Conformational studies of (2'-5') polynucleotides: theoretical computations of energy, base morphology, helical structure, and duplex formation⁺

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ABSTRACT

A detailed theoretical analysis has been carried out to probe the conformational characteristics of (2'-5') polynucleotide chains. Semi-empirical energy calculations are used to estimate the preferred torsional combinations of the monomeric repeating unit. The resulting morphology of adjacent bases and the tendency to form regular single-stranded structures are determined by standard computational procedures. The torsional preferences are in agreement with available nmr measurements on model compounds. The tendencies to adopt base stacked and intercalative geometries are markedly depressed compared to those in (3'-5') chains. Very limited families of regular monomerically repeating single-stranded (2'-5') helices are found. Base stacking, however, can be enhanced (but helix formation is at the same time depressed) in mixed puckered chains. Constrained (2'-5') duplex structures have been constructed from a search of all intervening glycosyl and sugar conformations that form geometrically feasible phosphodiester linkages. Both A- and B-type base stacking are found to generate non-standard backbone torsions and mixed glycosyl/sugar combinations. The 2'- and 5'-residues are locked in totally different arrangements and are thereby prevented from generating long helical structures.

INTRODUCTION

One of the unsolved puzzles of nucleic acid chemistry is why nature favors (3'-5') over (2'-5') phosphodiester linkages along the sugar-phosphate backbone. The latter connections are not only theoretically feasible but are well known to predominate over (3'-5') linkages under certain experimental conditions.¹⁻⁶ In the presence of various divalent metal ions in aqueous solution, for example, short (2'-5') linked oligoadenylic acids are formed more easily than (3'-5') linked chains.¹ The ApA produced by the reaction of adenosine 5'-phosphorimidazolidine with adenosine on a poly(U) template is also 95% (2'-5') linked.² Moreover, a large excess of the (2'-5') internucleotide form is invariably produced in the template-directed synthesis of oligoribonucleotides under simulated prebiotic conditions.⁴ Furthermore, a series of unique (2'-5') linked oligoadenylates of the general formula ppp5'A2'(p5'A)_m (1 ≤ m ≤ 15), is generated in interferon treated cells.⁷⁻⁹

A number of experimental and theoretical studies¹⁰⁻²⁶ have already probed the structural differences between (2'-5') and (3'-5') linkages in nucleic acid chains. The results are fairly limited and are somewhat mixed. There is some evidence from solution measurements, for example, that (2'-5') oligomers are as well if not more highly base stacked than

their (3'-5') analogs.¹⁰⁻¹⁴ On the other hand, the (2'-5') units are clearly more disordered than (3'-5') residues under certain conditions of chain length, temperature, and solvent.¹⁵⁻¹⁸ Moreover, the stacking of adjacent bases is not observed in the few available X-ray crystal structures of (2'-5') oligonucleotides,¹⁹⁻²² including the free dimers A2'p5'C and A2'p5'U and the complexed structure of C2'p5'A with the enzyme ribonuclease-S. Indeed, an extended structure is crucial for C2'p5'A to optimize its contacts at the active site of ribonuclease-S.

PCILO calculations²³ carried out on diribose monophosphates more than ten years ago further suggest the (2'-5') structures to be more flexible than their (3'-5') analogs. Early model building work by Dhingra and Sarma²⁴ also indicates that the (2'-5') linkage cannot form helical polynucleotide configurations with standard monomeric repeating units. More recent model building studies by Parthasarathy et al.²⁵ on the basis of the A2'p5'C crystal structure confirm this observation yielding a very tightly wound (2'-5') single-stranded helical model with only four nucleotides per helical turn. The proposed right-handed poly(G2'p5'C) helix repeats at the dinucleotide level with a 7.5Å rise per residue. The GpC moiety, which has minimal base stacking, conforms identically to the structure of ApC found in the crystal. The theoretically constructed internucleotide linkage between C and G, however, involves appreciable base stacking. The overwinding of the backbone and the positioning of the purine bases additionally prevent formation of a duplex with complementary base pairing. Extensive energy minimization studies carried out by Anukanth and Ponnuswamy²⁶ in the past year on a typical A2'p5'A nucleic acid fragment, on the other hand, lead to a double helical (2'-5') structure fairly similar in backbone torsion angles to the conventional A- and B-helical models. While not described in great detail, the structure appears to exhibit little, if any, adjacent base stacking. The relative stability of the (2'-5') duplex compared to a (3'-5') duplex on energetic grounds is also not clear. The reported energies describe only a single-stranded dimer model.

