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Crystal structure of the NS3 protease-helicase from Dengue virus

Dahai Luo[1], Ting Xu[2], Cornelia Hunke[3], Gerhard Gruber[3], Subhash G. Vasudevan[2], Julien Lescar[1]
[1]School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore, Singapore, 637551, Singapore, [2]Novartis Institute for Tropical Diseases, 10 Biopolis Road, Chromos Building, Singapore 138670, [3]School of Biological Sciences, Section of Structure and Function of Molecular Motors, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, E-mail：lu0001ai@ntu.edu.sg

Several flaviviruses are important human pathogens including dengue virus, a disease against which neither a vaccine nor specific antiviral therapies currently exist. During infection, the flavivirus RNA genome is translated into a polyprotein, which is cleaved into several components. The non-structural protein 3 (NS3) carries out enzymatic reactions essential for viral replication, including proteolysis of the polyprotein through its serine-protease N-terminal domain, with a segment of 40 residues from the NS2B protein acting as a cofactor. The ATPase/helicase domain is located at the C-terminus of NS3. Atomic structures are available for these domains separately but a molecular view of the full length flavivirus NS3 polypeptide is still lacking. We report two distinct crystallographic structures of a complete NS3 molecule fused to 18 residues of the NS2B cofactor: structure I, the protease domain sits beneath the ATP binding site, giving the molecule an elongated shape; structure II, the protease domain self-rotates 161 degree. The relative orientation between the protease and helicase domains is drastically different compared to the scNS3-NS4A molecule from hepatitis C virus (HCV), which was caught in the act of cis cleavage at the NS3-NS4A junction. The domain arrangements found in the crystal structures fit well into an envelope determined ab-initio using small angle X-ray scattering experiments in solution, suggesting a cluster of molecular conformations. The affinity for both ATP and ADP is approximately 10 fold higher for full length NS3 compared to the helicase domain, mdeasured using fluorescence correlation spectroscopy. It indicates that the protease domain plays an important regulatory role for NS3 NTPase and helicase activity.

Keywords: NS2B-NS3 protease, NS3 helicase, NS3 bifunctional enzyme crystal structure

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Understanding and controlling polymorphism

Joel Bernstein
Ben-Gurion University of the Negev, Chemistry, P.O. Box 653, Beer Sheva, Israel, 84102, Israel, E-mail：yoel@bgu.ac.il

In order to control polymorphism we must understand it－so understanding becomes the first priority. What do we want to understand and what do we want to control? Understanding requires addressing and answering questions regarding similarities and differences of structure, energetics, crystallization kinetics and thermodynamics. For instance, if we want to control polymorphism we have to be able to answer with reasonable confidence the question of why some simple molecules exhibit a propensity for polymorphic behavior, while other simple molecules, crystallized perhaps countless times, show apparently no proclivity to crystallize in more than one crystal structure. Control of polymorphism allows us to obtain consistently and robustly the polymorph with the most desirable properties, to avoid obtaining polymorphs with less desirable properties, and to prevent the appearance of new and less desirable polymorphs. While recent years have witnessed considerable and impressive advances in our understanding and control of polymorphism, especially for individual molecular systems, there are still major challenges to be met for many other individual systems and, more significantly, for the overall fundamental understanding and control of polymorphism. This talk will review some of the recent advances and current challenges.

Keywords: crystal form, crystal growth, structure-property relationships

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Dehydration process of lisinopril, investigated by *ab initio* powder crystal structure analysis

Kotaro Fujii[1], Hidehiro Uekusa[1], Gen Hasegawa[2], Etsuo Yonemochi[2], Katsuhide Terada[2]
[1]Tokyo Institute of Technology, H-62, 2-12-1, Ookayama, Meguro-ku, Tokyo, 152-8551, Japan, [2]Toho University, E-mail：kfujii@chem.titech.ac.jp

Lisinopril, which is a widely used ACE inhibitor, has three crystalline phases, dihydrate, monohydrate and anhydrate. The dehydration process of Lisinopril hydrates has been studied by thermal analysis and IR spectroscopy, however, the crystal structures have been unknown because of the difficulty in preparing single crystals.
In order to elucidate the dehydration mechanism, crystal structures of Lisinopril dihydrate and anhydrate were successfully revealed using *ab initio* powder crystal structure analysis from synchrotron X-ray powder diffraction data. Both structures are almost isostructural in

$P2_1$ with $Z = 2$. The molecules are stacking along the b axis(Figs.) to construct two types of water channel structures (I and II) in the dihydrate crystal. The hydrogen bond donor/accepter distances suggest that the channel I forms stronger hydrogen bonds than channel II. Therefore, the first dehydration from dihydrate may occur through the channel II to form the monohydrate crystal without major molecular conformation change. Then the water in channel I may be eliminated with rotation of the phenylethyl substituent of Lisinopril molecule.

