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How Flexible are DNA Constituents? The Quantum-Mechanical Study

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Abstract

Relaxed force constants (RFCs) and vibrational root-mean-square deviations have been evaluated by the original calculation method for conformational parameters of the DNA structural units and their constituents: nucleic acid bases (uracile, thymine, cytosine, adenine and guanine) and their 'building blocks' (benzene, pyrimidine, imidazole and purine molecules), as well as the DNA backbone structural units: tetrahydrofuran, 1,2-dideoxyribose, methanol and orthophosphoric acid. It has been found that the RFCs for nomenclature torsions β, γ, ε and sugar pseudorotation angle P in 1,2-dideoxyribose are sensible to the molecule conformation and their values are in the range of 1-25 kcal/(mole·rad^2) obeying the inequality $K_\gamma > K_\varepsilon > K_P > K_\beta$. The RFCs values for endocyclic torsions of nucleic acid bases six-member rings lie within 15-45 kcal/(mole·rad^2) in pyrimidines and within 20-60 kcal/(mole·rad^2) in purines. It is shown that the quantum zero-point motion effectively neglects the amino group non-planarity in cytosine, adenine and partially in guanine.

Introduction

Nucleic acids (NA) are essential components of all living organisms. They are responsible for such vitally important functions as reproduction (DNA) and protein synthesis (RNA) and it is the NA-protein interaction events being the molecular basis for these processes. The intrinsic NA features, crucial for NA-protein (1-7) and drug binding (8-12), are the NA polymorphism (13), *i.e.* their ability to adopt different conformations, and their flexibility (see (14-16) and references therein). Thus NA mechanical properties investigation is of significant biological importance (17-20). The key to understand these properties is, in turn, conformational flexibility of NA structural units – nitrogenous bases, sugar (21) and phosphate (22).

Even the nitrogenous bases – perhaps the most rigid NA component – have been shown to be rather flexible (23-25) since very little energy (less than 3 kcal/mole or about $5 \cdot k_B T$) is needed to change their endocyclic torsions by 30° (26).

Canonical 2'-deoxyribonucleosides have also been shown to have rather 'soft', low-lying vibrational mode (with frequencies below 200 cm^{-1}) responsible for the motion of sugar and base with respect to each other as well as the collective vibrations involving deformation of both the sugar and the base (27). Moreover, it has been stressed (27) that less than 30% of the molecules possess a near-equilibrium geometry at a given moment of time.

The aim of the present work is comparative study of the DNA structural units (Figures 1 and 2) mechanical properties. Namely, the relaxed force constants (RFCs, see below) being the quantitative measure of mechanical flexibility for such conformational parameters as torsion angles and sugar pseudorotation phase are

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calculated by original method (see *Method of Calculations* section). Vibrational root-mean-square (RMS) deviations (see below) of all the conformational parameters mentioned are also presented and discussed. For the best of our knowledge, this is the first time when explicit values of RFCs and vibrational RMS deviations for the DNA constituents conformational parameters are presented and compared.

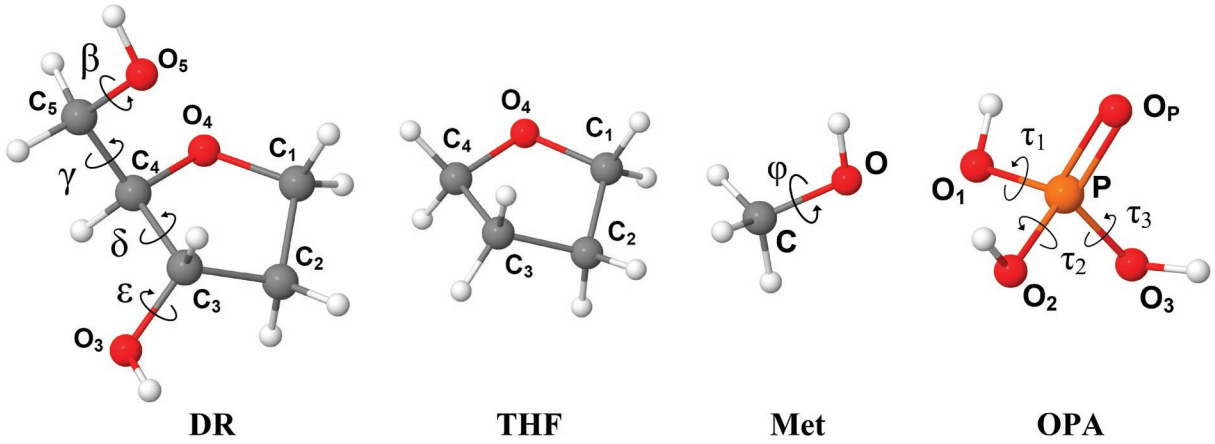

Figure 1: Model DNA backbone structural units (1,2-dideoxyribose (DR), tetrahydrofuran (THF), methanol (Met), ortophosphoric acid (OPA)).

The differentiation of molecular degrees-of-freedom (DOF) by their mechanical rigidity should provide a deeper insight into the qualitative understanding of conformational dynamics of biological molecules in general and of the DNA in particular.