In this work we report further theoretical information on the conformational character of (2'-5') nucleic acid chains. Our work includes semi-empirical energy estimates of the preferred conformational domains of the monomeric repeating unit. We also examine the local base morphology and the helix forming tendencies of the allowed torsion angle combinations. We find fairly limited conformational domains in which well stacked single-stranded (2'-5') helices can be generated. We have further attempted to construct (2'-5') duplex models with base pairs constrained to the morphology of standard A- and B-DNA models. While we find numerous (2'-5') dimer miniduplexes that link adjacent base pairs, we do not obtain solutions that link a series of base pairs into regular oligomeric or polymeric structures.

Figure 1.

Dinucleoside monophosphate segment of (2'-5') ApA illustrating chain atoms and torsion angles. Atoms are distinguished by open (oxygen), solid (phosphorus), shaded (carbon), and stippled (nitrogen) spheres.

METHODS

Torsional Variables

A computer generated representation of a (2'-5') linked dinucleoside monophosphate showing heavy atoms and torsion angles is depicted in Figure 1. The backbone conformation is described, following Parthasarathy et al.,²¹ by five acyclic torsion angles— ϕ'' (C3'-C2'-O2'-P), ω'' (C2'-O2'-P-O5'), ω (O2'-P-O5'-C5'), ϕ (P-O5'-C5'-C4') and ψ (O5'-C5'-C4'-C3')—and two ring dihedral angles— λ (C5'-C4'-C3'-C2') and ψ'' (C4'-C3'-C2'-O2'). The angles are measured in a right-handed sense relative to the *cis* conformation as 0°. The base-orientation is given by the glycosyl torsion χ relating atoms O1', C1', N9, and C8. Only an adenine base is considered in the current calculations.

The flexibility of the (2'-5') chain is taken, like that of (3'-5')-linked DNA and RNA, to reside primarily, in the chain backbone. Only the rotation ϕ about the C5'-O5' torsion is assigned a fixed value (180°) in these calculations. The glycosyl torsion is assumed to be restricted to the *anti* (-15 - 75°) conformation in the studies of base morphology and helix formation but is treated as a variable in the energy and duplex modeling studies. The sugar is taken in either a C2'-endo ($\lambda = -101^\circ$ and $\psi'' = -153^\circ$) or a C3'-endo puckered form ($\lambda = -152^\circ$ and $\psi'' = -90^\circ$), while the C2'-O2' torsion is allowed to vary between the *trans* or *t* ($180^\circ \pm 30^\circ$) and *gauche*⁻ or *g*⁻ ($-60^\circ \pm 30^\circ$) rotational ranges. The P-O torsions are varied at 10°-increments over their entire angular range (0-360°), while ψ is

held at g^+ (60°), t (180°), and g^- (-60°) rotational isomeric states.

Energy Functions

The total conformational energy $E(\psi, \phi'', \chi)$ of adenosine 2'-monophosphate (2'-Amp) is estimated from a standard sum of nonbonded and intrinsic torsional terms:

$$E(\psi, \phi'', \chi) = E_{6-12} + E_{\text{electrostatic}} + E_{\text{torsional}} \quad (1)$$

The various nonbonded energy components and the related constants are those introduced earlier.²⁷ The torsional potentials about the C5'-C4' (ψ) and C2'-O2' (ϕ'') bonds are assumed to be threefold with energy barriers of 2.8 and 3.0 kcal/mole, respectively. An additional *gauche* correction term²⁸⁻³⁰ with appropriate constants that reflect the known preferences of O-C-C-O and O-C-C-C sequences to be *gauche* rather than *trans* is employed for the ψ torsion. No terms are included to estimate the relative energies of the rigid sugar rings. As a first approximation, the electrostatic charge distribution in 2'-Amp is assumed to be that of a 3'-mononucleotide with the values on O2' and O3' simply interchanged. Such switching of charges is found by us in a classical Del Re³¹ analysis of 2'- and 3'-Amp. More refined comparisons of electron density in these systems, unfortunately, are not available in the quantum chemical literature. The net charge on the nucleotide unit is taken as -1.00 esu. The phosphate group is oriented in a favored staggered conformation and the oxygens bonded to phosphorus are assumed to be equivalent. The hydrogen atoms on the furanose are not included explicitly in the calculations but are treated in terms of extended carbon atoms on the sugar ring and extended exocyclic hydroxyl groups.

