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# Structure of poly 8-bromoadenylic acid: conformational studies by CPF energy calculations

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ABSTRACT

Poly 8-bromoadenylic acid [poly(8BrA)] is the only known all-syn polynucleotide. It shows a helix-coil transition with a melting curve centred around 55° C. Energy calculations based on classical potential functions have been used to explore the three-dimensional structure of this polymer in helix and random coil. It is concluded that the ordered state is a helix of two parallel strands with a two-fold rotation axis, and the duplex is stabilised by hydrogen bonds involving N1 and H6. Each strand has a conformation with C3' endo geometry, $\phi' = 216°$, $\omega' = 280°$, $\omega = 294°$, $\phi = 179°$, $\chi = 243°$ and $\psi = 57°$. Such a conformation leads to approximately 8 nucleotide units per turn of the helix and an axial rise of 3.9A°. The structure of poly(8BrA) has been compared with that of the related polymer poly(A) which forms a double helical structure in acidic conditions with bases in the anti conformation and with interstrand hydrogen-bonds between N7 and H6. This is the first time that a specific geometrical model of a novel polynucleotide structure has been produced initially by potential energy calculations, though such calculations on a number of known structures have been reported previously.

## INTRODUCTION

In recent years considerable information has accumulated on the conformation of nucleosides, nucleotides and other substructural units of DNA and RNA. Based on these studies several general conclusions on polynucleotide structure can be made. Fibre-diffraction techniques[1-5] and energy calculations[6-10] have shown that the conformational angles in ordered polyribonucleotides correspond within 20° to the values listed below[11] and illustrated in Fig. 1. The most generally used chemical notation for these dihedral angles is also given: $\phi' = 220°$ (H3' and P eclipsed along H3'-C3'-O3'-P), $\omega' = 290°$ ($g^-$ along C3'-O3'-P-O5'), $\omega = 290°$ ($g^-$ along O3'-P-O5'-C5'), $\phi = 180°$ (t along P-O5'-C5'-C4'; also called g'g' by NMR spectroscopists), $\psi = 60°$ ($g^+$ along O5'-C5'-C4'-C3'; also called gg), $\psi' = 83°$ (C3' endo ribose geometry; also called N type conformer) and $\chi = 20°$ (bases in anti conformation). Such a structure leads to a right-handed helical arrangement for polynucleotides with an axial rise of 2.5 - 3.5A per unit and bases almost perpendicular to the helix-axis and stacked one on top of the other with a unit twist of 30 - 45°. The bases lie in the inner core of the overall

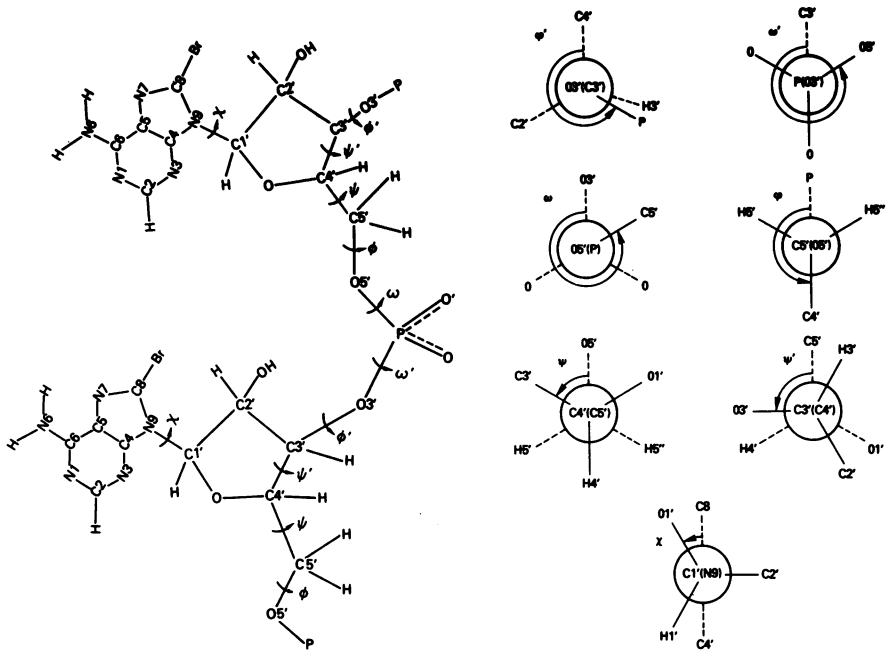

Fig. 1. The fragment pBrApBrAp on which the present calculations have been made, and the Newman projection diagrams of the various conformational angles indicating the preferred orientations observed in ordered RNA structures.

structure and the phosphates lie on the outer periphery. Analysis of NMR and other data[12-16] has shown that the torsion angles in a randomly-coiled single-stranded polynucleotide show preferences for values corresponding to those in individual strands in a helical polyribonucleotide with the exception that the two basic ribose conformations, C3' endo ($\psi'$ = 83°) and C2' endo ($\psi'$ = 150°) occur with almost equal probability[14]. Such generalisations hold for all known polyribonucleotide structures and generally include the conformations observed in single crystal measurements for low molecular weight analogs of RNA[10, 17-23].

Conformations other than the ones listed above have been observed, however, in nucleosides, nucleotides, and di- and trinucleoside phosphates. For example, among the nine known crystal structures[17-23] of di- and trinucleoside phosphates having 3'-5' linkage, UpA is known[19] to exhibit both ($g^+$, $g^+$) and (t, $g^-$) conformations with respect to O-P bonds while a part of the ApApA structure[23] has a ($g^+$, $g^+$) conformation

around these bonds. Variations in molecular conformation are relatively more common among nucleosides, although the conformations described above still occur in the majority of cases. In particular, conformational states with $\psi$ = 300° and 180° and bases in the syn conformation have been observed in nucleosides[10].

