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Intramolecular hydrogen bonds and structural nonrigidity of pyrimidine nucleosides

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Optimal structures and intramolecular web of H-bonds of cytidine, uridine, thymidine, their deoxyribo-analogues and some O5'-, O3'-deutero derivatives were studied by means of MNDO/H semiempirical quantum-chemical method. Effect of the intramolecular H-bonds on the stereochemical structure of nucleosides (particularly, on the stabilization of anti conformation), on the physico-chemical characteristics of nucleoside molecules (heat of formation, dipole moment, first adiabatic ionization potential and the charge distribution), and on the dynamical characteristics of pyrimidine nucleosides (barriers of the interconvertion, frequencies of the torsional vibrations) was elucidated. The intramolecular H-bonds in polynucleotides and their influence on the nucleic acid architecture and nonlinear dynamic properties are discussed.

Introduction. Nucleoside molecules exhibit many possibilities as model objects for biophysical investigations [1]. The structure of isolated nucleoside molecule is complicated and nonrigid [2, 3]. Pyrimidine nucleosides have in particular a higher barrier of rotation around glycoside bond as purine ones. While it is possible for pyrimidines to adopt the syn conformations, it is sterically difficult and rarely observed in nature (see [4—6] and references there) and leads to the prefering of *anti*-conformation of sugar. The optimal conformation of pyrimidine nucleoside is determined by the dipole-dipole interactions, torsion rigidity of glycoside C1'N1 bond and steric hindrances. But there are additional interactions between the base residue and the sugar moiety contributing in stabilization of the nucleoside molecule in certain conformation.

Effect of such interactions was found in some cases. For example, Emerson and Sundaralingam [7] in their study of dihydrouridine 3'-monophosphate hemihydrate pointed out that puckering of the base could influence the ribose puckering due to interactions of the C6 methylene proton with the ribose. Van Lier, Smits and Buck [4] in quantum-chemical study of tetrahydrofuryl-1-(5-methylcytosine) explain its unusually high *anti-syn* transition barrier in terms of an electronic effect of the *para*-substituted methyl group on the and C2' methylene fragment through the carbonyl C2 group.

Moreover, the hydrogen bonds (H-bonds) involving atoms of the base residue and the sugar moiety were supposed in several studies, such as NMR investigations of mononucleotides [8] (O2'H...O2), crystallographic refinement of tRNA [9] (O2'H...O2, C6H...O5'), refinement of cytidine crystal structure [10] (C6H...O5'), PM3 [11] and CNDO/2 [12] semiempirical quantum chemical calculations (C6H...O5'). Ts'o [13] in NMR investigation of poly(U) also assumed the contribution of hydrogen bonding in interactions of C6H proton with the nearby ribose oxygen.

The possibility of participation of CH groups in H-bonds in nucleic acid constituents was supported by the experimental [14, 15] and theoretical [12, 16] investigations. It also is known that the existence of C-H...O H-bond interactions in the nucleic acid polymers have a strong influence on their molecular structure [12, 16].

The present study was undertaken to elucidate by means of MNDO/H semiempirical quantum-chemical calculation method, what kinds of intramolecular H-bonds are in pyrimidine nucleosides and what is the influence of these H-bonds on the parameters of structural nonrigidity and dynamics of the nucleosides.

Methods. MNDO/H semiempirical quantum-chemical calculation method was shown to be useful for the investigation of the stereochemical nonrigidity of nucleotide bases [17] and in calculation of the parameters of hydrogen bonds [18]. It slightly underestimates the values of geometric and energetical characteristics of nucleotide base nonplanarity effects [1], allowing to consider more precisely many of phenomena having been earlier neglecting.

The structure, IR spectra and some physicochemical properties of pyrimidine nucleosides were calculated with full optimization of all geometric parameters with the gradient norm < 0.01. Starting geometries were obtained by the composition of fully optimized structures of components: nucleotide bases [19] and ribose (deoxyribose) molecules. Optimization of the structures of all pyrimidine nucleosides were started from *anti*-conformation of the sugar moiety with respect to base residue. Intramolecular H-bond enthalpies were obtained by comparing of two different heats of formation of the nucleoside molecule which were calculated in cases «with» and «without» intramolecular H-bonds. For more details of calculations see [17, 19].

Results and Discussion. The resulting lowest energy structures of calculated pyrimidine nucleosides cytidine (Cyd), uridine (Urd), thymidine (Thd) and their deoxyribo-analogues (dCyd, dUrd, dThd respectively) are shown in Figure. All the pyrimidine nucleoside sugar moieties are in *anti*-conformation with respect to the base residues and in *gauche-gauche* conformation around C4'-C5' bond. This is in accordance with the results of previous molecular modelling [5], so as semiempirical quantum-chemical MNDO [4] and CNDO/2 [12] calculations in which the preference of this conformation for pyrimidine nucleosides was shown. In Figure, calculated values of the χ (O4'C1'N1C2 dihedral angle), which characterizes the *anti*-conformation, are exhibited. As one can see, χ is somewhat higher in ribonucleosides than in deoxyribonucleosides. Besides, in cytidine nucleosides χ values are obviously higher than in uridine and thymidine ones.

