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Ab initio structure determination of two anhydrous forms of α-lactose by powder X-ray diffraction

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Abstract. Powder X-ray diffraction patterns of the stable anhydrous form of α–lactose and of a mixture of α-lactose monohydrate with hygroscopic anhydrous α–lactose were recorded at room temperature. The starting structural models were found by a Monte-Carlo simulated annealing method. The final structures were obtained through Rietveld refinements with soft restraints on interatomic bond lengths and bond angles and crystalline energy minimisation to locate the H atoms of the hydroxyl groups. The stable form of α–lactose is triclinic with space group P1, Z = Z'= 2, a = 7.6522 (2), b = 19.8637 (5), c = 4.9877 (1) Å, α = 92.028 (1), β = 106.261 (1), γ = 97.153 (1) °, V = 720.18 (3) Å3. For hygroscopic anhydrous α–lactose, the symmetry is monoclinic, space group P21, Z = 2, Z' = 1, a = 7.7795 (3), b = 19.6931 (7), c = 4.9064 (1) Å, β = 103.691 (2)°, V = 730.32 (4) Å3.

Introduction

Lactose (4-O-β-D-galactopyranosyl-D-glucopyranose, $C_{12}H_{22}O_{11}$), is a 'mixed' disaccharide containing a galactose and a glucose unit linked through a β–1,4 linkage. It exhibits two anomers (α–lactose and β–lactose) which differ in the configuration of the terminal hydroxyl group of the glucose unit. For the α–anomer, three crystalline forms have been characterised [1] : the α–lactose monohydrate (hereafter named αL-H_2O), the hygroscopic anhydrous α–lactose (αL_H) and the stable anhydrous α–lactose (αL_S). The β–anomer has only one crystalline form (βL); mixed compounds α-β-lactose have also been identified with different stoechiometries (α/βL) [2], [3]. The crystalline structures of the αL-H_2O form [4], [5], [6] and βL form [7] were solved from single crystal samples with an automatic X-ray diffractometer.

The aim of this article is to give the crystallographic structure of two anhydrous forms of α-lactose by powder X-ray diffraction: the αL_H and αL_S forms. The reason why we used powder data is given for each phase in the next parts.

Experimentation

We have followed the same procedure for the two phases:
1) The data were collected on the laboratory diffractometer equipped with an INEL curved sensitive detector CPS120. A bent quartz monochromator allows selection of the $K_{\alpha 1}$ wavelength of a Cu X-ray tube (λ = 1.54056 Å). The powder was introduced in a Lindemann glass capillary (diameter = 0.7mm), mounted on the axis of the diffractometer. It was rotated during the experiment in order to reduce the effect of possible preferential orientations.
2) The profiles of *n* reflections were individually refined with the program WINPLOTR [8] in order to obtain their exact 2θ positions. We used then the program TREOR [9] to find the unit cell and to index the reflections. A part of the X-ray diffraction pattern was refined with the cell found by TREOR and using the "profile matching" option [10] of the program FullProf [11], in order to determine the space group.
3) Lattice and profile parameters, zero point and interpolated background calculated with the previous refinements were introduced in the program F.O.X. [12] in order to get a starting structural model. A molecule of α-lactose, with C and O atoms only, is built with bond lengths, bond angles and torsion angles calculated from the atomic coordinates of Fries *et al*. [4]. This molecule is introduced randomly in the cell. The "parallel tempering" algorithm of this program was used.
4) The final structure was obtained through Rietveld refinements with soft restraints on interatomic bond lengths and bond angles (program Fullprof [11]) and crystalline energy minimisation to locate the H atoms of the hydroxyl groups: calculations are performed using the DL_POLY molecular modelling package [13] on a system of N = 80 (4 x 2 x 5 crystalline cells) lactose molecules using periodic boundary conditions. Each molecule is described by its 45 atoms which interact through the Ha force field [14] developed for carbohydrates. Electrostatic interactions are handled by the Ewald method. We work in the NVE statistical ensemble where the number of atoms (N), the volume (V) and the energy (E) are fixed. A cut-off radius of 10 Å is used. In order to determine positions of the hydroxyl H atoms, energy minimisation calculations (T = 0) are realised from the structure obtained experimentally. C–O–H angles are initially chosen to be 180°. No dihedral interaction is applied to the hydroxyl H. During minimisation, only H atoms of the hydroxyl groups are allowed to move in order to maintain the experimental structure as much as possible.