The interest in poly 8-bromoadenylic acid (poly(8BrA)) arises from the fact that in this case the anti conformation of the base is destabilised by short contacts between bromine and ribose[24-27]. Recently this polymer has been synthesised and its NMR and IR properties have been reported[24-26]. Poly(8BrA) shows several physico-chemical differences from the related system poly(A). It exhibits a cooperative melting and does not form a complementary complex with poly(U) under conditions identical to those resulting in complex formation between poly(A) and poly(U). Thus, while the structure of poly(8BrA) is interesting from the biochemical view-point, the polymer also offers a model system for investigating the effect of the base conformation on the structure of the nucleotide backbone. No fibre diffraction data on this polymer have been reported. The crystal structure of 8-bromoadenosine has been determined[27], however, and shows that the ribose conformation is C2' endo ($\psi'$ = 150°), the base is in a syn conformation ($\chi$ = 240°) and ($\phi$, $\psi$) values are (60°, 46°). The structure is stabilised by an intramolecular N3-----H(5')-O(5') hydrogen bond. Such a hydrogen bond cannot occur in a 5'-phosphate analogue, and consequently, this result does not necessarily throw light on the structure of poly(8BrA). The possibility that poly(8BrA) has a C2' endo ribose geometry has been suggested by Olson[28], who concluded that such a polymer should be incapable of intrastrand base stacking. Sarma et. al.[29], have also suggested a C2' endo geometry and a $\psi$ angle of 180° (gt structure) for poly(8BrA). Experimental data, on the contrary[24-26], indicate that poly(8BrA) forms an ordered helical structure with greater ease than poly(A), is considerably more stacked above the melting temperature ($\sim$55°C) and occurs in predominantly C3' endo geometry.

In this paper we propose a three-dimensional structure for ordered and randomly coiled poly(8BrA) based on energy calculations by classical potential function (CPF) and on model-building. The proposed structure is consistent with IR, UV, NMR and other physico-chemical properties of the polymer[26].

DETAILS OF THE CALCULATION

In the CPF approach the interaction energy between a nonbonded pair of atoms is partitioned into contributions from van der Waals (vW), electrostatic (es) and torsional (tor) potentials. Thus,

$$V = V\ (vW) + V(es) + V\ (tor)$$

where the first two terms are summed over atom pairs other than bonded and geminal and the third term is summed over all bonds. Van der Waals energies are estimated from a 6-12 Lennard-Jones potential:

$$V\ (vW) = V_m Z^6\ (-2 + Z^6)$$

$$\text{with } Z = R/r$$

where r is the interatomic distance between the interacting atoms and $V_m$ and R are Lennard-Jones parameters. Electrostatic interactions are estimated from monopole-monopole approximation by use of charge densities obtained from quantum chemical methods. Torsional potentials are evaluated from empirically assigned barrier heights and potential symmetries based on experimental or theoretical data of related molecules.

The basic application of the CPF method to nucleotide conformation has been reported in a number of papers[28-34]. We employ the same formalism except with regard to torsional potential for rotation around O-P bonds where a more recently suggested function[34] has been used:

$$V(tor) = V1\ (1 + \cos 3\theta) + V2(1 + \cos 2\theta)$$

with $\theta = \omega$ or $\omega'$ and V1=V2=1.5 kcal/bond. Previous calculations had used a three-fold potential for O-P bonds with V1=0.5 (V2=0.0), which gives a disproportionately high weight to conformers associated with trans states with respect to O-P bonds and is in conflict with the observed (g, g) structure of dimethyl phosphate. Qualitatively, however, most of the conclusions mentioned below are not significantly altered when one reverts to the earlier parameters. We have calculated the Lennard-Jones parameters for atom pairs involving Br using the standard formulae[30], based on bromine polarizability ($\alpha$ = 3.34), effective number of polarizable electrons ($\eta$ = 22.0) and van der Waal radius (1.85A°). The partial charge on Br is assumed to be zero (q = 0) since the electronegativity of C8 and Br are estimated to be almost equal.

The computer program for the calculations starts from a given set of bond lengths, bond angles and torsional angles for the molecule under study. One can then calculate V for such a geometry. Calculation of V as a function of torsional angles gives either potential energy curves or two dimensional isoenergy maps. Our program also has an option to

minimize energy by varying all the torsion angles. The minimization process is based on the method of steepest descent and reaches a local minimum energy conformer from a starting set of torsion angles by an iterative process. The minimization process is terminated when either the energy change between successive iterations is less than 0.1 kcal/mole or the changes in torsional angles are less than 1°. The molecular fragment used in these calculations is the single-strand dinucleotide (pBrApBrAp) shown in Fig. 1. The bond length and bond angles of the ribose, base and nucleotide backbone have been selected from published reports[6, 27]. Two sets of calculations were performed. First, we started from the conformational angles of other RNAs and by energy minimization, from conformational energy maps and from potential energy curves, searched for a structure related to known RNA structures. Second, starting with other reasonable values of the various conformational angles, we applied energy minimization procedures to arrive at all probable low energy conformations. Out of the thirteen low energy conformations thus obtained, only one is consistent with other physico-chemical considerations.

For comparison, we have also carried out some selected calculations on the fragment pApAp.

## CONFORMATIONAL SEARCH FOR A STRUCTURE SIMILAR TO OTHER RNA'S

A reasonable starting point for the conformational search is the structure of other polyribonucleotides. Energy minimization carried out with these conformational angles for the fragment in Fig. 1 converged rapidly to a nonbonded interaction energy of -66 kcal/mole. The minimum energy structure is defined by the following conformational angles: $\phi' = 216°$, $\omega' = 294°$, $\omega = 297°$, $\phi = 179°$, $\psi = 57°$ and $\chi = 245°$ (with C3' endo geometry, $\psi' = 83°$). The value of $\chi$ shows a large change after minimization, while other angles show marginal variations. The anti region gave prohibitively high energies, and irrespective of the starting value of $\chi$, the minimization process always led to a $\chi$-value of 245°. The single-strand structure with the above conformational angles leads to a right-handed helix with a twist of 55°, 6.6 units per turn of the helix, an axial rise of 4.1 A, stacked bases, and a helical diameter of the path described by phosphorus atoms of 8 A. Fig. 2 shows the arrangement of various atoms in this conformation viewed from two different planes.

