The effect of deuteration on polymorphic outcome in the crystallization of glycine from aqueous solution

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This paper reports the first systematic studies of the effect of deuteration on the polymorphic outcome of the crystallization of glycine from water, focusing on crystallization experiments carried out as a function of the level of deuteration in the system; the dependence of polymorphic outcome on the time of the crystallization process is also explored.

Glycine ($H_3N^+ \cdot CH_2 \cdot CO_2^-$) has been studied widely in the context of polymorphism research,[1–15] and has, in many respects, assumed the role of a prototypical system in this regard. Under ambient conditions, three polymorphs of glycine (denoted α, β and γ) are known[1–4] (we note that other polymorphs have been observed at high pressure[5]). The γ polymorph is thermodynamically stable under ambient conditions, with the order of stability: $\gamma > \alpha > \beta$.[6,7] The widely accepted consensus in the literature is that crystallization of glycine from aqueous solution at neutral pH leads reproducibly to the formation of the metastable α polymorph. The stable γ polymorph, on the other hand, is obtained on crystallization from sufficiently basic (pH > *ca.* 8.9) or sufficiently acidic (pH < *ca.* 3.8) aqueous solutions. Several other strategies have also been reported to induce the formation of the γ polymorph, including addition of sodium electrolytes to the crystallization solution[8] (*e.g.*, sodium chloride, sodium fluoride or sodium nitrate), application of polarized laser radiation,[9] application of a dc electric field,[10] evaporation of micro-droplets,[11] or thin-film evaporation from a glass surface.[12]

In 1961, Iitaka[3] noted that "*from heavy water solutions the γ-form crystallizes more easily without adding any substance except the seeding crystals*". Although this observation was mentioned subsequently on a few occasions,[7,13] it was not explored in more detail until recently,[14] as part of a wider study of several different aspects of glycine crystallization. In particular, it was shown in ref. 14 that seeding is not essential for observing this phenomenon, with the γ polymorph obtained for a fully deuterated system under normal conditions of crystallization without seeding. However, ref. 14 only considered the results from crystallization experiments carried out in the limiting cases of natural ^1H–^2H isotopic abundance (*ca.* 0.012% ^2H) in comparison with the fully deuterated system (greater than *ca.* 99% ^2H).[16]

In this letter, we report the first systematic studies of the effect of deuteration on the crystallization of glycine, by exploring the polymorphic outcome of crystallization experiments as a function of the ^1H–^2H isotopic ratio for the exchangeable hydrogen sites in the system.[16] Several different isotopic ratios between the limiting values of natural-isotopic-abundance and full deuteration have been considered and the dependence of the polymorphic outcome on the time of the crystallization process has also been explored.

The powder X-ray diffraction patterns of all samples obtained from our crystallization experiments were completely accounted for by the α and γ polymorphs of glycine, and there was no evidence for the presence of any other phase (including the β polymorph of glycine[2]) in these samples. To assess the relative amounts of the α and γ polymorphs, the powder X-ray diffraction data were analysed by Rietveld refinement[18] (using the GSAS program[19]) using the known crystal structures of the α and γ polymorphs[4] and with the refined value of the scale factor used to quantify the relative amounts of the α and γ polymorphs (in addition, unit cell and profile parameters were refined, but not the fractional atomic coordinates). The ratio of the amount of γ polymorph to the amount of α polymorph (established from the scale factor) is denoted F_γ. From such analysis, the results are quoted as the "polymorphic excess" (denoted p.e.), defined as:

$$\text{p.e.} = \frac{1 - F_\gamma}{1 + F_\gamma} \qquad (1)$$

Clearly, a p.e. value of +1 corresponds to pure α polymorph, a p.e. value of 0 corresponds to equal amounts of the α and γ polymorphs, and a p.e. value of −1 corresponds to pure γ polymorph.

Within all the samples obtained from the crystallization experiments, three different crystal morphologies were observed: relatively large, translucent crystals, smaller needle-like crystals (often radiating from a single point), and clusters of smaller, opaque crystals. In several cases, the three types of crystal were obtained concomitantly in the same crystallization experiment, whereas in other cases, only one type of crystal morphology was observed. The larger crystals were identified as the α polymorph, and the needle-like crystals and clusters of smaller crystals were identified as the γ polymorph.[20]

We consider first the results from seven sets of crystallization experiments (totalling 48 individual experiments) in which there was no delay between the cooling period and collecting the crystals. Fig. 1 shows the values of p.e. determined for these experiments as a function of percentage deuteration. Although there is appreciable spread in the

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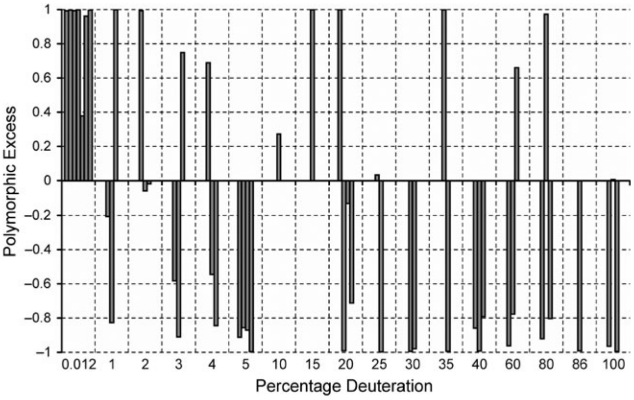

Fig. 1 Polymorphic excess (p.e.) as a function of percentage deuteration for 48 crystallization experiments, with no delay before collecting the crystals. Natural-isotopic-abundance corresponds to a percentage deuteration of 0.012%. For the other experiments, the values of percentage deuteration are rounded to the nearest integer, and the results of experiments at the same (rounded) percentage deuteration are grouped together on the plot.