Base Morphology

The relative orientation of adjacent bases is described in terms of the stacking parameters Λ , $\langle D \rangle$ and $\langle Z \rangle$.³² The parameter Λ is the angle formed by the normals to adjacent base planes, while $\langle D \rangle$, which is a measure of base overlap, is defined by the equation

$$\langle D \rangle = \sum_{i=1}^{N_I} \sum_{j=1}^{N_J} d_{ij} / N_I N_J \quad (2)$$

Here d_{ij} is the distance between atoms i and j , while N_I and N_J are the total number of heavy atoms on bases I and J . The parameter $\langle Z \rangle$ is the mean vertical displacement of base J with respect to base I . In computing $\langle Z \rangle$ the coordinates of the dimer are transformed into a new right-handed orthogonal system in which the xy plane coincides with that of base I . Then,

$$\langle Z \rangle = \sum_{j=1}^{N_J} z_j / N_J \quad (3)$$

where z_j is the z coordinate of atom j in base J . In standard (3'-5') A- and B-helices,³³ respective (A , $\langle D \rangle$, $\langle Z \rangle$) values are (11.6°, 4.7Å, 3.5Å) and (4.5°, 4.2Å, 3.5Å). In representative intercalated drug-oligonucleotide crystal structures^{34,35} average values of these parameters are (9.0°, 7.7Å, and 6.9Å).

Helical Parameters

Helical parameters— n , h , and r —describing regular repetition of the (2'-5') sugar phosphate backbone are computed using established procedures^{36,37}. The parameter n is the number of residues per helical turn, while h is the vertical step height between adjacent residues. The radial distance of phosphorus atoms is defined by the symbol r . In contrast to the earlier work of Parthasarathy et al.,²⁵ a monomer repeating unit is used to generate (2'-5') helical structures here. Such regular structures are more closely comparable to the well known (3'-5') A- and B-DNA fiber diffraction models.

Duplex Structures

The possible (2'-5') duplex geometries linking a given sequence of base pairs are obtained from an exhaustive search of the glycosyl and sugar conformations able to form a geometrically acceptable phosphodiester unit. The bases are held, as outlined recently,³⁸ in a preselected arrangement with the adjacent chain torsions treated as independent variables. The glycosyl torsions are varied at 10°-increments, the sugars allowed to range over 20 pseudorotational states P ,²⁸ and the exocyclic sugar torsion ψ incremented at 10°-values. All combinations of parameters that bring the O2'...O5' atom pair within the 2.47-2.56Å distance necessary for acceptable phosphate group formation are further tested for their ability to accommodate standard ($119 \pm 3^\circ$) P-O-C valence bond angles. The conformers that meet the valence angle criteria are then ranked on the basis of their potential energies at the dimeric level. The empirical function used to study the 2'-Amp monomer is employed with the addition of three new terms: one to account for the intrinsic pseudorotational preferences of the ribose ring,²⁸ a second to reduce the charge on the phosphate group to the predicted levels associated with counterion condensation,²⁹⁻³⁰ and a third to account for the interaction between the two lone pair orbitals in the phosphate unit.³⁹ In contrast to other constrained model building approaches,⁴⁰⁻⁴⁷ this procedure is not tied to an energy minimization function. A greater range of low energy structures can be identified with such an exhaustive conformational searching technique.³⁸

RESULTS

Energy Calculations

The conformational energy surfaces associated with simultaneous 30°-variations of ϕ'' and χ in 2'-Amp are reported in Figure 2. Energy contours (1-5 kcal/mole) are drawn at intervals of 1 kcal/mole relative to the absolute minimum located at $\times$. The chemical structure of 2'-Amp with appropriate sugar puckering is also indicated in the figure. The

C3'-endo puckered form is described in Figure 2(a) and the C2'-endo form in Figure 2(b). The exocyclic sugar torsion ψ is fixed at 60° in both studies. Conformational ranges are described by abbreviated symbols (t , g^+ , g^- for ϕ'' and a , ha , s , hs for χ) along the coordinate axes.

As evident from the figure, the location of the lowest energy conformation is extremely sensitive to the choice of sugar puckering with the C3'-endo form favoring a *syn* (-120°) glycosyl conformation and the C2'-endo form an *anti* (60°) arrangement. The C2'-O2' ϕ'' torsion, however, is found in its *trans* range at the lowest minimum on each surface (180° and -150° , respectively). In general there is much less freedom of rotation in ϕ'' than in χ on these surfaces. The glycosyl torsion is able to rotate at little energetic cost between *anti* and *syn* states in both cases, although the barrier is lower and the range of χ states wider with the C3'-endo sugar than the C2'-endo sugar. The allowed range of ϕ'' is somewhat broader but more dependent on the choice of χ for the C2'-endo than the C3'-endo sugar. The transition between *syn* and *anti* arrangements can be accomplished without variation of ϕ'' in the C3'-endo case but is associated with a sizable (60 - 90°) increase in ϕ'' in the C2'-endo case. Interestingly, the transition on the C3'-endo surface is more easily accomplished through the high *syn* ($-60 \pm 45^\circ$) range, while that on the C2'-endo surface is effected through the high *anti* ($120 \pm 45^\circ$) conformation.