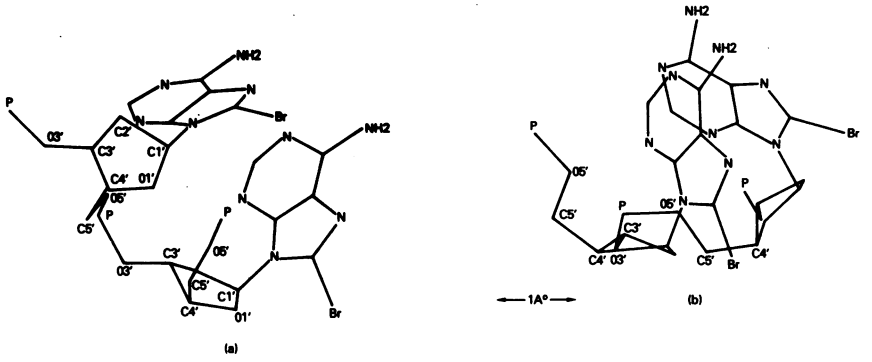


Fig. 2. The projection of structure IV (Table I) when viewed (a) normal to a plane described by C3', O3' and P and (b) perpendicular to the helix axis. The helix axis passes through a point close to N1.

The effect of Br on the stability of the structure can be estimated from the difference in the conformational energies of the fragments pBrApBrAp and pApAp, each in a syn conformation. With the torsion angles identical to the minimum energy conformer of pBrApBrAp, the fragment pApAp gives an energy of -63 kcal/mole compared to -66 kcal/mole for BrA fragment. However, the all-anti conformer for pApAp has a lower energy than the syn conformer. When no constraints are placed on the values of torsion angles, energy minimization on pApAp leads to a structure with nonbonded interaction energy of -80 kcal/mole and conformation angles of $\phi' = 220°$, $\omega' = 298°$, $\omega = 292°$, $\phi = 172°$, $\psi = 54°$ and $\chi = 21°$. These angles are remarkably close to the crystal structure[23] of the middle fragment in ApApA ($\phi' = 223°$, $\omega' = 283°$, $\omega = 298°$, $\phi = 162°$, $\psi = 56°$, $\chi = 28°$ and $\psi' = 82°$) and that of acid poly(A)[5] ($\phi' = 216°$, $\omega' = 293°$, $\omega = 285°$, $\phi = 168°$, $\psi = 69°$, $\chi = 5°$ and $\psi' = 83°$). Acid poly(A) has a twist of 45°, 8 units per turn of the helix, an axial rise of 3.8 A, stacked bases, and a diameter of 12 A for the locus of P atoms. The basic difference between the minimum energy conformers of acid poly(A) and poly(8BrA) is the orientation of the base with respect to the helix axis and the ribose moiety.

It is clear from the above discussion that the substitution of H8 by Br influences the energetics of ordered RNA-type structures by destabilizing the entire anti range and stabilizing the syn conformer by $\sim$ 3 kcal/mole.

Using the conformational angles for pBrApBrAp listed above, we have

calculated potential energy curves with respect to angles $\phi'$, $\chi$, $\phi$ and $\psi$ (Fig. 3). The two dimensional isoenergy map with respect to ($\omega'$, $\omega$) has also been obtained. This map is shown in Fig. 4.

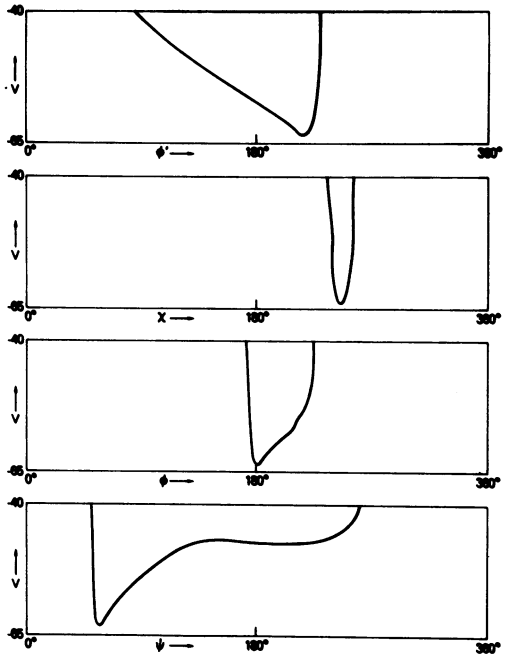


Fig. 3. Potential energy variation as a function of torsion angles $\phi'$, $\chi$, $\phi$ and $\psi$.

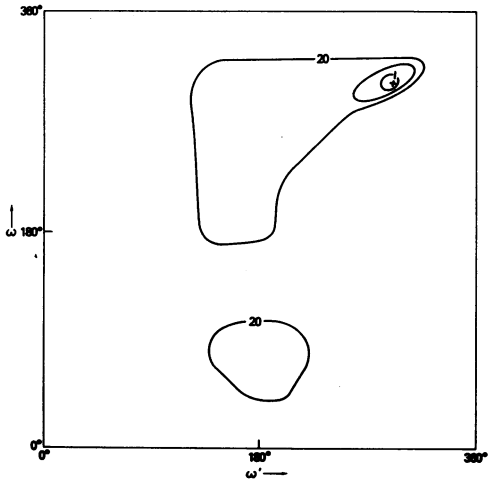


Fig. 4. Isoenergy contours as a function of rotation around O-P bonds.

We shall first discuss the ($\omega'$, $\omega$) map. As discussed in other papers[6-10], these angles show a fairly large flexibility in 3'-5' diriboside phosphate. For pBrApBrAp (Fig. 4) the global minimum lies in a very small allowed region enclosed by an isoenergy contour of 5 kcal, corresponding to a ($g^-$, $g^-$) arrangement. Other low energy states corresponding to one of the O-P bonds in a trans arrangement differ in energy from the global minimum by about 20 kcal/mole. In those cases where the (t, $g^-$) conformer has been observed in crystal structures[10] we find from the corresponding potential energy curves that such conformers lie within the contour of less than 3 kcal/mole. For example, we have carried out energy minimization on pApAp and pUpUp with the above parameters and find energy differences of 1.6 and 5.9 kcal/mole respectively, between the ($g^-$, $g^-$) and (t, $g^-$) conformers, the former state being more stable in both cases. With poly(8BrA) the conformational freedom around the O-P bonds is relatively more restricted and confined to the (290°, 290°) region.

The potential energy curves with respect to the other torsional angles are also characterised by only a single minimum. In every case the energy contours rise sharply on both sides of the minimum. Except for the angles $\phi'$ and $\psi$, a span of not more than about 20° lies within 20 kcal/mole relative to the minimum energy structure. These results suggest that the conformational freedom of RNA type structures for pBrApBrAp is restricted and that such structures should persist as a major fraction in random coil polynucleotide with limited internal mobility and with a high contribution from base stacking.

