Volume 5 Number 9 September 1978 Nucleic Acids Research

A model for the single stranded random coil form of polydeoxyadenylic acid from minimum energy conformations of the dimeric subunit

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Received 20 April 1978

Abstract

The minimum energy conformations of dApdA have been examined for their suitability as buildings blocks of the single stranded coil form of polynucleotides. Calculations of the characteristic ratio $C_\infty = \langle r_0 \rangle^2 / n\ell^2$ were made for a polymer generated from all the low energy conforrers, as well as for selected combinations. A polymer composed of a conformer with $\omega', \omega = t^*, g^+$, (skewed) $\psi = t$, C-(2')-endo type pucker, in combination with the 'B' form, has a C_∞ equal to that observed in coils of apurinic acid (6) when the fraction of 'B' form conformers is ~25% and ~91%. The t^*, g^+ conformer is the second lowest energy form in the C-(2')-endo puckering domain, following the 'B' form.

Introduction

The low energy conformations of deoxydinucleoside phosphates include conformers like helical DNA and others which may occur in DNA regions which are conformationally different from the 'A' and 'B' forms. Minimum energy conformations have been previously calculated for the deoxydinucleoside phosphates dApdA, dCpdC, dGpdG, dTpdT and dGpdC (1). In these calculations, all eight dihedral angles (see Fig. 1) and the sugar pucker were flexible. Helical and base stacking parameters of the 'A' and 'B' form conformations have also been calculated for these sequences (2). Here we address our attention primarily to the calculated low energy minima which are not like 'A' and 'B' DNA. The low energy conformations of dApdA are typical, and we focus on these.

An aim of the present work was to obtain some understanding of the conformational character of the random coil existing in solution at higher temperatures. Measurements of the radii of gyration in several poly rA fractions show that the low temperature stacked and ordered structure is rod-like, with a length of

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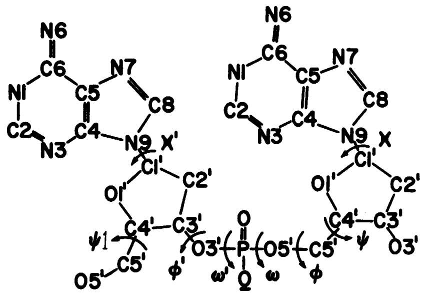

dApdA

FIGURE 1. Structure, numbering scheme and conformational angle designations for dApdA. The dihedral angles A-B-C-D are defined a as follows:

χ', χ: O1'-C1'-N9-C8
$\psi 1$, (the exocyclic C4'-C5') ψ: C3'-C4'-C5'-O5';
ϕ': P-O3'-C3'-C4';
ϕ: C4'-C5'-O5'-P;
ω': O5'-P-O3'-C3';
ω: C5'-O5'-P-O3'.

The angle A-B-C-D is measured by a clockwise rotation of D with respect to A, looking down the B-C bond.

about 3.2 Å/nucleotide (3). However there is evidence that the unstacked coil also possesses considerable order. The solution studies of Inners and Felsenfeld on polyribouridylic acid (poly rU) (4), which does not form stacked, single stranded structures led to the conclusion that the coil is highly extended and relatively restricted in conformation. We have evaluated the low energy conformers of dApdA as to their likely importance in the coil. To this end, the characteristic ratio $C_\infty = \langle r^2{}_o \rangle / n\overline{\ell^2}$, where $\langle r^2{}_o \rangle$ is the mean square unperturbed end to end distance, n is the number of bonds in the chain and $\overline{\ell^2}$ is the mean square bond length, was calculated for a polymer constructed with low energy conformers as buildings blocks, in order to compare our results with experimental findings on coils of poly rU (4), poly rA (5) and apurinic acid (6). Coils of the ribo and deoxy polymers have similar measured characteristic ratios. Our calculations are consistent with the view that the poly dA coil may be composed of a substantial fraction of nucleotides in the 'B' form, together with a conformer which has $\omega', \omega = t^*, g^+$; $\psi = t$.

