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pK_a Determination of D-Ribose by Raman Spectroscopy

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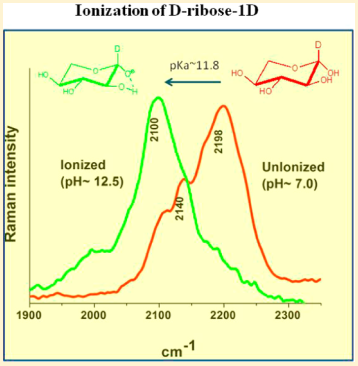
Supporting Information

ABSTRACT: Determination of the pK_a of OH groups present in D-ribose is crucial in order to elucidate the origin and mechanism of many catalytic processes that involve the ribose unit. However, there is hardly any reports about the experimental pK_a of the OH group due to the lack of an appropriate method. In this study we investigated the protonation state of OH groups in D-ribose by introducing C−D labeling and measuring the changes in the isolated C−D frequency in several isotopologues of the compound with pH. The large shift in the ν_{C-D} of D-ribose-C1-D in ionized condition compared to other deuterium-substituted D-riboses (e.g., D-ribose-C2-D, D-ribose-C3-D, etc.) confirmed that the C1−OH group preferred ionization, and the ionization pK_a was 11.8. Both the ionized and the unionized structures of D-ribose preferred the pyranose conformation, which was supported by ^{13}C NMR experiments. Electronic redistribution via resonance and intramolecular hydrogen-bond formation were proposed to account for the stabilization of the ionized structure.



INTRODUCTION

D-Ribose in aqueous solution largely remains as an equilibrium mixture of furanose and pyranose structures.[1−5] However, in many biological systems the ribose unit is incorporated with the ribofuranose structure such as in RNA, DNA, and cofactors.[6] This ribose unit is actively involved in the structure and many important biological functions such as molecular recognition, proteolytic processing, and catalytic activity of functional RNA and DNA molecules.[6] It is believed that, in many catalytic processes, a particular hydroxyl group of the riboside ring is involved in hydrogen-bond formation with the substrate. For example, the 2′-hydroxyl group in RNA has many structural and functional implications during ribonuclease catalysis, which is steered by different acidities/basicities of the 2′-OH group.[6] It is thus very important to address the acidity and pK_a of a particular OH group in the molecule.

After much time a team of German/Swiss-based researchers has presented a long-sought result: the crystal structure of ribose in the year 2010.[7] However, there is hardly any report about the experimental pK_a of D-ribose and its ionized structure. There are few conflicting reports about the pK_a of the ionizable hydroxyl group of the ribonucleosides and necleotides. Izzat et al., assuming one dissociable proton, established the pK_a of adenosine as ∼12.3.[8,9] Similarly, potentiometric titration of phosphorylated ribose gives a pK_a of 2′-OH to be 13.9.[10] However, they failed to report the exact pK_a value for D-ribose and the conformational preference of the ionized D-ribose.

In the present report we combined NMR, Raman spectroscopy, and DFT analysis to report the pK_a of D-ribose and the ionized structure of the molecule in solution state. NMR spectroscopy was used to demonstrate the content of different conformers of D-ribose in both ionized and unionized states. To determine the pK_a and identify the ionizable O−H group in the molecule, we used a unique method of Raman spectroscopy on deuterium-labeled D-riboses. In this method the ionization of the −OH groups in D-ribose was measured via vibration of a neighboring C−D bond (H−C$_\alpha$−O−H was replaced with D−C$_\alpha$−O−H) in a specific position of the molecule. Measurement of the C−D stretching vibration of a specifically labeled D−C$_\alpha$−O−H group avoided spectroscopic overlap with other C−H bond vibrations. C−D substitution also specified a particular O−H position, and ν_{C-D} appeared in a clear spectroscopic window (1800−2600 cm^{-1}). By monitoring the C−D bond vibration of labeled D-riboses at different pHs, we showed that C1−OH of D-ribose was the primary site of ionization and the estimated pK_a was 11.8. DFT analysis and NMR results further indicated that the pyranose conformation was the favored ionic structure in alkaline media.