The relative importance of the various conformational combinations of ϕ'' and χ is reported in terms of the statistical weights w at 298°K in Table I. The energy surfaces are arbitrarily divided into 12 domains (corresponding to all possible combinations of the t , g^+ , and g^- ranges of ϕ'' and the *anti*, high *anti*, *syn*, and high *syn* ranges of χ) and the relative contributions to the total partition function are determined numerically.⁴⁸ Only the major low energy domains are included in the table. The first and fourth entries are the data reported in Figures 2(a) and 2(b), respectively, while the remaining four lines are associated with energies found with alternate rotational isomeric combinations of the C5'-C4' ψ torsion. The latter surfaces are noticeably different from those where $\psi = 60^\circ$ in that the $\phi''\chi = t, \text{syn}$ conformer is overwhelmingly favored by a combination of steric and electrostatic interactions between the 5'-hydroxyl group, the 2'-phosphate residue, and the adenine base. The high *anti* barrier between *anti* and *syn* conformers in Figure 2 is completely eliminated from the C3'-endo energy surfaces and partially altered on the C2'-endo maps at $\psi = 180^\circ$ and $\psi = -60^\circ$. With steric factors thus removed, the relative importance of *anti* and *syn* states is then determined by electrostatic contributions between the base and phosphate. Simi-

Figure 2.

Contour diagram in the $\phi''\chi$ plane (with $\psi = 60^\circ$) of total potential energy of (a) C3'-endo and (b) C2'-endo 2'-Amp. Contours are drawn at intervals of 1 kcal/mole relative to the absolute minimum located at $\times$; those above 5 kcal/mole are omitted. A computer generated model⁵² of the low energy conformer found at $*$ is also provided.

Table I
Statistical Weights of Various ϕ'' χ Conformations of 2'-Amp as a Function
of Sugar Puckering and Exocyclic C5'-C4' (ψ) Torsion Angle

Sugar	ψ , deg	$\phi''\chi$							
		<i>t</i> , <i>anti</i>	<i>t</i> , high <i>anti</i>	<i>t</i> , <i>syn</i>	<i>t</i> , high <i>syn</i>	<i>g</i> ⁻ , <i>anti</i>	<i>g</i> ⁻ , high <i>anti</i>	<i>g</i> ⁻ , <i>syn</i>	<i>g</i> ⁻ , high <i>syn</i>
C3' -endo	60	0.24	0.27	0.46	0.03	0	0	0	0
"	180	0.01	0.01	0.98	0	0	0	0	0
"	-60	0.01	0.02	0.97	0	0	0	0	0
C2' -endo	60	0.43	0.40	0.01	0.08	0.04	0.03	0.01	0
"	180	0.07	0.05	0.80	0.02	0.01	0.01	0.04	0
"	-60	0.07	0.07	0.81	0.03	0	0	0.02	0

lar $\psi\chi$ interdependence has long been observed in (3'-5') nucleic acid chains,^{49,50} but the ψ , ϕ'' , χ interactions are unique to the 2'-mononucleotide. Only *anti* arrangements are considered in the structural analyses that follow. The absolute energy minimum is raised by ~ 1.2 kcal/mole when ψ is increased from 60° to 180° in these studies and by ~ 0.8 kcal/mole when decreased from 60° to -60°. The *syn* combinations are accordingly disfavored on this basis. Moreover, the *syn* conformer is no longer preferred at $\psi = 180^\circ$ and -60° when the sugar/base is flanked, as in a polynucleotide, by both 5'- and 2'-phosphate groups.

Base Morphology

Contour surfaces describing the local base morphology as a function of the phosphodiester torsions in dimeric (2'-5') ApA are reported in Figure 3. The sugars are assigned identical conformations in the dimers with ψ and ϕ'' fixed respectively at 60° and -135° and λ and ψ'' allowed to adopt either C3'-endo or C2'-endo combinations. The glycosyl torsions are rigidly fixed at 60° throughout. Attention is focused on the possible occurrences of stacked and intercalated base-base arrangements in the dimer. Acceptable angular arrangements are found within the areas enclosed by the dashed $\Lambda = 45^\circ$ and $\Lambda = 135^\circ$ contours. The bases in the former region are aligned in a roughly parallel fashion, while those in the latter domain are in an antiparallel orientation. Base stacked states are noted by the heavily shaded bands where $3.0\text{\AA} \leq \langle Z \rangle \leq 4.0\text{\AA}$ and $4.5\text{\AA} \leq \langle D \rangle \leq 5.5\text{\AA}$. Potential intercalation geometries are found in the lightly stippled areas where $6.5\text{\AA} \leq \langle Z \rangle \leq 7.5\text{\AA}$ and $7.0\text{\AA} \leq \langle D \rangle \leq 9.0\text{\AA}$. Contours of $\langle Z \rangle$ are indicated by solid lines, while contours of $\langle D \rangle$ are omitted to clarify the figure. C3'-endo dimers are described in Figure 3(a) and C2'-endo dimers in Figure 3(b).

