Conformations of Dinucleoside Phosphates in Aqueous Solution†

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ABSTRACT: The conformations of dinucleoside phosphates have been reexamined by semiempirical potential energy calculations. Conformations I, II, and III, proposed by Lee & Tinoco [Lee, C. H., & Tinoco, I., Jr. (1977) *Biochemistry 16*, 5403], are possible species after refinement of their structures by potential energy minimization. These three conformers can represent three types of dinucleoside phosphate species in solution. Dhingra et al. [Dhingra, M. M., Sarma, R. H., Giessner-Prettre, C., & Pullman, B. (1978) *Biochemistry 17*, 5815] had concluded that conformations of type II and III were unlikely or impossible. They favored conformations g^-g^- (equivalent to I), g^+g^+, g^+t, and tg^+; the last three conformations have little stacking and are calculated to be energetically less favorable by more than 5 kcal/mol. Common structures of the types I, II, and III are found for dinucleoside phosphates with different purine–pyrimidine sequences. The sequence dependence of the potential energy of these three conformers has been calculated. The experimental nuclear magnetic resonance data of dinucleoside phosphates are consistent with these three conformations.

A decade ago Ts'o et al. (1969) demonstrated the usefulness of nuclear magnetic resonance methods in elucidating the conformation of dinucleoside phosphates. Based on NMR data of the base protons and the ribose 1′ proton, they proposed right-handed stacked structures for dinucleoside phosphates. Recently, Lee et al. (1976) and Ezra et al. (1977) were able to solve the NMR spectra of all the ribose protons in dinucleoside phosphates. An attempt was made to explain the conformation of dinucleoside phosphates in solution. Unfortunately, except for the right-handed structure, they failed to obtain other conformations correctly because of the uncertainty of their assignment of the signals for H5′ and H5″ of the Np residue in dinucleoside phosphates. With the aid of a modified adenosine residue, εA (1,N^6-ethanoadenosine), in dinucleoside phosphates, Lee & Tinoco (1977) were able to assign correctly these NMR signals for H5′ and H5″ of the Np residue. This led them to propose three groups of conformations: (I) a right-handed conformation very similar to the ones earlier proposed, (II) another conformation with stacked bases, (III) a conformation with one base "stacked" on ribose. However, the torsional angles in their proposed structures, estimated from molecular models, were in some cases inaccurate. Therefore, Dhingra et al. (1978) concluded that conformations II and III were impossible and that the standard species g^-g^- ($\omega' = 285°$, $\omega = 295°$, right-handed stack), g^+g^+ ($\omega' = 80°$, $\omega = 80°$), and g^+t ($\omega' = 110°$, $\omega = 215°$) and extended forms (such as tg^+, $\omega' = 180°$, $\omega = 80°$) were the principal species present in solution. It should be noted that the ω' and ω values for the originally proposed g^-g^-, g^+g^+, g^+t, and tg^+ are 300° and 300°, 60° and 60°, 60° and 180°, and 180° and 60°, respectively (Govil, 1976; Yathindra & Sundaralingam, 1974).

We have carried out semiempirical potential energy calculations for all the proposed conformers (I, II, III, g^-g^-, g^+g^+, g^+t, and tg^+). For each conformer a minimum-energy structure is obtained. We have found that conformers qualitatively the same as II and III are indeed possible. In fact, they are calculated to have a lower energy than g^+g^+, g^+t, and tg^+. This is not surprising as the conformers g^+g^+, g^+t, and tg^+ [Figure 4 of this paper and Figure 6 of Dhingra et al. (1978)] show little stacking. Attractive London forces will favor stacked forms in our calculations as solvent is not considered. From the minimum-energy structures of conformers I, II, and III we have calculated NMR ring current shifts with the method of Giessner-Prettre et al. (1976). These calculated NMR shifts are consistent with the experimental data (Lee & Tinoco, 1977). We conclude that conformations I, II, and III, as proposed qualitatively in Figure 7 of Lee & Tinoco (1977) and as characterized quantitatively here, are reasonable conformers for interpreting experimental data on oligonucleotides. The proposal of any limited set of conformations is a simplification, as it is clear that there is a continuous range of conformations possible for a dinucleoside phosphate in solution and that much conformational mobility exists among the structures. We also recognize that other distinctly different conformations are possible and that variations of the ones discussed here may in fact be more stable in aqueous solution.

