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INFLUENCE OF HYDROGEN IONS ON DNA MOLECULE . SPECTROSCOPIC STUDY AND ELECTRON-CONFIGURATION ANALYSIS.

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Academic Secretary of the Dissertation Council
Candidate of Chemical Sciences **N. Japaridze**

SHORT DESCRIPTION OF THE WORK

THE SUBJECT ACTUALITY:

As it is known, nucleic acids are polyelectrolytes and therefore usually they are bound to different types of charged ligands, which significantly influence the parameters of their secondary and tertiary structures and physiological activity. The hydrogen ions differ from other ligands as they have no electron shell and represent actually a point charge without any electronic screening. As a matter of fact, they are just protons in the environment of water molecules ("wet" protons). This determines exceptionally high reactivity and mobility of hydrogen ions. On the other hand, hydrogen ions represent a product of water dissociation and they inevitably and ontogenetically exist in every structure of the living organisms. They can also enter organisms from the environment as certain amount of H^+ occurs in the atmosphere due to solar radiation and different kinds of chemical reactions taking place in our environment. So, DNA as well as other structures in living bodies is always surrounded by number of hydrogen ions. There are numerous buffer systems in that maintain equilibrium concentration corresponding to pH 7.4 in most of the tissues of organisms. It is obvious that accurate balance of the amount of H^+ ions is of a great importance for correct functioning of different cell structures and bio-macromolecules among them.

With the development of methods for intracellular pH-measurement, interesting data were found on variations of this parameter connected with cell division and cancer development (Marie Yeo, Dong-Kyu Kim, Helmlinger G, Yuan 1997; Stubbs M., McSheeh P., 1999). It was shown that change of pH just for 0.05 unit leads to serious consequences starting from cancer to osteoporosis and ageing (Yau-Huei Wei and Hsin-Chen Lee, 1998). Data exist that H^+ ions may cause damages similar to that by high energy lead ions. It is assumed that H^+ irradiation threat is still very much underestimated (Sutherland and Hada, 2006) and is actively used nowadays for cancer treating.

Following from the above said, study of influence of hydrogen ions on DNA structure and functioning, revealing of dependence of this influence on environmental conditions (ionuc strength, DNA sequence, existence of other ligands on DNA double strand) is of an actual problem of modern biology and byophysics.

THE TASKS AND GOALS OF THE PRESENTED WORK:

The presented work had set as a goal to study spectral and thermodynamic changes caused by hydrogen ions on DNA double strand and on the basis of phenomenological electronic-configuration analysis understand the mechanisms of influence of H^+ on DNA. In order to reach this goal, the following problems were set to solve:

- Study of variations of spectral and thermodynamic parameters caused by H^+ ions;
- Study of dependence of variations of spectral and thermodynamic parameters caused by H^+ ions on ionic strength of the solvent;

- Study of influence of dyes intercalated in DNA double strand (ethidium bromide and acridine orange in our case) on variations of spectral and thermodynamic parameters caused by H^+ ions;
- Study of influence of H^+ ions on fluorescence of intercalated in DNA ethidium bromide and acridine orange;
- Phenomenological electronic-configuration analysis of the atoms involved in hydrogen bonds between DNA base pairs and charge redistribution caused by H^+ ions;
- Evaluation of the risk of point mutations caused by H^+ ions on the basis of obtained results;

SCIENTIFIC NOVELTY OF THE WORK:

Scientific novelty of the presented work consists in the following:
- Anomalous high difference spectrum caused by H^+ ions comparing to transition metal ions of the same order of binding constants with DNA is illustrated and explained;
- Drastic decrease of H^+ ions' dependent spectral and thermodynamical changes with increase of ionic strength is explained;
- Mechanism of fluorescence quenching of intercalated in DNA dyes (ethidium bromide and acridine orange) by H^+ ions is explained;
- Double mechanism of thermal destabilization by H^+ ions is suggested;
- Destabilization of DNA by some transition metal ions at high concentrations is explained by protonation of the solution by these ions;
- The idea of DNA Stress is introduced, which reflects the risk-factor of transition type point mutations induced by H^+ ions.

SCIENTIFIC AND PRACTICAL VALUE OF THE WORK:

The obtained results provide considerable advance in understanding of thermal and structural properties of DNA double strand. They make certain contribution also in justification of DNA as a matrix for charge and excitation energy transmitter. Furthermore, anticipation of results of the presented work would help in elaboration of optimal regimes at cancer chemo- and chemo-phototherapy. Evaluation of H^+ dependent point mutations may help in understanding and development of theories of salutatory evolution of living species.

APPROBATION OF THE PRESENTED WORK:

The main results of the thesis were presented on international conference "Winter Conference on Plasma Spectrochemistry 2006", Arisona, Tucson, USA, January, 2006 and on the Board of Biological Systems in E. Andronikashvili Institute of Physics, Tbilisi, Georgia, July, 2006.

