Crystal Structure of the Trihydrate of the Neuraminidase Inhibitor $C_{15}H_{28}N_4O_4$ (Peramivir), a Potential Influenza A/B and Avian-influenza (H5N1) Drug

Egbert Keller and Volker Krämer

Kristallographisches Institut der Albert-Ludwigs-Universität, Hermann-Herder-Str 5,
D-79104 Freiburg i. Br., Germany

Reprint requests to Dr. E. Keller. Fax: 0049 761 203 6434. E-mail: egbert.keller@krist.uni-freiburg.de

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The crystal structure of the trihydrate of peramivir ($C_{15}H_{28}N_4O_4$), a potential influenza A/B and avian-influenza drug, has been determined. The structure, belonging to the tetragonal space group $P4_22_12$ with $Z = 32$, $a = 27.216(4)$, $c = 23.084(5)$ Å, $V = 17098(5)$ Å³, contains four organic molecules plus 12 partially disordered water molecules per asymmetric unit. 16 Organic molecules per unit cell form a kind of 1D infinite micelle separated from vicinal micelles by approximately planar water layers. During exposure to X-rays or under long-time storage on air peramivir trihydrate undergoes a phase transition to a structurally closely related phase with reduced water contents.

Key words: Crystal Structure, Peramivir, Neuraminidase Inhibitor

Introduction

Peramivir ($C_{15}H_{28}N_4O_4$) is a neuraminidase inhibitor developed by BioCryst Pharmaceuticals Inc. Its absolute configuration as given in (1) has been determined crystallographically from a complex of the molecule with neuraminidase (influenza A) [1]. The compound is currently tested as an influenza A/B [2] and an avian-influenza (H5N1) [3] drug.

Peramivir can be crystallized from methanol/water mixtures either as a dihydrate (needle-like crystals) of hitherto unknown structure or as a trihydrate (distorted octahedra), the latter being used as the phar-

maceutical form [4]. In the following, we report work done on the crystal structure of the trihydrate, $C_{15}H_{28}N_4O_4 \cdot 3 H_2O$. This work was already performed in the years 2000/2001. It is published now due to kind permission of BioCryst Pharmaceuticals Inc. [5].

Experimental Section

Colorless single crystals of $C_{15}H_{28}N_4O_4 \cdot 3 H_2O$ (distorted octahedra) were provided to us by CILAG AG, Schaffhausen (Switzerland). They had a fine (though slightly milky) appearance, but allowed not even to determine a unit cell by single crystal X-ray diffractometry. X-Ray rotation photographs showed their reflections to be split and smeared (Fig. 1). Crystals suitable for X-ray diffraction experiments were therefore grown by the following procedure.

Crystal growth

3.02 g (7.9 mmol) of the trihydrate was filled into a 50 mL Schlenk tube previously heated to a temperature of about 82 °C; the Schlenk valve of the tube was connected by a hose to a H₂O reservoir which was maintained at 82 °C. Distilled water (10 mL) was added to the trihydrate, and the suspension was stirred. Then methanol was added until all trihydrate was dissolved, *i. e.*, until the mixture turned clear (about 4 mL of methanol). Stirring was stopped, the tube was sealed with a stopcock and the Schlenk cock was opened to a min-

Fig. 1. X-Ray rotation oscillation photographs of peramivir crystals with [110] aligned parallel to the rotation axis. (a) Crystal of high optical quality as provided by CILAG AG; (b), (c) crystal of low optical quality as grown by the method given in the text; photograph taken initially (b) and after several hours of exposure to X-rays (c). Note that images (a) and (b) show superstructure layers owing to an approximate C centering while image (c) does not, owing to perfect C centering (rows of weak spots in image (c) are due to $\text{CuK}\beta$ radiation).

imal degree to let water from the reservoir diffuse in slowly. After three days, some crystal needles (typical for the dihydrate) had formed; after 13 d, more needles plus some precipitate had formed; after 20 d, the needles had disappeared and the precipitate had grown in volume. The mother liquor was decanted at 82 °C; the precipitate was poured onto a filter, washed with about 5 mL of H_2O and dried at 35 °C in the presence of a water reservoir. It turned out to consist of octahedrally shaped crystals of the trihydrate.