While there are large areas of $\omega''\omega$ torsion angle space where adjacent bases are aligned in parallel and separated by appropriate vertical distances on both surfaces, only a small

Figure 3.

Composite contour surface of the base stacking angles Λ (dashed curves) and distances $\langle Z \rangle$ (solid curves) as functions of the phosphodiester angles in (a) C3'-endo and (b) C2'-endo (2'-5') ApA. Base stacked states are found in the heavily shaded areas and intercalative geometries in the stippled regions. Remaining torsions are fixed at the following "preferred" values: $\chi = 60^\circ$, $\phi'' = -135^\circ$, $\phi = 180^\circ$, $\psi = 60^\circ$.

Table II
Local Base Morphology in 2'-5' ApA as a Function of Sugar Puckering
and Exocyclic Torsions

Sugar	ϕ'' ,deg	ψ ,deg	% Stacking		% Interaction	
			Parallel	Antiparallel	Parallel	Antiparallel
C3' -endo	180	60	.29	-	.21	-
		180	.03	-	.13	-
		-60	.02	-	.02	-
	-135	60	.05	-	-	-
		180	-	-	-	-
		-60	-	-	-	.06
	-90	60	.88	-	1.22	-
		180	.71	-	.80	-
		-60	.45	-	.66	-
C2' -endo	180	60	-	.23	.63	.18
		180	-	.93	-	-
		-60	-	.37	-	-
	-135	60	.06	.57	.99	.02
		180	.11	.60	.78	-
		-60	-	-	.32	-
	-90	60	.32	.75	1.12	.80
		180	-	.57	.78	-
		-60	.03	.08	.53	.39

proportion exhibits the necessary overlap for base stacking or ligand intercalation in Figure 3. There are examples of parallel base stacking (centered at $\omega''\omega = -30^\circ, -90^\circ$) for C3'-endo dimers in Figure 3(a) and examples of both parallel and antiparallel base stacking (centered at $\omega''\omega = -120^\circ, -60^\circ$ and $-60^\circ, 90^\circ$, respectively) and parallel and antiparallel intercalation (centered at $-150^\circ, 0^\circ$ and $-100^\circ, 120^\circ$, respectively) for C2'-endo dimers in Figure 3(b). Intercalated geometries may occur in C3'-endo sugars if the C5'-C4' and/or C2'-O2' torsions ($\psi\phi''$) are altered from the values used to generate Figure 3(a). Table II lists the relative proportions of the $\omega''\omega$ conformational plane that meet certain morphological criteria when the sugar is confined to selected rotational isomeric states.

According to this survey base stacking and intercalative opening are noticeably improved in C3'-endo dimers if the exocyclic C2'-O2' torsion ϕ'' is restricted to the *gauche* rotational range. When averaged over three rotational isomeric state combinations of ψ at $\phi'' = -90^\circ$ roughly 0.7% of the conformational combinations yield stacked geometries and another 0.9% intercalative arrangements. The C2'-endo dimers, in contrast, are found to adopt intercalative arrangements at all values of ϕ'' . The total number of intercalated (2'-5') combinations is increased nearly threefold in the C2'-endo form compared to the

Figure 4.

Helix forming domains of (2'-5') polynucleotide chains represented as a function of sugar conformation in the $\omega''\omega$ torsional plane. Right- (R) and left-handed (L) domains are reported for labeled rotational isomeric state combinations of ψ and ϕ'' and for C3'-endo (solid contours) and C2'-endo (dashed contours) ring puckering.

C3'-endo form. The extent of base stacking is also greater in C2'-endo dimers than in C3'-endo dimers. The C2'-endo stacked states, however, are principally antiparallel. The C3'-endo dimers are therefore more apt to adopt parallel stacked geometries typical of standard helical nucleic acid structures.

