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Abnormal shifts in Raman spectra of deuterated cytidine and 6-azacytidine

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Abstract. It was revealed that some peaks in Raman spectra of cytidine, 6-azacytidine (6-azaC), and cytosine dissolved in D_2O are shifted to high frequencies in respect to their positions in spectra of H_2O solution. Such "blue shifts" occur due to deuteration of the nucleoside molecule itself but not due to effect of deuterated solvent. This conclusion is deduced from observation of blue (abnormal) shift in Raman spectra of cytidine and 6-azaC microcrystals recrystallized from D_2O solution. Both normal and abnormal shifts close to the experimentally observed were obtained in the calculated spectra of 6-azaC and cytidine. We assume that abnormal shifts may be caused by substitution of intramolecular H-bonds with D-bonds.

Keywords: Raman spectra, cytidine, 6-azacytidine, blue shift, H-bonds, deuteration

1. Introduction

The cytidine is well-known canonical nucleoside and anomalous nucleoside 6-azacytidine (6-azaC) is a structural analogue thereof with N atom replacing of C–H group at the 6th position of pirimidine ring ([5] and references therein). The 6-azaC is an antimetabolite of nucleic acid exchange and considered as a prospective pharmaceutical component with a wide spectrum of therapeutic effects [1]. The 6-azaC is usually available in microcrystalline form but its valuable biological activity appears in solutions and not in crystalline state. Raman spectra of 6-azaC were measured for the first time both in microcrystalline form and in different particularly in water H_2O and D_2O solutions [6]. Shifts of some Raman peaks of 6-azaC dissolved in D_2O to high frequencies in respect to their position in H_2O solution and microcrystalline spectra were noticed. Similar blue shift was observed also in spectrum of cytidine dissolved in D_2O. In this paper we present results of experimental and computational study of blue shift in Raman spectra of cytidine and 6-azaC.

2. Materials and methods

Ar^+ laser with 200 mW at 488 nm was used to obtain Raman spectra at room temperature with Coderg T-800 triple monochromator equipped with a photon counting system. Solution samples were prepared by dissolving the crystalline 6-azaC, cytidine and cytosine in distilled deionized H_2O and in D_2O (99.9%).

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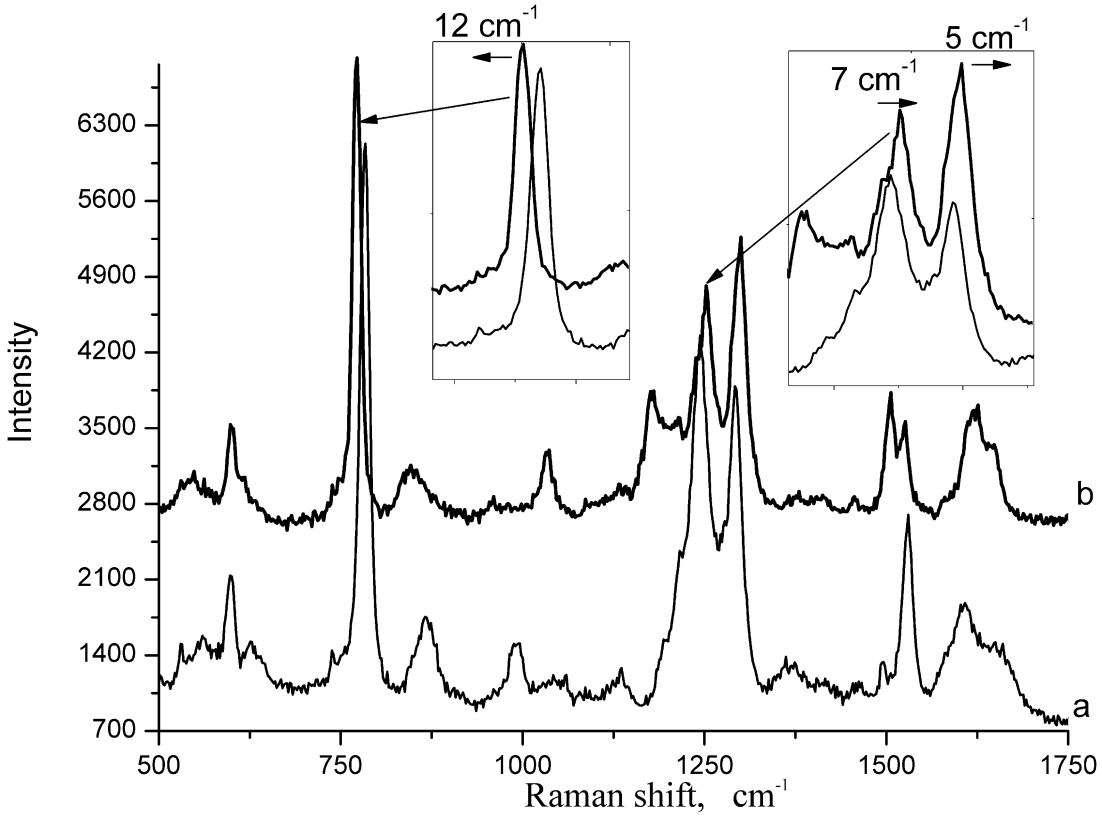

Fig. 1. Raman spectra of cytidine dissolved in H_2O (a) and D_2O (b).