METHODS

The characteristic ratio (C_∞) was computed by the method of Flory (7), expanded by Olson (8-12) for polynucleotides. $n=6x$ is the number of bonds along the chain backbone and

$$C_n = \frac{2}{n\ell^2} \;(1\ 0\ 0\ 0\ 0)\ \underset{\sim}{G}^{x} \begin{bmatrix}0\\0\\0\\0\\1\end{bmatrix} \qquad (1)$$

where $\underset{\sim}{G} = \Sigma w_i \underset{\sim}{G}_i / \Sigma w_i$ (w_i=weight of each low energy region)

and
$$\underset{\sim}{G}_i = \begin{bmatrix} 1 & \underset{\sim}{\ell}_i^T \langle \underset{\sim}{T}_i \rangle & \underset{\sim}{\ell}_i^2/2 \\ \underset{\sim}{0} & \langle \underset{\sim}{T}_i \rangle & \underset{\sim}{\ell}_i \\ 0 & 0 & 1 \end{bmatrix} \qquad (2)$$

The $\underset{\sim}{T}_i$ matrix is evaluated by the single virtual bond method of Olson (12), as is the virtual bond itself, $\underset{\sim}{\ell}_i$. $\underset{\sim}{\ell}_i^T$ is the transpose of the vector $\underset{\sim}{\ell}_i$, which is a constant for a given $\langle \underset{\sim}{T}_i \rangle$. The matrix $\langle \underset{\sim}{T}_i \rangle$, for the state i, was obtained using the energies and areas in the ω',ω energy contour maps for dApdA (1), up to energies of 3 kcal/mole. The weights (w_i) were the usual Boltzmann factor exp $(-\Delta E_i/RT)$, where ΔE_i is the energy of state i relative to the global minimum. The characteristic ratio C_∞ was found as the asymptotic limit of eq. 1, in units of $x = 2^N$ as $N \rightarrow \infty$. In practice N = 15 was sufficient. A representative example is given in Figure 2. The method of evaluating helical parameters follows Olson (2a), and has been described previously (2b).

RESULTS AND DISCUSSION

Base Stacking in the dApdA Building Blocks. In Table I are given low energy minima obtained for dApdA. We have noted previously (1) that the bases are stacked and close to parallel in all but one instance, the extended conformer 9, which has ω',ω = g^+,t, ψ = g^+, C-(3')-exo pucker. Table I presents the base stacking parameters for each conformation. Z, the mean perpendicular distance between base planes is the average of Z_a and Z_b, the perpendicular distance from the center of one base to the other. Z'

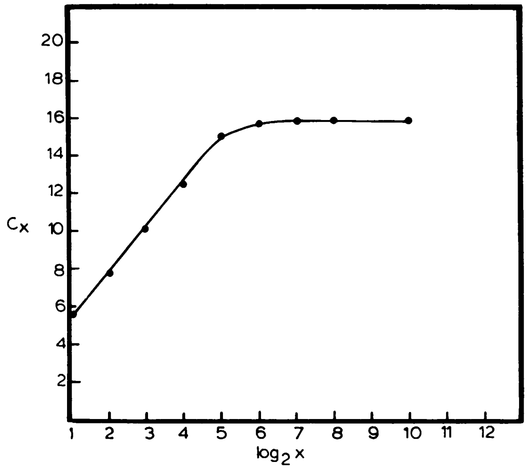

Figure 2. Characteristic ratio, C_x, as a function of degree of polymerization x=n/6 for a copolymer **composed of 90% 'B'** form, 10% ω',ω = t*,g+; ψ=t.

is the distance between base centers and by comparison with Z, is a reflection of the degree of base overlap (2b).In these conformers Z is between 2.9 and 3.4 Å. Z' deviates most from Z in conformer 9. Λ is the mean dihedral angle between neighboring bases. It is 12° or less in 9 of the 12 conformers. A large tilt angle of 47° is found only for the extended conformer 9. Flexibility in the other torsion angles, especially the glycosidic bonds, permits the bases to stack in a variety of favorable ω',ω backbone conformations.