MATERIALS AND METHODS

Chemicals. Deuteriated and ^{13}C-labeled D-riboses were obtained from Omicron Biochemicals, Inc. Compounds were used as obtained. Other chemicals were purchased from Sigma Chemicals. Milli Q water was used for most of the sample preparation.

EXPERIMENTS

NMR Measurements. ^{13}C NMR spectra of D-[^{13}C1] ribose were collected with a Bruker 600 MHz spectrometer at 24 °C with proton decoupling. One millimolar labeled D-ribose solution was prepared in H_2O and NaOH solution. NaOH

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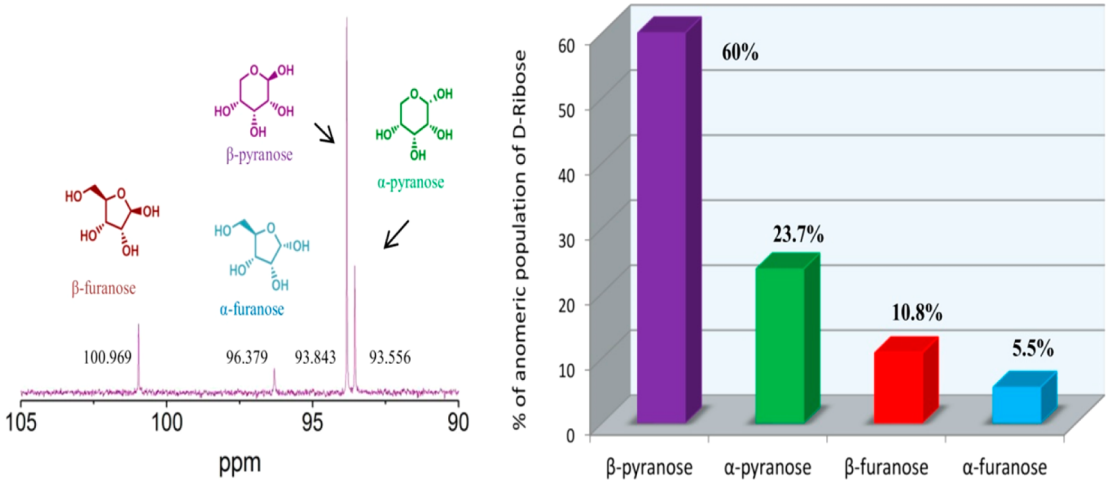

Figure 1. ^{13}C1 NMR spectrum of an aqueous solution of D-[^{13}C1] ribose at pH 6.85. Solution contained 1 mM labeled ribose. Chemical shift values are marked in the spectrum. Bar diagram shows the percentage of each furanose and pyranose anomers based on the integrated peak intensity of the ^{13}C1 NMR spectrum.

solution of different concentrations was prepared by dissolving solid NaOH in H_2O. Ten percent D_2O was added in each sample for locking purposes.

Computational Method. Different anomers of riboses in neutral structure in the gas phase were energetically minimized using Gaussian 09 and B3LYP methods with the 6-31G + +(d,p) basis set. Each molecule was ionized by removal of a proton from the hydroxyl, minimized again at the same level of theory, and subjected to frequency calculation using Gaussian 09W

Raman Spectroscopy. Raman spectra were recorded using the 647.1 nm laser beam from an Innova 400 krypton laser (Coherent, Inc.). A back-illuminated charge-coupled device (CCD) detector (model 1024EHRB/1, Princeton Instruments, Inc.) operating at 183 K was used. A Holospec f/1.4 axial transmission spectrometer (Kaiser Optical Systems, Inc.) was employed as a single monochromator as described previously.[11]

Raman spectra of deuterium-labeled riboses (~10 mM) were acquired in aqueous solution at different pHs. The sample (60 μL) was held in a 2 × 2 mm quartz cuvette under the 90° excitation/collection geometry, and Raman spectra were collected using a laser power of ~400 mW and CCD exposure times of 1 min. For each spectrum, generally 10 exposures were acquired. The Raman spectrum of the solvent was recorded for an equal time and subtracted from that of the ribose spectrum. Wavenumber calibration was performed by recording the Raman spectra of a 1:1 mixture of cyclohexanone and acetone-D_6, providing band positions to within ±1 cm^{-1} for sharp bands.