In the structures shown in the Figure the calculated intramolecular H-bonds are presented forming the special web around the each nucleoside molecule. We have found three common types of intramolecular H-bonds in pyrimidine nucleosides: H-bonds involving atoms of the base residue and the sugar moiety, H-bonds between the base fragment atoms and H-bonds between the furanose ring atoms. The geometric and energetical parameters of the calculated intramolecular H-bonds are presented in Table 1. For comparison, the intramolecular H-bonds of the different organic compounds have energies $2\div10$ kcal/mol [20]. It must be noted that all intramolecular H-bonds, except N4H'...N3 (here and below — H' is the proton of $\leq CNH_2$ fragment of the Cyt base residue involved in the NH...O intermolecular H-bond upon Gua:Cyt Watson-Crick base pairing and H'' is the another aminoproton) (in Cyd and dCyd) and C6H...O5', are considered in isolated pyrimidine nucleosides for the first time.

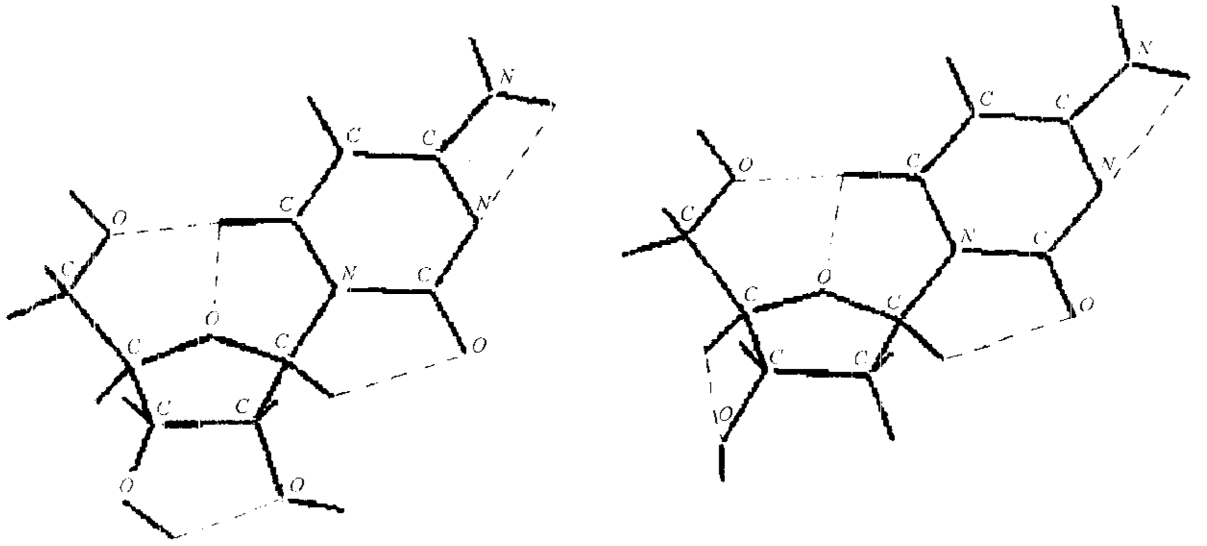
The bifurcated intramolecular H-bonds in organic chemistry are known to be mutually affected [20, 21]. In nucleosides, the web of the intramolecular H-bonds include not only bifurcated, but also the H-bonds which are competitors in formation of nucleoside conformation (for example, H-bonds with C6H and C2=O2 groups) (Figure). This is because they demonstrate pronounced cooperative effect: all H-bonds are mutually weakened by 0.29, 0.60 and 0.80 kcal/mol for Cyd, Urd and Thd respectively, and these values are much higher for dCyd, dUrd and dThd: 1.10, 2.10 and 2.16 kcal/mol respectively. Resulting enthalpy of all intramolecular H-bonds are 18.07 and 14.32 kcal/mol for Cyd and dCyd, 19.21 and 15.64 kcal/mol for Urd and dUrd, 18.97 and 15.41 kcal/mol for Thd and dThd.

Our results exhibit the influence of the intramolecular H-bonds on the physico-chemical characteristics of the nucleosides: heat of formation, dipole moment, first adiabatic ionization potential and the fundamental vibration frequencies — some of these parameters are shown in Table 2.

The data presented in Table 3 demonstrate the most prominent structural peculiarities of the nucleosides and the effect of the intramolecular H-bonds on the conformation and mutual orientation of their structural fragments.

