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COMMUNICATION

Resonant Acoustic Mixing-Induced Polymorphic Transformation of Glycine Observed by Solid-State NMR

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The potential of resonant acoustic mixing for inducing polymorphic transformations is assessed via real-time ^{13}C cross-polarization magic-angle spinning solid-state NMR. Conversion of metastable α-glycine to γ-glycine is sensitive to initial conditions, follows stepwise kinetics which depend on the filling factor of the vial used for RAM, and contrasts with previous results obtained via ball milling.

Mechanochemical approaches can effect a wide range of transformations including catalysis,[1-6] synthesis of complex molecules,[7-9] formation of known and novel cocrystals,[10-16] and more. From the perspective of developing and understanding the mechanisms of mechanochemical transformations, it is of particular interest when different approaches can yield different products or reaction pathways. These approaches include the physical method used, e.g., ball milling, resonant acoustic mixing (RAM), hand-grinding, SpeedMixing, etc., as well as studying the effects of additives, catalysts, and small amounts of liquid.[6,15] The number, size, and type of balls used in a mill can have determinative impacts on the products of a reaction.[17] Our own recent work has examined the potential of RAM for producing known and novel halogen-bonded and chalcogen-bonded cocrystals, and in particular how new products can be obtained with RAM.[15,16] In the present work, we turn our attention to the potential of RAM to effect polymorphic transformations. This is of particular interest given the importance of understanding and controlling polymorphism both from an academic perspective as well as an industrial perspective. Ball milling, for example, has been shown to cause the polymorphic transformation of the γ polymorph of glycine into the α form.[18] We report here the novel observation of RAM-induced polymorphic transformation of glycine monitored by real-time *ex-situ* ^{13}C cross-polarization magic-angle spinning (CP/MAS) solid-state nuclear magnetic resonance (SSNMR) spectroscopy.

α-Glycine is metastable and γ-glycine is known to be the most stable polymorph.[19,20] ^{13}C CP/MAS SSNMR spectroscopy has been used to study these polymorphs extensively.[21-24] The α polymorph can be identified by its carbonyl ^{13}C chemical shift of 176.4 ppm while the γ polymorph can be identified by a shift of 174.6 ppm. The β polymorph is less stable than γ and α, and has been observed via CP/MAS SSNMR. High-pressure δ and ε polymorphs have been reported as well.[25]

Herein, for most time-course experiments, α-glycine was obtained by dissolving commercially obtained glycine (≥99%, Sigma-Aldrich) in distilled water followed by slow evaporation inside a fume hood, leading to the formation of crystals. The crystals were then carefully collected using a metal spatula, followed by fine and even grinding using a mortar and pestle. A PharmaRAM™I resonant acoustic mixer was used to treat solid powder samples during each time-course experiment. The sample is first transferred to a borosilicate glass vial (51 mm height x 19 mm diameter; 7.4 mL total volume) with a plug-style polyethylene cap. The vial is placed inside a plastic vessel filled with Styrofoam material to hold the vial securely. The plastic vessel is then placed on the platform of the resonant acoustic mixer, held in place using the supplied hold-down fixture with the help of a hold-down knob and jam nut (Fig. 1). Three mixing settings were used: low (40 *g*), medium (60 *g*), or high (80 *g*). Faster conversion was noted at 80 *g*, and therefore this setting was used for all experiments reported below. In a typical experiment, glycine is subjected to resonant acoustic mixing for a specified period, then the powder is removed and packed into a zirconia NMR rotor and the ^{13}C CP/MAS NMR spectrum is acquired. This is repeated many times at increasing time increments to monitor possible polymorphic conversion. All ^{13}C CP/MAS SSNMR spectra were acquired using a Bruker Avance III

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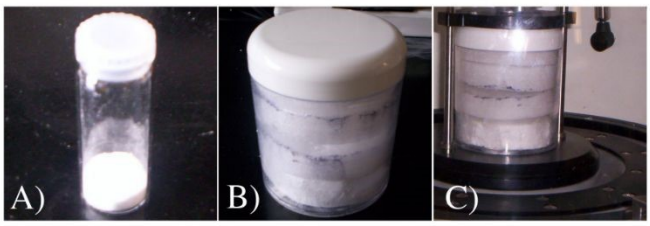

Figure 1. (A) Glass vial used for RAM experiments, here containing 1.54 g glycine. (B) The vial is placed in a plastic vessel and held in place with Styrofoam. (C) The plastic vessel is then placed on the platform of the resonant acoustic mixer.

200 NMR spectrometer equipped with a double-resonance (HX) 7-mm stretch rotor probe. Magic-angle spinning of the rotor was carried out at 3000 Hz. A standard CP pulse program with high-power proton decoupling was used with a $\pi/2$ pulse of 4 μs, a contact time of 3 ms, and a recycle delay of 4 s. Spectra were referenced to external adamantane with chemical shifts of 38.56 and 29.50 ppm.