## CONFORMATIONAL SEARCH FOR OTHER LOW ENERGY CONFORMERS

The conformational search by energy calculation in a multi-dimensional space poses some problems since the nonbonded interaction with respect to any particular angle depends on the value chosen for other angles, particularly those corresponding to neighboring bonds. Fortunately, in the case of nucleotides the conformational energy maps in the absence of a base are already available both from CPF and Molecular Orbital methods. A critical assessment of the usefulness of such results in predicting experimentally observed properties of polynucleotide chains is also available[12, 13]. It is important to note in this connection that the introduction of the base in either a syn or anti conformation cannot stabilise a structure which is rendered

unstable due to short contacts within the backbone itself. The presence of the base and its orientation can indeed change the relative stabilities of structures whose energies do not differ very much from the minimum energy conformations of diriboside phosphates. These structures therefore serve as good starting points in the global conformational search for poly(8BrA). The choice of initial conformations in energy minimization is discussed below.

(a) Ribose Conformation: Previous analysis of ribose conformation predicts two families of conformations: C3' endo ($\psi' = 83°$) and C2' endo ($\psi' = 150°$). In ordered polyribonucleotides only the former conformations have been detected. In randomly coiled poly(A) and poly(U), both conformations occur with almost equal probability. Both geometries have been used in the search although our NMR studies of poly(8BrA) above the melting point show a definite preference for C3' endo structure.

(b) Glycosidic Bond Rotation: Clearly for poly(8BrA) this is the most important angle to consider. As already stated, most purine nucleotides exist in anti conformation ($\chi = 20°$). However, such an angle results in steric hindrance in poly(8BrA) because of short contacts between ribose and Br on the base. In order to search for possible $\chi$ values for poly(8BrA) we performed CPF calculations on 8-bromoadenosine for three values of $\phi$ (60°, 180° and 300°) and used both C3' endo and C2' endo geometry. The results indicate that for $\phi = 60°$ and $\psi = 46°$ an intramolecular N3...H(5')-O(5') bond is formed with C2' endo geometry. Energy calculations indicate a preference for such a geometry for 8-bromoadenosine, which is indeed the observed geometry in single crystals of this molecule[27]. However, in the absence of a hydrogen bond (as in 5' phosphate) the structure with $\phi = 60°$ becomes energetically forbidden because of short contacts between N3 and 5' phosphate. Both energy calculations[29] and NMR data on 8-Br-5'AMP in aqueous solution indicate a mixture of C3' endo and C2' endo sugar geometries and $\phi = 180°$. Fig. 5(a) shows the behaviour of potential energy curves for C3' endo geometry and ($\phi$, $\psi$) = (180°, 60°) for 8-bromoadenosine. The results show three minima characterised by $\chi = 180°$, 240° and 340°. The corresponding curves for C2' endo geometry, on the other hand, indicate a relatively flat minimum in the range 180° - 360°. We have used $\chi = 180°$, 240° and 340° as starting points in energy minimization.

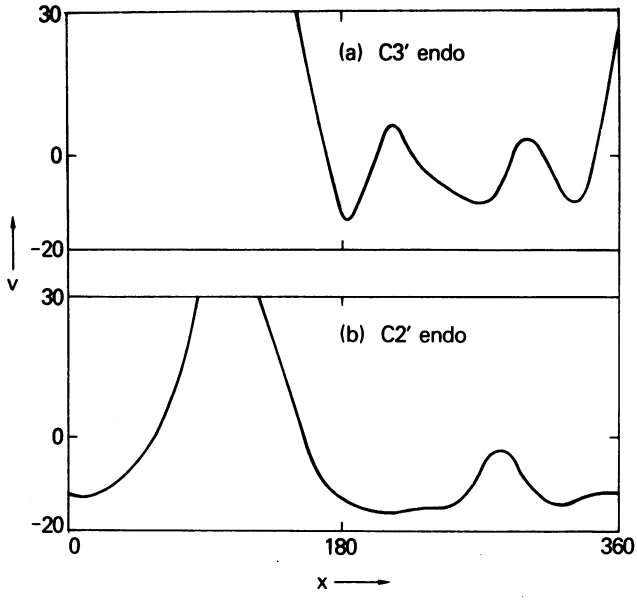


Fig. 5. Potential energy of 8-bromoadenosine as a function of the glycosidic bond angle ($\chi$). The values of ($\phi$, $\psi$) used in these calculations are (180°, 60°), which correspond to the values in a polynucleotide chain. The top curve is for C3' endo geometry of the ribose and the bottom curve corresponds to C2' endo.

(c) Angles ($\phi$, $\psi$): Previously obtained ($\phi$, $\psi$) maps on diriboside-phosphates[6-10] indicate that the region (180°, 60°) corresponds to the global minimum. Another minimum at (180°, 300°) is observed in the isoenergy maps obtained by MO calculations[10]. Previous CPF calculations[31] indicate that the region (180°, 180°) is also stable, although such a region is of fairly high energy in conformational maps obtained from CNDO and PCILO calculations[7, 8]. To make the search as general as possible, all three possible values of $\psi$ (60°, 180°, 300°) have been used. The value of $\phi$ is limited to 180°, as indicated by the previous calculations based on both MO and CPF methods, NMR and crystal structure data[1-13, 17-23].

(d) Angle $\phi'$: Only one possible starting point is indicated for polyribonucleotides from previous calculations[6-10], known crystal structures and NMR data. This corresponds to $\phi' = 220°$. Other values of $\phi'$ are precluded because of short contacts between 3' phosphate and atoms belonging to the ribose ring system.

(e) Angles (ω', ω): Energy calculations[6-10] have indicated a fair amount of flexibility with respect to these angles. The state (290°, 290°) corresponds to the global minimum and such a state has been observed in all known ordered polyribonucleotides. This state also occurs in the majority of the crystal structures of dinucleoside and trinucleoside phosphates. In addition (ω', ω) values of (180°, 60°), (180°, 180°), (180°, 300°) have low energies and the state (180°, 300°) has indeed been observed in some low molecular weight analogs of DNA and RNA. We have included all the nine staggered conformations with respect to O-P bonds in the conformational search.