RESULT AND DISCUSSION

D-Ribose in aqueous solution remains as an equilibrium mixture of furanose and pyranose structures.[1,2] Each ring structure has two different anomeric forms, α and β. Figure 1 shows the ^{13}C NMR spectrum of an aqueous solution of D-[^{13}C1] ribose (labeled at position C1 with ^{13}C) at pH 6.85. The assignment of C1 NMR signals for different anomeric conformations of the D-[^{13}C1] ribose was made based on the chemical shifts assigned in earlier reports.[1,2,12] The ^{13}C1 NMR band at 100.969 ppm was characteristic of β-furanose, the weakest peak at 96.379 ppm was the marker for α-furanose, while the strong peaks at 93.843 and 93.556 ppm, respectively, were assigned to the β and α conformers of the pyranose structure. The bar diagram in Figure 1 shows the percentage of each furanose and pyranose anomer based on the integrated peak intensity of ^{13}C1 NMR signal at pH 6.85. It consists of 60% β-pyranose, 23.7% α-pyranose, 10.8% β-furanose, and 5.5% α-furanose. At this pH the intensity patterns indicated that the overall pyranose population was higher than the furanose conformation, and the molecule remained unionized. The β-anomeric forms were predominant in the equilibrium condition for both the pyranoses and the furanoses. In equilibrium it may contain a very low concentration of open aldehyde conformation;[2] however, it was not detected.

The ionization behavior of D-ribose was investigated in aqueous NaOH solution. The ^{13}C NMR chemical shift of D-[^{13}C1] ribose at 24 °C as a function of the amount of NaOH added to a 1 mM solution of the ribose is shown in Figure 2, and the ^{13}C1 chemical shifts are listed in Table S1 (Supporting Information). ^{13}C1 chemical shifts indicated that all conformations of the molecule were present up to pH 7.3 (0.1 mM NaOH); however, the peak intensities of respective conformers varied and indicated changes in the concentration of different anomers. At greater than ~1 mM NaOH concentration (pH 8.4) the signature NMR signal for the α- and β-furanose conformations disappeared completely. Figure S1, Supporting Information, shows the plot of the ^{13}C1 peak intensity ratio of β-furanose and β-pyranose at different pHs and indicated that the concentration ratio was almost constant up to pH 7.3 (100 μM NaOH). At pH 10.4 the ^{13}C resonances of the furanose conformations disappeared completely; however, the pyranose forms (both α and β) remained in solution as reflected by their respective NMR peaks. It indicated that furanose forms the preferred initial ionization. Due to ionization, furanose population decreased and caused a lowering of the respective NMR peak intensity.

At higher NaOH concentrations (10 mM and above) the signature ^{13}C1 NMR signals for both anomers of the pyranose conformation disappeared and two closely spaced broad peaks appeared at lower field (higher ppm values). It indicated the start of the ionization of pyranose anomers. At 40 mM NaOH (pH 11.94) two closely associated broad bands appeared at 95.492 and 95.121 ppm, presumably due to complete ionization and formation of the ribopyranoside structure. The downfield

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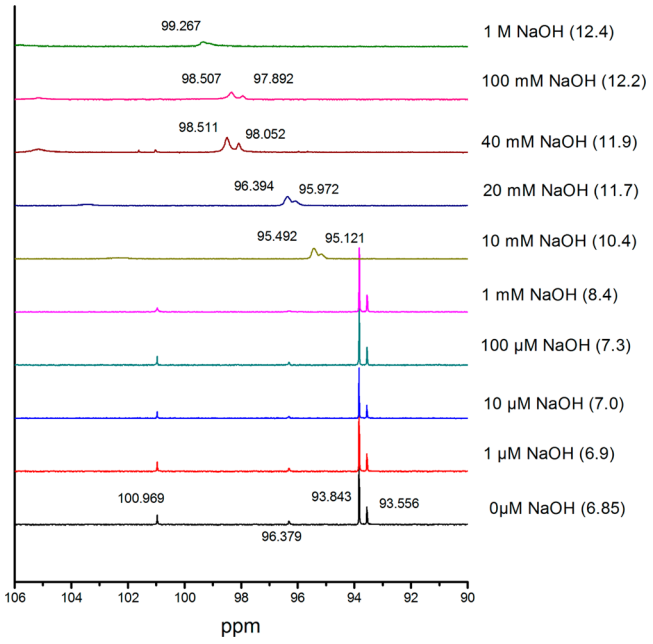

