On Spectral Identification of DNA-Base Pairs Polymorphism

V.M. KOMAROV

Institute of Cell Biophysics, Russian Academy of Sciences, 142292 Pushchino, Moscow region, Russia

Abstract. The results of MNDO-PM3 theoretical study of H-bonds liability of watson-crick base pairs are discussed. Some microwave and IR spectral criteria are suggested for identification of hidden polymorphism of DNA base pairing.

Key words: Hidden polymorphism, inertial defects, nitrous base pairs, normal modes analysis, propeller twisting, quantum-chemical calculations

For computer simulation of the internal linear or nonlinear dynamics of DNA molecule a precise knowledge of crucial conformational and energetic parameters determining spatial organization of this biopolymers [1–3] is of great importance. Rigorous geometrical isomorphism of complementary AT and GC base pairs is the most salient characteristic of the double-helical polynucleotide structures.

The assertion about the pairs isomorphism stability both in synthetic polydinucleotides and in native nucleic acid structures was postulated many years ago. It is based on unity and planarity of H-bonding form of the pairs. Recent comprehensive analysis of X-rays data reveals, however, a wide diversity of base pairing noncoplanarities in crystals of nucleotides and nucleosides [4–12]. This noncoplanarity, classified by propeller twisting angle ω and tilting κ of base planes in a pair (according to IUPAC/IUB nomenclature [13]) is rather significant and typical for almost all homo and hetero hydrogen bonded base pairs. As this take place the magnitude of ω angle turned out to be uniformly distributed between positive and negative values. The arguments presented in [5–6] indicate that base pairs nonplanarity, a shape desperateness of complementary pairs, is likely a peculiarity of the pairs themselves and not the crystal field effects.

In previous papers [14–18] we have theoretically shown that the noncoplanarity of Watson-Crick pairs as well as the non-uniqueness of their H-pairing form (i.e. their hidden polymorphism) is most probably a primordial, fundamental property of the pairs. It may be initiated by noncorrelated, spontaneous, inversion-symmetry movements of hydrogen atoms of pyramidal amino groups involved in bases H-binding.

It should be noted that there are many *ab initio* quantum chemical studies on the amino group pyramidal problem in the structure of single nitrous bases and

isolated base pairs [35, 36, 38, 39, 47–60]. Due to the estimated small energy difference ($\leq 1 \text{ kcal mol}^{-1}$, depending on the method used) between planar and nonplanar optimized geometry of the molecule (and of H-bonded complexes) it is widely accepted as a quasiplanar model of complementary base pairs and its constituents [34–36, 41, 44, 56–60].

He believes that the MP2/6-31G** approach is a more effective and reliable tool for this type of conformational and related spectral molecular calculations. Some overestimation of nonplanarity of DNA-bases amino group by this technique is mentioned [50]. Nevertheless, it is this good correspondence between the experimentally derived rotational constants A , B , C [25–26] and those calculated at MP2 level theory suggests [57] an essential C_s planar structure of nitrous bases with amino group.

No calculations, however, of an additional spectroscopic rotational constant such as the inertial defect, Δ' , are presented in [57]. (Parameter Δ' , as is known [23], is a measure of molecule nonplanarity.)

Our estimation of this parameter Δ' , based on the theoretical data of A , B and C from [57], gives us for adenine and cytosine molecules the value $\Delta' \leq -0,0001(u \cdot \text{\AA}^2)$. The experimental value is $\Delta' = -0,2(u \cdot \text{\AA}^2)$ [25–26]. The difference is about three orders! Thus, on our opinion, widely used *ab initio* calculations at correlated MP2 level probably underestimate the stability of amino group pyramidal structure in this type of molecular systems.

No precision experimental data (like a microwave spectroscopy or a gas-phase electronography data) are now available on the detailed geometry of single nitrous bases and H-bonded base pairs.

Because of this, both for the tasks of DNA internal dynamic and for the understanding of DNA structure-function' organization as a whole, it seems rather topical an elaboration of some additional proposals for experimental tests on non-planar character of base pairing geometry.

The main goal of the article is to demonstrate, by using semiempirical, quantum-chemical estimations, that some spectral criteria such as microwave and IR, could be suggested for identification of different geometric forms of Watson-Crick AT(WC) and GC(WC) base pairs.

Method

In the geometry and spectroscopy calculations we used a rather reliable quantum-chemical MNDO technique [19] (of AMPAC 2.1 program package [20]) with a modern, PM3 parametrization [21–22]. The optimization of all conformational degrees of freedom of molecular complexes with the program key 'PRECISE' was followed by spectral estimations. Standard numbering of atoms of nitrous bases in the pairs is presented in Figure 1.

This technique somewhat overestimates the energy of the barriers of intramolecular movements. This is a peculiarity of parametrization scheme employed, which

Figure 1. Watson-Crick base pairing.

must be taken into account in the discussion of appropriate properties of the system. First of all it concerns kinetical processes and rate constant estimations of under-barrier molecular rearrangements. The application of modified MNDO approach to some aspects of dynamical stereoisomerism of single nitrous bases and base pairs is discussed in [61–62].

Table I. MNDO-PM3 calculated and experimental [25–26] values of inertial defect, Δ' , ($u \cdot \text{\AA}^2$) of nitrous base molecules

Inertial defect	A	G	C	T	An
Δ' (theor.) nonplanar:	–0,500	–0,860	–0,500	–3,200	–0,620
planar (at NH_2^-):	–0,001	–0,001	–0,003	–	–0,002
(exper.)	–0,201		–0,220		–0,500

Results

ON THE MICROWAVE SPECTRAL CRITERION OF BASE PAIRS POLYMORPHISM

1. As is known, the value of inertial defect of a molecule is the most sensitive indicator of its nonplanar structure [23]. In the general case, for a molecular system as an asymmetric solid rotator the value of defect Δ' is determined from the microwave data through the rotational spectroscopic constants:

$$\Delta' = I_c - I_a - I_b,$$

where

$I_i = h/8\pi^2 c B_i$ – major moment of inertia of the system under study;
 B_i – rotational spectroscopic constant.