PUBLICATIONS:

The list of publications is available at the end of the summary.

THE THESIS INCLUDES:

The thesis includes introduction, 3 chapters, conclusions and list of references; it consists of 87 pages, 28 figures and 5 tables among them. The list of references contains 126 publications.

SHORT DESCRIPTION OF THE THESIS.

CHAPTER 1. DIGEST OF RELATED LITERATURE.

Electronic and polyelectrolitic properties of DNA is discussed based on Manning and Frank-Kamenetskiy theories. Pirson's conception of weak and strong acids and bases is also considered. Properties of hydrogen ions and their hydrated structures in water environment are described. Interaction of DNA with small size charged ligands is described. DNA absorption spectrum peculiarities and possible keto-enil and amine-imine tautomeric transitions is observed. Influence of environmental conditions on parameters of DNA double helix, its different configurations depending on those conditions and inner mobility of DNA are described. Influence of hydrogen ions on different biological objects and the processes taking place in there are considered. Based on literature data [Bregadze 2002, Gladchenko 1987] position of hydrogen ions in graphical arrangement of Pirson's conception of weak and strong acids is defined.

CHAPTER 2. EXPERIMENTAL METHODS AND MATERIALS.

In this chapter basic principles of absorption spectroscopy are described. We have used ultraviolet spectroscopy to register the influence of H^+ ions on DNA basis pairs (spectrophotometer SPECORD M40), Hungary).

The method of UDS, which allows to solve the problem of registration of little frequency shift induced by weak intermolecular interactions is described. The method is based on the fact that due to Gaussian shape of an absorption band, at little frequency shift ($\Delta\nu$) change of absorption value is ($\Delta\varepsilon$) quite great in the areas, where the slope of the curve is maximum i.e. at the points corresponding to the half-height of the peak (λ=294nm in case of DNA).Values of $\Delta\nu$ and $\Delta\varepsilon$ at little shift are related to each other by the following expression:

$$\Delta\nu = \Delta\varepsilon_s / K_s \quad (1)$$

where $\Delta\varepsilon_s = |\Delta\varepsilon(\nu_{\max})| + |\Delta\varepsilon(\nu_{\min})|$, $K_s = |\varepsilon^{(1)}(\nu_{\max})| + |\varepsilon^{(1)}(\nu_{\min})|$.

The concentrations in UDS studies were the following: $C_{DNA}=2\cdot10^{-4}$M (P); in the experomets, where H^+ concentration was not a constant parameter $C_{H3O+}/C_{DNA(P)}=0.25$; if the ionic strenght was a constant parameter $C_{NaCl}=2\cdot10^{-2}$M; the ratio of AO and EB were 1:10 relatively to DNA bais pairs..

For fluorimetric measurements the spectrometer SDL-1 ("LOMO" Russia) with double grating monochromator was used. Fluorescence excitation was obtained by double mochromator MDR-2 ("LOMO" Russia). As a light source an ultrahigh pressure mercury arc lamp (P=250 W) and tungsten ribbon filament lamp (2850^0K) were applied. The pH-mes were provided on precision pH-meter OP-212 (Radelkis, Hungary).

Experimental conditions and preparations are also described in this chapter.

CHAPTER III. THE RESULTS AND THEIR DISCUSSIONS.

III.1. EFFECT OF H^+ IONS ON DNA ULTRAVIOLET ABSORPTION SPECTRUM .

Absorption of DNA in ultraviolet area is determined by DNA base pairs. Transition metal ions cause shift of absorption band towards red part of the spectrum [Khutsishvili, 1997]. It is well established that this shift is conditioned mainly by change of absorption by GC pairs.

Study of UDS caused by hydrogen ions (Fig.1) has shown that the main difference between influence by H^+ and transition metal ions is of a quantitative character. Our study has shown, that H^+ related ultraviolet difference spectrum (UDS) at $2\cdot10^{-2}$M NaCl ionic strength is much more then that caused by transition metal ions although its binding constant (pK) to DNA is not more then that of the metal ions (Tab.1) [Bregadze, Khutsishvili, 1994].

One may conclude that such strong effect of H^+ on DNA ultraviolet spectrum is due to small size, which allows them to get very close to DNA nitrogen bases through the hydrated water shell. Although this is very considerable reason but we assume it not quite satisfying. Cu^{+2} also can get very close to N_7 of guanine and its binding constant is higher then that of H^+ but UDS induced by Cu^{+2} is mush less then that in case of hydrogen ions.

As it is known, the hydrogen ions in water environment exist in H_3O^+ form, which is associated by hydrogen bonds to hydrogen shell consisting mainly by three water molecules. In this structure exchange between $H_3O^+$and water molecule from its hydrogen shell is very fast process (Fig.2), i.e. H^+ is characterized by exceptionally high mobility (proton lifetime in water environment at room temperature is 10^{-11}sec. and in ice – 10^{-13}sec.). As a matter of fact, it is only charge transition and there is no necessity of leaving its hydration shell, or moving with it as it happens in case of metal (and any other) ions. Hydration layer of DNA represents ordered structure and once hydrogen ions get inside this structure, it can move along the double helix. So, we assume that H^+ ions are

characterized with mobile hydrogen ions are erased by mobile adsorption on DNA surface and each of them can affect several pairs of bases.