Methods

The method used for potential energy calculation was described by Cohen (1971). The computer program GEMO in Fortran IV, originally designed by Cohen (1971), was implemented for the PDP-10 computer at National Institutes of Health (Bethesda, MD) by Dr. N. E. Sharpless and W. Jennings of the Laboratory of Chemical Physics, National Institute of Arthritis, Metabolism and Digestive Diseases. The energy calculation procedures for conformations I, II, and III of ApA, ApU, UpA, and UpU may be summarized as follows. The conformational energy of a molecule is defined as

$$E_{conf} = E(l) + E(\theta) + E(\phi) + E(nb)$$

$E(l)$ and $E(\theta)$ are bond-stretching and valence bond angle bending energies. Each is quadratic in the deviations from reference bond lengths and angles. $E(\phi)$ is the torsional strain energy; it involves the usual sinusoidal threefold symmetry function of the torsion angle. The nonbonded energies or van der Waals energies, $E(nb)$, were evaluated from a Leonard–Jones potential with the parameters chosen by Scheraga and co-workers [see Scheraga (1968) for a review]. Nonbonded interactions between atoms separated by four or more bonds were considered; closer interactions are characterized by the bond stretching, bending, and torsion energies. However, Leonard–Jones interactions between the H6 of uracil and the O1′ and O5′ of the connected ribose were omitted. The interaction of the H8 of adenine with the O1′ and O5′ of its

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Table I: Structures[a] of Conformations I, II, and III of ApA, ApU, UpA, and UpU

compd	χ_1	ribose 1	ϕ'	ω'	ω	ϕ	ψ	ribose 2	χ_2
ApA I	15.5	3′ endo	184.0	298.7	291.5	172.3	57.1	3′ endo	28.2
ApU I	12.2	3′ endo	188.3	300.1	292.2	173.0	56.3	3′ endo	28.1
UpA I	8.6	3′ endo	184.0	301.0	290.6	172.5	55.2	3′ endo	25.9
UpU I	28.4	3′ endo	174.9	295.4	289.9	170.0	57.7	3′ endo	38.2
conformer I[b]	anti	3′ endo	203~211	300.0	290.0	$g'g'_{(180)}$	$gg_{(60)}$	3′ endo	anti
ApA II	32.2	3′ endo	166.8	54.7	69.3	199.5	74.2	3′ endo	32.4
ApU II	32.2	3′ endo	172.1	55.6	68.2	199.5	78.2	3′ endo	29.3
UpA II	29.8	3′ endo	170.9	54.3	67.8	199.1	79.2	3′ endo	26.4
UpU II	31.7	3′ endo	170.2	55.7	67.7	199.8	76.7	3′ endo	25.8
conformer II[b]	anti	3′ endo	203~211	30.0	100.0	$g'g'_{(180)}$	$gg_{(60)}$	3′ endo	anti
ApA III	15.3	2′ endo	295.7	61.5	181.5	192.0	66.5	3′ endo	99.7
ApU III	7.0	2′ endo	296.4	62.0	181.0	191.2	66.1	3′ endo	101.0
UpA III	16.1	2′ endo	295.9	61.4	181.4	191.4	66.5	3′ endo	99.6
UpU III	13.1	2′ endo	298.4	61.1	180.8	191.5	64.4	3′ endo	101.2
conformer III[b]	anti	2′ endo	260.0	50.0	220.0	$g'g'_{(180)}$	$gg_{(60)}$	3′ endo	$anti_{(100)}$

[a] The torsional angles in degrees are defined in Figure 1, following Kim et al. (1973). The conformation of the ribose is according to Altona & Sundaralingam (1973). [b] The originally proposed structure from Lee & Tinoco (1977).

ribose was also omitted. These protons are attached to electropositive carbons and may form hydrogen bonds to the oxygens, so the Leonard–Jones potentials are inappropriate. These interatomic distances were checked in the final structures to see that they were reasonable; the closest distance found was 2.18 Å between H6 and O1′ in the Up– of UpA I. In general no corrections for possible hydrogen bonding were applied. However, some calculations of Coulombic interactions were done by using point charges on the atoms; these approximate the hydrogen-bonding interactions.

The conformational energies characterize the energy factors which distinguish the conformers. We recognize the approximate nature of the energy functions. We also know that, because of the neglect of solvent, the calculated energies are most useful for choosing among similar structures. Nevertheless, these types of calculations have long been used [for recent applications to oligonucleotides and polynucleotides, see Broyde et al. (1974), Matsuoka et al. (1978), Olson (1978), and Thiyagarajan & Ponnuswamy (1979)]; they can definitely produce possible conformations.

The initial values of the bond lengths and valence angles of a dinucleoside phosphate were taken from the X-ray diffraction data of the ApU single crystal (Seeman et al., 1976). The starting values of the torsional angles for the ribose in 2′ endo and 3′ endo were taken from the standard geometries of Altona & Sundaralingam (1973). The starting torsional angles of the single bonds other than those in the ribose ring moiety characterize the conformation to be studied (I, II, III, g^-g^-, g^+g^+, g^+t, and tg^+). The structures of the bases adenine and uracil were kept constant at the values observed in ApU single crystals (Seeman et al., 1976) through the entire calculation. Thus, no contribution to the conformational energy was obtained from variations in the base structure. The potential energy was minimized by optimizing the bond lengths, valence angles, torsional angles, and van der Waals interactions, using the iteration procedure of the GEMO program.