Choice of Model Compounds

We have focused on the set of molecules (Figures 1 and 2) which includes all the classical DNA structural units and their constituents: nitrogenous bases (uracile (Ura), thymine (Thy), cytosine (Cyt), adenine (Ade) and guanine (Gua); benzene (Ben) and pyrimidine (Pyr) as primary building blocks for pyrimidine bases;

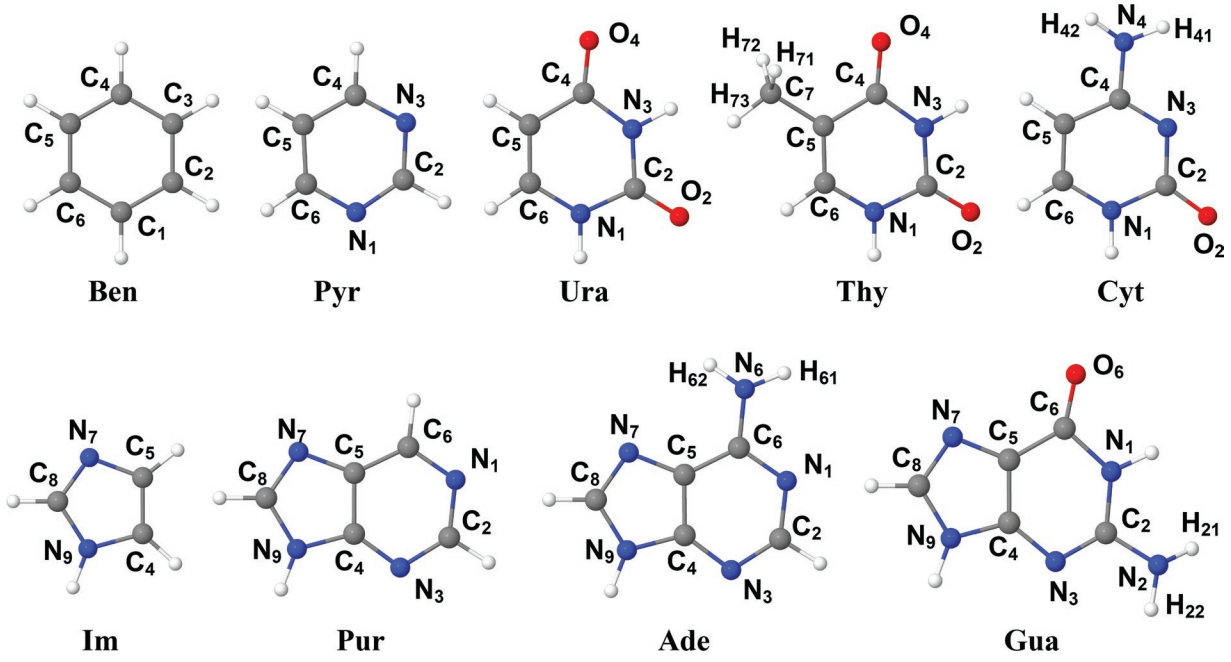

Figure 2: Nitrogenous bases – uracile (Ura), thymine (Thy), cytosine (Cyt), adenine (Ade), guanine (Gua) and their constituents – benzene (Ben), pyrimidine (Pyr), imidazole (Im), purine (Pur).

imidazole (Im) and purine (Pur) as building blocks for purine bases) tetrahydrofuran (THF) as the simplest model of the DNA sugar; 1,2-dideoxyribose (DR) representing abasic 2′-deoxyribonucleoside; ortophosphoric acid (OPA) and methanol (Met) as representative models for DNA backbone phosphate and nucleoside hydroxymethyl ($H–C_{5'}HH–O_{5'}H_{5'}$) groups respectively.

Method of Calculation

The physical quantity that describes the mechanical rigidity of conformational variable τ (the torsion angle for example) quantitatively is a force constant

$$K_\tau = \left(\frac{\partial^2 E}{\partial \tau^2} \right)_{F(\varphi_i)=0}, \qquad [1]$$

where E is the energy of the molecule (the sum of electron subsystem total energy in the field of nuclei and the coulomb interaction energy of the latter) and $F(\varphi_i) = 0$ represents constraints imposed on all DOFs except τ when the derivative [1] is evaluated. The typical way to obtain the value of the force constant K_τ is to perform the potential energy surface (PES) scanning, *i.e.* to evaluate $E(\tau)$ at the finite number of points and to differentiate it numerically. Depending on explicit constraints $F(\varphi_i) = 0$ involved in this process one would normally obtain different values of K_τ. In particular, when the *relaxed* PES scanning is performed (*i.e.* with the molecule energy minimization with respect to all DOFs except τ), one would get the minimal possible magnitude of K_τ, further on referred to as *relaxed force constant* (RFC).

The PES scanning is a time consuming method which can hardly be used in evaluating the force constant for 'collective' DOFs, *i.e.* those depending on Cartesian coordinates of a great number of atoms, the typical cases being pseudorotation phases angles of furanose (28) or pyranose (29) rings.

Another way to evaluate force constants is to calculate them using time-averaged values of molecule DOFs fluctuations obtained from molecular dynamics (MD) simulation (see, for example, (30)). However, this method requires great care in selecting the force field for reliable description of conformational preferences of the molecule (31) as well as of non-covalent interactions in it (32). Moreover, although only a MD simulation of considerable duration is desirable to achieve sufficient fluctuation statistics, this should be avoided since any change in the molecule conformation is unacceptable if force constants for definite conformation are of interest (for example, DNA-like conformations of deoxyribonucleotides).

As we have shown previously (21), all these difficulties can be avoided and RFCs can be easily calculated as soon as the molecule's harmonic normal vibration spectrum (normal modes frequencies, force constants and corresponding nuclei displacement vectors) is known. This spectrum may be efficiently calculated *ab initio* so no empirical force field is required. Furthermore, for 'collective' DOFs RFCs calculation a general formalism has been provided (21), which we use in the present work to investigate the mechanical properties of the DNA constituents.