X-Ray diffraction photographs

Although the optical appearance of the single crystals with respect to transparency and perfection of faces was worse than that of the original material, X-ray photographs were of much better quality. However, it was found that the crystal structure changed after several hours of X-ray exposure: the original diffraction pattern corresponding to a primitive tetragonal unit cell of about $27.2 \times 27.2 \times 23.1 \text{ \AA}^3$ changed to another one corresponding to a C-centered orthorhombic cell of $26.4 \times 26.9 \times 23.3 \text{ \AA}^3$ without affecting the ability of the crystal to interact with X-rays as a single crystal (Fig. 1). During the transition to this “phase II” the cell volume shrinks, probably corresponding to the loss of some water (see below). For further studies, freshly selected single crystals of the trihydrate were therefore mounted in sealed capillaries with some water enclosed such that the water did not touch the crystals. X-Ray photographs showed that crystals mounted in this manner did not undergo the transition to phase II upon exposure to X-rays. The following should be noted in this context. A later investigation of phase I crystals (which, in the meantime, had been stored on air for some months) showed that these crystals produced the phase II pattern even on the first X-ray photograph, *i. e.*, these

crystals had obviously undergone the transition to phase II already during storage.

Diffraction measurements

A crystal of the trihydrate (phase I), mounted in a sealed capillary as described above, was measured on a Bruker AXS SMART single crystal diffractometer equipped with a CCD detector. The measurement was performed at r. t. for two reasons: i) because of the water present in the sealed capillary, and ii) as the structure corresponding to the phase existent in material stored under normal conditions was to be determined. The measurement could be terminated without problems; a final check proved that the tetragonal unit cell of the crystal had not changed during X-ray exposure under these conditions.

Fig. 2. Atom numbering scheme for the peramivir molecules. Add $n \times 100$ to the numbers for molecule no. n of the asymmetric unit.

Fig. 3. ORTEP-3 drawings (50 % probability ellipsoids) of the four crystallographically independent molecules of peramivir trihydrate in the unit cell.

Structure determination

Numerous attempts to solve the phase problem by conventional Direct Methods [6] failed. However, employing Dual Space Methods [7] led to the solution of the structure with four independent molecules per asymmetric unit. Their absolute configuration was as shown in formula (1) [8]. Hydrogen atoms were added to the model in calculated positions. As molecules concomitantly containing guanidyl and carboxyl groups usually exist in a zwitterionic form [9], different from the molecular formula given in (1), the guanidyl group was assumed to be protonated while the carboxyl group was assumed to be deprotonated. The H_2O substructure could only be determined approximately due to disorder and/or thermal motion of the water molecules. After refinement of the complete organic part of the structure, all difference Fourier peaks above noise were assigned to whole, half, quarter, or eighth oxygen atoms. The assignment of the site occupancy factors was made such that in the final model the isotropic U values for all water O atoms were in the range $0.10(3) \text{ \AA}^2$. Finally, the water O atoms added up to 2.9 per organic molecule. Refinement of the complete structure [10] led to an R_1 value of 6.3 %. Fig. 2 demonstrates the numbering scheme for the atoms: to get the atom numbers for molecule n ($n = 1$ to 4) of the asymmetric unit, add $n \times 100$ to the numbers displayed in Fig. 2.