For comparison of the stability of the conformers g^-g^-, g^+g^+, g^+t, and tg^+ proposed by Dhingra et al. (1978) with that of conformations I, II, and III, the same energy minimization with and without consideration of the electrostatic interactions was carried out for these conformers of ApA with the torsional angles ω' and ω fixed at the values they proposed. The purpose of this was to obtain the minimum-energy structures available to conformers retaining essentially the g^-g^-, g^+g^+, g^+t, and tg^+ conformations.

For a test of the influence of monopole interactions (electrostatic interaction), separate calculations have been done for

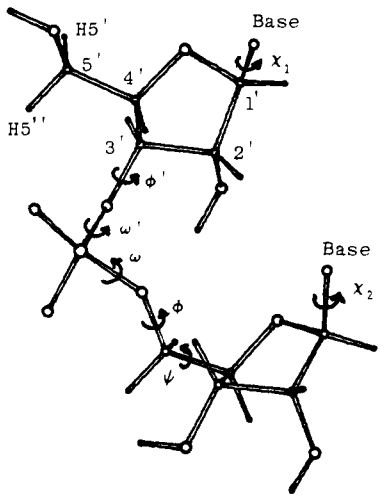

FIGURE 1: Structure of the sugar–phosphate backbone of dinculeoside phosphate with the nomenclature of the torsional angles and the numbering system of the sugar carbons.

ApA in conformations I, II, and III by incorporating the Coulombic energy of monopole interactions into the previously mentioned energy minimization. Various charge density distributions of the base (Mely & Pullman, 1969; Clementi et al., 1969; Renugopalakrishnan et al., 1971; Rein, 1973; Giessner-Prettre & Pullman, 1968), taken from Ornstein et al. (1978), have been used for this purpose. In the case of conformation III, the base of –pA fell on top of the ribose of Ap–. The charge density distribution for the ribose moiety of the Ap– residue is from Renugopalakrishnan et al. (1971).

The ring current shift of each conformer was calculated by using the method described by Giessner-Prettre et al. (1976) with the atomic coordinates generated from the potential energy calculations.

Results and Discussion

Structures of Conformations I, II, and *III for Dinucleoside Phosphates.* The energy-minimized structures of conformers I, II, and III of ApA, ApU, UpA, and UpU are listed in Table I. [The nomenclature of the structures is shown in Figure 1, and the definition of the torsional angles follows Kim et al. (1973).] For each conformer, the variation of each torsional angle is within 10° for the different base sequences in their energy-minimum states. In other words, each dinucleoside phosphate studied retains basically the same stable structures independent of base sequence. Stick-and-ball drawings of the

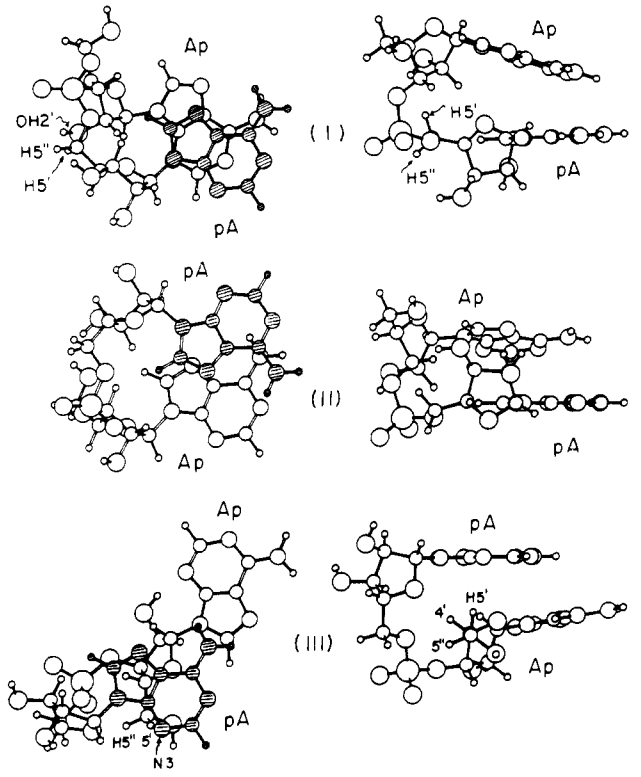

FIGURE 2: Conformations I, II, and III of ApA. The structures on the right-hand side are drawn parallel to the –pA base, while those on the left-hand side are perpendicular to the –pA base, which is shaded. The detailed structure is described in Table II.