Results

The hygroscopic anhydrous phase of α–lactose

α-lactose monohydrate annealed at 135 °C allowed to get *a mixture of this compound with hygroscopic anhydrous α–lactose*. A powder X-ray diffraction pattern of this mixture was recorded at room temperature. To determine the lattice parameters of the phases, the profiles of the 58 reflections with a 2θ angle lower than 40° were refined with the program Winplotr. Among the 58, 44 reflections were attributed to the αL-H_2O form but 14 of them ranging from 9 to 33° do not belong, unambiguously, to the αL-H_2O phase. Having isolated the αL_H phase, we could continue the procedure as described. We have found a monoclinic symmetry, a space group $P2_1$ with 2 molecules per cell (Z' = 1), and the following lattice parameters: a = 7.7795 (3), b = 19.6931 (7), c = 4.9064 (1) Å, β = 103.691 (2)°, V = 730.32 (4) $Å^3$. The final Rietveld plot is given on figure 1 (*Rp*=0.0657, *Rwp*=0.0733, *Rexp*=0.0222,

χ^2 = 10.9). The calculated specific percentage of αL_H phase contained in the powder is 83 (1) %. The projection of the unit cell is given on figure 3a.

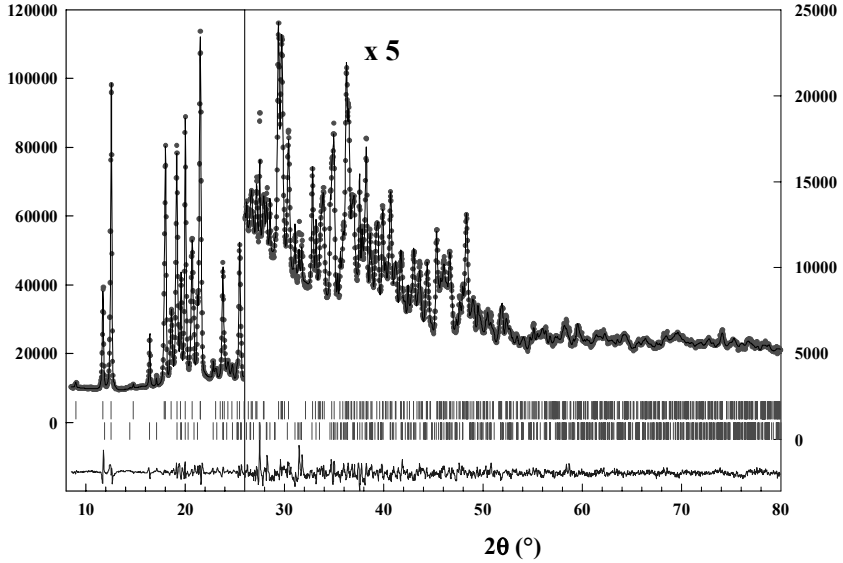

Figure 1. Final Rietveld plot of the hygroscopic phase of α-lactose. Observed data points are indicated by dots, the best-fit profile (upper trace) and the difference pattern (lower trace) are solid lines. The vertical bars correspond to the position of Bragg peaks: upper bars for αL_H, lower bars for $\alpha L\text{-}H_2O$.

The stable anhydrous phase of α–lactose.

The stable anhydrous α–lactose (αL_S) form, not commercially available, can be obtained from $\alpha L\text{-}H_2O$ either by heating at about 140°C or by dehydration in an hygroscopic solvent such as methanol [15], which we have used. To get single crystals to perform X-ray experiments with an automatic diffractometer, the αL_S powder must be dissolved in a solvent and, then, crystals grow either by temperature lowering or by evaporation. In solution, the molecule of lactose can undergo hydration to form $\alpha L\text{-}H_2O$ or mutarotation to form βL. For this reason, the structure of the αL_S form was solved *ab initio* from powder X-ray pattern using the Rietveld method. We have found a triclinic symmetry, a space group P1 with 2 molecules per cell, and the following lattice parameters: a = 7.6522 (2), b = 19.8637 (5), c = 4.9877 (1) Å, α = 92.028 (1)°, β = 106.261 (1)°, γ = 97.153 (1)°, V = 720.18 (4) Å^3. The final Rietveld plot is given on figure 2. (*Rp*=0.0555, *Rwp*=0.0624, *Rexp*=0.0159, χ^2=15.5). The projection along c* of the unit cell is given on figure 3b.

The reduced atomic coordinates, the bond lengths, the bond angles and the torsion angles for the two phases are given elsewhere [16, 17].