The nonbonded interaction energy of all the conformations generated by combination of the above torsion angles was calculated. Structures with initial energies of less than -58 kcal/mole were used for further energy minimization with respect to the angles φ', ω', ω, φ, ψ and χ. The final conformational angles and energies differ from the starting values owing to long range interactions not taken into account in selecting the initial conformations. Because of the wide choice in selecting starting points it is unlikely that we have missed any minimum energy conformers which may play a role in the structure of pBrApBrAp or poly(8BrA). The lowest energy structures have a nonbonded interaction energy of -72 kcal/mole. Thirteen structures were found within the energy range of -62 and -72 kcal/mole. These are listed in Table I.

TABLE I. MINIMUM ENERGY CONFORMATIONS OF POLY(8BrA)

| No. | φ' | ω' | ω | φ | ψ | χ | Energy(V) |
|---|---|---|---|---|---|---|---|
| | Structures with C3' endo geometry | | | | | | |
| I | 250 | 178 | 183 | 192 | 53 | 181 | -72 |
| II | 258 | 206 | 289 | 219 | 46 | 183 | -72 |
| III | 258 | 197 | 64 | 204 | 41 | 187 | -69 |
| IV | 216 | 294 | 297 | 179 | 57 | 245 | -66[a] |
| V | 253 | 183 | 294 | 181 | 46 | 347 | -65 |
| VI | 230 | 180 | 300 | 176 | 297 | 344 | -64 |
| VII | 250 | 180 | 61 | 180 | 56 | 344 | -63 |
| VIII | 249 | 180 | 180 | 180 | 54 | 344 | -63 |
| | Structures with C2' endo geometry | | | | | | |
| IX | 212 | 285 | 290 | 180 | 51 | 261 | -72 |
| X | 247 | 190 | 55 | 116 | 287 | 227 | -72 |
| XI | 248 | 181 | 305 | 122 | 304 | 234 | -71 |
| XII | 231 | 294 | 222 | 109 | 293 | 222 | -70 |
| XIII | 216 | 289 | 169 | 179 | 167 | 226 | -63 |

All angles in degrees. Energy in kcal/mole.
(a) Structure IV is the only one which leads to stacked bases. As noted in the text, only 50% of the nonbonded interaction energy from base-base stacking is included in the calculation.

There are eight low energy structures of pBrApBrAp having C3' endo geometry and five structures with C2' endo geometry. Several of these structures show close relationship with one another in terms of the values of conformational angles.

We have calculated the helical properties of all the low energy structures by using techniques similar to those discussed[35] for polypeptides. A computer program has been developed to generate several turns of the helix and to predict properties such as sense of helical chain (R or L), twist (t), units per turn (n), axial rise (h), and the relative position of the bases and phosphates with respect to the helix-axis. These parameters are listed in Table II.

The most interesting structures in Tables I and II are those numbered IV and IX. Structure IV is the one derived from conformational angles similar to other RNAs as discussed in the previous section. The two structures are closely related to one another, and the basic difference between them is just in the ribose geometry. Structure IX has a C2' endo geometry and does not lead to stacking of bases. Structure IV has a C3' endo geometry and has slightly higher nonbonded interaction energy (2.5 kcal/mole for each nucleotide unit) compared with IX. The backbone angles of IV and IX are very similar to those found in ordered RNAs and DNAs, although the base in this case has a syn conformation. Structure IV has a proper geometrical arrangement for base-base stacking. We recall that the present energy calculations on the fragment pBrApBrA

TABLE II. HELICAL PARAMETERS OF LOW ENERGY CONFORMERS OF POLY(8BrA)

| No. | Sense of helix[a] | Nucleotide units/turn n | Axial-rise h (in A)[c] | Unit twist(t) 360°/n | Distance from helix-axis (A)[b] P | N6 |
|---|---|---|---|---|---|---|
| I | L | 4.4 | 4.2 | 82 | 1.5 | 7.6 |
| II | R | 4.4 | 1.9 | 81 | 2.5 | 6.5 |
| III | R | 2.1 | 3.8 | 171 | 1.0 | 7.0 |
| IV | R | 6.6 | 4.1 | 55 | 4.1 | 2.3 |
| V | R | 4.9 | 0.5 | 73 | 4.0 | 10.1 |
| VI | L | 3.0 | 3.8 | 122 | 1.0 | 9.0 |
| VII | R | 2.1 | 4.4 | 166 | 1.5 | 8.0 |
| VIII | L | 4.4 | 4.6 | 82 | 1.0 | 8.0 |
| IX | R | 4.0 | 4.7 | 90 | 3.5 | 4.3 |
| X | R | 5.2 | 0.4 | 69 | 3.8 | 9.8 |
| XI | L | 4.5 | 3.6 | 81 | 2.2 | 8.5 |
| XII | R | 2.3 | 3.8 | 157 | 1.3 | 9.6 |
| XIII | R | 5.0 | 5.8 | 73 | 3.8 | 3.8 |

(a) L = left-handed helix, R = right-handed helix
(b) The relative positions of base and phosphate can be judged from these distances.
(c) See footnote (a) in the previous table.

include only 50% of the nearest neighbour stacking interactions present in a polymer where each base interacts with a top and a bottom unit. For the fragment under discussion, the lower base can interact only with the one on the top and vice-versa. Further, structure IV has phosphates on the exterior of the helix and the bases on the interior favoring hydration of phosphate groups and hydrophobic interactions. The intra-strand interactions and solvent-effects discussed above will further stabilise conformation IV relative to structures where the phosphates are on the interior of the helix or where the bases do not stack.

There are six other structures (I - III and IX - XI) which have energies lower than conformer IV. In all the six structures phosphate groups lie in the interior of the helix, while the bases are exposed to the solvent. A similar relative orientation of phosphates and bases is present in structures V - VIII and XII. While such structures have relatively low nonbonded interaction energies and may contribute to some extent to the randomly coiled structure of poly(8BrA), incorporation of a large proportion of nucleotide units with such conformations in a polymer structure will be prevented by intrastrand phosphate-phosphate repulsions and unfavorable disposition of phosphate groups for hydration.

Further evidence on the preferred conformations of random-coil poly(8BrA) in solution is provided by experimental data discussed below.