Shown in Figure 2 are representative ^{13}C CP/MAS SSNMR spectra of the carbonyl region of a pure α-glycine sample, a pure γ-glycine sample, and a physical mixture of the two obtained roughly halfway through a time-course experiment. Conversion ratios $\alpha/(\alpha+\gamma)$ were plotted using the integrals of the carbonyl peaks; this normalization approach circumvents the need to pack the NMR rotor with precisely the same sample mass for each experiment.

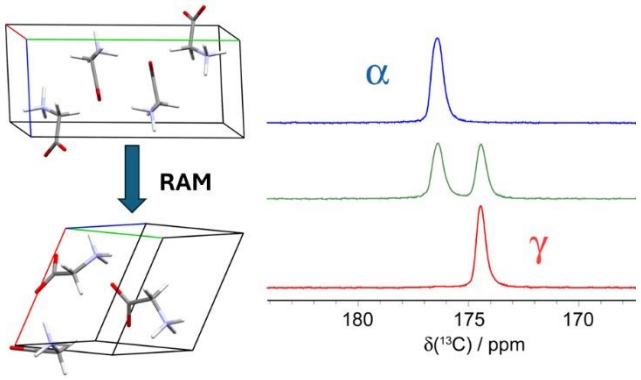

Figure 2. Left: Unit cells of α-glycine (top) and γ-glycine (bottom). Right: Representative ^{13}C CP/MAS NMR spectra of the carbonyl region of α-glycine (top), γ-glycine (bottom), and a physical mixture of the two, roughly halfway through a time-course experiment.

Several attempts were made to induce and monitor a transformation from α-glycine to γ-glycine. 1.51 g glycine taken directly from the bottle, without any treatment, proved to be the pure α form and did not show any transformation after 5 h at 80 *g* RAM. The same negative result was obtained if the sample was ground gently with a mortar and pestle prior to RAM. In a third experiment, the α-glycine was recrystallized at room temperature from water in a large rectangular glass dish. The harvested α-glycine crystals were gently grinded using a mortar and pestle before being subjected to RAM at 80 *g* (1.54 g of glycine). In this case, complete conversion from α-glycine to γ-glycine was observed over a period of 12 h (Fig. 3(A)). Two points should be made here. Firstly, because γ-glycine is the lower-energy polymorph, it is known that α-glycine will transform to γ-glycine given enough time. Therefore, at present we cannot conclude that RAM has caused the observed transformation, only that it has promoted or accelerated it. Secondly, it is not possible to rule out the presence of a seed crystal of γ-glycine which could have initiated the transformation only in the freshly recrystallized batch.

Following on this success, a further trial was attempted whereby the vial was more completely filled with 7.30 g of α-glycine. This attempt was motivated by recent work suggesting improved RAM efficiency with more highly filled vials, perhaps because there are more opportunities for intercrystallite collisions.[26] However, this trial resulted in less than 10% conversion to γ-glycine even after 7 h of RAM at 80 *g* (see ESI). It is known that the γ form of glycine can convert to the α form

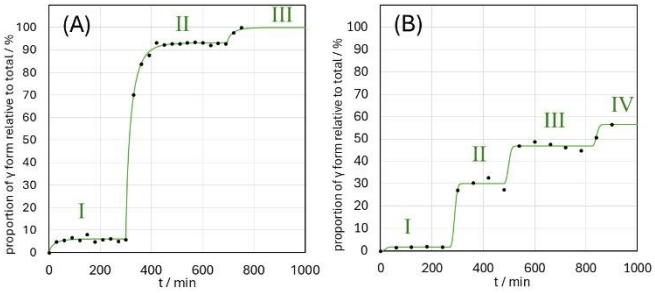

Figure 3. Proportion $\gamma/(\alpha+\gamma)$ of glycine as assessed by carbonyl NMR peak intensity plotted as a function of time for RAM products obtained using (A) 1.54 g of glycine in a vial and (B) 1.70 g of glycine in a vial. Data are fit to a modified, stepped Johnson–Mehl–Avrami–Kolmogorov (JMAK) equation (green line). Roman numerals indicate each step of the JMAK-based transformation.

at a temperature higher than 165°C; given that the surface of the full glass vial became quite hot to touch after it had been subjected to RAM, we speculate that the temperature is impeding the α to γ conversion when a fuller vial is used. The increase in temperature, particularly for a higher vial filling factor, is attributable to increased frictional heating between particles.[26]

An additional experiment was carried out with 1.70 g of α-glycine, a 10% increase in mass over the 100%-yielding experiment. This mass was chosen as an intermediate value to validate the hypothesis that the filling factor of the vial plays a key role in the success of the polymorphic transformation. Indeed, as shown in Figure 3(B), partial conversion was observed, thereby bolstering the validity of our hypothesis.