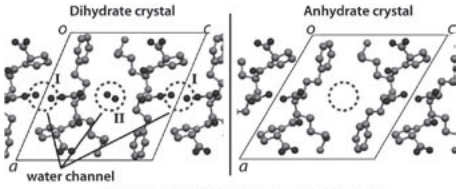

Figure : Crystal structure of Lisinopril

Keywords: powder structure determination, pharmaceutical compounds, crystalline hydrates

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What do polymorphs teach us about crystal nucleation and growth?

Lian Yu, Jing Tao, Ye Sun, Hanmi Xi, Jun Huang, Mark D Ediger
University of Wisconsin - Madison, School of Pharmacy and Department of Chemistry, 777 Highland Avenue, Madison, Wisconsin, 53705, USA, E-mail：lyu@pharmacy.wisc.edu

The ability of a liquid to crystallize as multiple polymorphs is a phenomenon of industrial importance and an opportunity to study crystal nucleation and growth. Using polymorphs to study crystallization follows the tradition of using polymorphs to test principles of thermodynamics and structure-property relations. Part 1 concerns the use of polymorphs to study the nucleation of one crystalline phase on the advancing growth front of another, a phenomenon of interest for controlling crystallization in polymorphic systems. The fastest-nucleating polymorph need not be the product of crystallization, but may nucleate another, faster-growing polymorph. The new polymorph may have higher or lower thermodynamic stability than the initial polymorph. The kinetics of such cross-nucleation were measured and compared with the kinetics of other types of nucleation (primary and growth-front nucleation) in the same liquid. Part 2 concerns the use of polymorphs to study the diffusionless crystal growth that is abruptly activated in certain fragile organic liquids near the glass transition temperature. The phenomenon is important for understanding the stability of amorphous solids. For the ROY system, currently the top system for the number of coexisting polymorphs of solved structures, diffusionless growth exists for some polymorphs but not others, with those showing the growth mode being denser and more isotropically packed.

Keywords: polymorph, crystal growth, nucleation

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Modeling single crystal diffuse scattering on polymorphs of the drug benzocaine

Eric J Chan, Thomas R Welberry, Andrew G Beasley, Aidan P Heerdegen, Darren J Goossens
Australian National University, Research School of Chemistry, Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia, Canberra, ACT, 0200, Australia, E-mail：echanj@rsc.anu.edu.au

Understanding and predicting the occurrence of polymorphism is of great importance, particularly for pharmaceuticals. Despite the attention that has been devoted to this problem, progress has been difficult and slow - a fact that may reflect the use of average (Bragg) crystal structures in the development of theoretical approaches. While efforts at crystal structure prediction, including the prediction of polymorphs, have been quite successful for rigid molecules, for conformationally flexible molecules success has been limited [1]. Diffuse X-ray scattering provides information over and above anything that can be learned from conventional crystallography and gives direct information of the local structure of materials and how the atoms and molecules are interacting. The present study is part of a research program in which we are using diffuse scattering methods to probe the local structure and dynamics of molecular systems that exhibit polymorphism, with particular emphasis on pharmaceuticals and molecules with conformational degrees of freedom. We describe a study of the diffuse scattering present in crystals of benzocaine (ethyl 4-aminobenzoate), which is commonly used as a topical local anesthetic. This has two polymorphs: form I is monoclinic $P2_1/c$; form II is orthorhombic $P2_12_12_1$. We have collected three dimensional diffuse X-ray scattering data for the two polymorphs on the 11-ID-B beamline at the Advanced Photon Source (APS). We describe the development of Monte Carlo simulation models used to interpret and analyse these data. Subsequent interrogation of the derived models provides details of the local structure of the two polymorphs and gives insight into the relationship between them.

[1] Day, G. M. et al (2005). Acta Crystallogr. Sect. B, 61(5), 511-8211;527.

Keywords: diffuse scattering, polymorphism, pharmaceuticals

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Computed crystal energy landscapes as an aid to understanding polymorphism

Sarah L Price
University College London, Chemistry, 20 Gordon Street, London, London, WC1H 0AJ, UK, E-mail：s.l.price@ucl.ac.uk

The fundamental scientific and industrial interest in controlling crystallisation is inspiring the development of methods of predicting which crystal structures are thermodynamically feasible. Frequently, computing this crystal energy landscape will reveal that there are many crystal structures that are approximately equi-energetic compromises between the various intermolecular interactions allowed by the conformational flexibility. Contrasting these crystal energy landscapes with the solid forms found experimentally shows the capacity to rationalise and predict polymorphism, disorder and a propensity for solvate formation. This will be exemplified by molecules such as uracils, carbamazepine, fluoroisatins, chloronitrobenzenes as well as the subjects of “blind tests”.

SL Price, From Crystal Structure Prediction to Polymorph Prediction: Interpreting the Crystal Energy Landscape. Phys.Chem.Chem.Phys. 2008, 10, 1996
PG Karamertzanis et al. The Thermal Stability of Lattice Energy Minima of 5-Fluorouracil: Metadynamics As an Aid to Polymorph Prediction. J.Phys.Chem.B 2007, 112, 4298
AJ Florence et al. An Automated Parallel Crystallisation Search for Predicted Crystal Structures and Packing Motifs of Carbamazepine.