ORTEP-3 [11] drawings of the four molecules of the asymmetric unit, all brought into the same orientation, are given in Fig. 3. The contents of one unit cell are displayed in Fig. 4. Table 1 contains crystal, measurement and refinement data. CCDC 641049 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

A structure determination of a crystal of phase II was also attempted. The structure obtained (orthorhombic space group $C222$ with $Z = 32$, $a = 26.416(2)$, $b = 26.939(2)$, $c = 23.260(2) \text{ \AA}$, $V = 16553(3) \text{ \AA}^3$) is very similar to the one of phase I. Water O atoms, localized and refined in the same manner as described for phase I added up to only two water molecules per organic molecule, thus suggesting that the trihydrate transforms into a dihydrate (different from the one mentioned in the introduction) upon exposure to X-rays or during long-time storage on air. However, data reduction and refinement led to high R values ($R_{\text{int}} = 0.20$, $R_1 = 0.12$). Owing to the poor data quality the corresponding results are therefore not presented here in more detail.

Results and Discussion

Peramivir trihydrate crystallizes in the tetragonal space group $P4_22_12$, $Z = 32$, $a = 27.216(4)$, $c =$

Table 1. Crystal and structure refinement data of peramivir trihydrate.

Empirical formula	C ₁₅ H ₂₈ N ₄ O ₄ · 3(H ₂ O)
Formula weight, g mol ⁻¹	382.46
Temperature, K	293(2)
Wavelength, Å	0.71073
Crystal system, space group	tetragonal, <i>P</i> 4 ₂ 2 ₁ 2 (no. 94)
Unit cell dimensions, Å	<i>a</i> = 27.216(4), <i>c</i> = 23.084(5)
Volume, Å ³	17098(5)
<i>Z</i> , calculated density, Mg m ⁻³	32, 1.189
Absorption coefficient, mm ⁻¹	0.093
<i>F</i> ₀₀₀ , e	6560
Crystal size, mm ³	0.4 × 0.3 × 0.3
θ Range for data collection, deg.	1.06–28.39
Limiting indices	–36 ≤ <i>h</i> ≤ 35, –36 ≤ <i>k</i> ≤ 35, –30 ≤ <i>l</i> ≤ 29
Reflections	
collected/unique/observed	162839/11590/5375
<i>R</i> (int)	0.071
Completeness to θ = 28.39°	0.99
Absorption correction	none
Refinement method	Full-matrix least-squares on <i>F</i> ²
Data/restraints/parameters	11590/0/954
Goodness-of-fit on <i>F</i> ²	0.956
Final <i>R</i> indices [<i>I</i> ≥ 2σ(<i>I</i>)]	<i>R</i> ₁ = 0.063, <i>wR</i> ₂ = 0.180
<i>R</i> indices (all data)	<i>R</i> ₁ = 0.137, <i>wR</i> ₂ = 0.220
(Δ/σ) _{max} (final cycle)	0.004
Largest diff. peak/hole, e Å ⁻³	0.366/–0.263

(about) 12 water molecules. Three of the peramivir molecules are also approximately identical with respect to substituent conformation, in the fourth one (molecule 2), the conformation of the isopentyl group differs from that of the other three. The asymmet-

ric unit (without water molecules) shows an approximate non-crystallographic twofold axis parallel [1 0 0] (or [0 1 0]), which relates molecules 1/4 and molecules 2/3, the symmetry being violated mainly by some methyl groups (Fig. 5). Hydrogen bonds (as far as can be judged from calculated H positions) establish a different scheme of pairing inasmuch as the two molecules 1 and 3 are connected by four H bridges (O14...N321, O142...N311, O341...N123, O342...N121) as are the two molecules 2 and 4 (O241...N423, O242...N421, O441...N221, O442...N211); there are also 4 additional H bonds between atoms *On*41 (*n* = 1, 2, 3, 4) and *Nm*23 (*m* = 2, 3) (O141...N223, O241...N323, O341...N223, O441...N323) which attenuate the zwitterion character of the molecules (as do the previously mentioned ones to N123 and N423). Other H bonds are N111...O1...N411 (Fig. 5) and O142...O151, O442...O451. The latter two (not shown in Fig. 5) are also *intermolecular* H bonds.

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