INVESTIGATION OF THE INTERMOLECULAR INTERACTION OF NITROGEN HETEROCYCLES OF THE PURINE AND PYRIMIDINE GROUPS IN AQUEOUS SOLUTIONS BY THE NMR METHOD

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The structure and functions of nucleic acids are largely determined by intramolecular interactions of nitrogen bases. These interactions can be divided into two types: hydrogen bonds between complementary base pairs and interplane interactions of neighboring nitrogen bases along the chain, the so-called stacking. To determine the nature of the interactions in the chain of nucleic acids, it is of interest to consider the interaction of the free bases. Moreover, an investigation of the influence of isomeric and tautomeric conversions of the nitrogen bases on their intermolecular interaction is important for an understanding of the mechanisms of spontaneous mutagenesis.

In aqueous solutions the proton acceptor and proton donor groups of nitrogen bases enter into an interaction with water molecules, forming hydrogen bonds. Therefore, the ability to form hydrogen bonds between nitrogen bases is substantially hindered; the interaction of the nitrogen bases in aqueous solutions is accomplished by means of interplane contacts of the bases on account of the action of dispersion forces [1].

Association of bases and nucleosides in aqueous solutions was first detected and studied by methods of osmometry [2]. However, the osmometric data do not permit a judgment of the mutual arrangement of the molecules in the complex, while by using NMR spectroscopy it is possible to obtain information on the structure of the associate.

When the concentration of the purine in aqueous solution was increased, substantial shifts of the PMR signals (Δ6) in the strong-field direction were observed [3], which are the result of the action of the field of the ring current of the π-electrons of the neighboring molecule [4]. However, the observable shifts are far more substantial than the usual Δ6 for aromatic molecules and cannot be explained by the effect of dilution. It has been shown [3, 4] that the cause of the concentration shift of the signals of the protons of the purine is association of bases in water. In this case the complex formed has a stacked structure, and the planes of the molecules in the stack are parallel.

A study of association of nucleic acid base in water is hindered on account of their low solubility; therefore the autoassociation of the readily soluble 6,9-dimethyladenine (I), simulating the basic tautomeric form of adenine; 1-methyl-4-methylaminopyrazolo-[3,4]d-pyrimidine (II), a pyrazole analog of adenine; as well as their heterodissociation with 1, 4-dimethylcytosine (III) and substances simulating the basic diketo form and the relatively improbable enol tautomeric forms of uracil: 1, 3-dimethyluracil (IV), 1,4-dimethyluracil (V), and 2, 4-dimethyluracil (VI), were investigated.

METHOD

The substances used (I)-(IV), were synthesized and identified according to the well known method of [6-9], respectively, while (V) and (VI) were synthesized according to [10]. The PMR spectra were measured...
RESULTS AND DISCUSSION

Four peaks are observed in the PMR spectra of solutions of the investigated compounds in D$_2$O (Fig. 1). The assignment of the signals from the H$_2$ and H$_8$ protons of (III)-(VI) was made according to [11]. The signals of the H$_2$ and H$_8$ protons of (I) were assigned on the basis of the ability of the H$_8$ proton to undergo exchange with the solvent [12]: when the temperature is raised to 100°, the intensity of the signal of the H$_8$ proton drops substantially. Exchange of the H$_8$ protons of (II) with D$_2$O is already observed at 34°. When the concentration of the dissolved substance is increased in the interval 0.05-0.4 M, a substantial shift of the signals from the protons of (I) and (II) in the strong-field direction is observed (Fig. 2), which indicates the formation of autoassociates of (I) and (II), possessing a stacked structure, in solution. At a constant concentration of (I) or (II) in solution, raising the temperature in the interval 34-100° causes a substantial shift of the resonance signals in the weak-field direction, which is evidence of exothermicity of the autoassociates. The chemical shifts of (I) and (II) vary linearly with changing temperatures.

Raising the temperature and varying the concentrations have no influence on the shifts of the protons for solutions of (III)-(VI) and their mixtures.

An analogous phenomenon was observed earlier [13] for uridine, thymine, and cytidine. This is due to the low diamagnetic anisotropy of the uracil and cytosine rings* and the weak ability of pyrimidine derivatives for autoassociation.†

However, in mixtures of (III)-(VI) with (I) or (II), when the concentration of the latter in solution is increased, there is a substantial shift of the resonance signals of the protons of (III)-(VI) into the strong-field region (Fig. 3 and Table 1), which is evidence of the formation of heteroassociates in solution, possessing a stacked structure. Raising the temperature weakens the concentration dependence of the chemical shifts of the protons of the pyrimidine derivatives. This indicates an exothermicity of the heteroassociation and serves as still another confirmation of the fact that the nature of the auto- and heteroassociatives is the same. The temperature dependence of the chemical shifts of the protons of (III)-(VI) in a mixture with (I) or (II) is linear.

Information on the mutual arrangement of the molecules in the stack can be obtained from the PMR spectra. As can be seen from Fig. 2, in the autoassociation of (I) a stronger concentration dependence of $\Delta \delta$ is observed for the H$_2$ proton of the pyrimidine ring in comparison with the H$_8$ proton of the imidazole ring. We should note that for all the nucleic derivatives investigated up to the present time [1], $\Delta \delta$ of the protons of the pyrimidine ring is greater than $\Delta \delta$ of the protons of the imidazole ring.*

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* According to quantum-mechanical calculations, the intensity of the $\pi$-electronic ring current is (benzene taken as the unit): uracil 0.05; cytosine 0.2; adenine 0.9 (five-membered ring) and 0.7 (five-membered ring) [14].
† Osmometric measurements [2] have shown that pyrimidine derivatives are inclined to autoassociation to a substantially lesser degree than purine and its derivatives.