The Fig.3 represents H^+ concentration dependence of UDS at $2\cdot10^{-2}$M NaCl ionic strength. Positive values of UDS in the whole range of observation (curve - 5) indicate hyperchromic effect due to double helix denaturation.

Study of UDS dependence on ionic strength of the solution revealed the same abnormal drastic decrease of it as was its initial high value (Fig.4). Except H^+ induced UDS curves, here are given also the ones induced by transition metal ions - Cu^+, Co^+, Cr^+ and Ni^+. The doted line in this figure shows decrease of surface potential (φ) determined by Na^+ ions [Bregadze 1996]. Obviously, the surface potential decreases in straight line with Na^+ concentration. The lines corresponding to transition metal ions are more or less close toφ. As for H^+, it is distinguished from the others. In this case, $\Delta\varepsilon$ reduces 10-fold at 2-fold reduction of the ionic strength. At the same time, the binding constant –K decreases just 1.3 fold [Gladchenko, 1987]. Issuing from these data we may conclude that due to their small size H^+ ions as distinct from transition metal ions, may adsorb not only on **GC** pairs of DNA, but also on **AT** pairs from the minor groove, which is out of reach of Na^+ ions as well. Hence, hydrogen ions stay on the chain, just their existence is not reflected in UDS (which is stipulated by **GC** pairs).

III.2. EFFECT OF H^+ IONS ON DNA THERMOSTABILITY.

We have studied dependence of the melting temperature of DNA on added to the solution H^+ ions. It appeared that this dependence is of the linear character in the investigated by us interval of H^+ concentrations (Fig.5) at $2\cdot10^{-2}$M NaCl. According to Manning polyelectrolyte theory, in wide range of ionic strength including that in our experiment, compensates just 75% of the negative charge of phosphate groups of DNA [Maning 1978]. At the first thought, addition of H^+ ions should increase the compensation rate and thus increase DNA stability, but it does not happen. Once more, it proves that they never adsorb on negatively charged phosphate groups and act only with the base pairs.

One way the H^+ ions cause DNA thermal destabilization is that they disturb the hydration layer due to their high affinity to water molecules involving them in hydrogen bonding with themselves. The other, and more important from our point of view, is recurrent opening (as the molecule is “breathing”) of complement base pairs of DNA in different areas [Bregadze, Khutsishvili … 2002, Crueger, Protozanova, Frank-Kamenetskiy 2006]. As we have noticed earlier, H^+ ions are of very high mobility and they can occupy the negatively charged sites involved in hydrogen between the helixes (N_1 of adenine, O_4 of thymine, N3 and O_2 of cytosine and O_6 of guanine). This prevents the double helix from recombination and of course, leads to thermal destabilization of the molecule in the whole.

Destabilization of DNA double helix by H^+ ions explains destabilization caused by some transition metal ions. We have noted in the introduction, that metal ions interact with water molecules and support their dissociation. We have investigated how they affect the water pH (Tab.2). Thus, we may conclude that destabilization of DNA observed at high

concentrations of ions Mn^{2+}, Cu^{2+} and Zn^{2+} should be ascribed to H^+ created by these ions, which causes the double helix destabilization and overlaps the stabilizing action of the mentioned ions.

III.3 EFFECT OF DYES INTERCALATED IN DNA ON INTERACTION BETVEEN H^+ IONS AND DNA .

Intercalated in DNA dyes (acridine orange (AO) and ethidium bromide (EB) in our case) affect the spectral changes caused by H^+ ions (Fig.6). In both cases the rate of intercalators was one molecule per ten base pairs. If we compare these data to the ones in Fig. 3, we can see that both of these dyes decrease the UDS. This is one more proof of our explanation of abnormal high value of UDS by mobile adsorption of H^+ ions on DNA surface. Obviously, the intercalation disturbs regular hydration layer the H^+ ions move through on one hand and increases the gap between neighboring base pairs, what prevents proton movement along the DNA chain. In addition, the positive charge of these molecules repels the protons leaving them at the isoelectric point.

We did not provide DNA melting in this case, but in contrary to Fig.3, one can see here that the double helix stays native even up to rate of H^+/DNA=4.

It is interesting that H^+ ions also affect the dyes. Fig.7 represents quenching of AO and EB fluorescence.

Effect of fluorescence quenching by metal ions is a known fact and it may be explained by absorption of emitted light by the ions. This mechanism is excluded in case of H^+ ions, as they have no electron shell and thus, they have no spectral absorption. Evidently, excitation energy transfer from the dye to DNA takes place with consequent capture of the excited electrons by protons and photoinduced reduction to the hydrogen atom.