As the experiment indicates for isolated, relatively small and near-planar molecules, this value is around zero, $\Delta' = -0,001(u \cdot \text{\AA}^2)$ [24]. In the case of gas-phase nitrous bases discussed the experiments give the value of inertial defect of about $\Delta' = -0,2(u \cdot \text{\AA}^2)$ [25–26].

We have compared some experimental and our theoretical MNDO-PM3 estimations of this defect Δ' both for nitrous base molecules and for their nearest amino aromatic analog-aniline [27] for purposes of checking the possibilities of the method employed (see Table I).

As it is seen from the table, the observed Δ' values are reproduced with confidence by the semi-empirical technique used. It turns out, however, that dominating contribution to the magnitude of the defect Δ' gives just the amino group pyramidalicity discussed, rather than purine or pyrimidine deformation as it would be expected [25–26]. One may explain somewhat heightened magnitude of the defect Δ' for thymine, $\Delta' = -3,20(u \cdot \text{\AA}^2)$, by the methyl group characteristic contribution to inertial constants of a planar molecule with rigid π -system of conjugation [28].

The results presented give us grounds to expect that an estimation of the value of the inertial defect for Watson-Crick pairs would really be a direct and reliable spectral criterion for identification of different nonplanar forms of the base pairings. Determination of this constants by microwave spectroscopy methods as it

Figure 2. Two-fold polymorphism of watson-crick AT(WC) pair.

Figure 3. Four-fold polymorphism of Watson-Crick GC(WC) pair.

was demonstrated by the example of single nitrous bases [25–26] is a problem been solved.

2. Earlier [17–18] we have pointed out that the six-fold degeneracy of the ‘complementarity’ of AT and GC base pairs is a special feature of Watson-Crick pairs. In accordance with our semi-empirical estimations, AT(WC) pair has two stable symmetrical propeller-like variants of complementary H-pairing, which depend on the orientation of N-H bonds of adenine amino group (‘up’ and ‘down’) relative to the purine plane of the molecule. Variant ‘a’ – AT(WCu), is related to the pair conformation with the angle of propeller twisting $\omega = +13,7^\circ$ and with tilting angle $\kappa = +9,0^\circ$. Variance ‘b’ – AT(WCd), is the configuration with the angles $\omega = -13,7^\circ$ and $\kappa = -9,0^\circ$ (Figure 2).

The GC(WC) pair is marked by the four-fold polymorphism of base pairing: ‘a’ – GC(WCdd), ‘b’ – GC(WCuu), ‘c’ – GC(WCdu) and ‘d’ – GC(WCud) in

accordance with the possible orientations of amino group N-H bonds in guanine and cytosine (Figure 3).

First two conformations with mirror symmetry, 'a' and 'b', are close to the two propeller structures of the AT(WC) pair mentioned above. But here they are more deformed and have the following parameters: for 'a' form $\omega = +11, 2^\circ$ and $\kappa = +13, 9^\circ$ and for 'b' form $\omega = -11, 2^\circ$ and $\kappa = -13, 9^\circ$. Another two symmetrical configurations, 'c' and 'd', are step-like structures. They are characterized by a nearly parallel shift at 1 Å of the base planes relative to each other.

3. Our MNDO-PM3 estimations show that polymorphism of hydrogen bonded canonical pairs is responsible for the following spectrum of possible values of the inertial defect:

- 1) for symmetrical watson-crick AT(WCu) and AT(WCd) forms which are varied in 'up' and 'down' orientation of amino group N-H bonds the defect Δ' should be closed to $\Delta' = -23, 0(u \cdot \text{\AA}^2)$;
- 2) for propeller-like structures of GC(WCdd) and GC(WCuu) forms with a substantial torsion deformation this values should be more large and have the order of $\Delta' = -32, 0(u \cdot \text{\AA}^2)$;
- 3) for slide-shifted structures of GC(WCdu) and GC(WCud) forms the defect Δ' should be minimal and have a magnitude of about $\Delta' = -12, 0(u \cdot \text{\AA}^2)$.

Thus, a significant difference in the inertial defect values obtained cause us to anticipate that the identification of nonplanar, polymorphic forms of the pairs through microwave spectroscopy data is a very promising procedure.

ON THE PECULIARITIES OF VIBRATIONAL SPECTRA OF NONPLANAR FORMS OF BASE PAIRINGS

As it is known, the IR spectra of normal modes are also highly sensitive to the framework distortion of the molecules. There are many examples of experimental IR and Raman spectroscopy studies of nitrous bases and pairs in various media and in liquid and solid phases [29–40]. Some theoretical interpretations of these data are now available [34–36, 38–39, 41–44, 57–60].

He believes that the most vibrational characteristics of isolated nitrous bases are retained in nucleotide pairs and are meaningful in the IR spectra of nucleic acids [29]. But a low volatility and a moderate thermal stability of bases [38] along with the liability of their tautomeric forms give rise to some difficulties in creation of gas-phase spectra of selected base pairs [45]. For this reason the discussion of the features of normal vibrations spectra of the canonical base pairs one can find in literature at a theoretical level for the most part [41, 42, 44, 58–60]. The vibrational analysis of pairs is applied only to the planar (C_s) model of the complex structure.