Figure 2. ^{1}H-decoupled ^{13}C NMR spectra of D-[^{13}C1] ribose in the presence of different concentrations of NaOH. pH value of the respective solution is shown in parentheses. Figure shows only the enriched carbon. Peaks are assigned to α- and β-ribofuranoses and α- and β-ribopyranoses. Each sample contained 1 mM labeled (^{13}C) ribose. Chemical shift values are marked in the spectrum based on a previously published report.

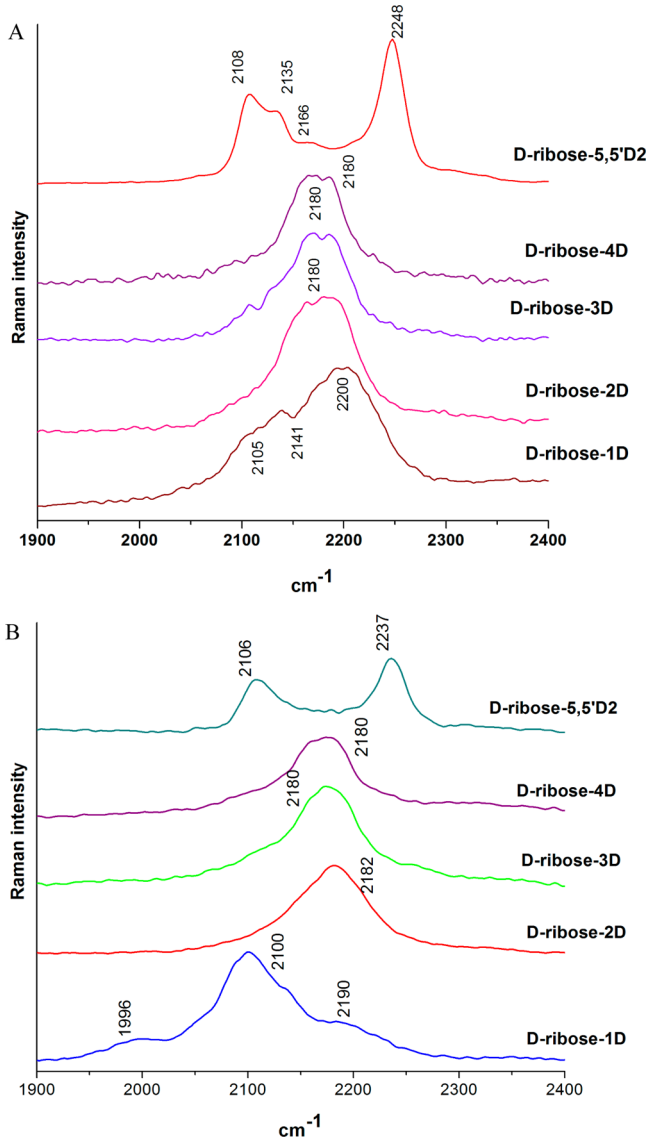

Figure 3. C−D stretching region of the room-temperature Raman spectra of different isotopologues of D-ribose (~10 mM). C−H bonds at C1, C2, C3, C4 C5, and C5′ positions are replaced separately with C−D bonds. (A) Raman spectrum of deuterium-labeled D-riboses in aqueous solution at neutral pH. (B) C−D bond vibrations of the labeled riboses in 1 M NaOH solution, i.e., in the ionized state. Raman band in the figures was due to vibration of C−D bonds. Ten millimolar D-ribose solution was prepared in water, and pH was adjusted with aqueous NaOH solutions.

shift was at least in part due a change in hydration on going from the neutral D-ribose to the D-ribose anion. Additional downfield shifts of the signals may be due to enhanced C═O bond character in the ionized structure.