III.4. ANALYSIS OF ELECTRONIC-KONFIGURATIONAL CHANGES INDUCED BY H^+ IONS.

The possible sites of proton adsorption for separate nucleotides are N_1, N_3 and N_7 in adenine; N_3, N_7 and O_6 in guanine; O_2 and O_4 in thymine and N3 and O_2 in cytosine. All these atoms carry negative charge of densities in range from _0.47e (O_2 of cytosine) to _0.65e (N_3 of cytosine). According to proton binding ability N_1>N_7 in adenine, O_4>O_2 in thymine, N_7>N_3 in guanine and N_3>O_2 in cytozine. At the same time, N_3A and O_2T are geometrically more accessible for ligands than N_3G. As for double helix, N_1A, O_4T, N_3C, O_2 G and O_6G are engaged in formation of hydrogen bonds between DNA strands. Quantum chemical evaluation of the left potential proton-acceptor arrangement both for unscreened DNA and the one screened by Na^+ ions is the following [Saenger, 1987]:

$N_7G – N_3G – N_3A – O_2T – O_6G – N_7A$

Hence, because of their small size, hydrogen ions can occupy sites in the major groove as well as in the minor one of DNA and on both types of base pairs. Of course, this leads to certain changes of common electronic configuration of the base pairs. The matter of our interest is to consider electronic configurations of the atoms that are engaged in hydrogen bindings between complementary base pairs.

Pyridine type unshared electron pair of N_7A (The one that does not take part in covalent binding) is located on *sp*^{2} hybrid orbital, which with certain probability is interlapped with π–electronic system of the indole ring of the guanine. Interaction of positively charged H^+ ions on N_7A decreases electron density on the indole ring including N_1. This leads to decrease of the depth of potential energy on N_1G side and increases probability of the proton tunneling to N_3C (Fig.8a). Consequently simultaneous proton transfer from N_4C to O_6G takes place (Fig.8b). As a result, selfcongruent double proton transfer (DPT) between the base pairs takes. In Watson-Crick base pairs two unshared pairs of this atom are located on *sp*^{2} hybrid orbital. After DPT, one pair of electrons stays on the hybrid orbital and another one dislocates to p_z orbital. From symmetry reasons p_z orbital id interlapped with π–electron system of guanine and so this pair becomes a bad donor for hydrogen bonding with the solvent molecules. Thus, DPT considerably lessens the donor-acceptor properties of guanine and its affinity to the water hydration layer.

The situation is similar for **AT** pair from the major groove side, where after DPT on *sp*^{2}hybrid orbital of O_4T remains one unshared electron pair (Fig.8c,d).

The base pairs after DPT are still Watson-Crick ones, but due to changes in their electronic configuration and thus, their donor-acceptor properties, they are called as Wrong Watson-Crick pairs (WWC) (Fig.9).

Thus, double proton transfer caused by H^+ ions changes donor-acceptor properties of **AT** and **GC** pairs of DNA in the major groove, what stimulates keto-enol and amino-imine tautomerization of GC base pairs and reduction of water activity surrounding DNA double helix. The first of these consequences is reflected in change of spectral properties of DNA and another – in change of its thermostability.

III.5.RISK-FACTOR OF MUTATIONS INDUCED BY HYDROGEN IONS.

DPT does not change geometry of Watson-Crick base pairs. Because of it, it is very difficult for reparation system to recognize the WWC pairs. Formation of wrong WWC pairs may lead to transition type point mutation due to change of donor-acceptor properties of the atoms engaged in hydrogen bonding of DNA complementary base pairs. So, when replication or transcription after DPT takes place, we may obtain transition type point mutation (Fig.10) that may appear catastrophic for the cell functioning. This is what makes H^+ ions very dangerous mutagenic agent. Amount of WWC pairs may be assumed as a risk-factor of H^+ induced point mutations. The number of WWC pairs is connected with the absorption band shift by expression:

$$n = \frac{\Delta \nu_{M^{n+}}}{700} N . \qquad (1)$$

where n is a number of wrong G-C base pairs per 10^6 base pairs of DNA , N - Number of H+ ions per 10^6 DNA b.p.; value 700 corresponds to 1 mole WWC **GC** pairs. Value of N basically depends on pH of the solution, $pK_{H^+\text{-}DNA}$, concentration of DNA (C_{DNA}) and the ionic strength (I) of the solution. In our case, pH =6.72, C_{DNA}= $2\cdot10^{-4}$M b.p., which means that there are 1000 H^+ ions per 10^6b.p. of DNA. On the other hand, we have I=10mM. In this condition, $pK_{H+\text{-}DNA}$=3.7 meaning that 860 H^+ ions out of each 1000 ones are bounded to DNA. So, we have 860 H^+ ions per 10^6 b.p. of DNA. In this conditions, $n_{H^+} = \frac{\Delta\nu_{M^{n+}}}{700} 860$ (1) and assuming that $\Delta\nu_{H^+}$ = 440 (Tab. 1), we can calculate $n_{H^+} \approx 541$. As the geometry of WWC pairs after DPT stays the same, they are not revealed by the reparation system.