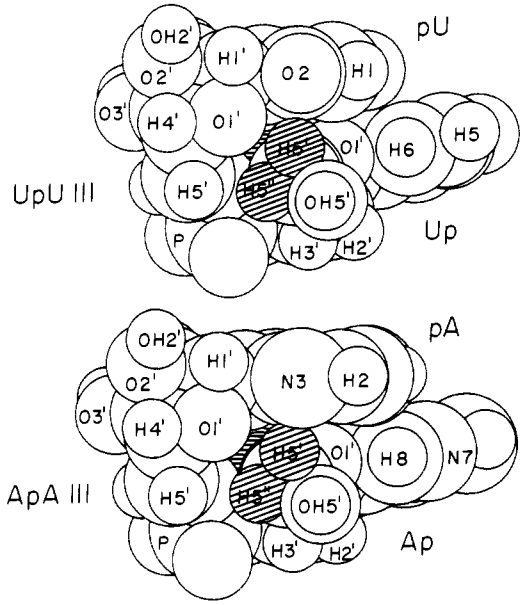

FIGURE 3: Conformation III of ApA and UpU drawn parallel to the –pN base. The size of the atoms is proportional to their respective van der Waals radii in order to show the compact structure of this conformation. The H5′, H5″, and H4′ (partially covered by the C4′ and C5′ atoms) of the Np residue are shaded.

three conformers of ApA are shown in Figure 2; Figure 3 shows a space-filling computer drawing of UpU and ApA in conformation III. The overlap of the molecules is clearly demonstrated by these structures. Compared with the originally proposed structures for conformations I, II, and III by Lee & Tinoco (1977), the refined structures show some changes for each torsional angle of each conformer (Table I). However, the overall feature of the molecular conformation remains essentially unchanged [compare Figure 2 of this paper with Figure 7 in Lee & Tinoco (1977)]. It should be recalled that the torsional angles of the originally proposed structures were estimated from CPK and Kendrew models. It is not surprising that those structures deviate from the structures calculated here on the basis of structural interactions.

Table II: Potential Energies[a] of Conformations I, II, and III of ApA, ApU, UpA, and UpU and Conformations g^-g^-, g^+g^+, g^+t, and gt^+ of ApA

	$E(l)$	$E(\theta)$	$E(\phi)$	E(nb)	E_{conf}
ApA I	0.03	13.8	9.4	−13.2	10.0
ApA II	0.14	14.3	9.8	−12.1	12.1
ApA III	0.11	11.3	9.7	−9.0	12.1
ApA g^-g^-	0.03	14.0	9.8	−13.4	10.4
ApA g^+g^+	0.09	14.6	11.7	−9.0	17.4
ApA g^+t	0.03	14.3	14.7	−3.8	25.2
ApA tg^+	0.03	14.4	9.4	−3.2	20.6
ApU I	0.03	14.0	9.5	−10.9	12.6
ApU II	0.09	14.1	9.9	−10.7	13.4
ApU III	0.12	11.5	9.6	−8.5	12.7
UpA I	0.04	14.3	9.3	−11.7	12.0
UpA II	0.11	14.1	10.1	−11.5	12.7
UpA III	0.11	11.3	9.7	−9.2	12.0
UpU I	0.06	13.7	9.4	−9.4	13.8
UpU II	0.10	14.2	9.9	−9.5	14.8
UpU III	0.06	11.2	9.3	−7.9	12.7

[a] In kilocalories per mole; $E(l)$ = bond-stretching energy; $E(\theta)$ = valence angle bending energy; $E(\phi)$ = torsional energy; E(nb) = van der Waals energy; E_{conf} = conformational energy.

Stability of Various Conformers. The potential energies of conformers I, II, III, g^-g^-, g^+g^+, tg^+, and gt^+ are listed in Table II. The positive energies for $E(l)$ and $E(\theta)$ are a measure of how much the bond lengths and bond angles differ from the reference values. The values for $E(\phi)$ characterize the deviations of the torsion angles from ±60° or 180°. All the conformers have essentially the same bond-stretching energy, $E(l)$. They all have very similar valence angle bending energies, $E(\theta)$, except for III which is ~3 kcal more favorable. The torsional energies, $E(\phi)$, are all similar except for ApA g^+g^+ and ApA g^+t, which are 1.5–5.3 kcal less stable than the other ApA conformers. The main difference among all the conformers is the values of the nonbonded energies, E(nb). The conformations with the bases stacked above each other (I, II, and g^-g^-) or with a base above a ribose (III) have significantly higher attractive forces than the three open forms (g^+g^+, g^+t, and tg^+).