The essence of the present RFC calculation algorithm is to approximate the molecule's energy increase $\Delta E = E - E_0$ resulting from its small deformation as (21)

$$\Delta E = \sum_{j=1}^{M} \frac{k_j x_j^2}{2}, \qquad [2]$$

where E_0 is the energy of the non-deformed (*i.e.* optimized) structure, M is the total number of normal vibrations of the molecule, x_j are their normal coordinates (equal to zero in non-deformed structure) and k_j are corresponding force constants.

According to (33), if the molecule's deformation is small, the increase $\Delta\tau = \tau - \tau_0$ of its conformational parameter τ (the torsion angle, for example) depends on the normal coordinates x_j as

$$\Delta\tau = \sum_{j=1}^{M} c_j^{\tau} \cdot x_j, \qquad [3]$$

where $c_j^{\tau} = \dfrac{\partial\tau}{\partial x_j}$ are 'permittivity coefficients', which can be calculated analytically for any structural parameter of interest when the molecule's geometry and normal vibrations displacement vectors are known. In particular case, when parameter τ corresponds to a torsion angle, $\tau = ABCD$, formed by four nuclei (designated as A, B, C, D) with coordinates $\vec{R}_A, \vec{R}_B, \vec{R}_C, \vec{R}_D$ we obtain (33)

$$c^{ABCD}{}_j = -\left|\vec{R}_{CB}\right| \cdot \left\{ \frac{\left(\vec{n}_{DCB} \cdot \vec{\xi}_{DC}^{\,j}\right)}{\left|\vec{n}_{DCB}\right|^2} - \frac{\left(\vec{n}_{ABC} \cdot \vec{\xi}_{AB}^{\,j}\right)}{\left|\vec{n}_{ABC}\right|^2} \right\} - \left\{ \frac{\left(\vec{R}_{AB} \cdot \vec{R}_{CB}\right) \cdot \vec{n}_{ABC}}{\left|\vec{n}_{ABC}\right|^2} - \frac{\left(\vec{R}_{DC} \cdot \vec{R}_{CB}\right) \cdot \vec{n}_{DCB}}{\left|\vec{n}_{DCB}\right|^2} \right\} \cdot \frac{\vec{\xi}_{CB}^{\,j}}{\left|\vec{R}_{CB}\right|}, \qquad [4]$$

where $\vec{n}_{DCB} = \left[\vec{R}_{DC} \times \vec{R}_{CB}\right], \vec{n}_{ABC} = \left[\vec{R}_{AB} \times \vec{R}_{CB}\right]$,

$\vec{R}_{a\beta} = \vec{R}_a - \vec{R}_\beta$ and $\vec{\xi}_{a\beta}^{\,j} = \vec{\xi}_a^{\,j} - \vec{\xi}_\beta^{\,j}$ $(a, \beta = A, B, C, D)$ and vector $\vec{\xi}_a^{\,j}$ gives the displacement of the nuclei $\vec{R}_a$ in j-th normal vibration; $\vec{\xi}_a^{\,j}$ should satisfy the normality condition $\sum_{a=1}^{N} \left(\vec{\xi}_a^{\,j}\right)^2 = 1$ for every normal vibration.

The key point of the present calculation method is minimization of [2] as the function of x_j under constraint [3] with $\Delta\tau$ being fixed. This leads (21) to interrelation between minimized ΔE and $\Delta\tau$ in the form $\Delta E = K_\tau \cdot (\Delta\tau)^2 / 2$ with the RFC

$$K_\tau = \frac{1}{\sum_{j=1}^{M} (c_j^{\tau})^2 / k_j}, \qquad [5]$$

where normal vibration force constants $k_j = \mu_j \omega_j^2$ and reduced masses μ_j depend on nuclei masses m_a as $\mu_j = \sum_{a=1}^{N} m_a \left(\vec{\xi}_a^{\,j}\right)^2$.

To take advantage of this algorithm we have performed the geometry optimization for all the molecules under investigation by the Gaussian 03 package (34) using the density functional theory (DFT) method at the B3LYP/cc-pVTZ theory level (35-38) with *tight* optimization criteria and *ultrafine* integration grid (consisting of 99 radial shells around each nuclei and 590 angular points on each of them). Vibrational spectra were calculated in the harmonic approximation at the same level of theory. Initial geometries of 1,2-dideoxiribose and ortophosphoric acid molecules were taken from (39) and (22) respectively while initial geometries of the others were built using standard bond lengths and valence angles.

The permittivity coefficients c_j^P for furanose ring pseudorotation angle have been calculated as follows (21)

$$c_j^P = \frac{1}{1+t^2} \left(\frac{c_j^{\nu_4} + c_j^{\nu_1} - c_j^{\nu_3} - c_j^{\nu_0}}{2\nu_2 \cdot (\sin 36^\circ + \sin 72^\circ)} - \frac{t}{\nu_2} \cdot c_j^{\nu_2} \right), \qquad [6]$$

where $t = \frac{v_4 + v_1 - v_3 - v_0}{2v_2 \cdot (\sin 36° + \sin 72°)}$, and $c^{v_i}_j$, ($i = 0...4$), are permittivity coefficients for furanose endocyclic torsions v_i (see (28) for their definition) respectively, given by [4].