Providing the similar calculations at close to physiological conditions (pH =7.4, I=0.18M NaCl), when according to Fig.4 $\Delta\nu_{H^+} \approx 30sm^{-1}$, and $pK_{H+\text{-}DNA}$=2.8 [Gladchenko 1997], at the same concentration of DNA we have obtained n≈7. The ratio of DNA and NaCl is about the same in living cells. So, we can assume that there are about 7 WWC pairs per every 10^6 base pairs in living cells. This is the evaluation of the risk-factor of spontaneous mutations, which naturally exists even without action of any outer damaging factors.

C O N C L U S I O N S :

1. It is established that H^+ ions induce anomalously great UDS of DNA double helix comparing to that caused by transition metal ions of the same order pK with DNA base-pairs. Cinsidering the nature of hydrogen ions, we conclude, that H^+ is characterized by mobile adsorption on DNA surface;
2. Two mechanisms of thermal destabilization by H^+ ions are considered. One consists in decrease of hydration rate due to double proton transfer and another – in direct bounding of the ions with N_3C, N_1A and O_6T during recurrent fluctuation opening of complement base pairs of DNA because of their fluctuation mobility;
3. Intercalated molecules of **AO** and **EB** increase resistance of DNA double helix to the action of hydrogen ions. This is caused by stretching of the helix in the sites of intercalation destroying the regular hydrogen layer preventing sliding of H^+ ions along the helix. In addition, these dyes prevent this motion because of their positive charge repulsing H^+ ions. At the same time, intercalated molecules of **AO** and **EB** prevent opening of complement base pairs of DNA direct bounding of the ions with N_3C, N_1A and O_6T (concl. 2);
4. **AO** and **EB** Fluorescence quenching under influence of H^+ ions takes place. The basis of this action consists in photoinduced reduction of H^+ ions, where double helix of DNA is an ordered environment, which plays a role of a matrix, through which excitation energy transfer takes place.
5. On the basis of phenomenological electronic-configuration analysis, it is shown that H^+ ions leads to destruction of **DNA** hydration layer and tautomeric transitions, which finally induces double proton transfer (DPT) between **GC** pairs. DPT shows

itself in shift of absorption spectrum of DNA in ultraviolet area to the red part of the spectrum.

6. On the basis of quantitative analysis of H^+ induced tautomeric shifts and connected with it formation of Wrong Watson-Crick pairs risk-factor of point mutations related to H^+ ions is evaluated, which depends on environment conditions. In case of pH =6.72, $C_{NaCl}= 2\cdot10^{-2}M$
   $n_{H^+} \approx 541$ per 10^6 b.p. of DNA. In close to the physiological conditions $n \approx 7$ per 10^6 b.p. This value characterises the risk-factor of spontaneous mutations in living cells.

THE LIST OF PUBLICATIONS RELATED TO THE THESIS:

1. V. Bregadze, I. Khutsishvili, K. Tsakadze. "Double Proton Transfer in DNA: the Role of Ions, Ethanol, Polyethilenglycol, Protamines and Histones". Bull. Georg. Acad. Sci. **173**, 1, 2006, 143-146;
2. V. Bregadze, K. Tsakadze. "The influence of Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} Ions on DNA Melting Temperature". Bull. Georg. Acad. Sci. **174**, 1, 2006, 440-442;
3. K. Tsakadze, V. Bregadze. "Adsorption of H^+ Ions on DNA Surface". Bull. Georg. Acad. Sci. **174**, 2, 2006;
4. V. Bregadze, E. Gelagutashvili, K. Tsakadze. "Glow Discharge Atomic-Emission Spectrometry. Application in Biology and Medicine". Materials of Winter Conference on Plasma Spectrochemistry, Tucson, Arizona, 2006, 365-367;

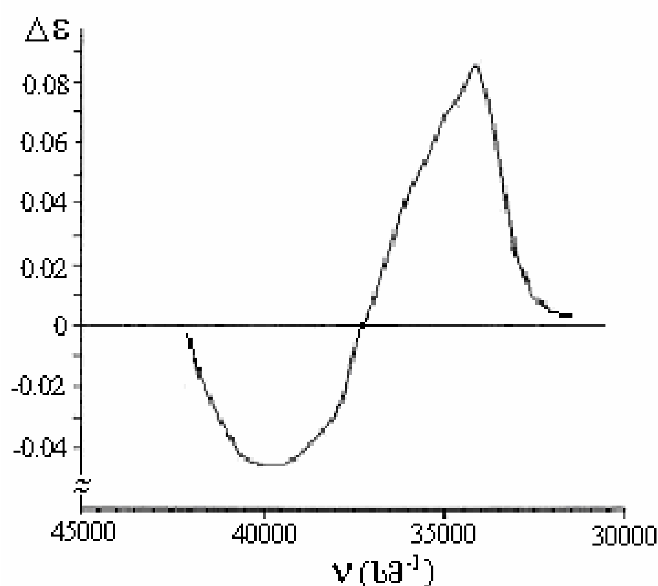


Fig.1 Ultrviolet different spectrum of calf thymus DNA complex with H_3O^+ ions ($C_{DNA}=2\cdot10^{-4}M(P)$; $C_{H3O+}/C_{DNA\ (P)}=0.25$; $C_{NaCl}=2\cdot10^{-2}M$)