3. Results and discussion

Overview Raman spectra of cytidine in H_2O and D_2O solutions in the range 500–1800 cm^{-1} are presented in Fig. 1. Comparison between them shows shifts of some peaks in spectrum of D_2O solution in two opposite directions in respect to their positions in spectrum of H_2O solution. Thus peaks which are shifted to high frequencies manifest so called blue shift. Similar situation were observed for 6-azaC dissolved in H_2O and D_2O (Fig. 2). In this paper we are focusing at the most prominent Raman peaks that demonstrate significant shifts in spectra of cytidine and 6-azaC water solutions (Table 1). Mentioned peaks in Raman spectra of studied compounds are assigned to vibrations of pyrimidine ring, specifically peaks in range 740–790 cm^{-1} are assigned to breathe ring vibration, in range 1240–1250 cm^{-1} are assigned to C5–H and C6–H out of phase bending vibration and peaks in range 1280–1305 cm^{-1} are assigned to C2–N3 stretching (molecular structure of 6-azaC see in Fig. 2).

In general, blue shifts in Raman spectra of dissolved compounds may occur due to change of the solvent (in our case H_2O for D_2O) or due to deuteration of solute molecule itself. Appropriate examples may be found for instance in [4] and [2] where Raman blue shifts are connected with transformation of intermolecular or intramolecular H-bonds into D-bonds, respectively. To discriminate effect of deuterated solvent and deuteration effect of the nucleoside molecule itself we have made recrystallization of 6-azaC and cytidine from their H_2O and D_2O solutions.

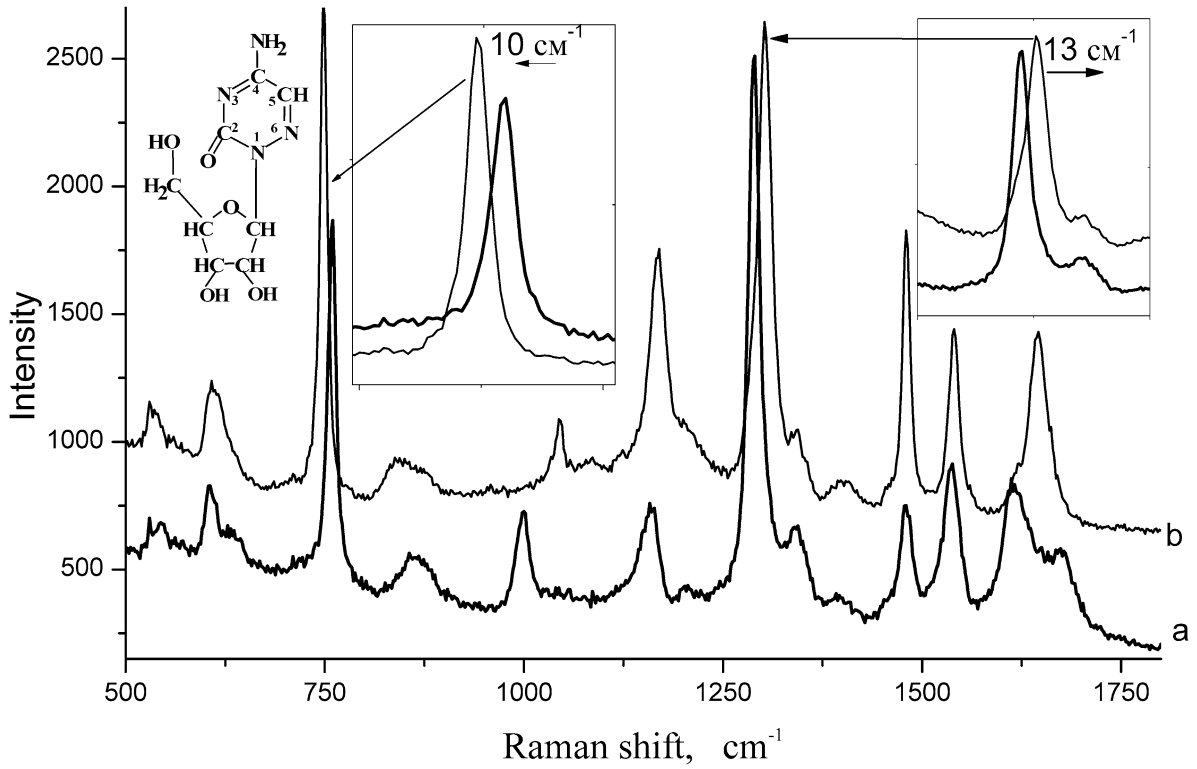

Fig. 2. Raman spectra of 6-azaC dissolved in H_2O (a) and D_2O (b).