The ionized species appeared to be in rapid equilibrium between the α- and the β-pyranoside forms, and complete ionization occurred at high alkaline condition. A similar ionization behavior and the pyronoside anionic form were reported for other sugars and hexoses.[13,14] The 2′-OH group possibly formed an H bond with C1−O$^-$ and may have contributed to the broadness, apart from the ionized structure at high NaOH concentration.[2,15−18] It may be noted that the occurrence of the 1,2-open chain configuration was also less probable since no signature NMR signal was obtained at ~204 ppm corresponding to the free aldehyde form of the molecule.[2,5] The ^{13}C chemical shifts of D-[^{13}C1] ribose in NaOH solutions thus indicated that ionization of the ribofuranose conformation started first and revealed the differences in the acid strength, the pK_a values between the furanose and the pyranose forms. NMR results further indicated that the pyranoside conformation was the most populated structure in the ionized state. Raman spectroscopic investigation as discussed below further established that in ionized condition (at high pH) the conformational heterogeneity of D-ribose was reduced; it also confirmed that only one −OH group (C1−OH) of D-ribose was ionized, and others were not ionized even at very high alkaline condition.

Raman spectra of different isotopologues of D-ribose (D-ribose-C1-D, D-ribose-C2-D, D-ribose-C3-D, D-ribose-C4-D, and D-ribose-C5,5′-D$_2$) in water in the ν_{C-D} region are shown in Figure 3A. D-Ribose-C1-D showed one strong C−D stretching band at ~2200 cm^{-1}; however, two shoulder bands were also observed at ~2141 and 2105 cm^{-1}. The presence of more than one C−D stretching band for a single deuterium substitution was due to the existence of both the α and the β anomers of pyranose and furanose conformers. The ^{13}C1 NMR spectrum of D-ribose (Figure 1) also confirmed the presence of different anomeric forms of the ribose molecule in aqueous media. The weak Raman band/shoulder arose due to conformers which were less populated in the solution. The characteristic C−D vibrations of D-ribose-C2-D, D-ribose-C3-D, and D-ribose-C4-D appeared at 2180−2190 cm^{-1}; the overall width of the bands was less than the C1−D bandwidth as observed in D-ribose-C1-D. C2, C3, and C4 were not the anomeric centers in the molecule. The differences in the C−D bond lengths in these positions among different anomers varied to a small extent. The C−D vibrations at these positions of

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different conformers, therefore, appeared with close frequency values, and the bands became relatively narrower. For D-ribose-C-5,5′-D_2, we observed four bands at 2248, 2166, 2135, and 2108 cm^{-1} due to symmetric and antisymmetric C−D stretching vibrations of different conformations. Thus, the C−D Raman band at different locations in the ring differed due to specific geometrical arrangements and the electronic interaction of the C−D bond with the OH groups.

Figure 3B displays the C−D stretching vibrations of deuterium-labeled D-riboses in alkaline solution (1 M NaOH). Table 1 summarizes the vibrational properties

Table 1. Calculated C−D Stretching Vibrations at Different Positions for β-Ribopyranose in Unionized (C1−OH) and Ionized (C1−O^-) Structure[a]

		γ (cm^{-1}, intensity)		
antisurrounding	bond	unionized	ionized	Δ
CH, lp, lp	C1D	2201	1973	228
CH, lp, CO	C2D	2231	2214	17
CC,CC, lp	C3D	2232	2180	52
CO, OH, CH	C4D	2268	2254	14
OC, CC	$C5D_{anti}$	2304	2235	69
lp, CH	$C5D_{gauch}$	2226	2156	70
	$C5D_2$ (symmetric)	2206	2141	65
	$C5D_2$ (asymmetric)	2326	2251	75

[a]lp, lone pair; Δ, difference of the frequency in ionized and unionized structure.

(calculated) of C−D at different positions before and after ionization of the C1−OH group. The C−D stretching band of D-ribose-C1-D peaked at ∼2100 cm^{-1}. It was ∼100 cm^{-1} lower in frequency compared to the ν_{C-D} band of D-ribose-C1-D in water. The DFT calculation also showed a large shift in ν_{C1-D} of the ionized molecule (Table 1). Thus, the Raman band at ∼2100 cm^{-1} became the signature band of ionized D-ribose-C1-D. A large shift in the stretching frequency of C−D bonds in primary and secondary alcohols was also observed due to ionization of the adjacent OH group.[19] This could be due to weakening of the C−D bond as a result of an increasing amount of orbital overlap of nonbonded oxygen electrons with the C−D/H antibonding orbital upon ionization of the geminal OH group.

