Angewandte International Edition Chemie

D-Ribose

DOI: 10.1002/anie.201002865

A Multi-Faceted Approach to Elucidate the Crystal Structure of D-Ribose: Similarities to Protein Structure Determination

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D-ribose · carbohydrates · DNA · nucleosides · structure elucidation

The chemical structure of open-chain D-ribose of composition $C_5H_{10}O_5$ looks trivial, yet D-ribose can occur in four different cyclic forms: two with five-membered α- or β-furanose, and two with six-membered α- or β-pyanose (see Scheme 1 of Ref. [1]). The β-furanoses are sugar constituents of the ribonucleosides with four different nucleobases (adenine, uracil, guanine, cytosine) in β-glycosidic linkage (Figure 1) and of ribonucleotides with additional phosphate group at O5′ that occur as parts of cofactors of enzymes or of the energy storage molecule ATP. More generally, the ribonucleotides are the building blocks of ribonucleic acid, RNA. The related 2′-deoxyribonucleotides (the OH group at the 2′ position is replaced by an H atom) are the building blocks of the genetic material 2′-deoxyribonucleic acid (DNA).

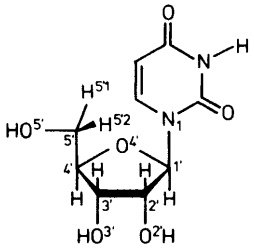

Figure 1. Uridine, a β-D-ribonucleoside occurring in RNA, and the 2′-deoxy form is found in DNA. This is the β-furanose form shown in Scheme 1 of Ref. [1] and found in many biological molecules, whereas the α- and β-pyranose forms were present exclusively in crystallized D-ribose.[1]

The crucial property of β-furanose or of 2′-deoxyribose in nucleic acids is that the five-membered ring is not planar but puckered, either in C2′-*endo* or C3′-*endo* envelope conformation, and the interconversion between the two forms follows the path of pseudorotation.[2] The energy barrier between the two forms is so small that they coexist in an equilibrium in solution, with one of the two favored depending on conditions. In DNA it can adopt these two states with C2′-*endo* conformation (B-DNA), which is the native state in chromatin. This contrasts the C3′-*endo* conformation (A-DNA) that is found when DNA is in complex with RNA during transcription (when the genetic information contained in the nucleotide sequence of DNA is copied into the complementary sequence of messenger RNA), because RNA with D-ribose can only occur in double-helical A-RNA with exclusive C3′-*endo* conformation.

The occurrence of β-furanose instead of β-pyranose in the nucleic acids is associated with the structural flexibility of β-furanose that is a hallmark of DNA. This contrasts with β-pyranose, which is more rigid because its six-membered ring occurs in a chair form that does not pseudorotate. If pyranose is substituted with the four nucleobases in β-glycosidic linkage and the formed hexose nucleosides are connected by phosphodiester links, this polymeric molecule can also form double helices similar to that known for DNA,[3] but cannot replace DNA biologically.

Because of the importance of D-ribose in nucleic acids, its structure was of considerable interest and the topic of an X-ray diffraction study in 1956. This investigation could, however, only determine the space group and crystal unit cell constants but not the three-dimensional structure because the crystals were of insufficient quality.[4] Earlier, Furberg had elucidated the first crystal structure of the nucleoside cytidine[5] that contains β-furanose linked to the nucleobase cytosine; the configuration of this nucleoside is analogous to that of uridine in Figure 1 and was crucial for the construction of the double helical structure of DNA by Watson and Crick.[6]

Let us return to the crystal structure of D-ribose. Since the first attempts to crystallize this molecule,[4] there must have been numerous unsuccessful (and consequently unpublished) trials to obtain crystals suitable for X-ray analysis. It took 50 years before a state-of-the-art and multifaceted approach was initiated to determine the crystal structure of D-ribose that encompassed powder and single-crystal X-ray diffraction and also solid-state ^{13}C MAS (magic angle spinning) NMR spectroscopy. As described in the work by Šišak et al.,[1] many attempts to crystallize D-ribose in a form suitable for single-crystal X-ray diffraction failed ("the compound

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(D-ribose) proved to be recalcitrant to crystallization"), and only microcrystalline samples, as already mentioned by Furberg et al.,[4] could be obtained that were investigated by powder diffraction using synchrotron X-radiation. As the asymmetric unit of the crystal is large enough to accommodate two linear D-riboses or two α- or β-furanoses or two α-or β-pyranoses or combinations of any two of these molecules, many trials of powder diffraction analysis were required to determine which two molecules occupied the crystal asymmetric unit.