According to nmr measurements of the proton coupling constants in (2'-5') model compounds,^{11-13,24} the sugar equilibrates rapidly between equally populated C3'-endo and C2'-endo states in ribose analogs but overwhelmingly favors the C3'-endo form in 3'-deoxyribose structures. The nmr data further support the above energy study which locks the C5'-C4' torsion into a g^+ conformation but, unfortunately, offer no information on the C2'-O2' rotation. If nmr data are combined with the computed energies in Figure 2, the principal conformations of $\psi\lambda\psi''\phi''$ ($60^\circ, -152^\circ, -90^\circ, 180^\circ$ and $60^\circ, -101^\circ, -153^\circ, -135^\circ$) generate very limited ranges of base stacked and intercalated structures. Similar analyses of base morphology in (3'-5') dimers, however, yield large families of stacked and intercalated states.⁵⁰ Recent experimental measurements,¹² nevertheless, suggest the (2'-5') compounds to form base stacked conformers more efficiently than their (3'-5') counterparts. This does not necessarily mean that the (2'-5') dimers form a greater number of stacked structures than corresponding (3'-5') dimers but rather that the few resulting

stacked arrangements are more stable than the many (3'-5') combinations.

Helical Parameters

Helical parameters associated with the regular repetition of selected (2'-5') sugar phosphate backbone conformers are reported in Figure 4. Potential duplex forming domains where the number of residues per turn n is high and the residue step height h is low are represented in the phosphodiester ($\omega''\omega$) torsional plane for selected combinations of the sugar puckering and exocyclic torsions. The C3'-endo regions are bounded by solid lines and the C2'-endo areas by dashed lines. Each helix forming domain is further subdivided by a diagonal (corresponding to values of $h = 0\text{\AA}$) that distinguishes the right-handed part R from the left-handed part L . Data are reported for three rotational isomeric states of ψ with ϕ'' fixed at 180° , -135° , and -90° .

According to Figure 4, (2'-5') helix formation is roughly independent of sugar puckering. The C3'-endo and C2'-endo helix forming domains are seen to overlap for all values of ψ and ϕ'' . The C3'-endo regions are more elliptical and larger in area than the corresponding C2'-endo domains. The boundaries defining the C3'-endo region, however, are given by the $n = 5$ contour, while those for the C2'-endo domain are given by the $n = 8$ contour. The C3'-endo chain is accordingly less likely to form duplex structures than the C2'-endo chain despite the larger helix generating areas in Figure 4. The maximum values of n , a measure of duplex forming capability, are found in this study to be 18.9 residues per turn in a C3'-endo chain with $\phi''\omega''\omega\psi = (-90^\circ, -120^\circ, 30^\circ, -60^\circ)$ and 69.5 residues per turn in a C2'-endo chain with $\phi''\omega''\omega\psi = (180^\circ, -140^\circ, 20^\circ, 60^\circ)$.

As noted previously with (3'-5') polynucleotides,⁵⁰ the helix forming domains of (2'-5') chains are interrelated by correlated torsional motions. Such correlated changes are useful in preserving the overall morphology of long chains while simultaneously allowing considerable flexibility in the (2'-5') backbone. As the exocyclic ψ torsion is decreased in value, for example, the ω rotation that precedes it in the chain is simultaneously increased. This is best illustrated in Figure 4 where the vertical (ω) coordinate of the helix forming domain is sensitive to the choice of ψ . The ω'' torsion is similarly affected by variations in sugar puckering, the C2'-endo helices adopting somewhat lower values of this parameter than the C3'-endo helices. Both ω'' and ω are influenced by variations of ϕ'' . A decrease in ϕ'' from -135° to 180° in C3'-endo helices is found to shift the helix forming domain by roughly -90° in ω'' , while an increase of the same magnitude in this angle is accompanied by a -60° shift in ω . The C3'-endo helix forming domain is also noticeably larger at $\phi'' = -90^\circ$. Domain size, however, is not altered with ϕ'' in C2'-endo helices, although the positions along both the ω'' and ω axes are affected.

The correlated motions associated with the (2'-5') nucleic acid helices are noticeably more limited than those found in (3'-5') chains.⁵⁰ Firstly, the (2'-5') helices are generally more tightly wound structures with lower values of n at $h = \pm 3.4\text{\AA}$ than the corre-

Figure 5.
Computer generated stereo representations⁵³ of (a) C3'-endo and (b) C2'-endo single-stranded (2'-5') poly(A) helices. Torsion angles and morphological parameters are described in the text.

