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INVESTIGATION OF THE INFLUENCE OF INTRAMOLECULAR ELECTROSTATIC INTERACTIONS ON THE CONFORMATIONAL EQUILIBRIUM IN PYRIMIDINE NUCLEOSIDES BY THE PMR METHOD

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The influence of solvents on the PMR spectra of uridine and cytidine has been studied. Because of intramolecular electrostatic interactions (IEIs) between the 2-keto oxygen and the freely rotating 2-hydroxyl, the position of the conformational equilibrium in the pyrimidine nucleosides but not in purine and deoxy nucleosides, depends substantially on the dielectric constant of the solvent and the size of the partial negative charge on the 2-keto oxygen of the base. It has been shown that an increase in the IEI leads to an increase in the 3'-endo (N) population of the ribose ring and to an increased influence of the temperature on the state of the conformational equilibrium.

Attempts are usually made to ascribe various conformational effects of nucleic acids and their components, nucleotides and nucleosides, mainly to the direct interaction between the base and the ribose and, in particular, between the 2-keto oxygen and the 2'-hydroxyl of the ribose in pyrimidine derivatives [1, 2]. However, such an approach does not permit an explanation of, for example, the difference in the states of conformational equilibrium of the ribose ring between corresponding derivatives of uridine (U) and cytidine (C) [2].

We have studied the influence of intramolecular electrostatic interactions (IEIs) on the state of the conformational equilibrium in U and C. With this aim we have investigated the PMR spectrum of U and C in a number of solvents: (dimethyl sulfoxide)-d_6 (DMSO-d_6), dimethylformamide-d_7 (DMFA-d_7), methanol-d_4, pyridine-d_5, and water-d_2, since, according to the theory of reaction fields [3], the energy of an IEI depends on the dielectric constant of the medium ϵ.

The conformation of the ribose ring of a nucleoside can be described on the basis of the idea of pseudorotation [4], making use of the phase angle of pseudorotation P and the degree of pucker τ. In solutions, however, there is a dynamic equilibrium between conformers of the N and S types (Fig. 1), which include the classical 2'-endo (S) and 3'-endo (N) conformations [2]. When the condition that P and τ are constant is observed, the populations p_N and p_S of conformers of type N and S, respectively, can be calculated by using the "direct method" [2]:

$$p_S = 10\ J_{1'-2'}, \quad (1)$$

$$p_N + p_S = 1. \quad (2)$$

It is considered that this condition is satisfied if $J_{2'-3'}$ and $\Sigma = J_{1'-2'} + J_{3'-4'}$ are constant. Unfortunately, it was impossible to check the fulfillment of this condition, since the H-2' and H-3' signals partially overlap, which considerably complicates the analysis of the spectrum. However, it is known [5] that IEIs have little effect on the positions of the minima in space of the geometric parameters characterizing the conformations of molecules (in this case, on P and τ) but substantially change the absolute values of the energy. Consequently, we have assumed that τ and P do not depend on the solvent.

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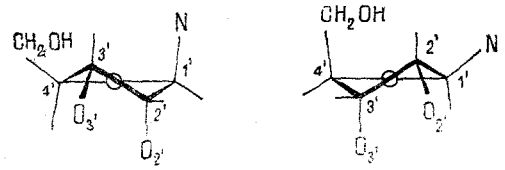


N type S type

Fig. 1. Conformers of types N and S.

TABLE 1. Influence of the Solvent on the Conformational Equilibrium of the Ribose Ring in Pyridine and Cytidine

Nucleoside	Solvent	ε	T, °C	$J_{1'-2'}$, Hz	p_S, %	$K_{S/N}$	ΔG°, kcal/mole
Uridine	Water-d_2	78,5	25	4,4	44	0,79	0,14
			80	4,4	44	0,79	0,17
	DMSO-d_6	48,9	25	4,9	49	0,96	0,02
			80	4,9	49	0,96	0,03
	DMFA-d_7	36,7	25	4,7	47	0,89	0,07
			80	4,6	46	0,85	0,11
	Methanol-d_4	32,6	25	4,0	40	0,67	0,24
			60	4,2	42	0,72	0,21
	Pyridine-d_5	12,3	25	3,3	33	0,49	0,42
			80	3,5	35	0,54	0,43
Cytidine	Water-d_2	78,5	25	3,6	36	0,56	0,34
			80	3,8	38	0,61	0,34
	DMSO-d_6	48,9	25	3,7	37	0,59	0,31
			80	4,0	40	0,67	0,28
	DMFA-d_7	36,7	25	3,5	35	0,54	0,37
			80	3,5	35	0,54	0,43
	Methanol-d_4	32,6	25	2,3	23	0,30	0,71
			60	2,6	26	0,35	0,69
	Pyridine-d_5	12,3	25	2,0	20	0,25	0,82
			80	2,9	29	0,41	0,63