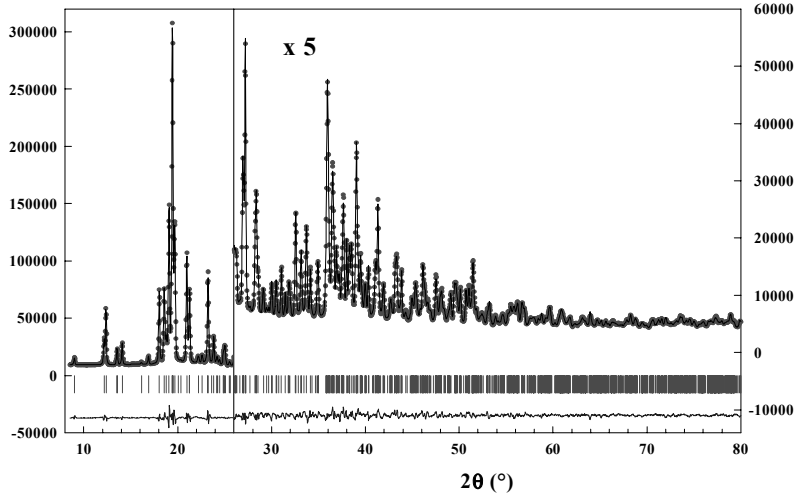

Figure 2. Final Rietveld plot of the stable anhydrous phase of α–lactose. Observed data points are indicated by dots, the best fit profile (upper trace) and the difference pattern (lower trace) are solid lines. The vertical bars correspond to the position of Bragg peaks.

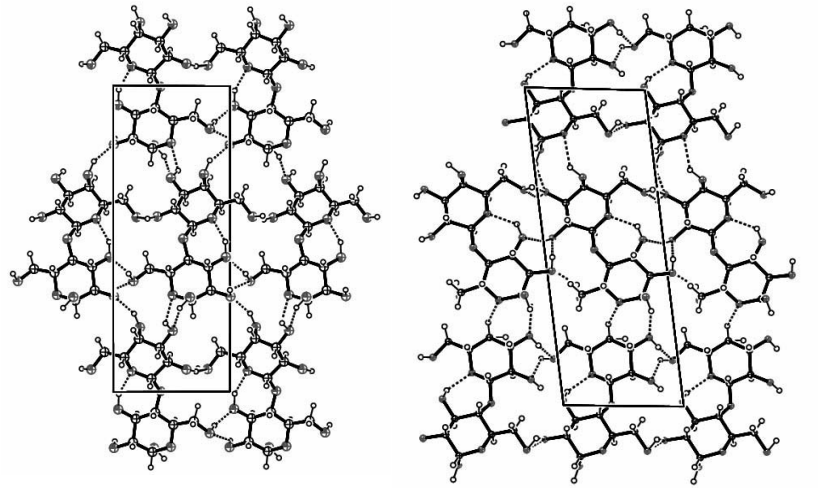

Figure 3. (a) Projection of the unit cell of the hygroscopic α-lactose along c. Dashed lines correspond to H bonds. (b) Projection along c* of the unit cell of the stable anhydrous form of α-lactose. Dashed lines correspond to H bonds.*

Discussion

Despite the different crystalline systems, very similar unit cells are observed between the αL_S form and the other two polymorphs of α–lactose. Only very small differences between the lattice parameters of the two anhydrous forms are seen : the relative changes are as follow : 1.7 % for *a*, -0.9 % for *b* and -1.63 % for *c*. The changes of the α and β angles equal 2.0 and 2.6°, respectively. The change in the γ angle is larger (7.2°) leading to a small 1.4% contraction of the cell volume of the αL_S form with respect to the αL_H one. For the three polymorphic forms of α–lactose, molecules are oriented with their long axis approximately along the ***b*** direction. For αL_S, molecules 1 (see figure 2b) of the cell lie in (***a***, ***c***) planes with the reduced coordinates of their mass centre at about $y \cong 0.5$; they correspond to the molecules generated by the twofold screw axis of the αL_H form. Molecules 2 are also in (***a***, ***c***) planes and their mass centre is at $y \cong 0.0$ and they correspond to the molecules of asymmetric unit of the αL_H form.

The crystalline cohesion of the two forms is achieved by networks of O–H···O hydrogen bonds different to those of the monohydrate phase. The detailed description of the hydrogen bonds networks of the two anhydrous forms of α–lactose and their comparison with those of the monohydrate form is made elsewhere [16, 17].

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