sponding (3'-5') helices. Secondly, the range of standard torsion angles which generate typical double helical conformations is much more restrictive with the (2'-5')-linked backbones than the (3'-5') chains. The combined analysis of base morphological data with helix generating characteristics, nevertheless, yields a limited number of arrangements which produce single-stranded (2'-5') helices with reasonably good base stacking. There are two such possibilities with C3'-endo chains occurring at $\phi'' = -90^\circ$ with $\psi = \pm 60^\circ$. The phosphodiester torsions are confined to g^-g^- and g^-g^+ combinations in these A-like analogs. A stereo representation of the lower energy form where $\phi''\omega''\omega\phi\psi = (-90^\circ, -100^\circ, -90^\circ, 180^\circ, 60^\circ)$ is shown in Figure 5(a). Helical parameters of the structure are $n = 8.4$, $h = 3.0\text{\AA}$, and $r = 10.3\text{\AA}$. With χ fixed at 60° , base stacking parameters — Λ , $\langle Z \rangle$, and $\langle D \rangle$ — are 24° , $3.8\text{\AA}$, and $4.9\text{\AA}$, respectively. The only example of a well stacked C2'-endo helix is found with $\phi'' = -135^\circ$ and $\psi = 60^\circ$. A stereo picture of this B-type analog where $\phi''\omega''\omega\phi\psi = (-135^\circ, -130^\circ, -20^\circ, 180^\circ, 60^\circ)$ is shown in Figure 5(b). Helical parameters of the structure are $n = 10.3$, $h = 3.1\text{\AA}$, and $r = 9.7\text{\AA}$. Adjacent bases are tilted at an angle Λ of 9.9° while $\langle Z \rangle$ and $\langle D \rangle$ are fixed at $3.6\text{\AA}$ and $5.7\text{\AA}$, respectively. Base stacking is more ideal in this model than in the preceding C3'-endo structure. Bases are roughly perpendicular to the helical axis but are shifted laterally compared to their positions in B-DNA. This particular sequence of torsions, however, is not able to form a duplex with a complementary antiparallel (2'-5') chain of the same conformation.

Duplex Structures

An exhaustive search of the intervening sugar and glycosyl torsions yields 2958 possible (2'-5') backbone linkages between successive A-type Watson-Crick base pairs and 3784 such arrangements between B-type residues. These numbers roughly match the numbers of (3'-5') structures (1945 and 3596, respectively) found to link the same configurations of base pairs in DNA and RNA.⁵⁰ Most of the (2'-5') A-type structures, however, are fairly high in energy with only four of the possibilities within 5 kcal/mole of the minimum energy state. In contrast, there are 177 A-type backbone solutions within 5 kcal/mole of the lowest (3'-5') backbone geometry, a state only slightly lower in absolute energy than the (2'-5') minimum. The number (23) of low energy (2'-5') linkages between B-type configurations, however, slightly exceeds the number of (3'-5') structures (18) found previously.³⁸ The B-type configurations are much less overlapped than the A-type base pairs and are apparently much less restrictive on the chain backbone structure.

The four low energy (2'-5') linkages between A-type base pairs fit into two distinct conformational categories. In one the phosphodiester torsions adopt the g^-g^- conformation typical of (3'-5') A-RNA chains, while in the other the P-O pair assumes a g^+g^+ arrangement. Both forms exhibit mixed sugar puckering with the 3'-residue adopting significantly lower pseudorotation angles and glycosyl torsions than the 5'-residue. Standard (3'-5') models, in contrast, repeat at the monomeric level with regular C3'-endo sugar puckering

Table III

Low Energy (2' - 5') Backbone Conformers Linking A-type Watson Crick Base Pairs

$P_{2'}$, deg	$\chi_{2'}$, deg	ϕ'' , deg	ω'' , deg	ω , deg	ϕ , deg	ψ , deg	$P_{5'}$, deg	$\chi_{5'}$, deg	ΔE , kcal/mole
0	0	-173	53	111	-126	70	144	120	0
36	90	-154	-82	-69	-156	70	180	70	4.26
0	-10	-159	53	95	-132	90	126	110	4.27
0	0	177	71	79	-116	80	72	100	4.29
18	21	-155(ϕ')	-68(ω')	-75	-179	59	18	21	A-DNA

and *anti* glycosyl torsions. In further distinction to (3'-5') structures, the (2'-5') linkage perturbs the C5'-O5' ϕ torsion from its energetically favored *trans* range to a negatively skewed conformation with the phosphorus eclipsing one of the 5'-hydrogens. The neighboring C5'-C4' torsion, however, remains in the preferred *gauche*⁺ range in the (2'-5') models. The observed *trans* conformations of the C2'-O2' ϕ'' torsion, while comparable in magnitude to the *trans* values of the C3'-O3' torsion in A-RNA, are the conformational equivalents of the *g*⁻ state of ϕ' in the (3'-5') backbone. Table III contains a detailed listing of torsions in the four low energy (2'-5') A-type backbones and in the standard (3'-5') model of A-RNA. None of the structures are potential (2'-5') A-RNA building blocks. The 2' and 5' sugar puckerings and glycosyl linkages must be identical in a monomerically repeating helix. In a dimerically repeating structure, the values of these parameters are interchanged in successive dimers.