Table 1

Raman peak positions ν in spectra of H_2O, D_2O solutions and respective shifts $\Delta\nu$, and the same for spectra of initial and recrystalized microcrystals

Substance		ν, cm^{-1}		$\Delta\nu$	ν, cm^{-1}		$\Delta\nu$	ν, cm^{-1}		$\Delta\nu$
		H_2O	D_2O		H_2O	D_2O		H_2O	D_2O	
6-azaC	exp	758	748	10	–	–	–	1289	1302	−13
	cal	775	762	13	–	–	–	1292	1300	−8
Cytidine	exp	784	772	12	1244	1251	−7	1292	1297	−5
	cal	786	774	12	1267	1270	−3	1311	1312	−1
Cytosine	exp	786	778	8	1225	–	–	1289	1290	−1
	cal	785	772	13	1211	–	–	1297	1303	−6

The Raman spectra of nucleosides recrystallized from H_2O solution are identical to the spectra of initial microcrystals. In the same time the Raman spectra of nucleosides recrystallized from D_2O solution contain both the peaks of initial microcrystals and the satellite peaks with the same spectral position as for D_2O solution. Thus, doublets appear in spectra of nucleosides recrystallized from D_2O (Fig. 3). It is important to note that relative intensity of doublet components under different recrystallization conditions is altered while spectral position of components do not change (Fig. 3(b), (b′)). It proves noticeable deuteration of the nucleoside molecule itself.

Under standard assumption if the type of vibrational mode and the force constants do not change under deuteration only decreasing of mode frequency is possible and results in low frequency shifts

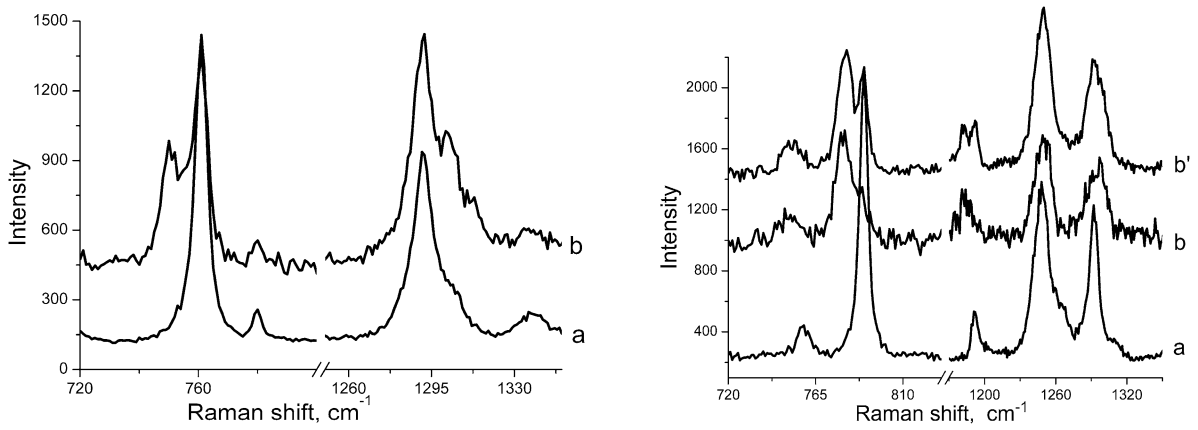

Fig. 3. Raman spectra of 6-azaC (left) and cytidine (right) microcrystals before (a) and after (b, b′) recrystallization from D_2O solution.

in Raman spectra. So in this approach observed Raman blue shift should be considered as abnormal. An increasing of mode frequency when reduced mass increases due to deuteration may be explained by advance strengthening of mode force constants. The last may be caused by substitution of intramolecular H-bonds with D-bonds.

This hypothesis is confirmed in part by calculations of Raman spectra performed both for free molecules of studied compounds and their water solutions using Gaussian 03 package. Both normal and abnormal shifts close to the experimentally observed were obtained in the calculated spectra of 6-azaC and cytidine (Table 1).

We should note that Raman blue shift of peaks corresponding to the same vibration types in spectra of 5′-CMP (cytidine 5′-monophosphat) and 5′-dCMP was explained by deformation of sugar ring near the glycosidic bond [3]. This interpretation does not coincide with our observation of blue shift in Raman spectrum of cytosine in D_2O solution (Table 1) because there is no sugar ring in cytosine molecule. Nevertheless one cannot exclude contribution of such mechanism to observed blue shift spectra of 6-azaC and cytidine.

4. Conclusions

Blue shifts of some peaks observed in Raman spectra of cytidine and 6-azaC dissolved in D_2O are caused by deuteration of the nucleoside molecule itself. An increasing of mode frequency under increasing of reduced mass due deuteration may be explained by advance strengthening of mode force constants. The last may be caused by substitution of intramolecular H-bonds with D-bonds. Strengthening of mode force constants matrix may be also accompanied with distortion of nucleoside molecule.

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