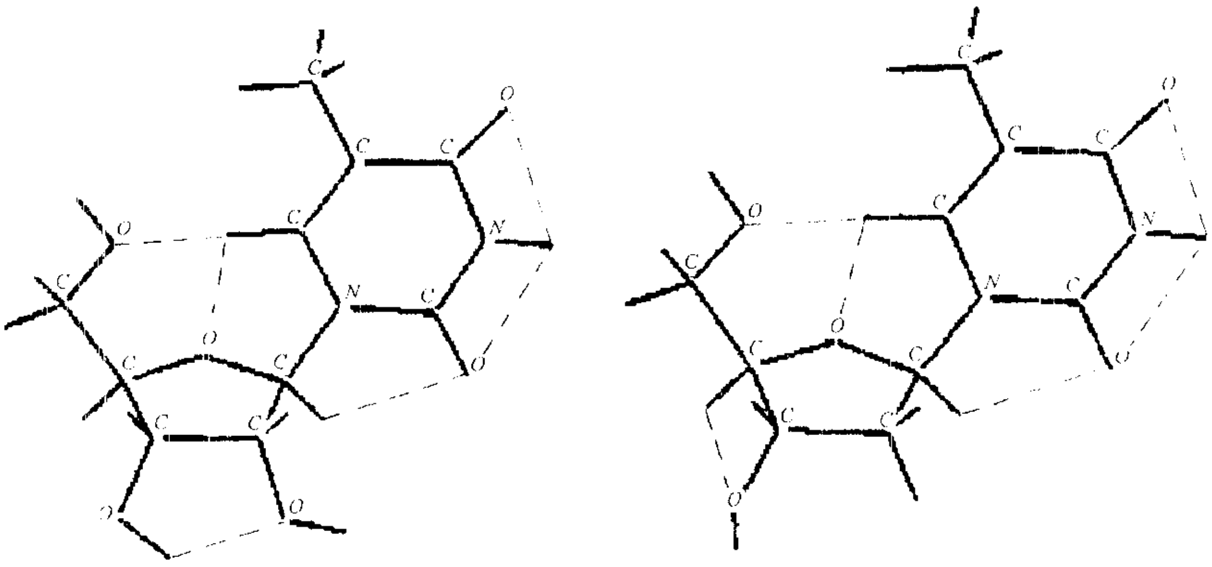
Intramolecular H-bonds between the base residue and the sugar moiety and nonrigidity of the pyrimidine nucleoside. C1'H...O2=C2 intramolecular H-bond. Earlier attention have not been put on the role of C1'H group in the hydrogen bonding between base residue and sugar moieties in nucleosides. There were assumptions about the participation of C2=O2 in the H-bond but they were concerned with O2'H group in pyrimidine nucleosides [4, 8, 9].

Recently we studied the effect of N1 methylation of pyrimidine nucleotide bases on their structural nonrigidity [22]. There the intramolecular H-bond between C2=O2 and C1H of methyl group have been found (in agreement with further *ab initio* investigations [23]) which serve us as the model for the C1'H...O2=C2 interaction in nucleosides. The exist-



Cyd, $\chi=139.3^{\circ}$

dCyd, $\chi=135.6^{\circ}$



Thd, $\chi=136.8^{\circ}$
(Urd, $\chi=136.9^{\circ}$)

dThd, $\chi=133.0^{\circ}$
(dUrd, $\chi=133.5^{\circ}$)

Fully optimized by MNDO/H structures of pyrimidine nucleosides with the network of the intramolecular hydrogen bonds (χ is the O4′C1′N1C2 dihedral angle) ence of C1′H...O2=C2 H-bond can be evidenced by diffuse low frequency band — satellite of fundamental stretching vibration of C1′H centered at ≈ 2785 cm^{-1} in the nucleosides IR spectra [24].

The enthalpies of the C1′H...O2 intramolecular H-bond was shown (Table 1) to be of the same order of value in uridine and thymidine nucleosides, but they are by 0.10÷0.16 kcal/mol smaller in cytidine ones: 3.47 kcal/mol (Cyd) and 3.57 kcal/mol (Urd and Thd); 3.67 kcal/mol (dCyd) and 3.83 kcal/mol (dUrd and dThd). Besides, one can see that in ribonucleosides C1′H...O2 H-bonds are by 0.20÷0.26 kcal/mol weaker than in their deoxyribo-analogues. These tendencies are in accordance with

Table 1
Energetic and geometric charecteristics of intramolecular hydrogen bonds in Cyd, Urd, Thd and their deoxyribo-analogues

Nucleoside	H-bond AH...B	Enthalpy, kcal/mol	Distance H...B, Å	Angle $\widehat{AHB}$ (degrees)
Cyd	N4H′...N3	2.92	1.29	74.6
	C1′H...O2	3.47	1.57	109.8
	C6H...O5′	4.36	1.70	172.3
	C6H...O4′	3.23	1.51	103.3
	O3′H...O2′	4.38	1.49	122.1
dCyd	N4H′...N3	2.76	1.29	74.8
	C1′H...O2	3.67	1.57	109.9
	C6H...O5′	3.78	1.70	171.6
	C6H...O4′	2.72	1.52	103.2
	C4′H...O3′	2.49	1.25	79.6
Urd	N3H...O2	2.21	1.27	69.4
	N3H...O4	2.19	1.26	68.2
	C1′H...O2	3.57	1.58	110.0
	C6H...O5′	4.48	1.68	172.7
	C6H...O4′	3.14	1.51	104.8
	O3′H...O2′	4.22	1.49	122.0
dUrd	N3H...O2	2.29	1.27	69.7
	N3H...O4	2.22	1.26	68.3
	C1′H...O2	3.83	1.59	109.8
	C6H...O5′	4.00	1.68	171.6
	C6H...O4′	2.73	1.51	104.7
	C4′H...O3′	2.67	1.25	79.7
Thd	N3H...O2	2.23	1.28	69.2
	N3H...O4	2.38	1.30	65.5
	C1′H...O2	3.57	1.58	110.2
	C1′H...O2	3.98	1.69	172.6
	C6H...O5′	3.35	1.50	106.6
	O3′H...O2′	4.26	1.49	122.0
dThd	N3H...O2	2.26	1.27	69.6
	N3H...O4	2.37	1.24	68.9
	C1′H...O2	3.83	1.59	110.0
	C6H...O5′	3.49	1.69	171.6
	C6H...O4′	2.94	1.50	106.4
	C4′H...O3′	2.68	1.25	79.7