The 23 low energy (2'-5') structures linking B-type base pairs are represented on the conformation wheels in Figure 6. The five independent parameters of the model building procedure are plotted in part (a) and the four dependent torsions in part (b). The observed torsions are represented by lines in the appropriate radial sectors of the wheel. The rings are scaled from 0° to 360° and the conformational categories are designated along the periphery of each ring. The data are compared with the torsions of the standard fiber diffraction model of B-DNA³³ noted by asterisks in the appropriate rings.

As evident from the figure, the sugar pucker in the low energy (2'-5') B-type dimers is noticeably different from the C2'-endo forms of conventional B-DNA helical models. The 2'-sugars of the sequence are forced to adopt less stable O1'-exo and C1'-endo pentose arrangements (where $P_{2'}$ = -90° to 0°) with the glycosyl torsion $\chi_{2'}$ concomitantly perturbed to the high *anti* range. The conformation of the 5'-residue is more standard with either C3'-endo or C3'-exo sugar pucker and the glycosyl linkage in the *anti* range. The exocyclic C5'-C4' torsion ψ is able to span a broad range of values between *gauche*⁺ and *trans* states. The four dependent torsions are highly flexible with some combinations near

Figure 6.

Conformation rings describing 23 low energy ($2'-5'$) conformers linking B-type base pairs. Torsions found in the standard B-DNA model³³ are noted by asterisks. Rotational ranges are designated along the periphery of the respective group of rings. (a) Independent and (b) dependent parameters of the calculation.

standard geometries and other varied by as much as 180° . The 23 low energy structures can be categorized into 9 different conformational states according to the two- and three-fold character of the chain torsions. Variations in ψ are generally accompanied by large correlated changes in the P-O5' torsion (ω) and by smaller fluctuations in the C2'-O2' (ϕ'') and O2'-P (ω'') variables. An unusual g^+g^+ combination of ϕ'' and ω'' is found in two instances where $\chi_2' < \chi_5'$. In all other cases the 2'-glycosyl rotation is greater in mag-

nitide than the 5'-glycosyl angle. A similar g^+ combination of ϕ' is known to generate sugar-phosphate contacts which are highly unfavorable in (3'-5') linked nucleic acids.⁵¹ The fact that such an unstable state is included among the lowest energy (2'-5') dimers may be indicative of the relative difficulty with which such chains form helices. As with the A-type structures, there are no examples of identical glycosyl rotations and homogeneous sugar puckering in the (2'-5') B-dimers and hence no simple (2'-5') B-DNA double helical structures. Moreover, despite the broad range of sugar puckerings and glycosyl angles, there are no low energy (2'-5') forms with overlapping dimeric torsions. As outlined above, such combinations are required to construct regular polymeric models with dimeric or higher order repeating units.

DISCUSSION :

The above conformational studies reveal several notable differences between (2'-5') and (3'-5') polynucleotide chains. There is a striking correlation between the glycosyl rotation χ and the C2'-O2' torsion ϕ'' not present in (3'-5') chains. The corresponding C3'-O3' torsion ϕ' and the attached 3'-phosphate are more distantly removed from the heterocyclic base in native DNA and RNA and are less apt to influence the preferred glycosyl rotation. There is also very little space in (2'-5') chains for intervening waters to mediate the electrostatic interactions between the 2'-phosphate group and the base. Only the 5'-phosphate has a significant effect on χ in (3'-5') polynucleotides.^{48,49} The combined interactions of the base with both the 2'- and the 5'-phosphate group accordingly limit the rotational freedom of the (2'-5') chain compared to the (3'-5') chain. There are smaller families of (2'-5') backbone conformations with similar base morphologies and helical parameters than (3'-5') combinations. It is also difficult to construct a (2'-5') equivalent of Z-DNA⁵² with the more restricted backbone.

The zigzag dislocation of the (2'-5') chain through the sugar ring additionally reduces the large degree of adjacent base overlap found in (3'-5') systems. It is therefore more difficult to form low energy dimer duplexes in the (2'-5') polymer than in the (3'-5') chain. The displacement factor also limits the numbers of well stacked and intercalated single stranded (2'-5') helices. According to the above constrained modeling studies, the stacking of adjacent bases forces mixed glycosyl and sugar puckering in the (2'-5') chain. A selected stacking pattern cannot be perpetuated beyond the dimeric level. The best way to construct a (2'-5') polymer duplex may be to vary the base stacking pattern along the chain. Indeed, it appears possible to construct a (2'-5') double helix with alternating A- and B-type stacking patterns from the dimeric models generated in this study.

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