In a similar alkaline solution (1 M NaOH), the ν_{C-D} band of D-ribose-C2-D, D-ribose-C3-D, and D-ribose-C4-D, however, appeared at ∼2180 ± 5 cm^{-1} (Figure 3B). The band position and bandwidth of the C−D Raman band of D-ribose-C3-D and D-ribose-C4-D were similar to the C−D Raman band of the molecules in water. Some broadening and a ∼10 cm^{-1} red shift were observed for D-ribose-C2-D in alkaline solution. However, the shift was not large enough to suggest ionization of C2−OH. This indicated that the hydroxyl groups at positions C2, C3, and C4 were not ionized. D-Ribose-C-5,5′-D_2 in a similar alkaline condition showed only two bands at 2106 and 2237 cm^{-1} due to symmetric and asymmetric stretching vibrations of the C−D bonds. The frequencies were close to the lowest and highest frequencies observed for this molecule at neutral pH (Figure 3A, Figure S2, Supporting Information). Thus, the Raman results strongly indicated that only the C1−OH group of the D-ribose and not other OHs at positions C2, C3, C4, and C5 was ionized in alkaline condition.

The pK_a of the ionization of D-ribose was measured following the Raman intensity of the C1−D stretching vibration of D-ribose-C1-D with pH as shown in Figure 4A. Intensity at ∼2200 cm^{-1} corresponds to the C1−D Raman peak of the

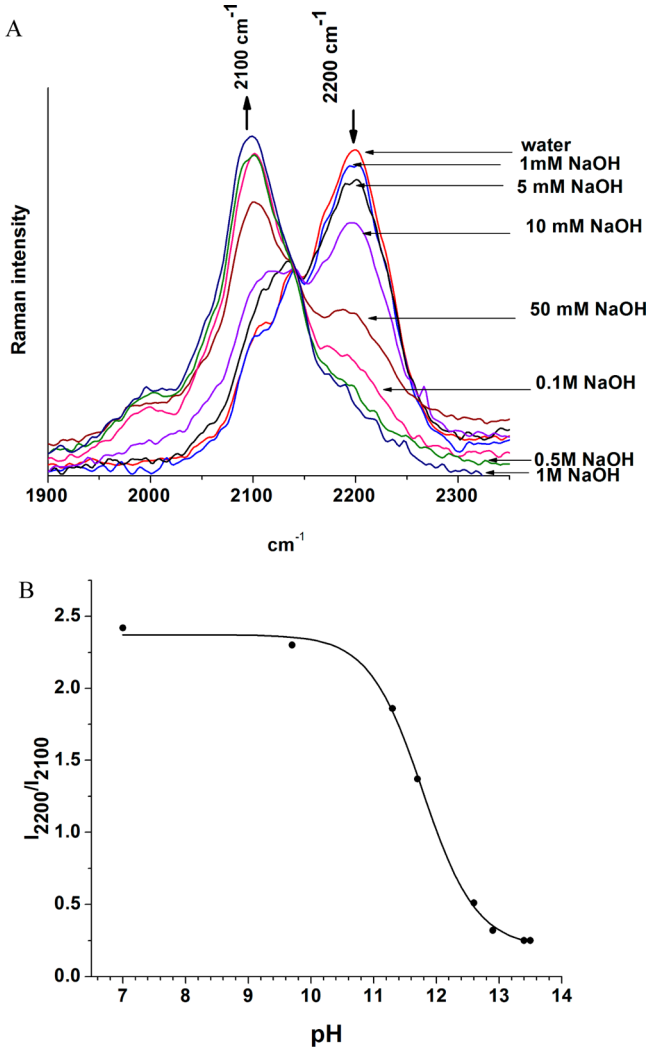

Figure 4. (A) C−D stretching region of the Raman spectrum of D-ribose-1D at different pHs. (B) Plot of intensity ratio (I_{2200}/I_{2100}) of the ν_{C-D} band at different pHs. Different data points are connected by a line, and pK_a of ionization was calculated from the slope.

major component of unionized D-ribose-C1-D. As the pH was increased, the Raman intensity at this position decreased and the intensity at peak position 2100 cm^{-1}, which was the marker band of the ionized ribose, was increased. Figure 4B shows the plot of intensity ratio (I_{2200}/I_{2100}) vs the pH of the solution. Above pH 11 the ratio started decreasing sharply and was not changed effectively above pH 12.5. From the midpoint of the slope of the graph the ionization pK_a value of D-ribose was measured to be 11.8. ^{13}C1 NMR experiments also indicated a similar pK_a value of the molecule (Figure 2).