Table 2
Some physico-chemical characteristics and frequencies of torsional vibrations of pyrimidine nucleosides in cases «with» (H ≠ 0) and «without» (H = 0) intramolecular hydrogen bonds

Nucleoside	Total energy, kcal/mol		Heat of formation, kcal/mol		Dipole moment, D		Ionization potential, eV		Torsion frequency, cm^{-1}	
	H ≠ 0	H = 0	H ≠ 0	H = 0	H ≠ 0	H = 0	H ≠ 0	H = 0	H ≠ 0	H = 0
Cyd	−82299.70	−82281.63	−201.37	−183.30	5.62	5.33	9.32	9.42	38	26
dCyd	−74864.20	−74849.89	−155.80	−141.48	7.02	5.14	9.16	9.46	36	22
Urd	−84630.60	−84611.39	−253.74	−234.53	4.56	4.16	9.65	9.79	33	26
dUrd	−77195.52	−77179.88	−208.59	−192.95	6.87	6.00	9.51	9.72	36	21
Thd	−88240.98	−88222.01	−261.02	−242.05	4.57	4.97	9.51	9.65	32	19
dThd	−80805.94	−80790.54	−215.91	−200.50	6.87	5.96	9.37	9.57	33	19

Table 3
Structural perturbation of pyrimidine ribo- and deoxyribonucleosides in cases «with» (H ≠ 0) and «without» (H = 0) intramolecular hydrogen bonds (the data obtained from OUP Molecular Modelling Package)

Dihedral angles (degrees)	Nucleoside					
	Cyd		dCyd		Urd	
	H ≠ 0	H = 0	H ≠ 0	H = 0	H ≠ 0	H = 0
H'N4C4N3	13.5	17.1	13.7	16.6	—	—
H''N4C4N3	−41.3	−34.7	42.4	−35.7	—	—
C1'N1C2N3	−4.4	−15.1	−0.8	−12.2	−4.3	−19.4
C1'C1'N1C2	−20.7	−28.8	−18.2	−5.7	−18.1	20.8
C1'C2'C3'C4'	0.8	10.3	−0.1	6.4	0.3	9.3
O4'C4'C3'C2'	0.5	−19.6	1.8	−17.2	0.8	−18.5
O4'C1'N1C2	40.7	34.7	44.5	56.9	43.1	42.6
O5'C5'C4'C3'	71.2	61.0	70.0	68.9	70.4	61.1
H5'O5'C5'C4'	3.5	−2.8	1.0	−88.8	2.9	−6.5
O2'C2'C3'C4'	63.2	70.2	—	—	63.0	69.3
O3'C3'C2'C1'	−58.1	−43.3	−64.1	−54.5	58.5	−44.3

Dihedral angles (degrees)	Nucleoside					
	dUrd		Thd		dThd	
	H ≠ 0	H = 0	H ≠ 0	H = 0	H ≠ 0	H = 0
C1'N1C2N3	0.5	−15.5	−4.1	−21.8	0.0	−17.3
C1'C1'N1C2	16.2	−3.7	−18.1	−16.4	−15.6	−6.0
C1'C2'C3'C4'	−0.6	−3.5	−0.4	−0.6	−1.8	3.4
O4'C4'C3'C2'	2.2	−12.9	1.3	−10.6	3.0	−12.9
O4'C1'N1C2	46.5	59.0	43.2	46.5	47.1	56.7
O5'C5'C4'C3'	69.5	60.5	70.4	60.8	69.6	60.3
H5'O5'C5'C4'	0.3	−15.9	3.7	−10.0	0.9	−16.0
O2'C2'C3'C4'	—	—	62.4	57.6	—	—
O3'C3'C2'C1'	−64.8	−57.6	−59.2	−61.2	−65.9	−57.7

the peculiarities of the base residue orientation: larger values of χ (Figure) and H1′C1′N1C2 dihedral angles (Table 3) correspond to molecules having weaker C1′H...O2 H-bonds.

On the other side, accordingly to our data in Cyd, Urd and Thd the O2′...O2 distances are respectively 3.57 Å, 3.70 Å and 3.82 Å and O2′HO2 angles values are 113°, 108° and 116°. Such interaction of O2′H with C2=O2 can reduce the energy of nucleoside not more than by 0.35 kcal/mol. This fact is in agreement with the same conclusion of Young and Kallenbach [25] that O2′H is unlikely to be involved in H-bond with base atoms because of the poor stereochemical fit.

