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Direct Observation of Transient Ostwald Crystallization Ordering from Racemic Serine Solutions

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Abstract: The Ostwald rule of stages describes the conjectured transitioning through metastable polymorphic crystal structures during crystallization. Direct observation of the Ostwald rule of stages using was performed using solutions of simple amino acids by second-order nonlinear optical imaging of chiral crystals (SONICC). SONICC, which is based on second-harmonic generation (SHG) imaging, enabled detection of homochiral microcrystals that survived only a few seconds before being converted to the more stable SHG-inactive polymorphic forms.

The Ostwald rule of stages states that crystallization may initially proceed through several metastable polymorphs, starting with the polmorph most closely matched in free energy with the amorphous solution and ending with most stable polymorph.[1] Such transitioning has the potential to significantly impact crystal nucleation and growth kinetics, as depicted in Figure 1. In turn, the significance of crystal nucleation and growth is perhaps best illustrated by its importance in pharmaceutical preparations, where it impacts dissolution rates, stability, and bioavailability. Factors affecting crystallization kinetics can have major impacts on the shelf life and dissolution kinetics of pharmaceutical formulations. However, experiments that provide insight into the early stages of crystal formation are generally quite challenging, particularly for relatively large organic molecules with significant conformational flexibility. The problems are largely practical. Crystal nucleation is generally a rare event, and polymorph transitions consistent with the Ostwald rule of stages are likely to be transitory. Thus, the readout method should allow for the rapid analysis of large volumes with high selectivity for early crystal formation. These daunting requirements have slowed progress in studies of the Ostwald rule to a relatively small number of model systems.

The Ostwald rule has been directly observed in a number of macromolecular systems.[2] In addition, evidence for Ostwald behavior has been observed in a number of small molecules, including glycine and various salt solutions.[3,4] X-ray diffraction, small-angle neutron scattering, high-resolution electron microscopy, and, in the case of colloids, optical microscopy have been used to make these observations.[2,5−8]

In this work, direct observation of transient polymorph changes during crystallization of serine, a molecule having a higher degree of conformational freedom than the previous subjects of direct observation of the Ostwald rule, from a racemic solution was observed using second-order nonlinear optical imaging of chiral crystals (SONICC). The intrinsic sensitivity of second-harmonic generation (SHG) to symmetry properties and molecular orientation make it well-suited for investigation of the possible rearrangements arising as crystallization progresses. In particular, both disordered media and molecular assemblies with inversion symmetry are SHG-inactive, allowing the presence of transient structures adopting non-centrosymmetric ordering to be detected selectively with a high signal-to-noise ratio (S/N).[9] Consequently, SONICC provides a sensitive means of detecting crystal formation at early stages in crystals containing a chiral unit cell (i.e., a unit cell with a nonsuperimposable mirror image). Amino acids were chosen initially to serve as model organic compounds. In addition, serine in particular has been implicated as a potential contributor to prebiotic chiral enhancement by its propensity to form stable homochiral octomers upon desolvation.[10] This observation appears to conflict with the established macroscopic room-temperature behavior, in which the racemic cocrystal is thermodynamically favored. One possible explanation for the discrepancy is the transient initial formation of homochiral structures.

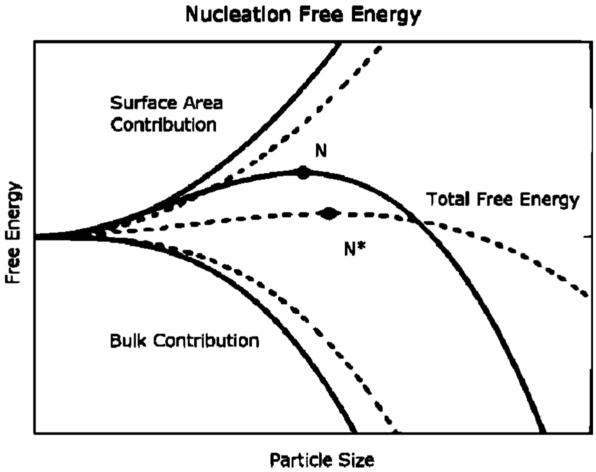

Figure 1. Free energy as a function of particle size, decomposed into bulk and surface-area contributions. For this example, the dashed-line polymorph has the lower free energy upon initial nucleation, but upon significant crystal growth, the solid-line polymorph is favored.

Solutions of homochiral 0.5 M L-serine (Aldrich) and racemic 1:1 DL-serine (Aldrich) were prepared using ultrapure water (16 MΩ cm). In each trial, a ∼3 μL drop was placed on a glass microscope slide and allowed to dry in air. SHG imaging was performed using an 800 nm, 50 mW, ∼100 fs laser (Mai Tai, Spectra Physics) focused using a 10× objective (Nikon, N.A. = 0.30). Doubled light was detected at 400 nm in both the backward (epi) and transmission directions, with collection in transmission performed using a matched 10× objective. Images were created using raster scanning with an 8 kHz resonant vibrating mirror (Electro-optics) on the fast scan axis and a galvanometer (Cambridge Technologies) on the slow axis to average ∼0.125 ms integration per pixel with the beam sweeping through the full 100 μm field of view at 60 Hz, corresponding to ∼8 s per frame for ∼250 × 250 pixel images. Rapid scanning over the full field of view was performed to minimize possible perturbation of the sample arising from local heating during crystallization. A more detailed description of the SHG microscope has been published previously.[11]

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Selected frames from SONICC movies of representative serine crystallization trials are shown in Figure 2 for both homochiral L-serine solutions and racemic DL-serine solutions. Initially, neither sample showed detectable SHG activity. Upon initiation of crystallization, the signal grew in rapidly for both but was localized in the case of the racemic DL-serine solution. Specifically, regions varying in size from 5 to 10 μm and surviving from 20 to 200 s were detectable with S/N greater than 40 for the racemic solution. In comparison, only growth was observed in the SHG activity of the crystals generated from the homochiral L-serine solution, which reached a plateau within a few minutes of initial crystal formation. Corresponding time traces of the SHG from a racemic solution measured at two locations are shown in Figure 3, together with a representative time trace for crystallization from a homochiral L-serine solution. The observations of transient SHG-active regions from racemic DL-serine solutions were reproducible over many trials and many months.

