Supporting information

Discovery of new polymorphs of the tuberculosis drug isoniazid

Keke Zhang,^{a,b} Noalle Fellah,^a Alexander G. Shukenberg,^a Xiaoyan Fu,^c Chunhua Hu,^a Michael D. Ward^{a, *}

^aDepartment of Chemistry and Molecular Design Institute, New York University, New York City, NY, 10003, USA; ^bSchool of Chemical Engineering and Technology, State Key Laboratory of Chemical Engineering, Tianjin University, Tianjin 300072, People's Republic of China.; ^cState Key Laboratory of Catalysis, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, People's Republic of China

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Methods and Materials

Isoniazid (INZ, $C_6H_7N_3O_2 \ge 99\%$) was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA,). Isoniazid was used without further purification. Controlled pore glass (CPG) was obtained from LGC Biosearch Technologies (Petaluma, CA, USA) with nominal pore diameters of 30 nm, 50 nm, 100 nm, 140 nm and 200 nm. Porous glass with nominal pore sizes of 4 nm and 8 nm were purchased from Sigma-Aldrich. The CPG beads were washed with boiling nitric acid prior to use to remove any possible contaminants, then washed with deionized water and dried for 12 h under a vacuum. The acid-washed CPG was stored under air in a desiccator. Isoniazid powder was mixed with CPG (with 30, 50, 100 and 140 pore sizes), and heated to 180 °C ($T_m = 169.75$ °C) for 10 minutes. Then the mixtures were quenched to room temperature. The embedded nanocrystals were characterized by powder X-ray diffraction. Approximately 5 mg of INZ powder was deposited on a glass slide of 25 mm x 75 mm x 1.2 mm and was covered by a 22 mm x 22 mm x 0.17 mm coverslip. The confined INZ was melted on a Kofler bench at 180 °C, then cooled to 100 °C, resulting in the nucleation of Form I. The sample then was moved to the 60 °C zone on the Kofler bench, resulting in the formation of Form II. Form III was nucleated by moving the sample to a metal heat sink at ambient temperature. The melting temperature of Form II was determined with hot-stage microscopy (HSM). HSM images and videos were recorded on an OLYMPUS SC50 microscope equipped with a METTLER FP82 hot stage system. Form II crystals were prepared by sublimation and placed between two glass slides.

SI 1. Raman Spectroscopy

Raman spectra were recorded using a Raman microscope (DXR, Thermo Fisher Scientific, Waltham, MA) equipped with a 532 nm excitation laser operating at 2 mW and 50 µm slit.



Fig. S1. Raman spectra of isoniazid polymorphs over the range of 250-3500 cm⁻¹.

SI 2. Powder X-ray diffraction

X-ray powder diffraction patterns were collected at room temperature with a Bruker AXS D8 DISCOVER GADDS microdiffractometer (Cu K α radiation, $\lambda = 1.54178$ Å) equipped with a VÅNTEC-2000 two-dimensional detector and a 0.5 mm MONOCAP collimator. Form I samples were loaded into a 0.8 mm Kapton capillary. Data for Forms II and III were collected directly on the glass slides on which they grew. Scans were performed with θ_1 (incident angle of the X-ray beam) = 5° and θ_2 (detector angle) = 14°, respectively. The sample-detector distance was 150 mm. One-dimensional (1D) diffraction patterns were integrated from the 2D XRD images over the entire range of azimuthal angles spanning 4° $\leq 2\theta \leq 40^{\circ}$ range using the XRD²EVAL program (version 2009.5-0; Bruker AXS Inc., Madison, WI, 2009).

SI 3. Sublimation of Form II

Isoniazid (100 mg) was placed in a glass Petri dish with a glass slide affixed to the dish cover (Figure S2). The Petri dish was kept on a hot plate at 90 °C for 24-48 h. The crystals sublimed onto the glass slide (Figure S3) were analyzed by Raman spectroscopy, which confirmed the presence of both Forms I and II.



Fig. S2. Sublimation experiment set-up.



Fig. S3. Crystals sublimated on glass slide. The rectangular crystal is Form I and needlelike crystals are Form II.

SI 4. Single-crystal structure determination of Form II

Single crystal X-ray data for INZ Form II was collected on a Bruker D8 APEX-II CCD system using graphite-monochromated and 0.5 mm MonoCap-collimated MoK α radiation ($\lambda = 0.71073$ Å) with the ω scan method at 100 K. The temperature was controlled by an Oxford Cryosystems 700+ Cooler. The datasets were processed with the INTEGRATE program of the APEX2 software for reduction and cell refinement. Multi-scan absorption corrections were applied by the SCALE program for the area detector. The structure was solved by intrinsic phasing methods (SHELXT) and the structure models were completed and refined using the full-matrix least-square methods on F^2 (SHELXL). Non-hydrogen atoms in the structures were refined with anisotropic displacement parameters, and hydrogen atoms on carbons were placed in idealized positions (C-H = 0.95-1.00 Å) and included as riding with $U_{iso}(H) = 1.2$ or 1.5 $U_{eq}(non-H)$. The selected crystallographic parameters are listed in Table S1.