Intramolecular H-bonds involving C6H group and the sugar moiety oxygens. Earlier, assumptions have been made about the interaction of C6H pyrimidine group with ribose oxygens in the experimental studies [7, 10, 26—28]. We have found two bifurcated intramolecular H-bonds with the C6H group participation in pyrimidine nucleosides: C6H...O5′ and C6H...O4′ (Figure, Table 1).

From the data in Table 1 one can see, that the enthalpies of C6H...O5′ H-bond in Thd and dThd (3.98 and 3.49 kcal/mol respectively) are by 0.29÷0.51 kcal/mol smaller than in other nucleosides correlating with the larger C6H...O5′ H-bond lengths. The PM3 calculated H...O5′ distances [11] show the same tendency: 1.851 Å in pdT is larger than 1.836 Å in pdC. Accordingly to results of CNDO/2 calculation [12] the C6H...O5′ H-bond in Urd is not weaker than 2 kcal/mol — it is the contribution of this H-bond to the energy of *gauche-gauche* conformation of nucleoside. Further stabilization of such conformation in polynucleotides was predicted due to the strengthening of C6H...O5′ H-bond when O5′ belongs to PO_4^- [12]. It may be the reason for a number of studies concerning the C6H...O5′ intramolecular H-bond interactions in nucleotides (see [6—9, 11, 12, 25, 26] and bibliography there).

The intramolecular H-bond C6H...O4′ have not been found in above mentioned studies. In thymidine nucleosides the largest enthalpics of C6H...O4′ H-bond are observed (Table 1), so the intramolecular H-bonds involving C6H group in Thd and dThd have the closer to each other enthalpy values ($\Delta E = 0.63$ and 0.55 kcal/mol respectively) than other nucleosides ($\Delta E = 1.06 \div 1.34$ kcal/mol). Besides, in ribonucleosides C6H...O5′ and C6H...O4′ are by 0.41÷0.58 kcal/mol stronger than corresponding H-bonds in deoxynucleosides. These features of the H-bond network of nucleosides have reflections in their structures: larger values of χ (Figure) and H1′C1′N1C2 angles (Table 3) are observed in molecules with weaker C1′H...O2 H-bonds and stronger C6H...O5′ and C6H...O4′ H-bonds.

From the Table 3 one can conclude, that the neglecting of the intramolecular H-bonds results in the essential changes in the dihedral angle values of nucleosides, especially consisting with glycoside N1C1′ bond, that reach 19° for nonhydrogen atoms and 90° for protons. Thus, intramolecular H-bonds involving atoms of the base residue and the sugar moiety (C1′H...O2, C6H...O4′ and C6H...O5′) put the important contribution in the stabilization of *anti*-conformation of pyrimidine nucleosides (and C6H...O5′ H-bond additionally support also *gauche-gauche* conformation [12]).

The intramolecular H-bonds between the base residue and the sugar moiety also affect the dynamics of pyrimidine nucleosides, particularly the torsion motion. In the Table 2 we have compared the calculated frequencies of torsion (libration) vibrations (ω_χ) in nucleosides in cases «with»($H \neq 0$) and «without» ($H = 0$) intramolecular H-bonds. According to our results, these low frequency torsional vibrations are not only anharmonical but also anisotropic: the direction of decreasing of the H1′C1′N1C2 dihedral angle value of nucleoside is preferred.

Intramolecular H-bonds and structural nonrigidity of the base residues. In the optimized structures of Cyd, Urd, Thd and their deoxyribo-analogues the intramolecular H-bonds between the base residue atoms are observed: N4H′...N3 H-bond in cytosine and N3H...O2, N3H...O4 in uracil and thymine bases (Figure, Table 1).

Cytidine ribo- and deoxyribonucleosides have the weaker N4H′...N3 H-bond (2.92 and 2.76 kcal/mol respectively) in comparison with isolated Cyt nucleotide base, where such H-bond have the enthalpy 2.99 kcal/mol [18]. Existence of the N4H′...N3 H-bond in anomalous nucleoside 6-azaCyd was fixed experimentally by means of PMR spectroscopy [29].

In Ura, the calculated enthalpy of N3H...O2 intramolecular H-bond (1.94 kcal/mol [18]) is smaller than and of N3H...O4 H-bond (2.22 kcal/mol [18]), while in Urd and dUrd N3H...O2 H-bond is somewhat stronger than N3H...O4 (Table 1) — it must be due to the sugar moiety presence and its H-bonding with the base, but the mechanism of this effect is rather complicated.

The base residues of Cyd, Urd and Thd nucleosides and their deoxyribo-analogues all have slightly puckering base rings, in accordance with the data of the previous quantum-chemical structural investigations of free nucleotide bases [19]. Maximal deviations from planarity are observed for the N1C1′ glycoside bond region of the pyrimidine nucleoside base rings with dihedral angle values not more than 2.6°, 3.7°, 3.6° for Cyd, Urd, Thd and 3.7°, 5.9°, 6.1° for dCyd, dUrd and dThd respectively. It would be noted that the neglecting of the intramolecular H-bonds in the base residues results in increasing of the base ring puckering: corresponding dihedral angle reach the values 11.7°, 9.6°, 9.3° for Cyd, Urd, Thd and 8.0°, 6.5°, 7.1° for dCyd, dUrd, dThd respectively. Such behavior of the base rings upon the influence of intramolecular H-bonds shows that in the nucleosides the bases are also stereochemically nonrigid fragments [19].