As shown in Figure 3(A) and (B), the transformations can be modelled using a modified, stepped Johnson–Mehl–Avrami–Kolmogorov (JMAK) equation[27-29]:

$$f(t) = \sum_{i=1}^{N} f_i(t)$$

where $N = 3$ or 4 (for 1.54 or 1.70 g respectively) and where each step i:

$$f_i(t) = \begin{cases} 0 & for\ t \le t_{0,i} \\ f_{max,i}\left[1 - e^{-(k(t-t_{0,i}))^n}\right] & for\ t > t_{0,i} \end{cases}$$

Here, each $f_i(t)$ represents the ratio $\alpha/(\alpha+\gamma)$; each $f_{max,i}$ represents the maximum value of $f_i(t)$ for a given step i; k is the effective rate constant for the transformation; n is the order of

the transformation; and $t_{0,i}$ is the effective time zero for each of the steps. Initially, a logistic curve and the standard JMAK equation were used to model the data in Figure 3(A) and the initial phase of (B). It appeared as if (A) has an induction period before ~300 minutes, to only then transform in an s-curve fashion. However, upon further inspection, we determined that both data sets exhibit stepped transformations of varying degrees, with the data points below 10% being the stalled plateau of the initial phases rather than the beginning of the subsequent transformations.

In order to avoid overfitting the data with too many unique parameters, the transformation rate and order, *k* and *n*, were held constant across the three or four steps of each data set. While this assumes identical kinetics along the whole transformation pathway, which is not always correct, fitting each parameter individually yielded mostly unphysical interpretations. Herein, a higher *k* leads to a steeper curve, a higher *n* results in a more s-shaped curve with a longer induction period, and a higher f_{max} represents a higher contribution of the respective transformation step. With 1.54 g (Figure 3(A)), k = 0.048 min^{-1}, n = 0.77, and the root-mean-square deviation (RMSD) is 0.78%. With 1.70 g (Figure 3(B)), k = 0.045 min^{-1}, n = 2.6, and RMSD = 1.2%. Despite the initial conditions (mass and/or filling factor) heavily influencing the exponent and the overall speed of the transformation, the rate parameters, *k*, remain very similar between the two data sets. These results tell us that despite the stalling effect resulting from a higher RAM filling factor (perhaps attributable to increased autogenous heating), the kinetics must remain fundamentally related as both experiments involve the same two crystal structures.

As for the f_{max} parameters, each step contributes to 6.12, 87.02, and 6.86% of the total transformation with 1.54 g of glycine. Given 1.70 g, f_{max} are 1.76, 28.43, 16.76, and 9.62%. In both cases, the central steps (A.II, B.II, and B.III) show more contribution towards the total conversion than the peripheral steps (A.I, A.III, B.I, and B.IV). This suggests that the polymorphic transformation is least hindered by the vial filling volume when near $t_{1/2}$. Moreover, each step of the transformation lasts between 200 and 400 minutes before the next, highlighting yet another interphase and intermass consistency, likely attributable to the fundamentally identical nature of both systems.

To confirm that the RAM effect is responsible for the observed conversion, a control experiment was carried out whereby α-glycine was loaded into 10 mL stainless steel milling jars with no balls or other milling media added. Following 5 h of oscillation in a Retsch MM400 mixer mill, no conversion to γ-glycine was observed.

It is emphasized that the different polymorphs are known to have different cross-polarization dynamics and different relaxation time constants, both of which diminish the degree to which the peak integrals can be used to quantitatively determine the amount of each polymorph present.[21,24] Proton relaxation time constants, T_1, have been reported to be 2 s for the α polymorph and 20 s for the γ polymorph; furthermore for the 3 ms contact time used in this work, the α polymorph is expected to give approximately double the carbonyl signal intensity compared to the γ polymorph. We did not attempt any *post hoc* data scaling to improve absolute quantitation given that the observed transformation follows a steep step function, i.e., the few data points which define the steep vertical parts of the curves would simply shift up and down the curve rather than shifting the inflection point significantly. Furthermore, preliminary T_1 measurements on some samples were far less than the values reported in the literature, perhaps in part due to the RAM treatment. This is consistent with the known effects of ball milling on lowering T_1 values.[30-32] The potential effect of RAM on relaxation time constants will be further explored in a forthcoming publication.

Finally, we note that the RAM-induced polymorphic conversion reported here is in complete contrast to that observed under planetary ball milling conditions.[18] Under such conditions, the reverse transformation is observed, from γ-glycine to α-glycine. The timescale of the transformation was also shown to be highly dependent on the number of balls used, as well as the rotation rate, with complete conversion taking from 1 h to > 30 h depending on the conditions used.

Conclusions

We have shown that resonant acoustic mixing can induce polymorphic transformations. Conversion of α-glycine to γ-glycine occurs in a stepwise fashion over a time scale of several hours, with a rate constant of approximately 0.05 min^{-1}. Achieving complete conversion is sensitive to the sample preparation method and to the filling factor of the vial used for RAM experiments. Notably, the conversion observed here is in clear contrast with what has been observed using ball milling. We conclude that RAM holds potential for effecting known and novel polymorphic transformations.

Author contributions

SS: Investigation, Methodology, Data Curation, Writing – review & editing. EB: Investigation, Methodology, Data Curation, Writing – review & editing. TI: Data Analysis, Writing – review & editing. DLB: Conceptualization, Funding Acquisition, Methodology, Supervision, Writing.

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Conflicts of interest

There are no conflicts to declare.

Data availability

Data are available as part of the Electronic Supplementary Information and upon reasonable request from the authors.

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