The structural-dynamical variability of the investigated molecules caused by their quantum normal vibrations at the given temperature have been characterized by the root-mean-square (RMS) deviations $\sigma^\tau = \sqrt{\left\langle \left(\tau - \langle\tau\rangle\right)^2 \right\rangle} = \sqrt{\left\langle \tau^2 \right\rangle - \langle\tau\rangle^2}$, which have been evaluated as follows (33)

$$\sigma^\tau = \sqrt{\sum_{j=1}^{M} \left(\sigma_j^\tau\right)^2}, \qquad [7]$$

where individual vibrational modes contributions σ^τ_j for the temperature $T > 0$ are given by (33)

$$\sigma_j^\tau = \sqrt{\frac{\hbar}{2\mu_j\omega_j} \cdot \left(c_j^\tau\right)^2 \cdot \coth\left(\frac{\hbar\omega_j}{2k_B T}\right.} \qquad [8]$$

and for $T = 0$ – by

$$\sigma_\tau = \sqrt{\frac{\hbar}{2\mu_j\omega_j} \cdot \left(c_j^\tau\right)^2}.$$

Results and Discussion

The DNA Sugar and Phosphate Group

The RFCs calculated according to [5] for AI-, BI- and ZI-DNA-like conformations of the 1,2-dideoxyribose (DR) molecule (Figure 1) are given in Table I (A, B). These Tables also contain data for tetrahydrofuran (THF) molecule (Figure 1) which differs from DR by the absence of substituents in the 3-rd and 4-th positions.

In all conformations of DR considered here $K_\gamma > K_\varepsilon > K_P > K_\beta$, so torsion β can be identified as the softest and γ – as the most 'rigid' one. Note, that the RFC values for γ and P depend on the C_5-O_5 bond orientation and furanose ring conformation respectively.

Pseudorotation angle RFC value in THF (both for $P = 0°$, v_{max}=36° and P= 180°, $v = 36°$ conformations) equals $K_p = 0.57$ kcal/(mole·rad)^2, which is much lower

Table I (A)

Relaxed force constants for backbone torsions and furanose pseudorotation angle in 1,2-dideoxyribose and tetrahydrofuran molecules.

	Conformation details		Force constants, kcal·mole^{-1}·rad^{-2}				
Conformation[a]	β / γ / ε[b]	P, deg.	K_β	K_γ	K_δ	K_ε	K_P
BI	t / g^+ / t	147.6	1.48	21.5	12.8	5.06	4.35
ZI$_+$	t / g^+ / g^+	154.4	1.41	20.7	15.3	4.44	4.14
AI	t / g^+ / t	330.3	1.47	23.9	**6.61**	3.94	**2.24**
ZI$_t$	t / t / t	105.6	**2.42**	15.7	**2.99**	5.13	**2.04**
THF	–	0.0	–	–	–	–	0.57

[a]These conformations correspond to the following 1,2-dideoxyribose conformer numbers in (39): 29 (AI), 14 (BI), 21 (ZI$_+$) and 44 (ZI$_t$).
[b]Torsion ranges: $g^+ = \{60° \pm 30°\}$, $g^- = \{-60° \pm 30°\}$, $t = \{180° \pm 30°\}$.

Table I (B)
Relaxed force constants for endocyclic torsions in 1,2-dideoxyribose and tetrahydrofuran molecules.

Conformation	Force constants, kcal·mole^{-1}·rad^{-2}				
	$K_{\nu 0}$	$K_{\nu 1}$	$K_{\nu 2}$	$K_{\nu 3}$	$K_{\nu 4}$
BI	13.8	**43.5**	39.2	13.7	9.41
ZI$_+$	12.2	34.7	**52.3**	16.3	9.79
AI	8.97	**39.4**	21.3	7.16	5.33
ZI$_t$	**52.4**	8.52	4.27	4.03	8.97
THF	1.53	4.21	**65.4**	4.21	1.53

than in any conformation of DR molecule. Thus, the furanose ring mechanical properties are sensitive to any modifications of its substituents (the hydroxyl and hydroxymethyl group in the case), so it is reasonable to expect that K_p should change when the nitrogenous base is attached to DR (we are going to publish corresponding investigation results soon).

The methanol (Met) molecule (Figure 1) has been investigated as the reference compound for $-C_5HH-O_5H$ and $-C_3HH-O_3H$ hydroxymethyl groups of DR. The RFC value for φ = HOCH torsion of Met is $K_\varphi = 4.57$ kcal/(mole·rad^2), which is close to K_ε in DR but is higher than DR's K_β. Hence, not only furanose ring properties are influenced by its substituents, but the 4-th position hydroxymethyl group mechanical rigidity is affected by the furanose ring as well, whereas this effect is less pronounced for the 3-rd position substituent. This conclusion is supported by the fact that K_γ in ZI$_t$ conformation of DR, when the $-O_5H$ hydroxyl group is the most distant from the furanose ring atoms, is evidently lower than in all other conformations of DR.

It should be noted that vibrational RMS deviations of β, γ and ε torsions in DR (Table II (A)) are less sensitive to its conformation than the RFCs for the same torsions. At the same time, vibrational RMS deviations of the furanose ring endocyclic torsions are substantially influenced by its conformation (Table II (B)).

Table II (A)
Vibrational RMS deviations (deg.) of backbone torsions and furanose pseudorotation angle in 1,2-dideoxyribose and tetrahydrofuran molecules.