The cytosine base in the nucleoside has the asymmetrically pyramidal aminogroup, so as Cyt in the isolated state [19, 30]. The N4H′...N3 intramolecular H-bond affects the parameters of $\geq CNH_2$ fragment pyramidality (Table 3). The character of potential energy hypersurface which determines stereochemical nonrigidity of Cyt base remains almost unchanged [31]. The base residues with aminogroups in nucleosides are usually considered as planar rigid structures [3, 7, 9—12]. In contrast with this approach, our data evidenced that the planar inversion barrier of $\geq CNH_2$ fragment of Cyt residue in nucleoside is 1.1 (Cyd)—1.2 (dCyd) times higher than in free Cyt (0.15 kcal/mol [32]) and consist 0.17 and 0.18 kcal/mol respectively. These data exhibit particularly that the $p\pi$-conjugation of lone electron pair of N4 with π-electron system of the base ring reduce upon the transition from nucleotide base to nucleoside.

The internal rotation barriers of the $\geq CNH_2$ fragment mainly decrease in nucleosides in comparison with free Cyt and consist 3.37, 10.56 kcal/mol for Cyd and 2.33, 10.46 kcal/mol for dCyd (3.72, 10.47 kcal/mol for Cyt [17]) for rotation to the N3 atom and C5H bond respectively. The intermediate state with two aminoprotons turned to the N3 atom (with planar base ring and plane symmetrical location of protons) is stabilized by the bifurcated pair of intramolecular N4H′...N3 and N4H″...N3 H-bonds with the resulting enthalpy 3.59 and 3.55 kcal/mol for Cyd and dCyd respectively. The internal rotation of aminogroup is the dipole active process, transition dipole moment (≈ 0.8 D) lies in the ring plane in 90° with respect to C4N4 bond.

Intramolecular H-bonds and nonrigidity of the sugar moiety. Analysis of a number of crystal structures of nucleosides shows that furanose ring is usually nonplanar conformationally nonrigid molecule [2]. Theoretical studies and several NMR experiments show for furanose two preferred ring puckering conformations, C2′-endo and C3′-endo, with almost equal energy and 2—5 kcal/mol barrier of interconversion through O4′-endo intermediate conformation (see [5, 6] and bibliography there).

According to our data, all energy differences between C2′-endo, C3′-endo and O4′-endo conformations of furanose ring in the pyrimidine nucleosides are find to be not more than 0.95 kcal/mol. This fact is in agreement with the results of the Levitt and Warshel [33] on the force field calculation of energetic profile of furanose ring in nucleosides, where such energy differences including repuckering barrier consist not more than 0.60 kcal/mol.

In pyrimidine nucleosides with either C2′-endo or C3′-endo sugar puckering modes the ribose atoms are involved into O3′H...O2′ H-bonds, while the 2′-deoxyribose atoms form C4′H...O3′ H-bonds, which are more than 1.5 times weaker than the O3′H...O2′ in ribose (Figure, Table 1). In spite the fact that in crystal structures of some nucleoside derivatives [27] O2′H...O3′ intramolecular H-bond occurs more frequently than O3′H...O2′, in free pyrimidine nucleosides the fully optimized conformation with O2′H...O3′ H-bond is 4.00÷5.95 kcal/mol higher in energy than conformation shown in Figure.

The intramolecular H-bonds involving the sugar moiety atoms reduce the furanose ring puckering parameters (Table 3) and cause the essentially free interconvertion at room temperatures. Increase of dihedral angle values, which characterized the furanose ring puckering, is observed when the amplitude of torsion motions around glycoside C1′N1 bond increase. This is due to the weakness of the base residue-sugar H-bonds caused by the torsion motions. Other studies using AM1 and PM3 semiempirical quantum-chemical methods [11], in which the full optimization process involve calculation of hydrogen bonding interactions between all accessible atoms, also show the unusually small values of the sugar puckering parameters. Influence of intramolecular H-bonds results in decrease of the interconvertion barrier of furanose ring in pyrimidine nucleosides.

O5′- and O3′-deprotonated nucleoside molecules. We have modelled the charge situation in nucleotides and polynucleotides (polyanions) by deprotonation of Cyd and dCyd molecules at O5′ and O3′ sites. This was done in order to elucidate the effect of negative charged phosphate groups on the stereochemical nonrigidity and intramolecular H-bond web parameters of nucleosides.