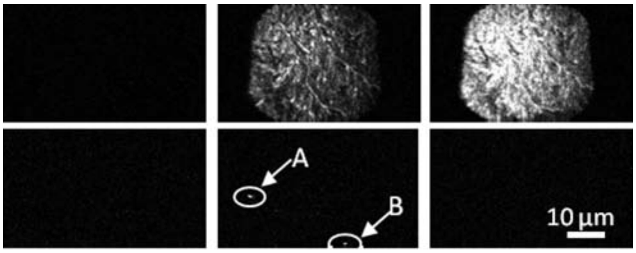

Figure 2. SHG images of (top row) L-serine and (bottom row) DL-serine as crystallization progressed. The middle column shows crystallization initiation, the left column 165 s previous to initiation, and the right column 165 s following initiation. Points A and B are small regions of likely homochiral domains.

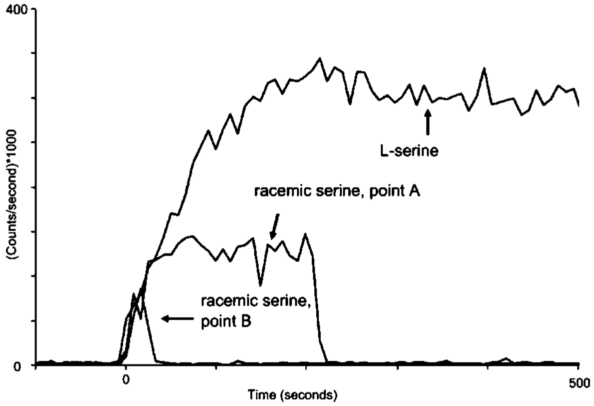

Figure 3. Crystallization of selected points within the field of view for L-serine and DL-serine over time. The L-serine showed clear, sustained non-centrosymmetric order, whereas DL-serine showed brief non-centrosymmetric order.

The SHG-active regions are attributed to transient formation of homochiral crystalline domains from the racemic solution. Other possible interferences from fluorescence and supercontinuum generation were excluded, as no significant background signals were detected from a third detection channel with optics designed to detect light over a range from 460 to 570 nm. Symmetry properties intrinsic to the SHG process make it insensitive to bulk centrosymmetric and amorphous materials. This fact makes the presence or absence of inversion symmetry in a crystal particularly straightforward to determine, primarily on the basis of the presence or absence of bulk-allowed SHG. With the exception of the relatively rare cubic gyroidal symmetry, all crystals that are homochiral in composition must fall into space groups that exhibit bulk-allowed SHG. For homochiral serine crystals, the previously determined space group is $P2_12_12_1$, which contains a number of nonvanishing SHG tensor elements.[12] Racemic DL-serine crystals have been determined to be in the $P2_1/n$ space group, which has an SHG tensor that completely vanishes because of symmetry considerations.[13,14] The most likely explanation for the observation of transient SHG is the presence of transient thermodynamically unfavored crystal polymorphs. It is difficult to determine on the basis of SHG alone whether these structures are non-centrosymmetric forms of the racemic cocrystal or homochiral crystals. However, it is reasonable to suggest that the transient crystals likely are homochiral in composition.

The observation of transient SHG-active serine crystals consistent with the Ostwald rule of ordering may help to explain two previously incongruent experimental results. Mass spectrometric and computational chemical studies of serine have reported strong preferences for homochiral "magic" octomeric clusters. However, bulk measurements of serine crystals clearly indicate that the racemic cocrystal is energetically favored over conglomerates of homochiral crystals under ambient conditions. A phase transition from the homochiral microcrystal to the racemic cocrystal consistent with the energy-level diagram described in Figure 1 could provide a viable explanation that connects these two observations. Furthermore, one might expect significant kinetic barriers for interconversion from homochiral crystals to racemic cocrystals, as the chemical composition within the lattice differs for the two forms. Consequently, homochiral crystals may be kinetically trapped sufficiently long enough to allow facile detection by SONICC. Studies using faster drying methods and functionalized surfaces will be the subject of future investigations and should provide additional insights into the mechanics of kinetic trapping of unfavorable homochiral polymorphs.

These observations provide compelling evidence for the ability of SONICC to directly monitor polymorph transitioning consistent with the Ostwald rule for complex molecular systems in near-real-time. To our knowledge, they provide the first direct evidence for rapid polymorph transitioning in organic crystals.

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Supporting Information Available: Movie files (AVI) containing the full sets of images obtained during crystallizations from both the racemic and homochiral serine solutions, from which the frames in Figure 2 were extracted, along with a file containing a description of the movie files. This material is available free of charge via the Internet at http://pubs.acs.org.

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