Conformation	T = 0 K					T = 298.15 K				
	σ_β	σ_γ	σ_δ	σ_ε	σ_P	σ_β	σ_γ	σ_δ	σ_ε	σ_P
BI	22.0	6.4	5.8	15.8	10.2	37.1	10.1	12.6	21.0	21.7
ZI$_+$	22.1	6.4	5.7	16.7	10.9	37.9	10.3	11.6	22.4	22.1
AI	21.9	6.2	6.6	17.0	11.5	37.2	9.6	17.4	23.5	29.9
ZI$_t$	19.3	6.0	7.9	15.7	9.9	29.4	11.4	25.7	20.9	31.1
THF	–	–		–	21.0	–	–		–	58.6

Table II (B)
Vibrational RMS deviations (deg.) of endocyclic torsions in 1,2-dideoxyribose and tetrahydrofuran molecule.

Conformation	T = 0 K					T = 298.15 K				
	$\sigma_{\nu 0}$	$\sigma_{\nu 1}$	$\sigma_{\nu 2}$	$\sigma_{\nu 3}$	$\sigma_{\nu 4}$	$\sigma_{\nu 0}$	$\sigma_{\nu 1}$	$\sigma_{\nu 2}$	$\sigma_{\nu 3}$	$\sigma_{\nu 4}$
BI	6.7	5.2	4.7	5.9	7.1	12.3	7.3	7.5	12.3	14.7
ZI$_+$	7.0	5.5	4.5	5.8	7.2	13.0	8.0	6.7	11.3	14.5
AI	7.1	5.4	5.0	6.5	7.6	15.1	7.6	10.0	16.7	19.4
ZI$_t$	5.3	6.2	7.0	7.1	6.1	6.8	15.4	21.5	22.2	15.0
THF	12.9	8.5	4.7	8.5	12.9	35.8	21.7	6.1	21.7	35.8

Phosphate group is another important structural unit of the DNA backbone and the orthophosphoric acid molecule (OPA, see Figure 1) can be considered as its simplest molecular model. We have investigated only 'right-handed' conformations of OPA since their mirror-symmetrical counterparts have the same mechanical properties.

The OPA molecule conformation can be identified by three torsions: τ_1, τ_2 and τ_3 (see Figure 1), which describe hydroxyl group rotations round single $P-O$ bonds. The RFCs (K_{τ_i}, $i = 1...3$) for these conformational parameters are given in Table III (A) while Table III (B) contains vibrational RMS deviations (σ_{τ_i}, $i = 1...3$) for these parameters. The RFCs are in the range of 2.2-4.7 kcal/(mole·rad^2) thus showing intermediate values between the K_ε of DR and K_φ of Met. It should be stressed again that the RFCs values are sensitive to the OPA molecule conformation.

Nitrogenous Bases

Now, when mechanical properties of the DNA backbone constituents have already been characterized, it is reasonable to focus on the nitrogenous bases, which are the elementary 'bits' of genetic information encoded in nucleic acids.

Nitrogenous bases (see Figure 2) are traditionally assumed to be planar rigid structures. However, as it has been shown in (26), very little energy (less than 3 kcal/mole) is needed to change their endocyclic torsions by 30°. In spite of being planar aromatic compound, Ben is also known to be relatively flexible molecule (40, p. 211). Therefore, it is interesting to investigate mechanical properties of nitrogenous bases endocyclic torsions and compare them with those of the DNA backbone.

In order to do this, we have evaluated RFCs for endocyclic torsions of nitrogenous bases (Cyt, Thy, Ura, Ade and Gua) and their building blocks (Ben, Pyr, Im, Pur). The values obtained for six-member rings of pyrimidines are presented in Table IV (A). It can be easily seen that the softest endocyclic torsions show the RFCs values of only 16 kcal/(mole·rad^2), which is even less than typical value of K_γ in DR. In this way Ura, Thy and Cyt differ drastically from Pyr, their 'parent' compound, having some of their RFCs about 3 times lower than Pyr. This clearly demonstrates the influence of side atomic groups on the mechanical properties of the rings. At the same time, Pyr shows mechanical properties similar to Ben (Table V) in spite of having different atoms (nitrogen instead of some carbon atoms) in its ring.

It should be noted, however, that the vibrational RMS deviations of endocyclic torsions in pyrimidine nitrogenous bases (Table IV (B)) do not differ much from molecule to molecule. Furthermore, they are close to the corresponding values in Pyr and even Ben (Table V) molecules and lie in the range of 7°-12° at temperatures from 0 K to 298.15 K (*i.e.* their rings are planar only 'on average').

Thus, it is convenient in this regard to consider Ben molecule as a typical case to get a closer look into partial contributions of each of its normal vibrations into

Table III (A)

Relaxed force constants (kcal·mole^{-1}·rad^{-2}) for torsion angles of ortophosphoric acid molecule.

Conformation	Conformation attributes (deg.)[a]			Force constants, kcal·mole^{-1}·rad^{-2}		
	τ_1	τ_2	τ_3	$K_{\tau 1}$	$K_{\tau 2}$	$K_{\tau 3}$
1	34.0	34.0	34.0	2.71	2.71	2.71
2	179.3	24.1	45.7	2.89	4.65	2.19

[a]Torsions τ_i are defined as $\tau_i = OPO_iH_i$, $i = 1 \ldots 3$.

Table III (B)

Vibrational RMS deviations (deg.) of torsion angles in ortophosphoric acid molecule.

Conformation	T = 0 K			T = 298.15 K		
	$\sigma_{\tau 1}$	$\sigma_{\tau 2}$	$\sigma_{\tau 3}$	$\sigma_{\tau 1}$	$\sigma_{\tau 2}$	$\sigma_{\tau 3}$
1	18.8	18.8	18.8	28.0	28.0	28.0
2	18.5	16.6	19.9	27.1	21.9	30.9

Table IV (A)

Relaxed force constants[a] (kcal·mole^{-1}·rad^{-2}) for endocyclic torsions of pyrimidine nitrogenous bases.

