended phosphodiester conformations causing the adjacent bases to drift apart.

**CONFORMATIONAL TRANSITIONS IN NUCLEIC ACID HELICES**

The apparent similarity in the conformational structures of B-DNA and C-DNA described above also gains support from the experimental observation of noncooperative B-DNA→C-DNA transition. It is clear that this transition would be expected to be necessarily noncooperative only if both the B-DNA and C-DNA structures occur in the type 1 or type 2 conformations (Figs. 3 and 4). In such a situation the B-DNA→C-DNA transition involve only minor local modifications in the phosphodiesters. But if B-DNA occurs in type 1 conformation and C-DNA in type 2 conformation, B-DNA→C-DNA transition would then involve relatively large rotations around the P-O bonds. Such a transition is expected to be cooperative in nature. It is
extremely interesting to note that the recent C-DNA model has the phosphodiester in the region suggested by the \( \langle n-h \rangle \) plots thus providing further confirmation to the above deductions from the \( \langle n-h \rangle \) plots. The B-DNA\( \rightarrow \)A-DNA transition is necessarily cooperative since the \( C(4')-C(3') \) torsion (sugar pucker) has to change from the gauche\(^+\) (C3'-endo pucker) to the trans (C2'-endo pucker) conformation domain involving a rotational change of nearly 80°. On the other hand the A\( ^R \)-RNA\( + \)A\( ' \)-RNA transition is expected to be noncooperative since it involves small adjustments in the internucleotide P-O torsions (Fig. 2).

LEFT-HANDED HELICES

One of the most interesting observations in the \( \langle n-h \rangle \) plot is that the negative values of \( h \), for instance \( h = -2, -3, -4 \) also intersect curves of \( n = 3 \) to 12 in the \( g'^-g^- \) domain suggesting the possibility of left-handed helical structures with helical parameters very similar to those observed for right-handed helices. For instance according to Fig. 2, phosphodiester torsions \( (\omega',\omega) = (290^\circ,300^\circ) \) generate a right-handed helical backbone with \( n = 12 \) and \( h = 3 \) whereas \( (\omega',\omega) = (265^\circ,275^\circ) \) lead to a left-handed helical backbone with \( n = 12 \) and \( h = -3 \). Thus relatively small changes in the internucleotide phosphodiester separate right-handed and left-handed helical polynucleotide backbones. It is therefore important to examine why only right-handed helices are found in naturally occurring nucleic acids.

Fig. 7 and Fig. 8 show a typical right-handed and a left-handed polynucleotide backbone having identical torsions along the backbone except for internucleotide phosphodiester. The right-handed helix has the internucleotide phosphodiester \( (\omega',\omega) = (290^\circ,300^\circ) \) with the helical parameters \( n = 10.5 \) and \( h = 3.2 \) \( (t = 3.2) \). The left-handed helix has the phosphodiester \( (265^\circ,275^\circ) \) and has the helical parameters \( n = 11.4 \) and \( h = -3.2 \) \( (t = -3.16) \). The glycosyl conformation is set at anti \( (\chi = 10^\circ) \) in right-handed helix while it is set at high-anti \( (\chi = 120^\circ) \) in the left-handed helix. The high-anti range is deliberately chosen in the latter to demonstrate that the base plane is nearly perpendicular only for this range of glycosyl torsion whereas the normal anti glycosyl conformation renders the base plane nearly parallel to the helix axis. Comparison of Fig. 8 with Fig. 7 shows that exactly the opposite is true for a right-handed helix. It should be noted that the high-anti glycosyl conformation shown in Fig. 8 is not meant to imply that it will automatically lead to left-handed helix. Complementary Watson-Crick hydrogen bonding with the bases of the opposing strand results in a left-handed ribbon or sheet like structure. It is clear that there is hardly any overlap.
Fig. 7: Illustration of a right-handed helical polynucleotide chain comprising the preferred C(3') endo nucleotides with successive phosphodiesters having \((\omega', \omega) = (290°, 300°)\). The backbone has the helical parameters \(n = 10.5\) and \(h = 3.2\) \((t = 34.2°)\). Note that the base is anti and its plane is perpendicular to the helix axis.

of the adjacent bases in the left-handed helix and furthermore the favorable short-range van der Waals interactions between the adjacent sugar residues are reduced. These factors at least in part, may be responsible for the preference of a right-handed rather than a left-handed double helix for nucleic acids in nature.
Fig. 8: Illustration of a typical left-handed helical polynucleotide chain comprising the preferred C(3') endo nucleotides with successive phosphodiester bonds having \((\omega', \omega) = (265°, 275°)\). The backbone has the helical parameters \(n = 11.4\) and \(h = -3.2\) \((t = -31.6°)\). Note that the base is high-anti and its plane is perpendicular to the helix axis. The base plane would be parallel to helix axis for the normal anti conformation.