The first conclusion to draw from Table II is that the optimized conformations I, II, and III are reasonable. The optimized structures were obtained with a Leonard–Jones potential to prevent unreasonably close contacts. The energy minimization ensured that most interatomic distances were in the attractive region, but a few contacts, particularly H–H interactions, were slightly repulsive. Short interatomic distances do occur between ribose protons of Np– and base atoms of –pN in conformation III, but they still lead to attractive energies. For example, H5′ or Up– in UpA III is only 2.5 Å from N3 of –pA and is 2.7 Å from C4 of –pA. Nevertheless, both interactions are attractive. Dhingra et al. (1978) ruled out structures in which any atom approached closer than 3 Å to the plane of a base. It is clear that this criterion is incompatible with standard functions for interatomic interactions (Scheraga, 1968). The criterion used by Dhingra et al. (1978), as originally defined by Giessner-Prettre et al. (1976), is that no atoms or groups are allowed to occupy the region of space $0 \leq z \leq 3$ Å and $0 \leq \rho < 4$ Å; the cylindrical coordinates z and ρ (in angstroms) of atoms are measured from the centers of the five- and six-membered rings of a base. The context of the criterion was calculation of chemical shifts for

Table III: Contact between H4′ and H5′ of Up- and the Nearest Atoms on the -pA Base of UpA III

protons on Up	nearest atoms on pA	distance (A)	van der Waals energy[a]
H4′	C4	3.2	−0.091
	C5	3.2	−0.091
	N7	3.0	−0.121
	C8	2.8	−0.104
	N9	2.9	−0.129
H5′	C2	3.1	−0.098
	N3	2.5	−0.078
	C4	2.7	−0.067
	N9	3.1	−0.104

[a] The van der Waals energy (kilocalories per mole) is estimated by using the Leonard-Jones potential of Scheraga (1968).

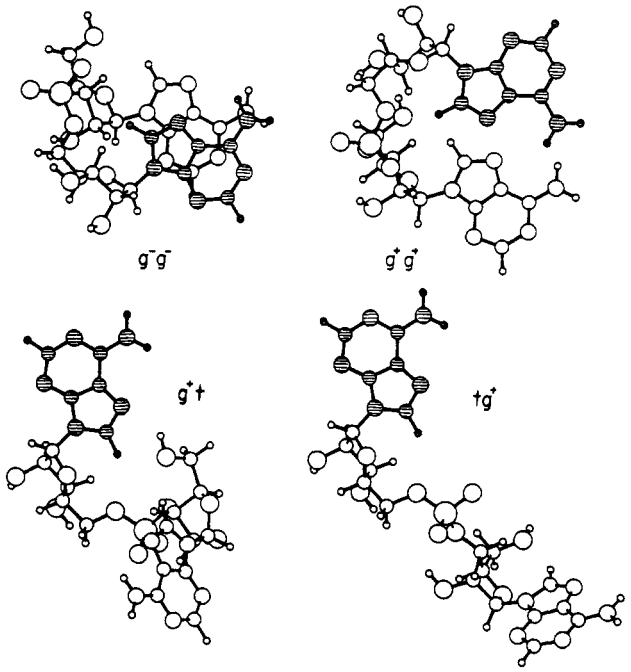

FIGURE 4: Structures of conformers g^-g^-, g^+g^+, g^+t, and tg^+ of ApA with the base of the pA residue shaded. The ribose moiety for all conformers is 3′ endo, and the torsional angles ω' and ω are the same as those proposed by Dhingra et al. (1978). The torsional angles are $\chi_1 = 25.6°$, $\phi' = 180.9°$, $\omega' = \omega = 295°$, $\phi = 171.3°$, $\psi = 56.8°$, and $\chi_2 = 30.7°$ for g^-g^-, $\chi_1 = 30.3°$, $\phi' = 173.6°$, $\omega' = 80°$, $\omega = 80°$, $\phi = 203.1°$, $\psi = 69.4°$, and $\chi_2 = 37.2°$ for g^+g^+, $\chi_1 = 22.4°$, $\phi' = 170.5°$, $\omega' = 110°$, $\omega = 215°$, $\phi = 178.1°$, $\psi = 62.6°$, and $\chi_2 = 28.1°$ for g^+t, and $\chi_1 = 23.7°$, $\phi' = 170.6°$, $\omega' = 180°$, $\omega = 80°$, $\phi = 180.4°$, $\psi = 63.5°$, and $\chi_2 = 31.6°$ for tg^+.

base protons in stacked bases; it is not valid in general. Table III gives the closest distances and the corresponding favorable energies for ribose protons–base atoms in UpA III. The cylindrical coordinates and calculated chemical shifts are given in Table V.