Comparison of Raman spectra of unionized D-ribose-C1-D (Figure 3A) with ionized D-ribose-C1-D (Figure 3B) clearly indicated that the peak population and overall bandwidths of ν_{C-D} decreased in ionized condition, indicating that the population heterogeneity in ionized condition decreased. D-Ribose-C-5,5′-D_2 showed only one symmetric and one asymmetric stretching vibration, respectively, at 2106 and 2237 cm^{-1} (Figure 3 and Figure S2, Supporting Information) due to the presence of the CD_2 group. The asymmetric

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stretching frequency was ~10 cm^{-1} less compared to the highest value observed for the unionized molecule. The decrease was due to possible changes in electronic coupling between the σ^* orbital of the C5−D_2 bonds and the electron lone pairs of the C5−O bond as the ionization may cause buildup of extra charge at the C1−O^- group. D-Ribose-C-5,5′-D_2 in neutral aqueous solution and semicrystalline material (data not shown) showed four stretching bands (Figure 3A). However, we observed only two C−D stretching bands as symmetric and asymmetric stretches, which indicated single/structurally very close population of the molecule in ionized condition.

All these observations and NMR experiments, as discussed earlier, indicated ionization of the C1−OH group and that the pyranoside conformation was the most favored structure in the ionized state. DFT analysis also showed that in ionized condition pyranoside was the most stable conformation (Table 2). The C2−D frequency of ionized D-ribose was ~10 cm^{-1} less

Table 2. Calculated Bond Energy (in au) of the Ribose Ring in Different Conformations and Anomeric Forms in Both the Ionized and the Unionized Conditions

	furanose		pyranose	
ionization state	α	β	α	β
unionized	−572.6865	−572.6806	−572.6858	−572.6871
ionized	−572.1362	−572.0130	−572.1335	−572.1243
unionized (solvated)	−572.7187	−572.6986	−572.7201	−572.7287
ionized (solvated)	−572.2328	−572.2267	−572.2361	−572.2347

than the unionized one (Figure 3A, Figure S3, Supporting Information). The bond became weaker due to the anomeric effect of C1−O.[20,21] Also, the C2−D band of D-ribose-C2-D in alkaline condition was slightly broader than the unionized ribose (Figure S3, Supporting Information) in water. It could be possible that C2−OH was involved in H-bond formation with C1−O^-. This H-bond formation could make the C2−D bond broader. The possibility that D-ribose may remain in its open chain form in the ionized condition was discarded based on the fact that if it was in an open chain there would not be a pronounced change (~100 cm^{-1}) in ν_{CD} at C1. The observed C5−D_2 frequencies in two solution conditions (neutral and alkaline pH) also indicated the ring conformation of the ionized D-ribose. ^{13}C NMR spectra at high pH showed no other peak at ~204 ppm and discarded the possibility of a substantial presence of the open aldehyde conformation in the ionic state.

CONCLUSION

By monitoring ν_{C-D} in the Raman spectra of different isotopologues of D-ribose at different pHs, the current investigation established that C1−OH of D-ribose was the primary site of ionization and the estimated pK_a was 11.8. ^{13}C NMR analysis further indicated that the pyranoside conformation was the preferred structure in the ionized state. The observed pK_a value and the understanding of the ionized conformation of D-ribose may assist greatly to explain many catalytic processes and its implication in structural biochemistry.

ASSOCIATED CONTENT

Supporting Information

Plot of the intensity ratio of NMR peaks that represent α- and β-anomeric conformers of D-ribose as a function of NaOH concentration/pH of the solution; figures showing the band position and width of the C−D vibration of different isotopologues in different solution conditions; calculated parameters related to the optimized geometry of D-ribose. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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