Helical Parameters.In a preliminary view the coil is pictured as composed of polymeric segments, each occupying one of the low energy conformations of dApdA. In this treatment the coil is composed of discrete conformers of moderate flexibility spanning ω' ,ω regions of ~20°, centered about these minimum energy conformations. To obtain quantitative information on the nature of these segments, we calculated helical parameters of the polymers **generated by a repetition of the exact backbone angles in the minimum energy conformers. These results are given in Table I. The** table lists n, the number of nucleotides per turn, (t, the turn angle per residue is 360°/n) h, the translation per residue along the helix axis, and r, the helix radius, which is the perpendicular distance of the phosphorus atoms from the helix axis. A negative value of t/h ($0^\circ \le |t| \le 180^\circ$) denotes a left handed helix.

Table I

Characteristics of the Low Energy Conformations of dApdA and the Helices Generated From These Building Blocks

#	χ'	ϕ'	ω'	ω	ϕ	ψ	χ	P^a	ΔE^b	n	h	r	Z_a	Z_b	Z	Z'	Λ
				degrees					kcal/mole		Å	Å				Å	degrees
								C-(3'-)endo region									
1	-12	186	305	201	252	72	36	16	0.	4.19	2.61	3.32	2.97	3.03	3.00	3.16	8.0
2	5	208	312	280	183	47	21	7	0.2	5.74	2.43	4.93	2.86	2.98	2.92	3.26	12.0
3	48	187	41	76	196	84	30	10	0.5	2.17	0.13	3.24	2.97	2.89	2.93	3.51	2.6
4	17	293	210	310	107	47	59	11	1.3	8.23	4.21	4.51	3.71	2.64	3.18	3.95	28.2
5	55	175	50	233	158	312	39	0	1.6	2.26	-1.30	2.69	3.06	2.97	3.02	3.43	12.2
6	25	283	203	86	263	163	32	-3	1.7	12.02	2.03	10.99	3.45	2.78	3.12	3.55	24.7
								C-(2'-)-endo region									
7	79	168	261	303	185	52	83	177	0.	6.51	3.10	6.55	3.10	3.03	3.07	3.12	4.8
8	16	183	234	85	185	190	-23	191	0.4	5.09	-3.92	5.72	3.00	2.93	2.97	3.39	3.0
9	-20	194	57	167	182	51	13	204	0.7	2.14	4.48	2.63	4.36	2.43	3.42	5.65	46.5
10	57	174	267	148	180	181	44	175	0.8	6.25	3.03	7.35	2.91	2.97	2.94	3.03	4.6
11	149	195	272	306	183	314	6	197	1.3	4.69	-2.18	4.96	3.34	3.11	3.23	3.54	9.2
12	123	201	297	182	177	68	17	155	2.3	3.66	-0.65	4.58	3.00	2.85	2.93	3.54	4.5

a. P is the pseudorotation parameter of the sugar pucker (notation of Altona and Sundaralingam, (23))

b. ΔE is the energy difference between the local minimum and the lowest energy conformation in the same puckering domain, i.e., C-3'-endo or C-2'-endo. The absolute energy of conformer 1 is 2 kcal./mole lower than that of conformer 7 (1).

For completeness, we show helical parameters for the $\omega',\omega = g^+,g^+$ conformer #3, and the $\omega',\omega = g^+,t$ conformers 5 and 9, although long range phosphate repulsion energetically disfavors higher polymers of these types (13, 14) when ϕ' is trans, as it is in these conformers. However with $\phi' = g^-$, a stereochemically acceptable polymer can be constructed having $\omega',\omega = g^+, g^+$, as in the model for poly rA in formamide (15). The 'A' and 'B' helices were described in detail previously (2b). We find a variety of other helices generated. Conformers 3, 5 and 9 produce narrow, tightly wound, two fold helices which reflects their steric infeasibility, while conformer 6, in the C-(3')-endo region produces a wide, 12 fold helix. Left handed as well as right handed helices are obtained. The approximately linear relationship between n and r existing in the 'A' and 'B' form helices (2) is also apparent here.