## EXPERIMENTAL RESULTS ON THE BACKBONE STRUCTURE OF POLY(8BrA)

We have recently carried out extensive NMR, UV and IR studies to investigate the properties of random-coil poly(8BrA), poly(A) and poly(U) and helix-coil transitions in these systems[26]. While details of these investigations will be the subject of a subsequent paper, we briefly review findings which throw light on the structure of poly(8BrA) in random-coil and in ordered helix. The high resolution $^{1}H$ and $^{13}C$ NMR of poly(8BrA) at 70°C is characterised by fairly sharp lines permitting an estimate of three-bond $^{1}H$-$^{1}H$, $^{1}H$-$^{31}P$ and $^{13}C$-$^{31}P$ coupling-constants. Using standard techniques[36-39], we have calculated distributions in conformational populations with respect to C3'-O3', C4'-C5', C5'-P bonds as well as puckering of the ribose ring. The population of N type conformers (I to VIII) is estimated to be 65% [J (H1'-H2') = 3.5 Hz, J (H3'-H4') = 6.3 Hz, J (H2'-H3') = 5.3 Hz. The percentage population of the g'g' state ($\phi \sim 180°$) is found to be 64% [J (H5'-P) = 4.5 Hz, J (H5''-P) = 6.0 Hz, J (C4'-P) $\sim$ 10 Hz], while the gg state ($\psi \sim 60°$) is

present to the extent of almost 50% [J (H4'-H5') = 1.7 Hz, J (H4'-H5'') = 7.2 Hz]. The angle $\phi'$ shows an overall preference for $\phi' \sim 220°$ [J (H3'-P) = 8.7 Hz, J (C2'-P) $\sim$ 4 Hz, J (C4'-P) $\sim$ 4 Hz]. Finally, the population of the stacked conformer (IV) has been estimated to be around 50% by comparing the chemical shifts of H2 in the random-coil, that in an ordered helix (fully stacked), and in 8-Br-5'AMP (unstacked). The remainder consists of a blend of several conformations where one or more of the backbone angles differ from the values found in ordered RNAs.

A NMR study in the transition region of helix-coil (20-70°C) shows that the percentage of stacked conformer increases with lower temperature and a complete ordering occurs below $\sim$ 25°C. The behaviour of the stacking curve as monitored by the H2 chemical shift parallels that obtained previously by UV spectroscopy[24-26]. Both $^{1}H$ and $^{13}C$ NMR lines broaden at lower temperature and information on coupling-constants becomes obscured. However, the line-shape and line-width of the H1' resonance in the temperature range 20-70°C rule out any substantial shift in the N (C3' endo) $\rightleftarrows$ S (C2' endo) equilibrium to the right. Thus, the ordered structure maintains a C3' endo geometry.

Previous experimental studies have indicated that the bases in ordered poly(8BrA) are hydrogen bonded[24-26]. There is also strong evidence to show that the structure consists of two strands held together by base-base hydrogen bonds rather than a single strand forming a hair-pin loop. Three possible hydrogen bonding schemes have been considered previously[24-26] (Fig. 6), but it had not been possible to establish which of the three structures exist in ordered poly(8BrA). One possible explanation of the doublet (1632, 1617 $cm^{-1}$) observed for the adenine ring vibration of poly(8BrA) in $D_2O$ solution had been the existence of a non symmetrical structure[24]. We have since observed[26] in $H_2O$ solution that both the ring vibration (1601 $cm^{-1}$) and $NH_2$ deformation (1652 $cm^{-1}$) are single bands and now conclude that the alternative Fermi resonance explanation accounts for the doublet observed in $D_2O$[24]. These evidences favor a symmetrical structure.

## STRUCTURE OF ORDERED POLY(8BrA)

In an ordered polynucleotide duplex the two strands may be either parallel or anti-parallel. The two strands must have a common helix axis in order to maintain the geometrical requirements for hydrogen bonding throughout the entire length of the polymer chains. The ability

to bring the axes of separate single-strand helices into coincidence is therefore a necessary condition for duplex formation, and the inability to achieve such coincidence (with acceptable hydrogen bonding distances and angles) is sufficient grounds to exclude a possible duplex structure. Modelling of double-helices is facilitated by the assumption that the strands forming the duplex have identical conformation angles. With such an assumption (often made in the analysis of fibre-diffraction data) the locations of atoms in two parallel strands of a duplex are related by a rotation around the helix axis. For structures formed from anti-parallel chains, the two strands have a symmetry involving a dyad axis perpendicular to the helix-axis. Using the above symmetry operations for parallel and anti-parallel chains, we have attempted to build a model for a double helix starting with the minimum energy conformers in Table I and thus determine whether base-base hydrogen bonding is possible. For example, a hydrogen-bonded structure as shown in Fig. 6(a) is possible if the two strands are parallel and have their common helix axis aligned near the centre of the hydrogen-bonded ring.

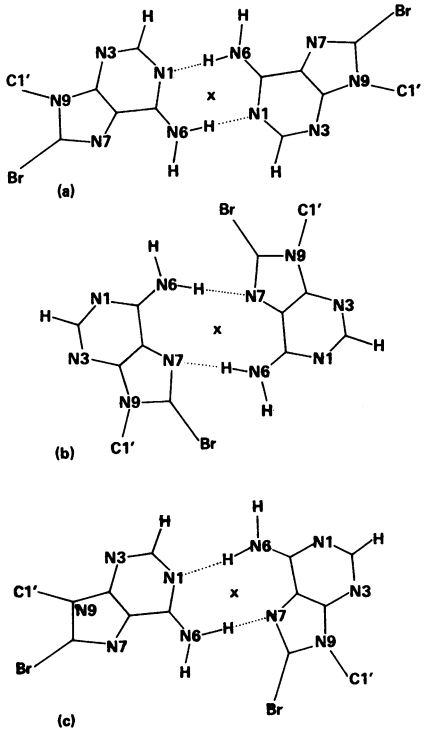


Fig. 6. Possible hydrogen bonding schemes suggested for poly(8BrA). The X indicates the position where the helix axis intersects the plane containing the base pairs to achieve hydrogen bonding.