Table II. MNDO-PM3 calculated and experimental (in Ne gas matrice [34]) spectrum of normal mode frequencies (in cm^{-1}) of isolated adenine molecule*

No. mode	exper.	theor.	interpretation	No. mode	exper.	theor.	interpretation
1	3569s	3538	(NH ₂) asym.str.	20	1008	1005	(Pur + NH ₂) def.
2	3503s	3462	(N9H) str.	21	928	916	(Pur) def.
3	3453s	3440	(NH ₂) sym.str.	22	887	894	(Pur) def.
4		3055	(C8H) str.	23	862	855	(Pur + NH ₂) def.
5		2898	(C2H) str.	24	849	826	(Pur) def.
6		1765	(Pur) str.	25	804	795	(Pur + NH ₂) def.
7		1710	(Pur) comb.str.	26	722	756	— // —
8	1641vs	1663	(NH ₂) scis.	27	716	716	— // —
9	1613vs	1589	(Pur) str.	28	608	602	(NH ₂) wag.
10	1598	1570	(Pur) str. (NH ₂) scis.	29	583	601	(Pur) def.
11	1476s	1494	— // —	30	566	559	— // —
12	1420	1420	(Pur) str.	31	508	504	— // —
13	1390	1388	— // —	32	497	496	— // —
14	1329	1343	(Pur) str. (NH ₂) scis.	33		491	— // —
15	1290	1276	(Pur) str.	34		375	(NH ₂) tors.
16	1241	1192	(Pur) str.	35	311	324	(Pur) def.
17	1133	1159	(N9H) bend.	36	274	278	(NH ₂) tors.
18	1061	1078	(NH ₂) rock.	37	261	254	(Pur) def.
19	1023	1050	(C8H) bend.	38	239	200	(Pur) def. (NH ₂) tors.
19	1023	1050	(C8H) bend.	38	239	200	(Pur) def. (NH ₂) tors.
				39	207	151	(Pur) def.

* Pur – denotes a purine part of the molecule. Vs and s denote a very strong and strong experimental IR intensity of the mode. The other lines are either weak or very weak;

However, no normal mode analysis of nonplanar base pairings in the context of their possible intrinsic polymorphism is now available.

In our studies we have calculated first of all the normal mode absorption spectra of MNDO-PM3 optimized structures of single nitrous bases in harmonic approximation. The IR vibrational frequencies of the molecules with mode interpretation and experimental data are presented in Tables II–V.

As can be seen the results obtained agree satisfactory with spectroscopy data available [34–36, 40] though in the computational technique we did not use a fitting procedure of force constants scaling which is, however, conventional for most similar pure, ab initio quantum-chemical calculations [32, 34–36, 38–39]. Some exceeding of the estimated frequency of the C=O stretched mode over the experimental one is a known salient feature of PM3 parametrization.

One general conclusion here is the following:

Table III. MNDO-PM3 calculated and experimental (gas-phase [40]) spectrum of normal mode frequencies (in cm^{-1}) of isolated thymine molecule*

No. mode	exper.	theor.	interpretation	No. mode	exper.	theor.	interpretation
1		3390	(N1H) str.	20		1007	(Pyr) def.
2	3446	3365	(N3H) str.	21		996	— // —
3		3167	(CH ₃) str.	22		958	— // —
4		3077	— // —	23		888	(C6H) def.
5		3069	— // —	24		820	(C4C5) bend.
6		3008	(C6-H) str.	25		751	(Pyr) def.
7	1750	1942	(C4=O) str.	26		716	(C4O,Pyr) def.
8	1670	1901	(C2=O) str.	27		620	(C2O,Pyr) def.
9		1821	(Pyr) str.	28		554	(Pyr,C4O) def.
10		1500	(Pyr,C4O) str.	29		506	(Pyr,C2O) def.
11		1430	(Pyr) str.	30		467	(N3H) dend.
12		1396	— // —	31		450	(N1H,Pyr) def.
13		1385	— // —	32		413	(N1H) dend.
14		1378	— // —	33		373	(Pyr) def.
15		1357	(N3H) bend.	34		341	(Pyr,2(CO)) def.
16		1336	(Pyr) str. (N1C2) str.	35		303	(Pyr) def.
17		1262	(Pyr) str.	36		268	— // —
18		1206	(N3C4) bend.	37		88	(Pyr,C2O) def.
19		1127	(Pyr) str.	38		85	(CH ₃) def.
				39		74	— // —

* Pyr – denotes a pyrimidine part of the molecule.

For amino substituted purines and pyrimidines of interest a correct reproducing the low-frequency spectrum of normal modes is impossible within the forced planar model of the amino group. The given model one can meet elsewhere in many applications. According to our estimations a valence-force field of such a planar group always gives an imaginary frequencies in spectra of out-of-plane modes of bases. The other authors also mention this circumstance. It does not depend on the method used whether it is a semi-empirical MNDO approach [41, 46] or *ab initio* calculation with different levels of approximations [36, 39, 41, 44, 46–47, 58–60]. Only a deviation from the planarity in the model, i.e. taking into account a small but stable amino group pyramidalicity in nitrous bases, eliminates this artifact.

As for Watson-Crick pairs two more general points need to be made for the results obtained.

First, there is a high density of normal modes in IR vibrational spectra. They are 84 modes for the AT(WC) pair and 81 modes for the GC(WC) pair at the same spectral range from tens cm^{-1} up to 3500 cm^{-1} as for the bases themselves. There-

Table IV. MNDO-PM3 calculated and experimental (polycrystalline-phase [36]) spectrum of normal mode frequencies (in cm^{-1}) of isolated guanine molecule*