Table 1 shows the population of the S conformation, the equilibrium constants $K_{S/N}$, and the values of the differences in the standard free energies ΔG° of the nucleosides investigated in dependence on the solvent and the temperature, calculated by the "direct method." As can be seen from Table 1, the solvent affects the conformational equilibrium of the ribose ring of pyrimidine nucleosides far more strongly than the temperature. The absence of an influence of the solvent on the SSCCs of the protons in deoxynucleosides and purine nucleosides has been shown previously [6]. Apparently, the influence of the solvent on the conformation of the equilibrium in pyrimidine nucleosides is due to electrostatic repulsion between the 2-keto oxygen and the 2'-hydroxyl, which increases with a decrease in ε. This leads to an increase in the population of the N conformation, since an analysis of models shows that in this case the distance between the 2-keto oxygen and the 2'-hydroxyl increases. With an increase in the contribution of the IEI, the influence of the temperature on the conformational equilibrium rises (see Table 1). Considering that the 2-keto oxygen in C bears a larger partial negative charge than in U [7], the difference in the states of conformational equilibrium on the ribose ring between the corresponding derivatives of U and C can be explained to a considerable degree by the IEI between the 2-keto oxygen and the 2'-hydroxyl.

It must be mentioned that the formation of a $C_2{=}O\bullet\bullet\bullet HO_2$, intramolecular hydrogen bond is also possible. However, as has been established [8-10], in polar solvents the formation of such a bond is unlikely, since intermolecular interactions with the molecules of the solvent predominate, as a consequence of which free rotation of the 2'-hydroxyl is observed. This rotation is apparently the main reason for the appearance of forces of electrostatic repulsion.

In aqueous solutions, the value of $J_{1'-2'}$ is considerably smaller than should be expected on the basis of the general dependence on ε. Here the expected values ($J_{1'-2'}$ = 5-6 Hz) are close to the values in the absence of an IEI between the base and the 2'-hydroxyl in the mononucleotide nicotinamide ($J_{1'-2'}$ = 5.4 Hz [11]) and in the purine nucleosides (about 6 Hz

[6]). Such an intensification of the IEI is possible in the case of incomplete solvation of the interacting groups, which is equivalent to a decrease in ε [3]. Furthermore, it is not excluded that an increase in the population of 3'-endo (N) conformations in aqueous solutions of pyrimide nucleosides is a consequence of the formation of an intramolecular hydrogen bond between the 2-keto oxygen and the 2'-hydroxyl, stabilizing the 3'-endo (N) conformer [10]. Thus, the total energy of the IEI and the hydrogen bond, which, in particular, depends on the degree of solvation and may play an important role in conformational transitions of nucleic acids.

The conformation of the exocyclic $-CH_2OH$ group (see Fig. 2) was investigated with the aid of the $J_{4'-5'}$ and $J_{4'-5''}$ SSCCs. The assignment of the signals of the methylene protons and the calculation of the populations of the gauche-trans and trans-gauche rotamers p_{gt} and p_{tg}, respectively, were performed according to Davies and Rabczenko [12]. The population of the gauche-gauche rotamer p_{gg} was determined by using the equations [13]

$$p_{gg} = \frac{12 - \Sigma}{10}, \tag{3}$$

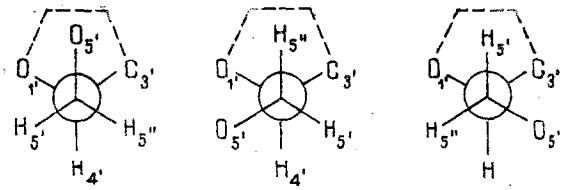
where $\Sigma = J_{4'-5'} + J_{4'-5''}$. The figures obtained are given in Table 2. It is known [14] that in the 3'-endo (N) conformation the gauche-gauche rotamer predominates, and in the 2'-endo (S) conformation the gauche-trans rotamer. This law is also observed, in general, in the present case. However, with a decrease in ε the IEI between O-1' and O-5' in the gauche and gauche-trans rotamers rises, which is is possibly the reason for the absence of a clearer expression of the law mentioned. The influence of the temperature on the state of the conformational equilibrium rises considerably with a decrease in ε. As we shall see, a rise in the temperature leads to some equalization of the population of the rotamers. This also shows an increase in the influence of the IEI on the state of the conformational equilibrium in weakly polar solvents.