It was found that the change of the charge state of nucleoside molecule by the deprotonation of its sugar moiety does not destroy the intramolecular H-bond web. The deprotonation results in essential perturbation of the balance of intramolecular H-bonds in nucleoside (Table 4) and increasing of their cooperativity ($\Delta = -2.10 \div 3.64$ kcal/mol). In the nucleosides deprotonated at the O5′ site C6H...O5′ H-bond becomes much stronger, that results in the weakness of C1′H...O2 and other H-bonds (Table 4). This is in accordance with the predicted in [12] strengthening of C6H...O5′ H-bond when O5′ belongs to PO_4^-. In the case of O3′ deprotonation site C6H...O5′ becomes weaker (Table 4) because of the turning of O5′ proton to C6H (Table 5), which reduces the interaction of O5′ atom with C6H group. The data in Table 5 describe the changes of most prominent structural parameters — dihedral angles in case of neglecting of intramolecular H-bonds in the anions under discussion.

The structural perturbation of cytidine nucleosides under the deprotonation can be considered particularly by comparison of χ values of anions: 134.6° ($Cyd_{O5'}^-$), 133.6° ($dCyd_{O5'}^-$), 140.1° ($Cyd_{O3'}^-$) and 121.2° ($dCyd_{O3'}^-$) with corresponding angles in Cyd and dCyd (Figure). The deprotonation of the sugar moiety affects also the stereochemical nonrigidity of the base residue in nucleoside molecule. In Cyd^- and $dCyd^-$ the aminogroups become more pyramidal, the base ring puckering parameters decrease (Table 5), their planar inversion barriers are 1.7 (Cyd^-)—1.8 ($dCyd^-$) times higher and the internal anisotropic rotation barriers are reduced by 20—40 %. These effects can be explained particularly by the reducing of pπ-conjugation in the base residue upon the influence of the surplus negative charge in the sugar moiety. The reverse is also true: possible intermolecular interactions which change the structural and dynamic properties of the base, especially in the vicinity of ≤ CNH_2 fragment attachment, cause the changes in charge distribution in the sugar moiety.

Thus the local change of the charge state of polynucleotide, for example, in the protein-nucleic

Table 4

Energetic and geometric characteristics of intramolecular hydrogen bonds in deprotonated Cyd and dCyd ($Cyd_{O5'}^-$, $Cyd_{O3'}^-$, $dCyd_{O5'}^-$, $dCyd_{O3'}^-$ anions)

Anion	H-bond AH...B	Enthalpy, kcal/mol	Distance (H...B), Å	Angle $\hat{ABC}$ (degree)
$Cyd_{O5'}^-$	N4H′...N3	2.36	1.30	76.0
	C1′H...O2	2.51	1.60	104.3
	C6H...O5′	16.39	1.54	173.3
	C6H...O4′	2.07	1.53	101.3
	O3′H...O2′	2.28	1.46	123.1
$dCyd_{O5'}^-$	N4H′...N3	3.08	1.30	76.1
	C1′H...O2	3.37	1.61	109.2
	C6H...O5′	15.73	1.54	174.7
	C6H...O4′	2.35	1.54	100.5
	C4′H...O3′	1.96	1.35	77.9
$Cyd_{O3'}^-$	N4H′...N3	3.09	1.30	76.3
	C1′H...O2	3.44	1.76	110.1
	C6H...O5′	3.07	1.68	175.7
	C6H...O4′	2.49	1.51	102.8
	C4′H...O3′	1.56	1.30	75.4
$dCyd_{O3'}^-$	N4H′...N3	3.09	1.30	76.3
	C1′H...O2	3.52	1.64	109.9
	C6H...O5′	0.37	2.49	121.0
	C6H...O4′	2.97	1.56	100.2
	C4′H...O3′	1.81	1.32	75.1

Table 5

Perturbation of geometric structure of deprotonated Cyd and dCyd in cases «with» (H ≠ 0) and «without» (H = 0) all intramolecular hydrogen bonds (data from the OUP Molecular Modelling Package)

Dihedral angles (degree)	Anion							
	CydO5′$^-$		dCydO5′$^-$		CydO3′$^-$		dCydO3′$^-$	
	H ≠ 0	H = 0	H ≠ 0	H = 0	H ≠ 0	H = 0	H ≠ 0	H = 0
H′N4C4N3	13.4	18.4	12.9	18.1	11.5	16.7	11.8	16.5
H″N4C4N3	−46.4	−38.1	−47.2	−38.8	−48.5	−40.7	−47.8	40.9
C1′N1C2N3	−5.6	−13.6	−2.3	−11.3	−1.4	−12.8	1.8	−7.0
H1′C1′N1C2	−16.0	−27.8	−15.8	−22.2	−26.7	−15.2	−5.7	9.6
C1′C2′C3′C4′	−7.1	0.9	−2.1	4.4	9.8	15.2	11.2	11.3
O4′C4′C3′C2′	11.3	−4.7	6.6	−8.7	−2.0	−23.0	−11.0	−15.6
O4′C1′N1C2	45.4	36.0	46.4	41.0	39.9	49.7	58.8	73.4
O5′C5′C4′C3′	64.0	57.7	63.3	55.4	66.9	59.5	−86.0	89.9
H5′O5′C5′C4′	—	—	—	—	81.2	—65.1	69.8	63.5
O2′C2′C3′C4′	55.0	59.8	—	—	69.2	74.8	—	—
O3′C3′C2′C1′	−65.1	−52.0	−59.4	−52.6	−43.6	−37.8	−44.1	−44.0

acid recognition processes, can affect its structural and dynamical properties, particularly the local curvature [34], through the disturbance of pπ-conjugation of base residue.