The relative stabilities of the conformers (Table II) for a given sequence are of great interest; the conformers considered here are shown in Figures 2 and 4. In ApA the stacked forms I, II, III, and g^-g^- are all significantly more stable than the forms g^+g^+, g^+t, and tg^+. Conformers I and g^-g^- have similar structures and conformational energies for ApA, as shown in Table II. The other sequences also have similar calculated structures and energies for I and g^-g^-. We will consider these conformations essentially equivalent from now on. The g^+t and tg^+ are open forms and have small van der Waals energies as expected; they are presumably stabilized by solvent interactions. The g^+t conformer, however, has the highest torsional energy; it could certainly be improved. The g^+g^+ is supposed

Table IV: Calculated Ring Current Shifts[a] and Dimerization Shifts[b] of ApA, ApU, UpA, and UpU

	Np-							-pN						
	H1′	H2′	H3′	H4′	H5′	H5″	H2 or -5	H8 or -6	H1′	H2′	H3′	H4′	H5′	H5″	H2 or -5	H8 or -6
ApA I	0.095	−0.107	−0.070	−0.007	0.002	−0.018	0.946	0.047	−0.021	0.118	0.172	0.004	−0.022	0.026	−0.122	0.633
ApA II	−0.039	−0.194	−0.107	−0.033	−0.005	−0.025	−0.106	0.082	0.062	−0.140	−0.065	0.006	0.025	−0.019	−0.016	0.579
ApA III	0.298	0.244	0.329	1.107	1.175	0.386	−0.034	0.178	−0.014	−0.006	−0.038	−0.021	−0.033	−0.031	−0.040	−0.017
ApA, 20 °C[b]	0.254	0.170	0.110	0.097	0.009	0.024	0.287	0.152	0.154	0.212	0.007	0.015	−0.253	−0.058	0.127	0.245
ApU I	0.014	−0.005	−0.007	0.000	0.001	−0.002	0.035	0.010	0.034	0.144	0.197	0.017	−0.014	0.035	0.753	0.669
ApU II	−0.003	−0.026	−0.014	−0.003	0.000	−0.003	−0.009	0.021	0.058	−0.129	−0.082	0.003	0.019	−0.023	1.713	0.474
ApU III	0.056	0.021	0.031	0.193	0.070	0.042	−0.002	0.005	−0.018	−0.006	−0.036	−0.022	−0.033	−0.031	−0.089	−0.013
ApU, 20 °C[b]	0.051	−0.009	0.123	−0.006	−0.103	−0.034	0.079	0.035	0.196	0.139	0.026	0.026	−0.201	−0.057	0.340	0.218
UpA I	0.089	−0.131	−0.091	−0.011	−0.002	−0.022	0.040	0.048	−0.011	0.003	0.013	−0.002	−0.002	0.003	−0.009	0.071
UpA II	−0.031	−0.242	−0.099	−0.028	0.005	−0.020	0.576	0.137	0.014	−0.009	0.016	0.005	0.006	0.001	−0.003	0.090
UpA III	0.306	0.247	0.334	1.111	1.149	0.385	−0.083	0.247	−0.001	0.000	−0.004	−0.002	−0.004	−0.003	0.004	−0.001
UpA, 20 °C[b]	0.237	0.203	0.107	0.102	0.124	0.069	0.128	0.121	0.025	0.036	−0.051	0.018	−0.129	−0.036	0.033	0.066
UpU I	0.037	0.010	0.002	0.006	0.004	0.001	0.004	0.012	−0.005	0.005	0.012	0.000	−0.002	0.002	0.107	0.044
UpU II	−0.001	−0.028	−0.008	−0.002	0.002	−0.001	0.094	0.032	0.010	−0.012	0.005	0.003	0.005	0.000	0.128	0.078
UpU III	0.051	0.022	0.031	0.156	0.070	0.037	−0.007	0.003	−0.001	0.000	−0.004	−0.002	−0.004	−0.003	−0.018	0.000
UpU, 20 °C[b]	0.068	0.002	0.019	−0.028	−0.014	−0.003	0.046	−0.026	0.040	0.021	0.032	0.023	−0.116	−0.050	0.054	−0.055

[a] The ring current shifts are calculated according to the method of Giessner-Prettre et al. (1976). [b] The dimerization shifts for ApA, ApU, and UpA at 20 °C are taken from Lee & Tinoco (1977), and those for UpU (20 °C) are from Lee et al. (1976).