No. mode	exper.	theor.	interpretation	No. mode	exper.	theor.	interpretation
1		3524	(NH ₂) assym.str.	22	950s	924	(NH ₂) comb.rock.
2		3465	(N9H) str.	23	884s	895	(NH ₂) comb.rock.
3		3412	(NH ₂) sym.str.	24	850s	808	(C8H) bend.
4		3369	(N1H) str.	25	790s	791	(Pur) def. (NH ₂) rock.
5		3063	(C8H) str.	26	778s	761	(Pur,C=O) def.
6	1702vs	1975	(C=O) str.	27	703	649	— // —
7		1717 (Pur) str.	28	654		632	(Pur,C=O) def.
8	1675	1684	(NH ₂) scis.	29	645	620	(C=O) bend.
9	1638	1645	(NH ₂) comb.scis.	30	601	582	(NH ₂) def.
10	1565	1602	(Pur) str.	31	557	548	(Pur) def.
11	1550s	1563	— // —	32	496	485	— // —
12	1477s	1463	— // —	33		457	— // —
13	1464s	1437	— // —	34		431	(N7H) bend.
14	1375s	1355	— // —	35	404s	390	(N1H) bend.
15	1362	1308	(N1H) bend.	36	348	317	(Pur) def.
16	1261s	1289	(Pur) str.	37		316	— // —
17	1216	1176	(N7H) bend.	38		306	(NH ₂) tors.
18	1174	1137	(Pur) str.	39	243	248	— // —
19	1118	1108	(NH ₂) rock.	40	175s	185	(Pur) def.
20	1042	1053	(NH ₂) comb. rock.	41		117	— // —
21		1047	(C8H) bend.	42		112	— // —

See abbreviations of Table II and Table III.

fore, in this short paper it is advisable to discuss the changes of characteristic mode spectra only for some active atom groups when passing from isolated nitrous bases to their H-bonded complexes. The more so general conformational differences of the pairs are concentrated at the hydrogen bonding region.

The second note is analogous to the above comment on single molecules of bases. No correct description of low-frequency spectra of base pairs is possible within the forced planar model of bases H-binding. So, the fact of simple observation of full low-frequency spectrum of normal modes of isolated pair is a justification of its nonplanarity form.

The detailing AT(WC) and GC(WC) IR spectra we shall draw at normal mode spectral changes of the groups involved in bases hydrogen bonding, i.e. at N1-H and C6=O fragments of purines and at N3-H and C4=O (C2=O) pyrimidine fragments of the molecules and at the amino groups.

Table V. MNDO-PM3 calculated and experimental (in Ar gase matrice [35]) spectrum of normal mode frequencies (in cm^{-1}) of isolated cytosine molecule*

No. mode	exper.	theor.	interpretation	No. mode	exper.	theor.	interpretation
1	3565	3534	(NH ₂) asym. str.	18		953	(Pyr) def.
2	3441s	3431	(NH ₂) sym.str.	19		943	— // —
3	3471s	3399	(NH) str.	20	818	839	(Pyr) def. (NH ₂) rock.
4		3082	(C5H) str.	21	781	795	— // —
5		3019	(C6H) str.	22	747	759	— // —
6	1720vs	1930	(C=O) str.	23	637	678	(C=O) bend.
7		1760	(Pyr) str.		623		
8	1656vs	1670	— // —	24	614	611	(NH ₂) wag.
9	1595	1663	(NH ₂) scis.	25	575	544	(Pyr + NH ₂) def.
10	1539	1510	(Pyr) str. (NH ₂) scis.	26	537	491	(Pyr) def.
11	1475s	1429	(Pyr) str.	27	532	452	(Pyr,C=O) def.
	1422			28		451	(NH) bend.
12	1337	1296	— // —	29	409	353	(NH ₂) tors.
13	1244	1271	— // —	30	360	314	— // —
14	1192	1169	— // —	31	330	273	(NH) bend.
15	1124	1124	(C5H) bend.	32	232	184	(Pyr) def. (NH ₂) tors.
16	1090	1100	(NH ₂) rock.	33	197	120	(Pyr) def.
17	1087	1023	(Pyr) str.				

* See abbreviations of Table II and Table III.

IR SPECTRAL FEATURES OF AT(WC) PAIR

In Table VI we presented calculated frequencies of some characteristic modes of the Watson-Crick AT pair and of its constituents – adenine and thymine.

As we pointed out the AT pair possesses a mirror symmetry of two conformational variants of H-pairing. As a consequence, IR spectra of the forms are identical. So, there is no trivial possibility to suggest an appropriate simple spectral criterion for identification of the pair internal polymorphism. Nevertheless, a spectrum evolution of characteristic modes of the groups active in nonplanar bonding of the bases is well traced here.

NH₂ – asymmetric and symmetric valent vibrations of pyramidal amino group of adenine ($\nu_1 = 3538 \text{ cm}^{-1}$ and $\nu_3 = 3440 \text{ cm}^{-1}$) undergo a long-wave shift in the IR spectrum as it does under N6-H...O hydrogen bond formation with thymine. Corresponding estimations of relative intensity changes show only a minor increasing the asymmetric mode intensity in contrast to rather large (at an order) increasing the intensity of the symmetric mode under this process.

Table VI. Calculated (MNDO-PM3) and experimental characteristic normal mode frequencies (in cm^{-1}) of isolated adenine and thymine and of their Watson-Crick variant of pairing, AT(WC)

Mode type		AT(WC)					
		A		A	T	T	
		exper. [34]	theory	theory	theory	theory	exper. [40]
-N6H ₂	‘asym’	3569	3538	3511			
	‘sym’	3453	3440	3370			
	‘scis’	1641	1663	1647			
	‘scis’ (comb.)	1598	1570	1561			
		1484	1494	1504			
		1346	1343	1352			
	‘rock’	1061	1078	1087			
	‘wag’	583	602	613			
	‘tors’	311	375	465			
	‘tors’ (comb.)	274	278	397			
N3-H	‘str’				2953	3365	3446
	‘bend’				1384	1357	
	‘bend’ (comb.)				729	467	
C2=O	‘str’				1908	1942	1750
	‘bend’				619	620	
C4=O	‘str’				1885	1901	1670
	‘bend’				718	716	
		39 modes		84 modes		42 modes	

NH_2 – *scissoring vibration* of the main tone in adenine ($\nu_8 = 1663 \text{ cm}^{-1}$) is somewhat increased in intensity as well as in the hydrogen complex. And some decreasing mode frequency arises. On the contrary to this vibration the combined, scissoring modes of low frequency are a little changed in transition to hydrogen bonded complex.

NH_2 – *rocking vibration* ($\nu_{18} = 1078 \text{ cm}^{-1}$) is weakly shifted to short-wave region in the infra-red spectrum of the pair without changing the intensity.