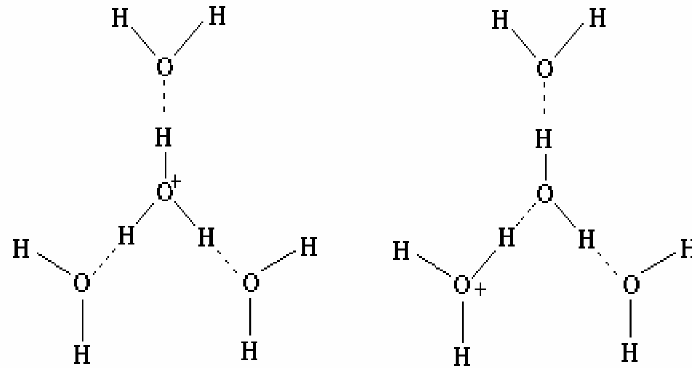


Fig.2 Exchange of hydrogen ions between H_3O^+ and a water molecule bound with hydrogen bond to it.

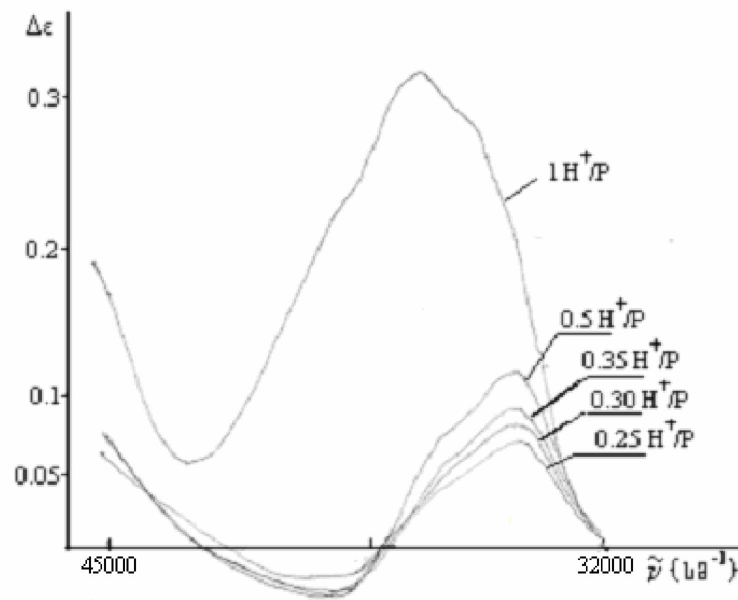


Fig.3 Dependence of UDS of DNA complex with H_3O^+ ions on amount of H^+ ions ($C_{DNA}=2\cdot10^{-4}M(P)$; $C_{NaCl}=2\cdot10^{-2}M$);

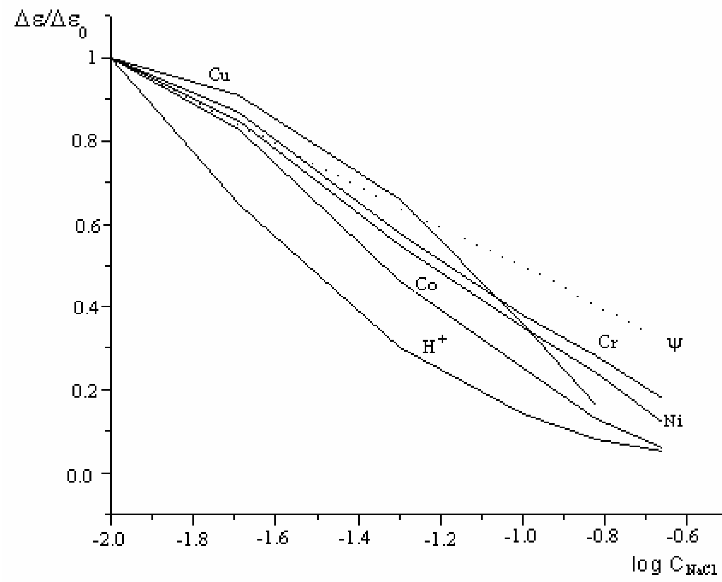


Fig.4 Dependence of relative UDS of DNA complex with H_3O^+ ions on ionic strength of the solution.
(C_{DNA}=2·10^{-4}M(P); C_{H3O+} / $C_{DNA\ (P)}$ =0.25;)