Table V: Cylindrical Coordinates z, ρ_6, and ρ_5 and the Corresponding Calculated Ring Current Shifts for the Up Protons of UpA III

atom	z	ρ_6	ρ_5	δ_6 calcd	δ_5 calcd	$(\delta_5 + \delta_6)$ calcd
H1′	2.6	3.5	2.1	0.034	0.272	0.306
H2′	5.3	2.6	2.1	0.147	0.100	0.247
H3′	5.6	1.5	0.5	0.200	0.133	0.334
H4′	2.7	2.5	0.9	0.277	0.834	1.111
H5′	2.5	1.6	2.4	0.973	0.175	1.149
H5″	3.9	2.5	2.5	0.247	0.138	0.385
H5	2.4	5.1	5.9	−0.056	−0.027	−0.083
H6	2.8	2.7	3.6	0.231	0.016	0.247

to be a base-stacked form, but it is not (see Figure 4) and it has a much smaller van der Waals energy than the other base-stacked forms; it also has the second highest torsional energy. We conclude on the basis of energy calculations (Table II) that conformers I (or g^-g^-), II, and III can represent stacked forms of ApA. There are many unstacked (or open) forms which include conformations like g^+t and tg^+.

The sequence dependence of the calculated potential energies for ApA, ApU, UpA, and UpU is informative (Table II). The magnitudes of the valence angle bending energies, $E(\theta)$, of conformations I and II (14.0 ± 0.3 kcal/mol) are invariably larger than those for conformation III (11.3 ± 0.2 kcal/mol), suggesting that the valence angles in conformations I and II are more distorted from "natural" angles than those in conformation III.

The van der Waals energy, E(nb), scatters over a wide range from −9.4 to −13.2 kcal/mol, as a result of different degrees of structural overlap for the conformers of these molecules. For conformation III the van der Waals attractive energy is greater when the base of the −pN residue is a purine than a pyrimidine (−9.1 vs. −8.2 kcal/mol). For conformations I and II, the magnitude of the van der Waals attractive energy is in the order of ApA > UpA > ApU > UpU. Conformations I and II have stronger van der Waals attractive energy (2.1–3.5 kcal/mol) than conformation III. This difference arises from greater base–base stacking in conformations I and II.

The magnitude of the conformational energy, E_{conf}, thus generally reflects the combination of the valence angle-bending energy, $E(\theta)$, and the van der Waals energy, E(nb), since the bond-stretching energy, E(l), and the torsional energy, $E(\phi)$, are nearly constant. Despite its smaller van der Waals attractive energy, conformation III has improved its conformational energy by its more relaxed valence angles [with smaller valence angle bending energy, $E(\theta)$]. Conformation III, as expected, has smaller total potential energy when the base of the −pN residue is a purine, in contrast to a pyrimidine (12.0 vs. 12.7 kcal/mol). For conformations I and II, the sequence-dependent stability of the dinucleoside phosphates is in the order of ApA > UpA > ApU > UpU. The order of the stability for conformers of each dinucleoside phosphate is I > II = III for ApA, I ≥ III > II for ApU and UpA, and III > I > II for UpU.

Generally speaking, ApA has the most stable stacked forms, UpA and ApU have the second most stable, and UpU has the least. This is consistent with the experimental NMR results (Lee & Tinoco, 1977; Lee et al., 1976; Ezra et al., 1977; Watts & Tinoco, 1978) and the experimental optical studies (Watts & Tinoco, 1978; Davis & Tinoco, 1968; Brahms et al., 1967).

Effect of Coulombic Interaction on the Structures of Dinucleoside Phosphates. We made calculations in which a Coulombic potential among monopoles on each atom was added to the nonbonded interactions; a dielectric constant of 4 was used. Different charge density distributions of the bases have been obtained (Mely & Pullman, 1969; Clementi et al., 1969; Renugopalakrishnan et al., 1971; Rein, 1973; Giessner-Prettre & Pullman, 1968). It is interesting, therefore, to examine the effect of the monopole interactions on the energies and the structures of dinucleoside phosphates at their energy-minimum states with these different charge densities. With the charge densities of Renugopalakrishnan et al. (1971), the conformational energies for conformers I, II, III, g^-g^-, g^+g^+, g^+t, and tg^+ of ApA are 10.2, 11.4, 12.2, 10.7, 17.0, 25.3, and 20.6 kcal/mol, respectively. Comparison with Table II shows that adding Coulombic interaction has a small effect on the calculated energies and does not change the relative stabilities of any of the conformers. Conformation I or ApA was calculated by using five different charge densities for adenine (Ornstein et al., 1978). The minimum-energy structures obtained show only small differences from that of ApA I obtained without monopole interaction. Each torsional angle except for χ varies within ±6°; χ varies by up to 17°. Similar small perturbations of the structures by a variety of charge density distribution are observed for conformations II and III of ApA. We thus consider the potential energy minimization calculations without including monopole interactions to satisfactorily represent dinucleoside phosphate structures.