Characteristic Ratios. The characteristic ratio $C_\infty = \langle r^2_o \rangle / n\ell^2$ calculated for a polymer composed of an energy weighted assembly of all conformers listed in Table I is 3.9. This is similar to the values derived from the results of quantum mechanical conformational calculations by Tewari, et al (16). However, they are much lower than the experimental results of 17-18 obtained for polyribonucleotide coils (4,5) and apurinic acid (6). Rather than a large assembly of states, the measurements indicate that the coil may be conformationally quite restricted. (When conformers 3, 5 and 9 are omitted, $C_\infty = 10.9$, which is still low.) As the next step, we chose from our low energy conformers those that might be most plausible in the coil on energetic and other grounds, and calculated characteristic ratios for selected combinations.

Olson (17) has examined the dependence of the unperturbed dimensions of polynucleotides on the orientation of the phosphodiester bonds. She concludes that the experimentally observed high values of C_∞ can be matched theoretically if conformers with ω' trans make an important contribution to the coil form. Accordingly, we used this as a criterion in choosing coil candidates. We also examined the low energy conformations for other extended forms that could lead to high values of C_∞. The extension of the polymer is reflected in h, the rise per nucleotide residue along the helix axis. (However, where the conformational domains of left and right handed helices intersect, small variations in conformation yield overall polymer dimensions reflecting the average of negative and positive h's). Conformer 9 has the largest h. However, the steric difficulty in polymerizing it led us to eliminate it from further consideration. Two conformers with relatively large h and $\omega' = t$ are seen. Conformer 4 with C-(3')-endo pucker has $\omega', \omega = t, g^-$ and a low value of $\phi = 107^\circ$. (It was obtained using the following angles as the starting conformation for the minimization:

$$\chi' = 26^\circ,\ \psi 1 = 58^\circ,\ \phi' = 229^\circ,\ \omega' = 274^\circ,\ \omega = 288^\circ$$
$$\phi = 128^\circ,\ \psi = 77^\circ,\ \chi = 12^\circ,\ \underline{P} = 18^\circ,$$

This conformation was not found as a low energy form when the conventional staggered domains were used.) Conformer 8, with C-(3')-exo pucker has $\omega', \omega = 234^\circ, 85^\circ$, which we will designate t^*, g^+, (the asterisk denoting that it is skewed) and $\psi = t$. As a model for the helix-coil transition that occurs on elevating the temperature, we considered a simple two state system composed of the 'B' form as the "helix" and either of the above conformers as the "coil". C_∞ was calculated as a function of fraction of subunits in 'B' form, in combination with the coil candidate. This was done by changing the energy weightings to achieve different populations. Results for both cases are shown in Fig. 3. We see that the $\omega', \omega = t, g^-$ conformer together with the 'B' form always has a C_∞ above the measured values of 17-18 (4-6) obtained for the coils. However, the $\omega', \omega = t^*, g^+$; $\psi = t$ conformer in combination with the 'B' form does reproduce the measured characteristic ratio, at ~25% and ~91% 'B' form. The former corresponds, on the average to three nucleotides in the $\omega', \omega = t^*, g^+$ conformation for each one in the 'B' form. The latter corresponds to a t^*, g^+ conformer occurring at intervals of ~9-10 'B' form nucleotides.

These results indicate that the coil may have a substantial fraction of 'B' form subunits. Tewari et al (16) used Monte Carlo methods to generate a model for the coil which reproduced the experimentally observed characteristic ratio. Their model had 90% of the subunits in the $\omega', \omega = g^-, g^-$ conformation (corresponding to the 'A' or 'B' forms). This was obtained by employing the low energy regions calculated via quantum mechanical