This proved to be a formidable task, because all the possible structures had to be considered and tested against the collected powder X-ray diffraction spectra. This involved the direct-space-optimization program FOX in combination with powerful difference Fourier techniques,[1] and after many cycles of structure refinement a consistent picture emerged. It has to be stressed that these techniques are so new and promising that Lynne B. McCusker of ETH Zürich, one of the co-authors of the paper,[1] was invited to present a lecture at the 25th European Crystallographic Meeting at Istanbul last August.

Although powder X-ray diffraction had indicated that the crystal asymmetric unit of D-ribose crystals harbors pyranoses in the $^4C_1$ chair form, namely one β-pyranose with an equatorial OH at the anomeric C1 atom and one α-pyranose with an axial OH group,[1] the ultimate proof for this structure had to be provided by single-crystal X-ray diffraction.

As only microcrystalline powder could be obtained by recrystallization of D-ribose, the group of Boese at the University of Duisburg–Essen was taken on board, who specialize in zone-melting procedures to obtain single crystals from microcrystalline specimens.[7] Indeed, they succeeded in obtaining single crystals that permitted an X-ray analysis to be carried out and showed unambiguously that the results obtained from powder diffraction were correct. However, there is one difference between the methods concerning the α-pyranose, which was shown to be a mixture of β- and α-pyranose at a molar ratio of 1:1, yielding an overall β/α-pyranose ratio of 3:1 in the asymmetric unit of the crystal.

It is known from solution NMR spectra since 1976[8] that D-ribose dissolved in water exists as a mixture of the molecules given in Scheme 1 of Reference [1], with β-pyranose predominating. Now it was possible to collect solid-state ^{13}C MAS NMR spectra of microcrystalline D-ribose powder at 20 °C, at the melting temperature of crystals at 90 °C, at 20 °C after the same sample was cooled, and one month later at 4 °C. The integrated peak ratio of the anomeric ^{13}C signals at 94.9 and 92.5 ppm for β- and α-pyranoses indicated an initial ratio of 2:1. In the melt, a mixture of furanose and pyranose forms was obtained, with β-pyranose at 46 % predominating. After cooling to 20 °C, the melt showed two broad and featureless peaks that after one month had transformed and appeared like the original spectrum taken at 20 °C, with a molar ratio of 2.5:1 for β- and α-pyranoses, in good agreement with the single-crystal ratio of 3:1.

The NMR data and the single-crystal structure suggested a re-examination of the powder diffraction analysis that now indicated a β/α ratio of 0.27:0.73 at the α-pyranose site, whrease the β-pyranose site was not changed. This afforded a β/α-pyranose ratio of 1.7:1, in agreement with 2:1 ratio from the NMR study described above but at variance with the 3:1 ratio of the single-crystal data that may be associated with the zon-melting procedure.

The approach described herein for the structure determination of D-ribose is a prime example for the solution of a scientific problem that resisted clarification for many decades. The breakthrough was possible because during the past two decades, methods have improved considerably for powder and single crystal diffractometry using synchrotron X-radiation (with zone melting to change the microcrystalline material to single crystal) and solid-state ^{13}C MAS NMR spectroscopy.

The situation is comparable in some aspects with the field of macromolecular (protein) structure determination. Owing to the large unit-cell volume of protein crystals, powder diffraction methods are obsolete even with synchrotron X-radiation, and suitable only for low-resolution (ca. 5 Å) studies.[9] Solid-state ^{13}C MAS NMR spectroscopy of proteins is becoming more powerful and requires microcrystalline or two-dimensional crystalline samples.[10] In an interdisciplinary approach, electron or cryoelectron microscopy of an individual protein or of a multiprotein complex can provide an initial low-resolution structure that can be used to arrive at a higher resolution structure if the crystal structures of part or of all of the relevant proteins are known and can be combined with the electron microscopic picture.

Another approach will become available in the near future with the construction of free-electron lasers (such as at DESY/Hamburg) that provide coherent X-rays and permit a medium- to low-resolution resolution picture to be obtained of a molecular structure of whatever size and complexity at very short timeframes before the high energy of the laser beam destroys the molecule.[11,12] This picture may be sufficiently detailed to be used in "molecular replacement", a routine method in protein crystallography, to finally arrive at the three-dimensional structure of the macromolecule under study with the help of conventional X-ray diffraction data, or individual protein structures may be fitted to the picture as described for electron microscopy.

Received: May 12, 2010
Published online: July 29, 2010

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