NH_2 – *out of plane, wagging mode* of adenine ($\nu_{28} = 602\text{ cm}^{-1}$) has a marked combined nature with mixing torsion deformations of a purine framework. This vibration exhibits a small short-wave shift of line absorption and some decreasing in intensity under H-bonding.

NH_2 – *weak intensity, torsing vibrations* ($\nu_{34} = 375\text{ cm}^{-1}$ and $\nu_{36} = 278\text{ cm}^{-1}$) are subjected to the most perturbations in IR spectrum. Nonplanar base pairing at the pyramidal amino group produces the 100 cm^{-1} frequency raising of the modes and, in addition, a four-fold for the first mode and an eight-fold for the second mode intensity increasing in the absorption spectrum.

Large deformation of the complementary pair structure is also much affected on spectral activity of another group, namely of N3-H group of thymine. It forms a central N3-H...N1 hydrogen bond of the complex.

So, *N3-H valent stretching vibration* ($\nu_2 = 3365\text{ cm}^{-1}$) of thymine undergoes a large, of about 400 cm^{-1} , bathochromic shift and also a large (of an order) increasing of its intensity in the IR absorption spectrum of the pair.

N3-H main bending vibration ($\nu_{15} = 1357\text{ cm}^{-1}$) and a combined, low-frequency bending mode ($\nu_2 = 467\text{ cm}^{-1}$) of this group are found to be rather ‘blue’-shifted with the marked intensity decreasing in spectrum of the pair.

Participation of *thymine C4=O group* in H-bonding leads mainly to 20 cm^{-1} decreasing the stretching vibration frequency and to three-fold increasing the intensity of its bending ($\nu_{26} = 716\text{ cm}^{-1}$) mode.

C2=O group does not take part itself in base pairing. But, due to extensive coupling of the vibrations in the deformed molecular complex, there is some alteration of spectral characteristics of the group. A frequency of its valent stretching mode is lowered at 35 cm^{-1} and the intensity of the mode is decreased at 1,5 times under base H-bonding.

The results presented here illustrate, mainly, rather an overall picture of a strong mutual influence of normal modes in the IR spectrum of the nonplanar structure of the Watson-Crick AT(WC) pair. An original cause of that is a stable pyramidal structure of the amino group which initiates the nontrivial, left and right propeller-twisting of base plans under H-pairing.

IR SPECTRAL FEATURES OF NONPLANAR FORMS OF GC(WC) PAIR

In Table VII are listed the changes of some IR vibrational frequencies of bases in two different forms (according to Figure 3) of the watson-crick GC base pairing – GC(WCdd)[GC(WCuu)] and GC(WCud)[GC(WCdu)].

Noncoplanarity of the GC(WC) pair leads in a whole to similar spectral effects, as in the case of the AT(WC) pair, of bases vibrational modes activity under H-bonding.

So, the *frequencies of NH_2 -group asymmetric and symmetric stretching vibrations* of guanine and cytosine in 3400 cm^{-1} – 3500 cm^{-1} region are ‘red’-shifted

Table VII. Calculated (MNDO-PM3) and experimental characteristic normal mode frequencies (in cm^{-1}) of guanine and cytosine and of their Watson-Crick variants of pairing

Mode type		GC(WCdd)			GC(WCud)				
		G		C	G		C	C	
		exper. [36]	theory	theory	theory	theory	theory	theory	exper. [35]
-N6H ₂	‘asym’		3524	3497	3512	3498	3514	3534	3565
	‘sym’		3412	3326	3311	3334	3320	3431	3441
	‘scis’	1675	1684	1661	1664	1661	1664	1663	1656
	‘scis’ (comb.)	1638	1645	1647		1647			
		1565	1602	1591	1523	1591	1525	1510	1475
	‘rock’	1118	1108	1124	1099	1124	1096	1100	1090
	‘rock’ (comb.)	1042	1053	1073		1071			
	‘wag’	601	582	594	648	594	650	611	614
	‘tors’	348	306	440	411	439	396	353	330
		214	248	432	497	431	496	314	
N1-H	‘str’		3369	3048		3047			
	‘bend’	1362	1308	1330		1330			
C6=O	‘str’	1702	1975	1913		1913			
	‘bend’	654	620	594		594			
C2=O	‘str’				1878		1878	1930	1720
	‘tors’				621		620	678	637
		42 modes		81 modes		81 modes		33 modes	

in spectrum under N-H...O hydrogen bonding as well as in the AT pair. And the intensities of symmetric modes, as calculations show, are also increased at an order.

It is particularly remarkable that a shifted to 3311 cm^{-1} symmetric mode vibration of cytosine in a propeller-like GC(WCdd) [or GC(WCuu)] structure is at 1,5 times more intensive than an appropriate mode (at 3320 cm^{-1}) in another, step-like GC(WCud) [or GC(WCdu)] structure.

Main NH₂ – scissoring mode of guanine ($\nu_8 = 1684\text{ cm}^{-1}$) is equally frequency lowed in both GC forms and is somewhat increased in the intensity.

On the contrary, a region of *NH₂ – scissoring vibration of cytosine* ($\nu_9 = 1663\text{ cm}^{-1}$) is rather informative for the GC polymorphic rearrangements. The

mode intensity increasing in the step-like GC(WCud) [or GC(WCdu)] form under hydrogen bonding is at 1,5 times as much as that in the twisted GC(WCdd) [or GC(WCuu)] form.

NH_2 – *rocking vibration of guanine* ($\nu_{19} = 1108\text{ cm}^{-1}$) is slightly increased in frequency and at three times is increased in intensity in both the nonplanar forms. At that time analogous rocking mode of a cytosine amino group, $\nu_{16} = 1100\text{ cm}^{-1}$, did not change its spectral characteristics in the pair.