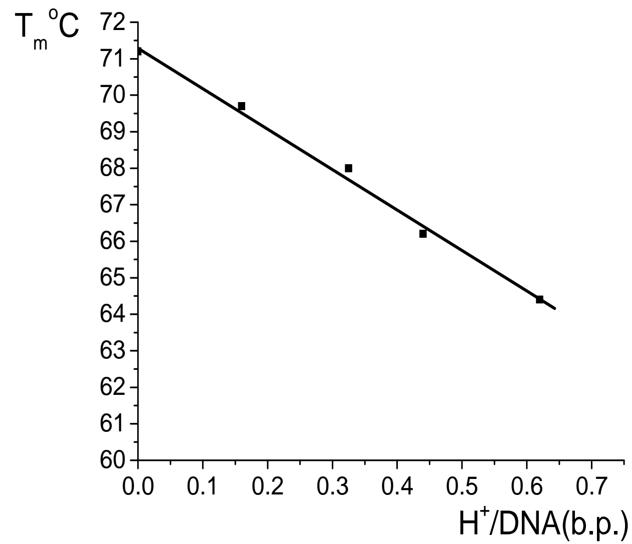


Fig.5 Dependence of DNA melting temperature on the rate of H^+ ions.

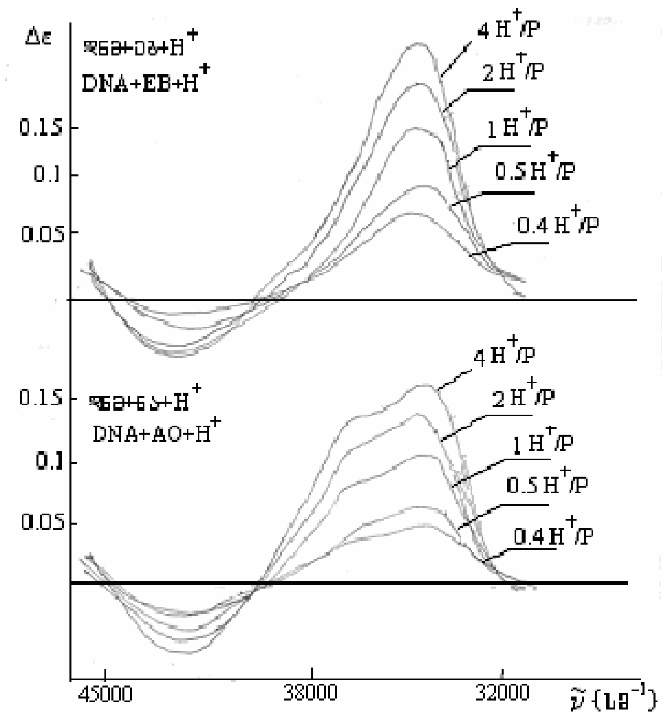


Fig.6 Influence of **AO** and **EB** on UDS of DNA complex with H_3O^+ ions.
(C_{DNA}=2·10^{-4}M(P); C_{Int} / $C_{DNA\ (P)}$ =0.1; C_{NaCl}=2·10^{-2}M)