	Torsion					
Base	$N_1C_2N_3C_4$	$C_2N_3C_4C_5$	$N_3C_4C_5C_6$	$C_4C_5C_6N_1$	$C_5C_6N_1C_2$	$C_6N_1C_2N_3$
Pyr	45.5	**53.8**	**45.8**	45.8	53.8	45.5
Ura	**15.8**	16.2	28.4	**40.3**	25.0	19.7
Cyt	22.0	32.9	32.8	**43.4**	25.7	**15.9**
Thy	**17.0**	17.2	30.6	**41.1**	23.3	19.6

[a]Their highest and the lowest RFC values for each base are marked with **bold.**

Table IV (B)

Vibrational RMS deviations (deg.) of endocyclic torsions of pyrimidine nitrogenous bases.

	Torsion					
Base	$N_1C_2N_3C_4$	$C_2N_3C_4C_5$	$N_3C_4C_5C_6$	$C_4C_5C_6N_1$	$C_5C_6N_1C_2$	$C_6N_1C_2N_3$
T = 0 K						
Pyr	7.4	7.0	7.2	7.2	7.0	7.4
Ura	8.5	8.5	7.2	7.6	8.4	8.0
Cyt	7.6	7.7	7.4	7.2	8.2	8.3
Thy	8.3	8.3	6.9	7.5	8.6	7.9
T = 298.15 K						
Pyr	8.2	7.6	8.0	8.0	7.6	8.2
Ura	12.0	12.0	9.3	8.4	10.1	10.9
Cyt	10.4	9.1	9.0	8.0	10.0	12.0
Thy	11.7	11.6	9.0	8.4	10.5	11.0

the endocyclic torsions vibrational RMS deviations. Table VI provides σ_j^τ values obtained by [8], showing the RMS deviation for the $\tau = CCCC$ torsion of Ben caused solely by its j-th normal vibration.

These data imply that it is one of the lowest frequency normal vibrations (415 cm^{-1}) that gives the maximum contribution ($\sigma^\tau = 5.3°$ at T = 298.15 K) into the overall vibrational RMS deviation (7.7° at T = 298.15 K) of τ. As for 727 cm^{-1}, 989 cm^{-1} and 1022 cm^{-1} normal vibrations, they give smaller contributions while for the rest of normal vibrations σ_j^τ are close to zero.

It is interesting to compare mechanical properties of pyrimidine bases with a single six-member ring with the properties of purine bases being two-ring structures. It is natural to expect *a priori* that the presence of 'additional' closed-ring structure should make the whole molecule more rigid.

Indeed, when the RFC values for endocyclic torsions of Ade, Gua, Im and Pur presented in Table VII (A) are compared with corresponding data for pyrimidines (see Table IV (A)) it can be easily seen that the softest torsions of Pur and Ade six-member rings (K > 40 kcal/(mole·rad^2)) are close to the most rigid endocyclic

Table V

Flexibility parameters for benzene molecule.

		RMS deviation, deg.	
Torsion	Relaxed force constant, kcal·mole^{-1}·rad^{-2}	T = 0 K	T = 298.15 K
$C_1C_2C_3C_4$	52.1	7.1	7.7
$C_1C_2C_3H_3$	39.4	9.6	9.9
$H_1C_2C_3H_3$	27.9	12.7	12.9

Table VI

Contributions of the benzene normal vibrations into thermal RMS deviations of the endocyclic CCCC torsion at T = 298.15 K[a].

j	1	**2**	3	4	5	**6**	7	8	**9**	10
ν_j, cm^{-1}	414,5	**414,6**	624,2	624,2	691,2	**726,9**	867,6	867,7	**988,6**	988,6
σ_j, deg.	0,78	**5,33**	0,00	0,00	0,00	**4,75**	0,10	0,00	**1,17**	1,60
j	11	**12**	13	14	15	16	17	18	19	20
ν_j, cm^{-1}	1015,3	**1021,9**	1030,6	1062,2	1062,2	1176,5	1200,4	1200,4	1335,1	1389,8
σ_j, deg.	0,00	**1,97**	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
j	21	22	23	24	25	26	27	28	29	30
ν_j, cm^{-1}	1518,8	1518,8	1637,4	1637,5	3156,6	3166,4	3166,4	3182,1	3182,1	3192,2
σ_j, deg.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

[a] j –normal vibration number, ν_j–normal vibration unscaled harmonic frequency, σ_j –the CCCC torsion root-mean-square deviation from its equilibrium value caused by j-th normal vibration.

torsions of Ura, Thy and Cyt (K < 44 kcal/(mole·rad^2)). However, Gua gives an exception: its $C_5C_6N_1C_2$ and $C_6N_1C_2N_3$ torsions RFCs (K ~ 23 kcal/(mole·rad^2)) are twice lower than the corresponding values in Pur and Ade, making the six-member ring of Gua the most flexible one among purine bases. This is caused by the presence of the oxygen atom in the 6-th position of Gua, lowering the C_6–N_1 bond order from 2 to 1 so that N_1 atom becomes 'free'.

The typical values of endocyclic torsions RFC in purine base five-member rings (K ~ 100 kcal/(mole·rad^2)) do not differ much from the same values of isolated Im molecule and they are twice higher than in their neighbouring six-member rings (K ~ 50 kcal/(mole·rad^2)). So, it can be concluded that it is the purine base five-member ring that is the main structural factor responsible for their overall higher mechanical rigidity compared to pyrimidines.