The two strands are related by a twofold rotation around the helix axis. Structure 6(b) likewise would require both the strands to be parallel, but the common helix axis in this case would lie between N7 and N6 instead of N1 and N6. Again the two strands will be related to one another by a rotation of 180° around the helix axis. Structure 6(c) can be formed from antiparallel chains and requires an asymmetry of the conformational structure of the two chains. In this case one of the two strands should have its common helix axis in a region between N6 and N7 and the other between N1 and N6.

Having decided on the atoms involved in the hydrogen-bonding scheme, we achieve further optimization of the N-H...N hydrogen-bonds through a computer program called GEOM. The program minimizes the function

$$\phi = \left[\sum_i \left\{(d_{N\cdots N} - 2.9)^2 + (d_{H\cdots N} - 1.9)^2 + \theta^2\right\}\right]^{1/2}$$

with respect to the torsion angles $\phi'$, $\omega'$, $\omega$, $\phi$, $\psi$ and $\chi$. Here $d_{N\cdots N}$ and $d_{H\cdots N}$ are the respective hydrogen-bond distances and $\theta$ is the angle between N···N and N···H vectors in radians. The summation is carried out over both the hydrogen bonds.

From the theoretical and experimental results discussed in the previous section, IV is the most likely candidate for the structure of each strand in ordered poly(8BrA). The duplex model with such a structure as input will be discussed first. In structure IV, the helix-axis lies between N1 and N6, and a hydrogen-bonded structure of the type 6(a), with parallel strands and twofold axis of rotation, is suggested from the considerations above. Application of the hydrogen-bond optimization process led to the following values of conformational angles: $\phi'$ = 216°, $\omega'$ = 280°, $\omega$ = 294°, $\phi$ = 179°, $\psi$ = 57° and $\chi$ = 243°. A computer graphic representation of the optimised duplex structure, viewed along the helix axis is shown in Fig. 7. The polymer structure forms an 8-fold helix with an axial rise of 3.9 A°, N1···N6 distance of 2.9 A°, N1···H distance 2.3 A° and the deviation of N1···H vector from N1···N6 vector is 16°. Each base pair forms two hydrogen bonds with identical geometry. The bases are tilted with respect to the plane normal to the helix-axis.

With the conformational angles as found in ordered RNAs and the purine base in anti conformation, the helix axis passes between N6 and N7. Application of the program GEOM with such a starting

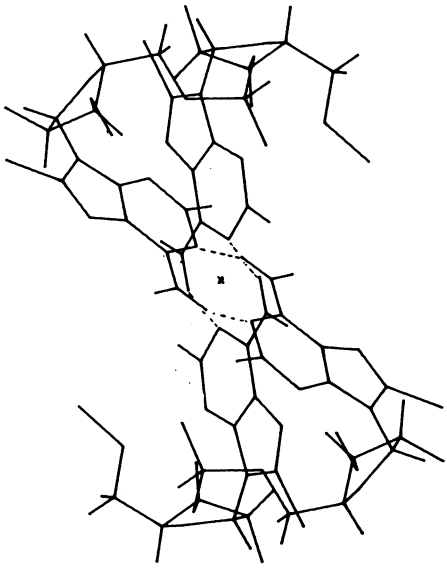

Fig. 7. A section of the poly(8BrA) double helix showing the base-base hydrogen bonds in the ordered structure. The X shows the position of helix axis.

conformation gives very satisfactory hydrogen-bond geometry: both N1···N7 distances of 2.9 A and almost linear hydrogen bonds. Indeed, such a hydrogen-bonding pattern has been observed in acid poly(A)[5]. Scheme 6(b) is not possible when the bases are syn and other conformational features remain unchanged. Therefore, such a structure is ruled out for poly(8BrA) where the anti range is forbidden due to hard contacts. We note that structure 6(b) has been excluded for poly(8BrA) previously[25] on the basis of steric repulsion between the two bases in such an arrangement. We have reached the same conclusion with an alternative reasoning.

Hydrogen-bonding scheme, 6(c) can be conceived by use of anti-parallel strands with both strands in the syn conformation. However, application of the program GEOM did not lead to a satisfactory hydrogen-bond geometry. Further, experimental data on poly(8BrA) rules out an asymmetrical hydrogen bonding scheme for ordered duplexes.

We next consider other structures. The distances of N6 from the helix axis for structures I, II, III, V, VI, VII, VIII, X, XI and XII are more than 6 A°, while those of the phosphate groups are less than 4 A°. Thus, one cannot conceive base-pairing without interference from phosphate groups in these conformations. In a double helical arrangement, such structures will lead to repulsive interactions between charged phosphate groups. Further, these models do not lead to base-base stacking, one of

the experimental observations for ordered poly(8BrA). An attractive alternative low energy conformer from the view of inter-base hydrogen bonding is IX. In this structure, the distances of base and phosphate from the helix axis are not widely different. Although the bases can be made to stack and hydrogen bond, for example by changing $\phi'$ to 150° (DNA-B type structure), energy calculations on pBrApBrAp with conformational angles of DNA-B, show several hard contacts. Moreover, NMR results show an equilibrium towards C3' endo conformations at all temperatures, and these rule out the possibility of structures IX - XIII for poly(8BrA).

MODELS OF HETEROPOLYMER DUPLEXES INVOLVING POLY(8BrA) AND POLY(A)

The model proposed above for ordered and random-coil poly(8BrA) allows us to consider heteropolymer duplexes and to speculate on the possible reasons for the inability of poly(8BrA) to form complementary structures with poly(A). One model for the poly(A)·poly(8BrA) duplex would consist of all syn residues, parallel strands, and hydrogen bonds as in 6(a). However, it can be readily seen that the energetics for the reaction

pApAp (anti) + $\frac{1}{2}$ [pBrApBrAp (syn)·pBrABrAp (syn)] $\rightleftarrows$ pApAp (syn)·pBrApBrAp (syn)

is unfavorable. The nonbonded interaction energy for the two strands on the left-hand side is -146 kcal/mole (-80-66), while it is -129 kcal/mole for the right-hand side. Thus, even with a stabilization from N1···N6 hydrogen bonds, the energetics will not favor formation of heteropolymer duplexes.