Transformation of the intramolecular H-bond web in polynucleotides. The intramolecular C1′H...O2′, C6H6...O4′, C6H6...O5′ H-bonds are expected to be also in polynucleotides because there the pyrimidine base and sugar are in *anti*-conformation [4, 6]. Moreover, the C6H...O5′ H-bond in the polynucleotide structures [9, 25] is predicted to be strengthened when O5′ belongs to PO_4^- [12]. It seems to be likely that O2′H groups in polynucleotides are involved in H-bond with 3′-PO_4^- group (directly or through water molecules) [35] and with O4′ of neighbour furanose ring along the polynucleotide strand [9, 25, 36].

Indeed, it is known that poly(dT) and poly(dU) don't form single strand helical structure and on the same conditions that poly(rT) poly(rU) does (see [6] and bibliography there). And study of CD spectra leads Ts'o [13] to the conclusion that poly(rC) single strands have more secondary structure (stacking) than poly(dC) in the same conditions. Hydrogen bonding of O2′H groups with 3′phosphate and neighbour furanose can successfully explain such relative stability of RNA strands with respect to DNA ones.

Conclusions. The structural and dynamical properties of pyrimidine nucleosides are shown at first to be formed with participation of the web of cooperative intramolecular H-bonds with enthalpies ≈ 2—4 kcal/mol. Involving of any site of the nucleoside into intermolecular hydrogen bonding (Watson-Crick pairing, specific solvent, crystal packing and so on) can perturb its intramolecular H-bond web that results in changes of structural and dynamical parameters of nucleoside.

Pyrimidine nucleosides, their base residues and sugar moieties are stereochemically nonrigid structures. The aminogroup pyramidality and plane inversion barrier increase in nucleoside in comparison with the nucleotide base and these parameters also depend on the charge situation on the sugar moiety. The intramolecular H-bonds effect the stereochemical structure of nucleosides, conformation and mutual orientation of their fragments, particularly, intramolecular H-bonds involving atoms of the base residue and sugar moiety put the important contribution in the stabilization of *anti*-conformation of pyrimidine nucleosides.

Intramolecular H-bonds affect the physico-chemical characteristics of pyrimidine nucleosides (heat of formation, dipole moment, first adiabatic ionization potential and the charge distribution), and they also can change the dynamical characteristics of nucleoside: barriers of the base residue and the sugar moiety interconvertion, frequencies of the torsional vibrations in nucleoside.

Intramolecular H-bonds in polynucleotides take part in the formation of the nucleic acid architecture and nonlinear dynamic properties. Local change of the charge distribution in the sugar region of polynucleotide, for example, in the protein-nucleic acid recognition processes, can affect its structural and dynamical properties through the disturbance of $p\pi$-conjugation of base residue.

Taking into account of the intramolecular H-bonds is important for the NMR and IR spectra interpretation, for molecular modelling, for the elucidation of mechanisms of structural and dynamic changes under molecular recognition processes.

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Внутрішньомолекулярні водневі зв'язки та структурна нежорсткість піримідинових нуклеозидів

Резюме

Оптимізовані структури цитидину, уридину і тимідину, їхніх дезоксирибо-аналогів та деяких O5'-, O3'-депротонованих похідних отримані за допомогою напівемпіричного квантово-хімічного методу MNDO/H. Виявлено сітки внутрішньомолекулярних водневих зв'язків піримідинових нуклеозидів та вивчено їхній вплив на стереохімічну структуру молекул (зокрема, на стабільність anti-конформації), фізико-хімічні параметри (теплоту утворення, дипольний момент, потенціал іонізації та розподіл зарядів), а також на динамічні характеристики піримідинових нуклеозидів (бар'єри інтерконверсії, частоти торсійних коливань). Обговорюється присутність внутрішньомолекулярних водневих зв'язків у полінуклеотидах та їхнє значення у формуванні структури та нелінійної динаміки нуклеїнових кислот.

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Внутримолекулярные водородные связи и структурная нежесткость пиримидиновых нуклеозидов

Резюме

Оптимизированные структуры цитидина, уридина и тимидина, их дезоксирибо-аналогов и некоторых O5'-, O3'-депротонированных производных получены с помощью полуэмпирического квантовохимического метода MNDO/H. Обнаружены сетки внутримолекулярных водородных связей пиримидиновых нуклеозидов и исследовано их влияние на стереохимическую структуру молекул (в частности, на стабильность anti-конформации), физико-химические параметры (теплоту образования, дипольный момент, потенциал ионизации и распределение зарядов), а также на динамические характеристики пиримидиновых нуклеозидов (барьеры интерконверсии, частоты торсионных колебаний). Обсуждается наличие внутримолекулярных водородных связей в полинуклеотидах и их значение в формировании структуры и нелинейной динамики нуклеиновых кислот.

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