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Abstract

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IONIZATION OF BASES AND PROTON TRANSFER IN NUCLEIC ACIDS AND THEIR COMPONENTS

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In the present work, using the spectrophotometric method, we determined the dissociation constants of nucleic-acid components over a wide temperature range. For this purpose we recorded the ultraviolet absorption spectra of bases and nucleosides at pH values from 1.8 to 12.5 and at temperatures from 20 to 80°.

Table 1

Thermodynamic characteristics of ionization in the ground and excited states of bases and ribosides at 25° C and $\mu = 0.1$

Compound	pK	pK^*	ΔH	ΔH^*	ΔF	ΔF^*	ΔS
Cytosine	4.5	6.78	5000	8100	6100	9200	−3.7
Cytosine	11.82	7.78	11000	5500	16100	10600	−17.1
Adenine	4.12	4.71	4200	5000	5600	6400	−4.7
Adenine	9.72	7.3	9500	6200	13200	9900	−12.4
Guanine	9.42	–	10100	–	12800	–	−9.1
Hypoxanthine	8.8	6.45	7200	3200	12000	8800	−16.1
Cytidine	4.22	6.86	4400	8000	5700	9300	−5.0
Adenosine	3.55	2.6	3800	2500	4800	3500	−3.4
Guanosine	1.6	3.07	1000	3000	2200	4200	−4.0
Guanosine	9.24	7.77	8600	6600	12600	10600	−13.0
Inosine	8.9	7.57	7200	5600	12100	10300	−16.4
Uridine	9.51	9.81	8000	8400	13000	13400	−16.8

Notes. ΔH, ΔH^*, ΔF, and ΔF^* are expressed in cal/mole; ΔS, in entropy units. pK^*, ΔH^*, and ΔF^* refer to the excited state. The error in determining pK does not exceed ±0.02, and in determining ΔH, ±500 cal/mole.

On the basis of the data obtained, thermodynamic parameters of ionization at 25° and ionic strength $\mu = 0.1$ were calculated. The corresponding results are given in Table 1. In the same table are shown the values of these quantities in the first excited state of the molecules studied. The values of the thermodynamic parameters in the excited state were calculated with the aid of Hess' s law from the displacement of the absorption bands in the ultraviolet region upon transition from the molecular to the ionic form. Obviously, $\Delta E + D^* = \Delta E' + D$. ΔE and $\Delta E'$ are the quanta of energy absorbed by the molecular and ionic forms, respectively; D and D^* are the dissociation energies of the molecule in the ground and excited states. If one takes into account that the ΔS of ionization changes little upon transition to the excited state, then pK^* can be calculated from the formula

$$pK^* = pK - \frac{\Delta E - \Delta E'}{2.3RT}.$$

The ΔH of ionization in the ground state, as is evident from Table 1, is considerably smaller in the acid pH region than in the alkaline region. This becomes understandable if one considers that, in acid media, dissociation of protonated molecules takes place:

$$AH^+ + H_2O \rightleftharpoons A + H_3O^+,$$

whereas at alkaline pH values the process takes place

$$A + H_2O \rightleftharpoons A^- + H_3O^+.$$

It is obvious that, in the bases and nucleosides we investigated, it is considerably more difficult to remove a proton from a neutral molecule than from a positive ion.

From a comparison of the ΔH values given in Table 1, the sharp difference in the ΔH values for the dissociations of guanine and guanosine in the alkaline pH region is noteworthy; this is apparently due to different tautomeric states of guanine. Investigation of the infrared and ultraviolet spectra of guanine led us to the conclusion that guanine exists predominantly in a tautomeric form in which the hydrogen atom of the imidazole ring is in position N_7. In guanosine, however, the guanine base is present in a tautomeric form with the hydrogen of the imidazole ring at N_9. Similar results were also obtained in the investigation of xanthine and xanthosine. Consideration of the thermodynamic parameters of dissociation in the excited state shows that, upon excitation of molecules, evidently owing to redistribution of electron density between atoms and bonds, in a number of cases there occurs a sharp change in acid–base characteristics, with enhancement of both the acidic and basic properties of the compounds. This is illustrated in Fig. 1 for cytosine. Apparently, such

Fig. 1. Energy scheme of protolytic reactions of cytosine in the ground and excited states

Figure 1: Fig. 1. Energy scheme of protolytic reactions of cytosine in the ground and excited states

effects may play a major role in the photochemistry of purines, pyrimidines, and other biologically important conjugated molecules. It should be taken into account that, during exothermic dark reactions, a perturbation accompanied by a similar redistribution of electron density may also occur, which likewise should lead to a change in acid–base properties.

Fig. 1. Energy scheme of protolytic reactions of cytosine in the ground and excited states