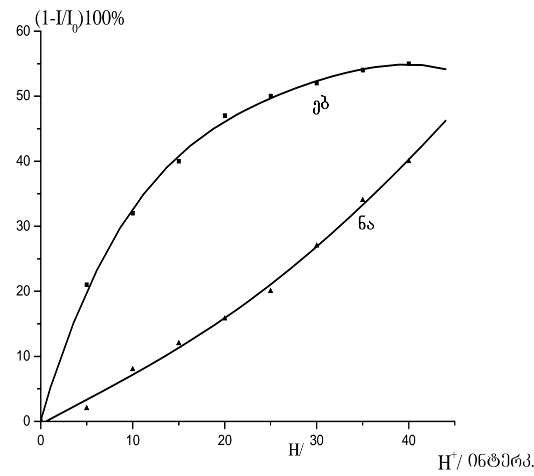
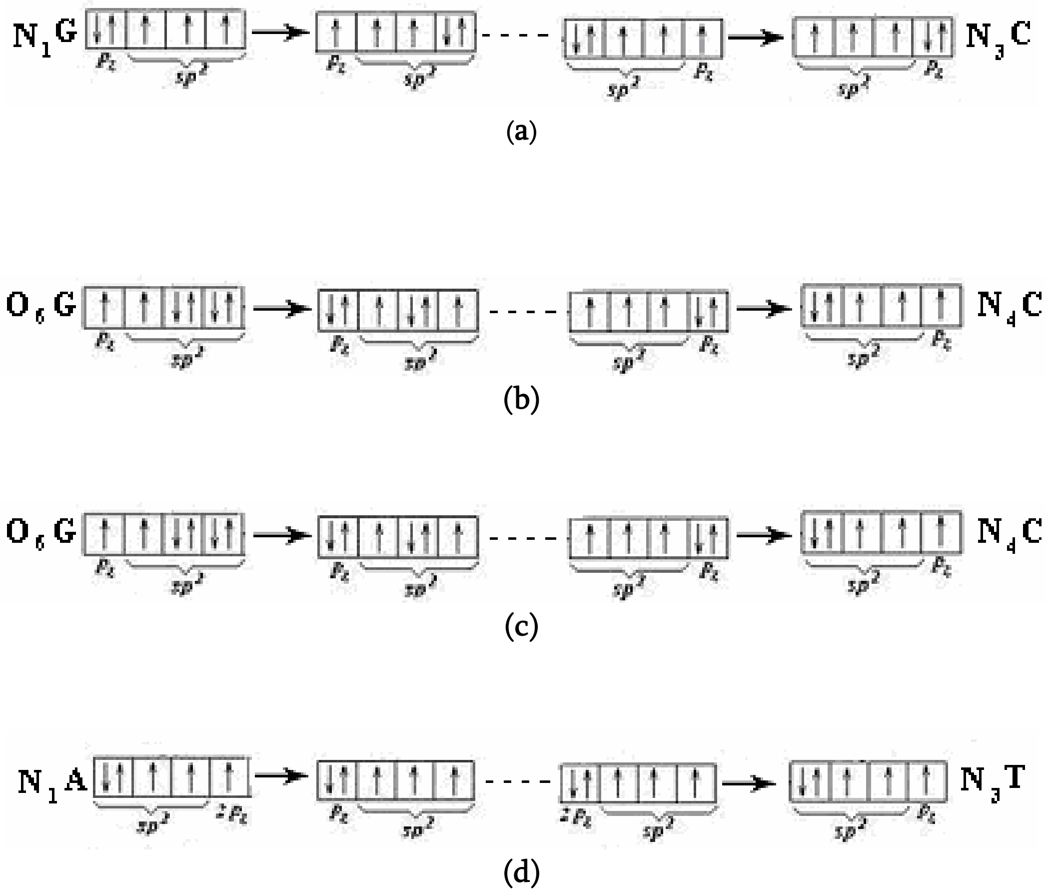


Fig.7 Quenching of fluorescence caused by H^+ ions
(C_{DNA}=2·10^{-4}M(P); C_{Int} /$C_{DNA\ (P)}$ =0.1; C_{NaCl}=2·10^{-2}M)



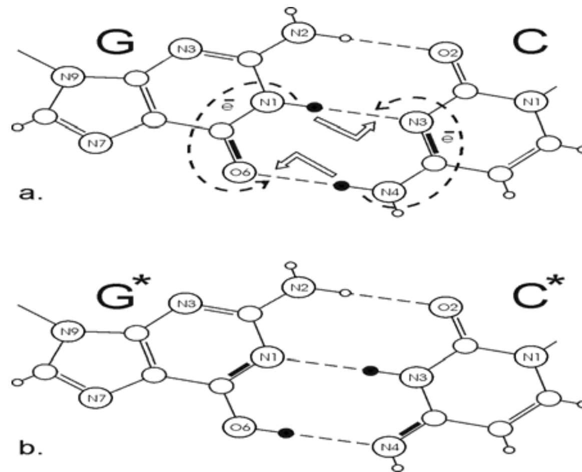


Fig.9 Self-congruent double proton transfer in GC pair (a). Wrong Watson-Crick pair (b)

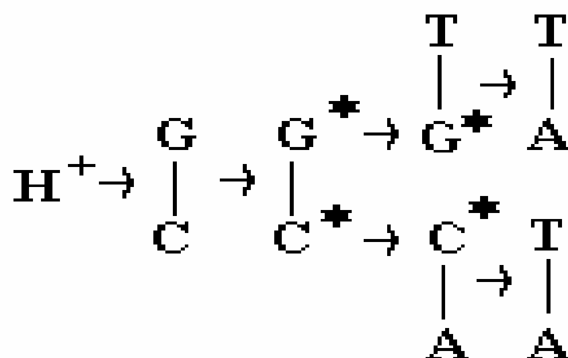


Fig.10 Scheme of point mutation

Tab.1 Rate of DNA UDS caused by H^+ and transition metal ions and their pK.

Ions	The total xtinction Δεs	Δυ(cm^{-1}) calculated	pK
H_3O^+	440	300	3.7
Mg^{2+}	25	15	4.9
Mn^{2+}	100	6	3.9
Co^{2+}	170	110	4.3
Ni^{2+}	220	150	4.6
Cu^{2+}	290	190	5.1
Zn^{2+}	130	85	4.2

Tab.2 pH values after addition of metal ions in the solvent ($C_{Me^{2+}}=10^{-2}M$, $C_{NaCl}=10^{-2}M$).

Me^2	-	Mg^{2+}	Mn^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
pH	6.72	6.42	5.97	6.12	6.27	4.78	5.67