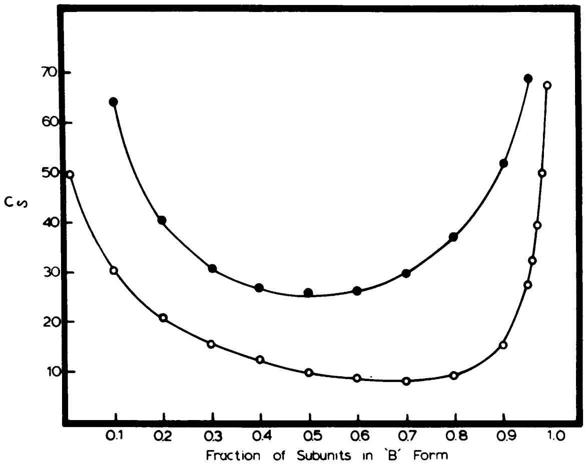

Figure 3. Cω for a polymer composed of 'B' form subunits and subunits with ω′,ω =t*,g^+, ψ=t (conformer 8, Table I) as a function of fraction of subunits in the 'B' form (o-o-o), (●-●-●) is for 'B' form in combination with conformer 4, ω′,ω =t,g^-.

methods with an added stabilization of 2 kcal/mole for the g^-,g^- conformation. Gorenstein (18) et al. used ^{31}P NMR to study the helix-coil transition in a number of ribo and deoxy nucleic acids at the dimer level and above. From the small size in the chemical shift on raising the temperature compared to theoretical predictions and studies on model phosphate esters, thay concluded that only about 20 ± 10% of the subunits are not in the g, g conformation in the coil. In addition, NMR studies of Cheng and Sarma (19) on dApdA show that there is an increase of 11% in the fraction of conformers with ψ = trans on elevating the temperature from 27° to 72° C. They also believe that ω′ is trans at higher temperature, but in association with ω = g^-.

The coil is characteristically identified by the absence of base stacking. A copolymer of the 'B' type with the ω′,ω = t*,g^+, ψ =t conformer would be unstacked at the 'B' form - coil form junction. This may be seen from the Kendrew wire model constructed to illustrate this junction. Figure 4a is a single stranded poly (dGpdC) in its 'B' form minimum energy conformation (1), while Figure 4b is the same polymer incorporating a dApdA unit in the ω′,ω =t*,g^+, ψ =t conformation. In this model the glycosidic torsion angles of the adenines were adjusted from their calculated values to prevent collisions with the preceding and

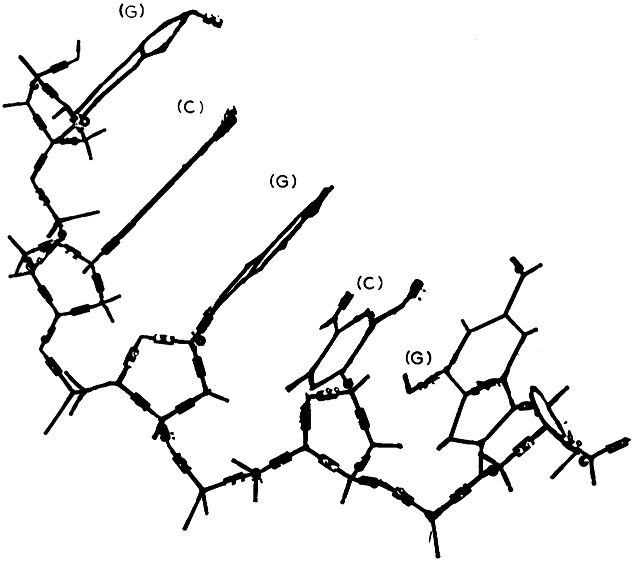

Figure 4a. Kendrew wire model of single stranded poly (dG-dC) in its calculated 'B' form minimum energy conformation (1), $\chi=65^{\circ}$, $\psi=46^{\circ}$, $\phi'=172^{\circ}$, $\omega'=257^{\circ}$, $\omega=302^{\circ}$, $\phi=191^{\circ}$, $P=169^{\circ}$.