Combined, NH_2 – *rocking vibration of guanine* ($\nu_{20} = 1053\text{ cm}^{-1}$) is shifted only at 20 cm^{-1} to higher frequencies without changing the intensity.

NH_2 – *out-of-plane, wagging modes of guanine* ($\nu_{30} = 582\text{ cm}^{-1}$) and cytosine ($\nu_{24} = 611\text{ cm}^{-1}$) are also little increased in frequency and in intensity under H-bond formation.

Low-frequency, NH_2 – *group torsing vibrations* of guanine ($\nu_{38} = 306\text{ cm}^{-1}$ and $\nu_{39} = 248\text{ cm}^{-1}$) and that of cytosine ($\nu_{29} = 353\text{ cm}^{-1}$ and $\nu_{30} = 314\text{ cm}^{-1}$) are subjected to many changes in spectra of this two deformed forms considered. The increasing up to 180 cm^{-1} of the vibration frequency of all this modes is observed. But the intensity of the high-frequency torsing mode of guanine, $\nu_{tors} = 440\text{ cm}^{-1}$, is in more deformed, propeller-like GC(WCdd) form three times as large as that in the step-like GC(WCdu) form.

The intensity difference of similar NH_2 – *group torsing vibrations* of cytosine in two forms is less significant in comparison to previous ones.

$N1-H$ *valent stretch vibration* of guanine ($\nu_4 = 3369\text{ cm}^{-1}$) undergoes, as it must, a large long-wave shift in the absorption spectrum of the GC pair. However, no pronounced intensity difference is obtained for two variants of the complementary pairing.

In contrast to the valent mode, an $N1-H$ *bending mode* of guanine is found to be sensitive to conformational differences of the forms. So, a mode at $\nu = 1300\text{ cm}^{-1}$ is characterized by a more intensive (at 1,5 times) absorption in the IR spectrum of the GC(WCdd) structure than in spectrum of GC(WCud) form.

Another, low-frequency $N1-H$ *group bending mode* ($\nu_{30} = 390\text{ cm}^{-1}$) of guanine exhibits only a 180 cm^{-1} frequency raising in both forms without any marked intensities difference.

$C=O$ *groups* of bases are active in H-bonding. A 60 cm^{-1} decreasing the frequency of their stretching modes and rather large intensity changes are the manifestations of the base pairing. However, according to our estimations, there is no marked spectral difference in these two forms. Only a $C=O$ *group bending vibration* of cytosine ($\nu_{bend} = 620\text{ cm}^{-1}$) enables us to detect some conformational dissimilarity of this pairings. As calculations show, the absorption intensity of this mode in the step-like GC(WCdu) form is about twice as large as that for the rather deformed, propeller-like GC(WCdd) structure.

The results presented in this part are displayed, as in the case of the AT(WC) pair, as mixing up of the base normal vibrations in highly deformed valent-forced fields of GC forms. However, the difference in the intensities delivery between

some modes of the bases in different pair conformations under H-pairing allows us to specify the regions of IR spectra which are very promising for identification of the forms.

More informative vibrations for this purposes are, from our point of view, a) the amino group vibrations of cytosine in the region of symmetric stretching mode ($\nu = 3320 \text{ cm}^{-1}$); b) NH_2 – scissoring vibrations at 1664 cm^{-1} and c) wagging modes of NH_2 -groups at 650 cm^{-1} region.

The region of C=O bending mode of cytosine at 620 cm^{-1} is also informative.

For guanine vibrations this is the case of NH_2 -torsing mode at 440 cm^{-1} region.

Conclusion

Theoretically revealed close relation between nonplanarity and non-uniqueness of Watson-Crick base pairs is most probable a fundamental, intrinsic property of the pairs. It should be taken into account not only in the tasks of the internal linear or nonlinear dynamic of double-helical polydinucleotides but also in adequate description of DNA ‘structure-function’ organization as a whole.

MNDO-PM3 estimations of the inertial defect values of nonplanar forms of base H-pairings show a rather high reliability of this spectral criterion for microwave identification of internal structural polymorphism of the complementary pairing.

Some regions of the IR spectrum of normal modes of an isolated Watson-Crick GC(WC) pair is also promising for differentiation of its hidden propeller-like and step-like forms.

For the AT(WC) base pair the observation of its full spectrum of low-frequency, out-of-plane vibrational modes is a simple indication of propeller-like nonplanarity of the complementary base pairing structure.

References

1. Yakushevich, L.V.: Nonlinear Physics of DNA, John Wiley & Sons, Chichester, New York, Brisbane, Singapore, Toronto, 1998.
2. Gaeta, G., Reiss, C., Peyrard, M. and Dauxois, T.: Simple models of nonlinear DNA dynamics, *Rev. Nuovo Cimento* **17** (1994), 1–48.
3. Yakushevich, L.V.: Nonlinear dynamics of biopolymers: theoretical models, experimental data, *Quart. Rev. Biophys.* **26** (1993), 201–223.
4. Saenger, W.: Principles of Nucleic Acid Structure, Springer-Verlag, Berlin, 1984.
5. Wilson, C.C.: An analysis of conformational parameters in nucleic acid fragments, *Nucleic Acid Res.* **15** (1987), 8577–8591.
6. Wilson, C.C. and Tollin, P.: Propeller twisting in single crystals of nucleosides, *Nucleosides & Nucleotides* **6** (1987), 643–653.
7. Wilson, C.C.: The effect of exocyclic substituents on base-pair propeller twist, *Nucleosides & Nucleotides* **9** (1990), 479–488.
8. Wilson, C.C.: Analysis of conformational parameters in nucleic acid fragments. III. Very short chain oligonucleotides. The effect of base stacking, *Nucleic Acid Res.* **16** (1988), 4752–4759.