A more likely model for the pApAp·pBrApBrAp duplex consists of adenine residues in anti and BrA residues in syn conformations and hydrogen-bonding as in 6(c). Application of GEOM for such a scheme led to satisfactory hydrogen-bond geometry with minor modifications in backbone angles: N···N distances around 3.0 A, N···H distance of 2.2 A, angle between N···N and N···H vector of 10°. Such a structure is particularly interesting since purine (syn)·purine (anti) hydrogen bonding schemes have been suggested as a possible source of wobble in codon-anticodon interactions between inosine and adenine[40, 41]. On geometrical considerations, the stability of such hydrogen-bonded base pairs will be comparable to those formed in the proposed model of poly(8BrA). The fact that poly(A)·poly(8BrA) duplexes are not formed, indicates that even in this case homopolymer interactions are much stronger than those involving poly(A)·poly(8BrA).

## CONCLUSIONS

One of the most interesting aspects of the results described in this paper is that although the orientation of the base and the hydrogen-bonding scheme are different in case of poly(A) and poly(8BrA), their backbone conformations are very similar, both in the ordered and random-coil forms. These results provide further evidence that (a) the basic nucleotide structure is stabilised by short-range interactions within the backbone itself, (b) intra- and inter-strand base-base interactions (stacking and hydrogen bonding) provide additional stabilisation to the structure corresponding to the conformational angles found in ordered RNA, and (c) a major fraction of a randomly coiled single-strand has a conformation similar to that of each strand in a double helix.

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## REFERENCES

1 Arnott,S.(1971) Prog.Biophys. and Mol.Biol. 21,265-319
2 Saenger,W.(1973) Angewandte Chem. 12,591-601
3 Arnott,S. and Hukins,D.W.L.(1973) J.Mol.Biol. 83,107-122
4 Arnott,S., Hukins,D.W.L. and Dover,S.D.(1972) BBRC, 48,1392-1399
5 Rich,A., Davies,D.R., Crick,F.H.C. and Watson,J.D.(1961) J.Mol.Biol. 3,71-86
6 Govil,G. and Saran,A.,(1971) J.Theor.Biol.33,399-406
7 Perahia,D.,Saran,A. and Pullman,B.(1972) Biochim.Biophys. Acta 269,1-14
8 Tewari,R.,Nanda,R.K. and Govil,G.(1974) J.Theor.Biol.46,229-239
9 Yathindra,N. and Sundaralingam,M.(1974) Proc.Nat.Aca.Sci. U.S. 71,3325-3328
10 Pullman,B. and Saran,A.(1976) Prog.Nuc.Acid Res. and Mol.Biol. 18, 215-322
11 Except for $\chi$, Nomenclature and Conventions used for torsional angles in this paper are those recommended in "Conformation of Biological Molecules and Polymers", Bergmann, E.D. and Pullman,B. Eds., Academic Press, New York (1973)
12 Tewari,R., Nanda,R.K. and Govil,G.(1974) Biopolymers 13,2015-2035
13 Nanda,R.K.,Tewari,R. and Govil,G.(1974) Proc. Ind. Nat. Sci. Aca. 40A,226-238
14 Sundaralingam,M. and Altona,C.(1973) J.Am.Chem.Soc. 95,2333-2344
15 Eisenberg,H. and Felsenfeld,G.(1967) J.Mol.Biol. 30,17-37
16 Inness,L.D. and Felsenfeld,G.(1970) J.Mol.Biol. 50,373-389
17 Sundaralingam,M.(1969) Biopolymers 7,821-860
18 Shelter,E., Barlas,M., Sparks,R.A. and Trueblood,K.N.(1969) Acta.Cryst. B25,895-909

19 Rubin,J., Brennan,T. and Sundaralingam,M.(1972) Biochem. 11, 3112-3128
20 Sussman,J.L., Seeman,N.C., Kim,S.H. and Berman,H.M.(1972) J.Mol.Biol. 66,403-421
21 Day,R.O., Seeman,N.C., Rosenberg,J.M. and Rich,A.(1973) Proc.Nat. Aca.Sci. 70,849-853
22 Rosenberg,J.M., Seeman,N.C., Kim,J.J.P., Sudath,F.L., Nicholas,H.B. and Rich,A.(1973) Nature 243,150-154
23 Suck,D., Manor,G., Germann,G., Schwalbe,C.H., Weismann,G. and Saenger,W.(1973) Nature N.B., 246,161-165
24 Howard,F.B., Frazier,J. and Miles,H.T.(1974) J.Biol.Chem. 9,2987-2990 and references contained therein
25 Howard,F.B., Frazier,J. and Miles,H.T.(1975) J.Biol.Chem. 10,3951-3959
26 Govil,G., Fisk,C., Howard,F.B. and Miles,H.T.(1976) Abstracts 7th International Conference on Magnetic Resonance in Biological Systems (St. Jovite, Canada) p.127. A full paper on the spectroscopic properties of poly(8BrA) is under preparation and will be published elsewhere
27 Tavale,S.S. and Sobel,H.M.(1970) J.Mol.Biol.48,109-123
28 Olson,W.K.(1973) Biopolymers 12,1787-1814
29 Sarma,R.H., Che-hung Lee, Evans,F.E., Yathindra,N., and Sundaralingam,M. (1974) J.Am.Chem.Soc.96,7337-7348
30 Lakshminarayanan,A.V. and Sasisekharan,V.(1969) Biopolymers 8,475-512
31 Olson,W.K. and Flory,P.J.(1972) Biopolymers 11,1-56
32 Yathindra,N. and Sundaralingam,M.(1973) Biopolymers 12,297-304
33 Hingerty,B., Broyde,S.B., Subramanian,E., Sato,T., and Langridge,R. (1973) Biopolymers 12,273-280
34 Govil,G.(1976) Biopolymers 15,2303-2307
35 Sasisekharan,V. and Ramachandran,G.N.(1968) Adv.Prot.Chem. 23,283-439
36 Govil,G. and Smith,I.C.P.(1973) Biopolymers 12,2589-2598
37 Davies,D.B. and Danyluk,S.S.(1974) Biochem.13,4417-4434
38 Davies,D.B. and Danyluk,S.S.(1975) Biochem.14,543-554
39 Lee,C., Ezra,F.S., Kondo,N.S., Sarma,R.H. and Danyluk,S.S.(1976) Biochem.15,3627-3639
40 Topal,M.D. and Fresco,J.R.(1976) Nature 263,285-293
41 Sakore,T.D. and Sobell,H.M.(1969) J.Mol.Biol. 43,77-87