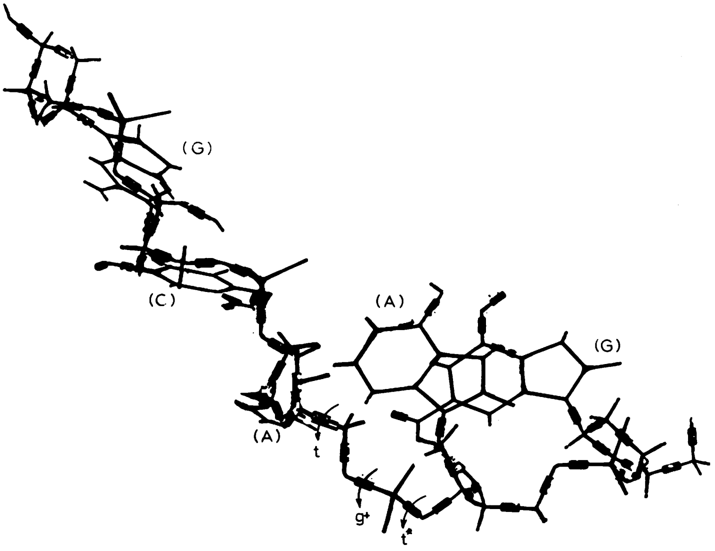

Figure 4b. Same as Figure 4a, incorporating a dApdA subunit with $\omega', \omega = t^{*}, g^{+}$, $\psi = t$, conformer 8, Table I.

following bases, and were reset to maximize coplanarity. Since our calculations show that base stacking is a major element in determining low energy conformations (1) this is a reasonable procedure. A polymer consisting of ~9-10 'B' form conformers for each $\omega', \omega = t^*, g^+$ conformation would have the bases primarily stacked if all the conformational angles remained at the 'B' form minimum energy conformation. However, we have shown recently (20) that the extent of base stacking in the $\omega', \omega = g^-, g^-$ region is very sensitive to changes in conformational angles within this domain. For example, the extent of intrastrand stacking is very much diminished when dApdA is altered from its calculated 'B' form minimum energy conformation (2b) to the 'B' DNA conformation of duplex poly (dA) • poly (dT) fibers (21), although both are 'B' type. Thus, it is possible for the polymer to remain largely in the 'B' domain with little or no intrastrand stacking. A polymer with three $\omega', \omega = t^*, g^+$ conformers for each one of the 'B' type would be primarily unstacked if the building blocks are randomly distributed.

CONCLUSION

These calculations indicate that the poly dA coil may be composed of a polymer which has a substantial fraction of 'B' type subunits. On the average, three conformers in the region $\omega', \omega = t^*, g^+$, $\psi = t$ to one in the 'B' form region $\omega', \omega = g^-, g^-$, $\psi = g^+$ or 9-10 'B' to one $\omega', \omega = t^*, g^+$, $\psi = t$ conformer, is consistent with our results. These combinations are composed of the two lowest energy conformers of dApdA in the C-(2')-endo puckering domain, and they reproduce the characteristic ratios observed experimentally in random coils. A large C_∞, agreeing with the hydrodynamic data, may be produced by other helical conformational segments present in large proportions. However, we demonstrate (Fig. 3) that all combinations will not do so. When the search is restricted to low energy conformers with ω' trans (17), only one of the two candidate conformations matched the experimental results. It is possible that different sequences will have differing conformers predominating in the coil, although these may have similar characteristic ratios. Preliminary studies on poly rU indicate that the lowest energy C-(2')-endo conformer of UpU with $\omega', \omega =$

t,g⁻ (22), in combination with the 'A' form can yield a characteristic ratio of 17-18, as measured in poly rU (4) for a polymer composed of 25% or 95% 'A' form. The two state model employed here is certainly a simplified view. There is evidence of greater flexibility in the phosphodiester rotations (24) and of contributions by C-(3')-endo conformers (25, 19) at high temperature. We are currently working on improved multi-state coil models that agree with NMR data on the actual conformational blend present (19).

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to the National Institutes of Health for support of this work under Public Health Service Grant 22765-02, awarded to S.B. B.H. would like to thanks the National Institutes of Health for a Postdoctoral Fellowship, 1F22CA02210-01 and 1F32CA06223-01.

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