CONCLUSIONS

Although the results reported here provide direct information only on the helical structures of single stranded polynucleotide chains, they also serve as a first step toward understanding the possible double, triple and multistranded helical structures of polynucleotides. A knowledge of these would be particularly helpful in predicting the helical structures of nucleic acids in such giant molecules as viruses, chromatin, certain RNA-DNA hybrid complexes and circular DNA. The results also provide useful details concerning the helical conformations of nucleic acids after
unwinding due to intercalations. In double helical nucleic acids, at least in the naturally occurring ones, the two backbone strands are necessarily related by a diad symmetry. In such cases, the two strands have the same helical parameters, but instead of having separate helical axes, the two strands share the common helix axis. Therefore the helical parameters determined for single stranded polynucleotide chain are especially relevant for the understanding of the double helical nucleic acid conformations. Model building studies clearly indicate that the necessity of complementary base-pairing between the opposing strands places stringent restrictions on the number of possible double helical conformations. Studies are currently in progress to examine in detail the possible double helical conformations in nucleic acids.

The present analysis shows that there is a striking correlation between the helical parameters and the geometry of the nucleotide building block, especially the nature of the sugar pucker (C(4')-C(3') torsion) and the exocyclic C(4')-C(5') torsion. There is only one broad, principal helix forming domain in the (ω',ω) surface regardless of the sugar pucker and the C(4')-C(5') torsion. For C(3') endo nucleotides it occurs at the g−g−, g+t and g−g+ phosphodiester domains when the C(4')-C(5') torsion assumes the gauche+, trans and gauche− conformations respectively (Table 1). It is most interesting that the adjacent nucleotide bases tend to overlap only for these phosphodiesters which also represent the important helix forming domains. This suggests that the 'stacking' interactions between the adjacent bases appears to be the fundamental property of nucleic acid helices. For C(2') endo (C3'-exo) nucleotides, the helix forming domain occurs at the tga, tt and tgg phosphodiester s for g+, t and g− orientations of C(4')-C(5') bond respectively. Although the adjacent bases lie on the same side of the phosphodiester, there is hardly any base overlap in the tt and tgg phosphodiester due to the extended nature of the nucleotide as well as phosphodiester conformations. Some of these helical domains seem less likely because the mononucleotide as well as the internucleotide phosphodiester conformations corresponding to them are energetically unfavorable. The C(3') endo and C(2') endo nucleotides with the gauche+ conformation around the C(4')-C(5') bond are energetically the most favored, and are the only conformations that have so far been observed in single, double and multistranded helices.25,26 Short segments of helices comprising the less favored nucleotide conformations may become important in certain nucleic acids possessing complex tertiary structures.
For each class of nucleotides, three broad types of helical structures could be defined depending on the C(U')-C(5') torsion. Within each type, of course, several families of helical structures differing in the n and h values could be generated by small variation in the internucleotide P-0 torsions. Even though the phosphodiesters generating these helices are energetically favored, relative stabilities of these helical structures are determined by the helix energy (energy per nucleotide residue) which takes into account all the interactions of neighboring residues in one turn of the helix. The rapid variation of n and h in the helical domains (see for eg. Figs. 2 and 3) and consequent close proximity of occurrence of the possible helices is suggestive of noncooperative transition between the different but closely related helical structures. As already pointed out only relatively small changes in P-0 torsion as necessary (Fig. 2) for A-RNA --> A'-RNA transition which is therefore expected to be noncooperative.

There are two possible phosphodiester conformations for the same n- and h- values leading to two different helical structures having identical n and h. The phosphodiester closer to the g-g domain is preferred and is favored in all double helical polynucleotides except C-DNA. It is argued from the (n-h) plots that C-DNA can also display the favored phosphodiester in the g-g domain thus possessing conformational structure very similar to B-DNA.

Another interesting observation of this analysis is that small variations in the internucleotide phosphodiester in the helical domains can also lead to left-handed helical polynucleotide backbone with helical parameters similar to those observed in right-handed nucleic acid structures. Model building studies suggest that the decreased short-range van der Waals attractive interactions between the adjacent residues of the sugar-phosphate backbone and the concommitant loss of stacking interactions do not apparently favor left-handed helical conformations in nucleic acids.

ACKNOWLEDGMENTS

We gratefully acknowledge support of this research by a grant GM-17378 from the National Institutes of Health of the United States Public Health Service.

References

    Biophys. Jour. 6, 849-872.
    J. Mol. Biol. 3, 71-86.
    J. Mol. Biol. 27, 507-524.
    J. Mol. Biol. 88, 523-533.
    11, 537-543.
    92, 181-192.