Nuclear Magnetic Resonance. The calculated ring current shifts (Giessner-Prettre et al., 1976) for conformations I, II, and III of ApA, ApU, UpA, and UpU are shown in Table IV along with the experimental dimerization shifts for these dinucleoside phosphates at 20 °C. The calculated values are reasonable and provide support for the existence of species such as I, II, and III in solution. Quantitative comparison cannot be made because of the presence of a wide range of other species and because of contributions to chemical shifts other than ring currents.

The values of the measured spin–spin splittings can be interpreted to give some of the torsion angles. The angle ϕ' can in principle be obtained from the dihedral angle H3′–C3′–O3′–P which effects the coupling constant, $J_{H3'P}$. With the usual Karplus-type equation (Lee et al., 1976; Ezra et al., 1977; Lee & Tinoco, 1977), a range of ϕ' equal to 240 ± 37° is obtained. Conformations I, II, and III all have ϕ' values outside this range, close to 180° for I and II and close to 300° for III. Steric hindrance is expected to favor $\phi' = 180°$ or 300°, so the values obtained from energy minimization are not surprising. It may be that the approximations in the Karplus equation used, plus the large number of conformers actually present, preclude interpretation of $J_{H3'P}$ in terms of a single useful angle. The coupling constants $J_{4'5'}$ and $J_{4'5''}$ are usually interpreted to give the percent of a gg ($\psi = 60°$) rotamer for C4′–C5′ and $J_{5'P}$ and $J_{5''P}$ give the percent g′g′ ($\phi = 180°$) for C5′–O5′. The experimental coupling constants (Lee et al., 1976; Ezra et al., 1977; Lee & Tinoco, 1977) indicate a high proportion of both gg and g′g′ in solution. This is consistent with conformation ($\psi \simeq 56°$, $\phi \simeq 172°$); conformations II ($\psi \simeq 77°$, $\phi \simeq 200°$) and III ($\psi = 66°$, $\Phi = 192°$) are significantly different.

Conclusions

The conformation of a dinucleoside phosphate can be approximately characterized by specifying seven torsional angles plus two ribose conformations (Table I). In addition, each bond length and bond angle must be specified and the torsion angles not in the main chain (three hydroxyl groups and a CH_2OH group) should be specified. In solution a wide range of conformations is present and there is rapid interconversion of the species. A measurement such as NMR or circular dichroism depends on a weighted average of all the species

present. It is obviously impossible to obtain a unique conformation (which in any case does not exist) from one such measurement. One can attempt to find *possible* conformations which will serve as guides for the types of species present in solution. These conformations can be used to help understand the experimental data and their dependence on base sequence, temperature, solvent, etc. We have found on the basis of semiempirical energy calculations that two base–base-stacked conformations ($I \simeq g^-g^-$ and II) and one base–sugar-stacked conformation (III) should be added to the previously used conformations of g^-g^- and g^+g^+ (slightly base–base stacked) and g^+t and tg^+ (open).

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Cloning of Synthetic Deoxyribonucleic Acid That Codes for Embryonic Cardiac Myosin Light-Chain Polypeptide†

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ABSTRACT: Double-stranded complementary deoxyribonucleic acid (cDNA) transcribed in vitro from a partially pure myosin light-chain messenger ribonucleic acid (mRNA) of the chick embryonic heart was cloned in *Escherichia coli* strain χ1776 by using the *Hin*dIII cleavage site in the plasmid pBR322. The insertion of essentially full length DNA was achieved by repeated selection of large-size cDNA transcripts. Of the 12 transformants that contained large-size DNA inserts, the clone pML10 insert was 950 base pairs in length, almost the same size as myosin light-chain mRNA (980 nucleosides). The clone pML10 was identified by hybridization with a highly pure cDNA probe and by hybrid-arrested translation assay. pML10 was further characterized by partial restriction enzyme mapping. The availability of a cloned DNA probe for myosin light-chain facilitates the analysis of the mechanism underlying the induction of cardiac muscle specific gene transcription in presumptive heart-forming cells of the chick blastoderm.

Heart muscle formation, which is an early event during chick embryonic development (Romanoff, 1960), is an attractive model for studying the molecular basis of induction of early embryonic gene functions. The apparently homogeneous population of cells, located on the lateral sides adjacent to Hensen's node in the postgastrulation stage [stage 5, Hamburger & Hamilton (1951)] of the chick embryo, is already programmed to develop into cardiac myoblasts which differentiate eventually into cardiac muscle tissue (DeHaan et al., 1970; Rosenquist, 1970). The availability of these primitive heart-forming cells offers a unique opportunity to probe into the mechanism of gene expression in the early embryo with the aid of cloned DNAs.

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