Let us now consider the possibility of proton transfer between pairs of components of nucleic acids that differ in acid–base characteristics (the discussion may also concern identical components of ampholytic character). The reaction of formation of a complex with proton transfer, for example, from uracil to adenine,

$$\mathrm{U} + \mathrm{A} \overset{K}{\rightleftharpoons} \mathrm{U}^- - \mathrm{AH}^+$$

may be represented as consisting of two stages

$$\mathrm{U} \overset{K_1}{\rightleftharpoons} \mathrm{U}^- + \mathrm{H}^+,$$

$$\mathrm{AH}^+ \overset{K_2}{\rightleftharpoons} \mathrm{A} + \mathrm{H}^+.$$

Then the equilibrium constant of the reaction of formation of the complex with proton transfer is

$$K = \frac{K_1}{K_2}.$$

Using the data given in Table 1, we calculated the thermal effects and constants of formation of the corresponding ion pairs. The results of these calculations are given in Table 2, from which it is seen that formation of a complex with proton transfer in a mixture of bases (or ribosides) cannot occur in any appreciable amounts. On the other hand–

protons, in the excited state the formation of complexes with proton transfer is often energetically favorable: this may facilitate electron transfer, since, in comparison with neutral molecules, the electron affinity (A) increases for the

protonated form, while the ionization potential (I) decreases for a negatively ionized structure. At the corresponding values of I and A for a molecular pair, hydrogen transfer may occur with the formation of two radicals. This should be taken into account when considering photoinitiated ionic and radical processes. The energetic disadvantage of proton transfer between separate free bases does not exclude proton transfer between purine and pyrimidine bases arranged in an ordered fashion along the double-helical DNA chain. Proton transfer between bases becomes possible owing to the energy gain due to the electrostatic interaction of the charges formed in the process. For ordered regions of a DNA or RNA molecule, where the distance between the resulting charges is close to 3 Å, the energy of electrostatic interaction exceeds, in absolute magnitude, the ΔH of complex formation at an effective intramolecular value of the dielectric constant $\varepsilon < 20$. It is obvious that ε inside the macromolecule certainly satisfies these conditions.

Table 2

Thermodynamic parameters of formation of complexes with proton transfer in aqueous solution at $\mu = 0.1$

Complex	K	K^*	ΔH	ΔH^*
$^{+}$–A^{-}	$6 \cdot 10^{-6}$	0.3	4500	−1900
$^{+}$– $^{-}$	$6.9 \cdot 10^{-6}$	10^3	4100	−8300
$^{+}$– $^{-}$	$3.8 \cdot 10^{-6}$	85	4900	−5200
$^{+}$– $^{-}$	$5.1 \cdot 10^{-6}$	$1.1 \cdot 10^{-3}$	3600	400
$^{+}$– $^{-}$	$9.5 \cdot 10^{-6}$	0.12	4200	−1400
$^{+}$– $^{-}$	$1.1 \cdot 10^{-6}$	$6.2 \cdot 10^{-8}$	4200	6100

Note. For the ionic pairs $^{+}$– $^{-}$ and $^{+}$– $^{-}$, the data given pertain to uracil in the ionic form with hydrogen in position N_3. The ionic pairs $^{+}$– $^{-}$, $^{+}$– $^{-}$, and $^{+}$– $^{-}$ refer to ribosides.

Proton transfer between bases in DNA occurs along the line of one of the hydrogen bonds, and in this case the other hydrogen bond, apparently, is strengthened because of charge redistribution in the molecule. The idea of proton transfer can be supported by data on the infrared spectra of complexes of synthetic polynucleotides. In work ([1]) it was shown that, when polyuridylic and polyadenylic acids are mixed in a 1:1 ratio, a two-stranded complex is formed whose infrared spectrum differs from the summed spectrum of the components constituting this complex.

Upon formation of the complex, the highest frequency of the components in the multiple-bond region, $\nu = 1661$ cm^{-1}, shifts to 1672 cm^{-1}, and the intensity of the band with $\nu = 1627$ cm^{-1} decreases significantly. Complex formation between polyinosinic and polycytidylic acids also leads to the appearance, in the high-frequency region of multiple bonds, of a new band with $\nu = 1697$ cm^{-1}.

Before formation of the complex, the highest frequency of the components in this region was 1677 cm^{-1}. Apparently, these changes in the spectra of the complexes indicate protonation of the adenine and cytosine bases. Indeed, in the spectra of adenosine and cytidine the highest frequencies in the multiple-bond region are, respectively, 1660 cm^{-1} and 1654 cm^{-1}. Addition of a proton to these bases is accompanied by the appearance in the spectra of intense bands with $\nu =$ 1724 cm^{-1} for cytidine and $\nu = 1685$ cm^{-1} for adenosine ([2,3]). These frequencies are assigned to vibrations of the C=O and C=N bonds of the protonated bases. Ionization of uracil and of the inosine base leads to a lowering of the frequency in the multiple-bond region.

In the high-frequency region of multiple bonds in DNA spectra at neutral and acidic pH values, according to our data, a band with $\nu = 1713$ cm^{-1} appears, which disappears at pH > 10.8. This fact also supports proton transfer. Earlier ([4]) it was shown that the energy of DNA denaturation, referred to one pair of nucleotides, is ~ 11

kcal/mole. For proteins, the denaturation energy per one hydrogen bond does not exceed 1.5 kcal, which evidently corresponds to the difference in the energies of hydrogen bonds in the polymer and of the polymer with water. It is probable that the increase in denaturation energy on going from proteins to nucleic acids can be associated with an additional strengthening of the structure due to proton transfer and the formation of charge on the bases.

The data presented above compel us to arrive at the following model of the DNA molecule. In ordered double-helical regions, in the ground state of the system, proton transfer between the bases occurs. The direction of this transfer and, consequently, the resulting distribution of charges along the chain is uniquely determined by the nucleotide sequence. In disordered regions of the macromolecule, proton transfer does not occur and the bases are neutral. It is possible that regions of different types also perform different biological functions. It is quite obvious that the presence of a system of charges in the ordered regions of DNA cannot fail to affect the chemical and physical properties of the molecule.

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Note: Figure translations are in progress. See original paper for figures.