	Form I (INICA03)	Form II		
Formula	C ₆ H ₇ N ₃ O	$C_6H_7N_3O$		
M_w	137.15	137.15		
Crystal system	Orthorhombic	Orthorhombic		
Space group	$P2_{1}2_{1}2_{1}$	$Pca2_1$		
<i>T</i> [K]	90(2)	100(2)		
<i>a</i> [Å]	3.7601	34.428(5)		
<i>b</i> [Å]	11.2911	3.8120(6)		
<i>c</i> [Å]	14.6877	19.787(3)		
Ζ	4	16		
Z'	1	4		
V [Å ³]	623.58	2596.8(7)		
Radiation	$\mathrm{Mo}_{K^{lpha}}$	$\mathrm{Mo}_{K^{lpha}}$		
Crystal size	$0.25\times0.24\times0.14$	$0.54 \times 0.03 \times 0.02$		
Absorption coefficient [mm ⁻¹]	0.11	0.101		
Number of measured reflections	83442	18380		
Number of independent reflections	6960	2728		
Number of obs. $(I > 2\sigma(I))$ reflections	6442	1998		
$R_{\rm int}$	0.028	0.1332		
$(\sin\theta/\lambda)_{\rm max}$ [Å ⁻¹]	1.100	0.500		
$R[F^2 > 2\sigma(F^2)]$	0.015	0.0515		
$wR(F^2)$	0.017	0.1202		
S	1.02	1.025		
Number of reflections used	6253	2728		
Number of parameters	344	397		

Table S1. Crystallographic parameters for isoniazid Form I (ref. 2) and Form II.



Fig. S4. Face indexing of isoniazid Form II single crystal.



Fig. S5. Simulated (black) and experimental (red) X-ray diffraction patterns of Form II.

SI 5. Synchrotron powder diffraction of Form III

High signal-to-noise synchrotron powder diffraction data was collected for Form III on the 17-BM beamline of the Advanced Photon Source, Argonne National Laboratory, at a wavelength of 0.45240 Å using a PerkinElmer PE1621 area detector. The sample-detector distance was 1000 mm and the measurements spanned an angular resolution of $2\theta = 0.5 - 11.9^{\circ}$. Approximately 5 mg of INZ powder was deposited on a glass slide and covered with a coverslip. The confined INZ was melted on a hot plate at 180 °C, then quenched to room temperature with an aluminum block. When quenched, the sample was not fully crystallized and was subsequently heated to *ca*. 35 °C to crystallize Form III. The coverslip was detached and the powder of Form III was carefully scraped with a needle. The powder was loaded into a Kapton 1.0 mm capillary and measured at 100 K using Oxford 700+ Cooler. Most of Form III converted to Form I during the sample preparation process and only six peaks were reliably observed in the PXRD pattern in Figure S6. Synchrotron data was also collected for Form I to distinguish the reflections attributed to Form III. Form I was prepared as previously described and it was measured in the same way as Form III. These six reflections of Form III can be indexed with an orthorhombic cell with a = 3.931(2) Å, b = 9.754(5) Å, c = 8.568(4) Å, V = 328.5 Å³, Z = 2.

Five of these reflections are 0kl reflections, indicating that periodicities along *b* and *c* are well determined with $\alpha = 90^{\circ}$. However, the only available $h \neq 0$ reflection does not allow reliable determination of the lattice constants *a* and cannot distinguish if the unit cell is orthorhombic ($\beta = \gamma = 90^{\circ}$) or monoclinic ($\beta \neq 90^{\circ}$ or $\gamma \neq 90^{\circ}$).



Fig. S6. (A) Synchrotron diffraction data of Form I (red) and Form III that mostly converted to Form I (black). (B) Enlarged region of A from 2.5° – 8.5°. Blue arrows indicate peaks that correspond only to Form III.

SI 6. Torsion angles in INZ structures

Table S2. Torsion angles (see Scheme, in degrees) for INZ molecules in the crystalline state, measured from experimental crystal structures (τ^{exp}) and calculated by DFT (τ^{DFT}). Letters *a*, *b*, *c*, *d* denote the four different conformers in Form II (Z' = 4).

Polymorph	τ_1^{exp}	${ au_1}^{ m DFT}$	τ_2^{exp}	$ au_2^{DFT}$
Form I	-18.07	-22.06	-5.63	-6.33
Form IIa	-25.81	-27.92	-5.54	-4.30
Form IIb	17.15	17.90	3.72	-2.47
Form IIc	-32.57	-33.87	-6.64	-2.77
Form IId	-21.78	-24.34	2.65	0.33



References

1. G. Rajalakshmi, V. R. Hathwar, P. Kumaradhas, Acta Cryst., 2014, B70, 331-341.