The vibrational RMS deviations (Table VII (B)) of endocyclic torsions, which have been shown to be less sensitive to mechanical rigidity than RFCs, are also lower

Table VII (A)

Relaxed force constants (kcal·mole^{-1}·rad^{-2}) for endocyclic torsions of purine nitrogenous bases.

	Torsion										
Base	$N_9C_4C_5N_7$	$C_4C_5N_7C_8$	$C_5N_7C_8N_9$	$N_7C_8N_9C_4$	$C_8N_9C_4C_5$	$N_1C_2N_3C_4$	$C_2N_3C_4C_5$	$N_3C_4C_5C_6$	$C_4C_5C_6N_1$	$C_5C_6N_1C_2$	$C_6N_1C_2N_3$
Im	110.5	**118.8**	115.1	**98.4**	108.9	–	–	–	–	–	–
Pur	105.1	**119.7**	104.3	**84.1**	101.4	51.4	53.4	**45.5**	49.8	**56.2**	50.2
Ade	106.8	**121.6**	111.6	**89.2**	104.6	**51.9**	51.9	**40.7**	43.7	47.4	44.6
Gua	105.5	**118.0**	110.8	**91.1**	104.7	**47.9**	40.0	32.9	35.4	**22.9**	23.5

Table VII (B)

Vibrational RMS deviations (deg.) of endocyclic torsions of purine nitrogenous bases.

	Torsion										
Base	$N_9C_4C_5N_7$	$C_4C_5N_7C_8$	$C_5N_7C_8N_9$	$N_7C_8N_9C_4$	$C_8N_9C_4C_5$	$N_1C_2N_3C_4$	$C_2N_3C_4C_5$	$N_3C_4C_5C_6$	$C_4C_5C_6N_1$	$C_5C_6N_1C_2$	$C_6N_1C_2N_3$
T = 0 K											
Im	5.3	5.3	5.3	5.6	5.2	–	–	–	–	–	–
Pur	5.1	5.1	5.4	5.7	5.1	7.2	6.9	7.1	6.8	6.9	7.4
Ade	5.2	5.1	5.3	5.6	5.0	7.1	6.9	7.3	6.6	6.9	7.7
Gua	5.2	5.2	5.3	5.6	5.1	6.9	7.5	7.8	6.5	7.7	8.4
T = 298.15 K											
Im	5.5	5.5	5.5	5.8	5.5	–	–	–	–	–	–
Pur	5.5	5.4	5.7	6.1	5.5	7.8	7.6	8.1	7.7	7.4	8.0
Ade	5.5	5.4	5.5	6.0	5.5	7.8	7.7	8.4	8.0	7.8	8.4
Gua	5.6	5.4	5.6	6.0	5.5	7.8	8.5	9.2	8.4	10.2	10.5

for endocyclic torsions of purines' five-member rings ($\sigma \sim 6°$) when compared with their six-member counterparts ($\sigma \sim 8°$).

In general, not only the endocyclic torsions rigidity determines overall mechanical properties of nitrogenous bases, but their side atomic groups mobility is important as well. The latter can be even more important when, for example, the hydrogen-bonded pairs of nitrogenous bases are considered.

An interesting conclusion may be drawn when the vibrational RMS deviation σ_θ (Table VIII) of torsion $\theta = XC_iN_iH_i$ (where X = C or N is the ring atom neighbouring C_i) describing out-of-plane motion of amino group hydrogen atoms in Cyt, Ade and Gua is compared with this torsion's equilibrium value, corresponding to the minimum on the molecule's PES. Namely, in Cyt and Ade $\sigma_\theta \geq 14°$ at $T = 0\,\mathrm{K}$ whereas in equilibrium $|\theta| < 13°$, which indicates that quantum zero-point motion effectively neglects the amino group non-planarity. This finding agrees well with the fact that in spite of its non-planar equilibrium configuration (41) amino group in nitrogenous bases has relatively low planarization barrier, which is less than 0.1 kcal/mole for Cyt and Ade and about 0.7 kcal/mole in Gua (42).

In Gua this is true only for H_{22} atom of its amino group while for its counterpart, H_{21}, the equilibrium value of $N_1C_2N_2H_{21}$ ($-32°$) is not overcame by its vibrational RMS deviation ($\sigma_\theta = 15°$ at 0 K).

However, not only Gua gives an example of the amino group hydrogen atoms different mobility. Comparison of RFC for corresponding torsions (Table VIII) reveals that in Cyt as well as in Gua one of these atoms needs twice smaller work to be done to turn it by 1 deg round the N–C bond than the other does. At the same time, the RFCs for both hydrogen atoms in Ade are very close (they differ no more than by 35%).

The data obtained for $\varphi = C_6C_5C_7H_{72}$ torsion angle (Table VIII) that describe the rotation of methyl group hydrogen atoms round C_5-C_7 bond in Thy gives a good opportunity to test the suitability of simple torsion oscillator approximation (43, p. 52). According to this approximation, the dispersion of the φ torsion angle (assuming that $\langle\varphi\rangle = 0$) may be obtained as follows

$$\left\langle \varphi^2 \right\rangle = \frac{\hbar}{2I\omega} \cdot \coth\left(\frac{\hbar\omega}{2k_BT}\right), \qquad [9]$$

Table VIII

Flexibility parameters for exocyclic torsions describing nitrogenous bases side atomic groups positions.