9. Wilson, C.C.: Analysis of conformational parameters in nucleic acid fragments. II. Co-crystal complexes of nucleic acid bases, *Nucleic Acid Res.* **16** (1988), 385–393.
10. Dickerson, R.E.: Base sequence and helix structure variation in B and A DNA, *J. Mol. Biol.* **166** (1983), 419–441.
11. Alexandresen, A., Drendel, W.B., Sundaralingam, M.: A highly propeller-twisted adenine-adenine base pair in 8-tret-butyladenine, *Acta Crystallog. C* **47** (1991), 1041–1044.
12. Jursa, J. and Kypr, J.: Geometries and energies of Watson-Crick base pairs in oligonucleotide crystal structures, *Gen. Physiol. Biophys.* **12** (1993), 401–409.
13. Dickerson, R.E., et al.: Definitions and nomenclature of nucleic acid structure parameters, *EMBO Journal* **8** (1989), 1–4.
14. Komarov, V.M. and Polozov, R.V.: On the propeller structure of isolated Watson-Crick base pairs, *Z. Naturforsch. B* **45c** (1990), 1080.
15. Komarov, V.M., Polozov, R.V. and Konoplev, G.G.: Non-planar structure of nitrous bases and non-coplanarity of Watson-Crick pairs, *J. Theor. Biol.* **155** (1992), 281–294.
16. Komarov, V.M.: Non-coplanar H-binding of the Hugstein base pairs. PCILO conformational evaluations. I. Adenine-adenine and adenine-thymine pairs, *Biophysics* **39** (1994), 863–868.
17. Komarov, V.M. and Mevkh, N.G.: A model of plurality of Watson-Crick base pairing forms, *Russian J. Phys. Chem.* **69** (1995), 1281–1283.
18. Komarov, V.M.: Quantum-chemical semi-empirical study of polymorphism of Watson-Crick base pairing, *Biofizika* **43** (1998), 967–974 (in Russian).
19. Dewar, M.J.S. and Thiel, W.: Ground states of molecules. 38. The MNDO method. Approximations and parameters, *J. Am. Chem. Soc.* **99** (1977), 4899–4907.
20. Liotard, D.A., Healy, E.F., Ruiz, J.M. and Dewar, M.J.S.: QCPE, Bloomington, Indiana, 1989, program 506, AMPAC (Version 2.1).
21. Stewart, J.J.P.: Optimization of parameters for semi-empirical methods. I. Method, *J. Comput. Chem.* **10** (1989), 209–220.
22. Stewart, J.J.P.: Optimization of parameters for semi-empirical methods. II. Applications, *J. Comput. Chem.* **10** (1989), 221–264.
23. Herzberg, G.: Molecular spectra and molecular structure. Electronic spectra and electronic structure of polyatomic molecules, Toronto-New York-London, 1966.
24. Brown, R.D., Godfrey, P.D. and Kleybomer, B.K.: The conformation of formamide, *J. Molec. Spectrosc.* **124** (1987), 34–35.
25. Brown, R.D., Godfrey, P.D., McNaughton, D. and Pierlot, A.P.: A study of the major gas-phase tautomer of adenine by microwave spectroscopy, *Chem. Phys. Lett.* **156** (1989), 61–63.
26. Brown, R.D., Godfrey, P.D., McNaughton, D. and Pierlot, A.P.: Tautomers of cytosine by microwave spectroscopy, *J. Am. Chem. Soc.* **111** (1989), 2308–2309.
27. Roussy, G. and Nonat, A.: Determination of the equilibrium molecular structure of inverting molecules by microwave spectroscopy: Application to anilone, *J. Mol. Spectrosc.* **118** (1986), 180–188.
28. Ogata, T. and Sugimoto, K.: Microwave spectrum, barrier to internal rotation and dipole moment of methoxuallene, *J. Molec. Struct.* **190** (1988), 61–67.
29. Hester, R.E. and Girling, R.B.: Spectroscopy of Biological Molecules. The Royal Society of Chemistry, Cambridge, 1991.
30. Sheina, G.G., Radchenko, E.D., Plokhotnichenko, A.M. and Blagoj, Yu.P.: IR spectra of associated and hydrated pyrimidine bases of nucleic acids in Ar matrix, *Biofizika* **33** (1988), 741–746 (in Russian).
31. Ivanov, A. Yu., Plokhotnichenko, A.M., Radchenko, E.D., Sheina, G.G. and Blagoj, Yu.P.: FTIR spectroscopy of uracil derivatives isolated in Kr, Ar and Ne matrices: Matrix effect and fermi resonance, *J. Molec. Struct.* **372** (1995), 91–100.
32. Nowak, M.J.: IR matrix-isolation studies of nucleic acid constituents – The spectrum of monomeric thymine, *J. Molec. Struct.* **193** (1989), 35–49.