An investigation of the influence of the IEI on the position of the conformational equilibrium relative to the glycosidic bond by the PMR method is made difficult by the overlapping of the H-2' and H-3', and also the H-1' and H-5, signals. In addition, an analysis of the changes in the chemical shifts is complicated by the differences in the magnetic anisotropy of the solvents used. However, the use of the ^{13}C NMR method [15] has shown that the IEI between the 2-keto oxygen and the 2'-hydroxyl also has an influence on the rotational state around the glycosidic bond.

Thus, it has been established that because of intramolecular electrostatic interactions the conformational equilibrium in pyrimidine nucleosides depend to a considerable degree on

TABLE 2. Influence of the Solvent on the Population of Rotamers of $-CH_2OH$ Groups

Nucleoside	Solvent	ε	T, °C	$J_{4'-5'}$, Hz	$J_{4'-5''}$, Hz	p_{gg}, %	p_{gt}, %	p_{tg}, %
Uridine	Water-d_2	78,5	25	2,6	4,4	0,60	0,33	0,07
			80	3,1	4,4	0,55	0,33	0,12
	DMSO-d_6	48,9	25	2,4	3,5	0,71	0,24	0,05
			80	2,9	3,7	0,64	0,26	0,10
	DMFA-d_7	36,7	25	2,4	3,1	0,75	0,20	0,05
			80	2,6	3,5	0,69	0,24	0,07
	Ethanol-d_4	32,6	25	2,6	3,1	0,73	0,20	0,07
			60	2,7	3,3	0,70	0,22	0,08
	Pyridine-d_5	12,3	25	2,6	2,9	0,75	0,18	0,07
			80	3,1	3,1	0,68	0,16	0,16
Cytidine	Water-d_2	78,5	25	2,3	4,3	0,64	0,32	0,04
			80	2,5	4,0	0,65	0,29	0,06
	DMSO-d_6	48,9	25	2,4	3,3	0,73	0,22	0,05
			80	2,4	3,5	0,71	0,24	0,05
	DMFA-d_7	36,7	25	2,4	3,0	0,76	0,19	0,05
			80	2,7	3,5	0,68	0,24	0,08
	Ethanol-d_4	32,6	25	2,2	2,9	0,79	0,18	0,03
			60	2,6	3,3	0,71	0,22	0,07
	Pyridine-d_5	12,3	25	2,6	2,9	0,75	0,18	0,07
			80	3,1	3,3	0,66	0,22	0,12



gauche-gauche gauche-trans trans-gauche

Fig. 2. Conformations of the exocyclic $-CH_2OH$ group.

the magnitude of the partial negative charge on the 2-keto oxygen and on the dielectric constant of the solvent.

EXPERIMENTAL

PMR spectra were taken on a Bruker WH-90 spectrometer at a frequency of 90 MHz. The solvents used were DMSO-d_6, DMFA-d_7, and pyridine-d_5 dried over 4 Å molecular sieves. The accuracy of the measurement of the SSCCs was ±0.2 Hz and of the temperature ±1°. The concentration of solutions depended on the solubility, but was not greater than 0.1 M.

SUMMARY

The influence of solvents on the SSCCs in the PMR spectra of uridine and cytidine has been studied. Because of intramolecular electrostatic interactions (IEIs) between the 2-keto oxygen and the freely rotating 2'-hydroxyl, the position of the conformational equilibrium of the ribose ring of a pyrimidine nucleoside depends substantially on the size of the partial negative charge on the 2-keto oxygen and the dielectric constant of the constant. An increase in the IEI leads to a shift of the equilibrium in the direction of the 3'-endo (N) conformation, which is accompanied by an increase in the influence of the temperature on the position of the equilibrium.

In aqueous solutions, an additional stabilization of the 3'-endo (N) conformation arises through the formation of an intramolecular hydrogen bond between the 2-keto oxygen and the 2'-hydroxyl and/or the incomplete solvation of the interacting groups.

The influence of IEIs on the rotation of the exocyclic $-CH_2OH$ group and of the base has also been shown.

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