Base	Atomic group	Torsion	Equilibrium value, deg.	Relaxed force constant, kcal·mole^{-1}·rad^{-2}	RMS deviation, deg.	
					T = 0 K	T = 298.15 K
Thy	$-CH_3$	$C_4C_5C_7H_{72}$	180.0	5.80	12.5	19.3
		$C_6C_5C_7H_{72}$	0.0	5.33	13.5	20.4
Cyt	$-NH_2$	$N_3C_4N_4H_{41}$	−8.0	**6.50**	15.3	19.3
		$C_5C_4N_4H_{41}$	173.0	**7.88**	14.0	17.5
		$N_3C_4N_4H_{42}$	−168.4	3.35	18.1	25.4
		$C_5C_4N_4H_{42}$	12.6	2.85	19.7	27.6
Ade	$-NH_2$	$N_1C_6N_6H_{61}$	−8.6	3.42	18.0	25.4
		$C_5C_6N_6H_{61}$	172.3	4.14	16.4	23.0
		$N_1C_6N_6H_{62}$	−171.9	3.73	17.0	24.2
		$C_5C_6N_6H_{62}$	9.1	3.05	18.9	26.8
Gua	$-NH_2$	$N_1C_2N_2H_{21}$	−31.7	8.12	14.9	17.6
		$N_3C_2N_2H_{21}$	150.6	8.72	14.1	16.8
		$N_1C_2N_2H_{22}$	−169.4	**15.8**	11.9	13.3
		$N_3C_2N_2H_{22}$	13.0	**15.6**	12.5	13.7

where I is the reduced moment of inertia and ω is the vibration frequency. Substituting $I = 3.08$ a.m.u·Å^2 (reduced moment of inertia about the C_5–C_7 axis in Thy) and $\omega = 151\,cm^{-1}$ (the frequency of normal vibration corresponding to methyl group rotation) into [9], we obtain $\sqrt{\langle\varphi^2\rangle} = 10.9°$ at T = 0 K and $\sqrt{\langle\varphi^2\rangle} = 18.5°$ at T = 298.15 K. These values are naturally lower than the corresponding values from Table VIII (13° at 0 K and 20° and 298.15 K) since only the contribution of a single normal vibration is taken into account [9]. For the same reason the corresponding rotational force constant estimated in accordance with the torsion oscillator model, $k = I\omega^2 = 5.94$ kcal/(mole·rad^2), is slightly higher than the value given in Table VIII. Thus, although the single mode torsion oscillator model (43, p. 52) gives reasonable approximation, it can be used only when it is possible to detect single normal vibration responsible for changing the specified torsion angle. At the same time, the calculation method used in the present work is free from this limitation.

Conclusions

Original easy-to-implement but powerful calculation method capable to obtain relaxed force constant values for any degree of freedom of the molecule has been described. Conformational flexibility of the DNA constituents, namely relaxed force constants and vibrational RMS deviations for conformational parameters of nitrogenous bases and model DNA backbone structural units, has been characterized with this method for the first time.

Mechanical properties of furanose ring as well as nitrogenous bases rings have been shown to be influenced by their side radicals.

In 1,2-dideoxyribose molecule the relaxed force constant values K_X for $X = \beta$, γ, ε torsions and furanose pseudorotation P lie in the range of 1-25 kcal/(mole·rad^2) and have been found to be conformation-dependent. In addition, in all DNA-like conformations of 1,2-dideoxyribose the following hierarchy has been revealed: $K_\gamma > K_\varepsilon > K_P > K_\beta$.

The relaxed force constants for endocyclinc torsions of nitrogenous bases six-member rings have been found to be in the range of 15-45 kcal/(mole·rad^2) for pyrimidines and 20-60 kcal/(mole·rad^2) for purines, supporting the viewpoint that nitrogenous bases are the most rigid NA constituents. However, all bases except Ade have been shown to have the following endocyclic torsions with relaxed force constant values smaller than for NA backbone: $N_1C_2N_3C_4$ in uracile (15.8 kcal/(mole·rad^2)), $C_6N_1C_2N_3$ in cytosine (15.9 kcal/(mole·rad^2)), $N_1C_2N_3C_4$ in thymine (17.0 kcal/(mole·rad^2)) and $C_5C_6N_1C_2$ in guanine (22.9 kcal/(mole·rad^2)). The softest endocyclic torsions in adenine, $N_3C_4C_5C_6$, is about twice more rigid having the relaxed force constant of 40.7 kcal/(mole·rad^2).

It has also been found that quantum zero-point motion effectively neglects the non-planarity of the amino group in cytosine and adenine since even at $T = 0$ K vibrational RMS deviations of *XCNH* torsions describing out-of-plane motion of amino group hydrogen atoms exceeds its equilibrium value.

The mobility of two hydrogen atoms in amino groups of cytosine and guanine has been found to differ essentially, whereas this is not the case for adenine.

Vibrational RMS deviations of NA constituents conformational parameters have been found to be less conformation-sensitive than corresponding relaxed force constant values. The typical vibrational RMS deviations for DNA backbone torsions are 5°-25° at 0 K and 9°-40° at 298.15 K while for nitrogenous base endocyclic torsions their values are within 5°-10° at 0 K and 7°-12° at 298.15 K.

The interrelation between the six-member rings flexibility and their low-lying vibrational modes has been studied with an example of benzene molecule. Four normal vibrations of benzene giving notable contributions into the overall vibrational RMS deviation (7.7° at $T = 298.15$ K) of the CCCC endocyclic torsion angle have been pointed out.

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