33. Duguid, J.G., Bloomfield, V.A., Benevides, J.M. and Thomas, G.J.Jr.: DNA melting investigated by differential scanning calorimetry and Raman spectroscopy, *Biophys. J.* **71** (1996), 3350–3360.
34. Nowak, M.J., Lapinski, L., Kwiatkowski, J.S. and Leszczynski, J.: Infrared matrix isolation and ab initio quantum mechanical studies of purine and adenine, *Spectrochim. Acta* **47A** (1991), 87–103.
35. Szczesniak, M., Szczepaniak, K., Kwiatkowski, J.S., KuBulat, K. and Person, W.B.: Matrix isolation infrared studies of nucleic acid constituents. 5. Experimental matrix-isolation and theoretical ab initio SCF molecular orbital studies of infrared spectra of cytosine monomers, *J. Am. Chem. Soc.* **110** (1988), 8319–8330.
36. Florian, J.: Scaled quantum mechanical force fields and vibrational spectra of solid-state nucleic acid constituents. 6. Guanine and guanine residue, *J. Phys. Chem.* **97** (1993), 10649–10658.
37. MacPhail, R.A., Williams, L.D., Jones, D.A. and Shaw, B.R.: Variable temperature infrared-spectroscopy of cytosine-guanine base-pairs-tautomerism versus polarization, *J. Biomol. Struct. Dynamics* **9** (1992), 881–898.
38. Gould, I.R., Vincent, M.A., Hiller, I.H., Lapinski, L. and Nowak, M.J.: A new theoretical prediction of the infrared spectra of cytosine tautomers, *Spectrochim. Acta* **48A** (1992), 811–818.
39. Kwiatkowski, J.S. and Leszczynski, J.: Molecular structure and vibrational IR spectra of cytosine and its Thio and Seleno analogues by density functional theory and conventional ab initio calculations, *J. Phys. Chem.* **100** (1996), 941–953.
40. Tsuboi, M.: IR and Raman spectra, in O.P. T's'o (ed.), *Basic Principles in Nucleic Acid Chemistry*, v1. AP, NY, London, 1974.
41. Hroda, V., Florian, J. and Hobza, P.: Structure, energetics, and harmonic vibrational spectra of the adenine-thymine and adenine*-thymine* base pairs: Gradient nonempirical and semi-empirical study, *J. Phys. Chem.* **97** (1993), 1542–1557.
42. Cornell, W.D., Cieplak, P., Bayly, C.I., Gould, I.R., Merz, K.M., Fergusson, D.M., Spellmeyer, D.C., Fox, T., Caldwell, J.W. and Kollman, P.A.: A second generation force field for simulation of proteins, nucleic acids, and organic molecules, *J. Am. Chem. Soc.* **117** (1995), 5179–5197.
43. Govorun, D.N., Danchuk, V.D., Misuchuk, Ya.R., Kondratyuk, I.V., Radomsky, N.F. and Zeltovsky, N.V.: AM1 calculation of the nucleic acid bases structure and vibrational spectra, *J. Mol. Structure* **267** (1992), 99–104.
44. Santamaria, R. and Vazquez, A.: Structural and electronic property changes of the nucleic acid bases upon base pair formation, *J. Comput. Chem.* **15** (1994), 981–996.
45. Verkin, B.I., Yanson, I.K., Sukhodub, L.F. and Teplytski, A.B.: Interactions of biomolecules. New experimental approaches and techniques; Naukova Dumka, Kiev, 1985 (in Russian).
46. Lowen, J.N. and Jenneskens, L.W.: Comparison between some semi-empirical and ab-initio Hartree-Fock models for the description of amides (Formamide revised), *J. Phys. Organic Chem.* **3** (1990), 711–722.
47. Leszczynski, J.: Are the amino groups in the nucleic acid bases coplanar with the molecular rings? Ab initio HF/6-31G* and MP2/6-31G* studies, *Int. J. Quant. Chem.: Quant. Biol. Symp.* **19** (1992), 43–45.
48. Sponer, J. and Hobza, P.: Nonplanar geometries of DNA bases: ab initio second-order MP study, *J. Phys. Chem.* **98** (1994), 3161–3164.
49. Sponer, J. and Hobza, P.: DNA Base amino groups and their role in molecular interactions: Ab initio and preliminary density functional theory calculations, *Int. J. Quant. Chem.* **57** (1996), 959–970.
50. Sponer, J., Leszczynski, J. and Hobza, P.: Hydrogen bonding and stacking of DNA bases: A review of quantum-chemical ab initio studies, *J. Biomol. Struct. Dynamics* **14** (1996), 117–135.

51. Sponer, J., Leszczynski, J. and Hobza, P.: Structure and energy of hydrogen-bonded DNA-base pairs – A nonempirical study with inclusion of electron correlation, *J. Phys. Chem.* **100** (1996), 1965–1974.
52. Sponer, J. and Hobza, P.: Nonempirical ab initio calculations on DNA-base pairs, *Chem. Phys.* **204** (1996), 365–372.
53. Sponer, J., Florian, J., Hobza, P. and Leszczynski, J.: Nonplanar DNA base pairs, *J. Biomol. Struct. Dynamics* **13** (1996), 827–833.
54. Gould, I.R. and Kollman, P.A.: Theoretical investigation of the hydrogen bond strength in guanine-cytosine and adenine-thymine base pairs, *J. Am. Chem. Soc.* **116** (1994), 2493–2499.
55. Dive, G., Dehareng, D. and Ghuysen, J.M.: Energy analysis on small to medium sized H-bonded complexes, *Theor. Chim. Acta* **85** (1993), 409–421.
56. Leszczynski, J.: Electron correlation effects in the ab initio study on tautomerism of guanine, *Chem. Phys. Lett.* **174** (1990), 347–349.
57. Stewart, E.L., Foley, C.K., Allinger, N.L. and Bowen, J.P.: Ab initio calculations with electronic correlation (MP2) on nucleic acid bases and their methyl derivatives, *J. Am. Chem. Soc.* **116** (1994), 7282–7286.
58. Florian, J., Leszczynski, J. and Johnson, B.G.: Intermolecular vibrational modes of the G-C, A-T and formamide-formamide H-bonded dimers, *J. Mol. Structure* **349** (1995), 421–426.
59. Florian, J. and Leszczynski, J.: What changes occur in vibrational spectra of guanine and cytosine when they form the Watson-Crick base pair? A quantum chemical SCRF/6-31G* study, *Int. J. Quant. Chem.: Quant. Biol. Symp.* **22** (1995), 207–225.
60. Spirko, V., Sponer, J. and Hobza, P.: Anharmonic and harmonic intermolecular vibrational modes of the DNA base pairs, *J. Phys. Chem.* **106** (1997), 1472–1479.
61. Govorun, D.N., Misuchuk, Ya.R., Kondratyuk, I.V. and Zeltovsky, N.V.: Dynamic stereo isomerism of Watson-Crick nucleotide base pairs, *Dopovidi NAN Ukrainy* **11** (1995), 121–123.
62. Govorun, D.N., Mishchuk, Ya.R. and Kondratyuk, I.V.: Topological properties of potential energy hypersurfer of canonical nucleotide bases, *Biopolimeri i Kletka* **12** (1996), 13–17 (in Russian).