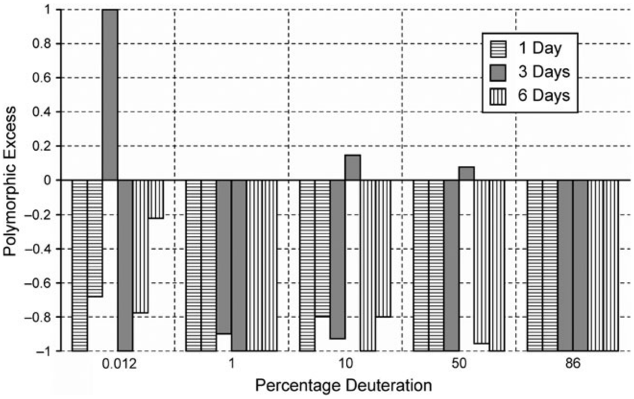

Fig. 2 Polymorphic excess (p.e.) as a function of percentage deuteration for 30 crystallization experiments, with delays before collecting the crystals of 1 day (horizontal lines), 3 days (grey) and 6 days (vertical lines). The grouping of results from different experiments is the same as that defined for Fig. 1.

data, an overall trend is nevertheless apparent, with the crystallization experiments carried out at higher percentage deuteration having a greater tendency to produce the γ polymorph.

For the natural-isotopic-abundance system, the crystallization experiment was carried out seven times (Fig. 1). In six cases, the p.e. was greater than 0.99, corresponding to essentially pure α polymorph, whereas in the other case, the p.e. was 0.38, representing concomitant formation of the α and γ polymorphs[21] but still with a significant excess of the α polymorph. Thus, all crystallization experiments at natural-isotopic-abundance led to an excess of the α polymorph.

In marked contrast, however, of the 41 crystallization experiments carried out with deuteration levels between 1% and 100%, 29 experiments (*i.e.* more than 70% of the total number of experiments) led to an excess of the γ polymorph and only 12 experiments led to an excess of the α polymorph. Among the 12 experiments that led to an excess of the α polymorph, only six experiments produced pure or nearly pure (p.e. $> +0.95$) samples of the α polymorph. Among the 29 experiments that led to an excess of the γ polymorph, 11 experiments produced pure or nearly pure (p.e. < -0.95) samples of the γ polymorph.

As a reflection of the general trend observed—*i.e.*, the probability of forming the γ polymorph increases as the percentage deuteration increases—the average values of p.e. for different ranges of percentage deuteration were: natural-isotopic-abundance system, 0.90 (standard deviation [s.d.] = 0.23); 1–5% deuteration, −0.26 (s.d. = 0.74); 10–40% deuteration, −0.34 (s.d. = 0.81); 60–100% deuteration, −0.48 (s.d. = 0.75). Although some specific experiments clearly behave as "outliers" with respect to this trend (see Fig. 1; for example, a pure sample of the α polymorph was obtained in one experiment at 80% deuteration and a pure sample of the γ polymorph was obtained in one experiment at 5% deuteration), the results nevertheless suggest that there is a general tendency to promote the formation of the γ polymorph by increasing the level of deuteration of the system above natural isotopic abundance. The existence of outliers reflects the inherent stochastic nature of crystallization processes and the fact that the time at which nucleation occurs under a given set of crystallization conditions is not necessarily reproducible on repeating the crystallization experiment under ostensibly identical conditions.

We now consider the results of the experiments involving a time delay between the end of the cooling period and the time of collecting the crystals. In total, 30 crystallization experiments were carried out, divided into three sets according to the length of delay (1 day, 3 days or 6 days). Fig. 2 shows the values of p.e. determined for these experiments as a function of percentage deuteration. Clearly there is much less spread in the data in comparison with the results discussed above (Fig. 1), with most experiments resulting in either pure γ polymorph (in 19 cases) or a significant excess of the γ polymorph (in 8 cases). Only three experiments produced an excess of the α polymorph: in one case, a pure sample of the α polymorph was obtained in crystallization from a natural-isotopic-abundance system, whereas in the other two cases (at higher deuteration levels) a small excess of the α polymorph was observed. These results suggest that the tendency to obtain the γ polymorph becomes significantly enhanced when the crystallization process is allowed to proceed for a longer time before collection of the crystals, even when the percentage deuteration of the system is low.

Another significant observation is that, in the crystallization experiments carried out with a delay before collecting the crystals for the natural-isotopic-abundance system, five of the six experiments actually produced an excess of the γ polymorph. In contrast, as discussed above, all seven experiments carried out for the natural-isotopic-abundance system with no delay before collecting the crystals produced an excess of the α polymorph (in fact pure α polymorph in six cases). It is clear from these observations that the preferential formation of the α polymorph on crystallization of glycine from neutral aqueous solution at natural isotopic abundance, which has long been the consensus in the polymorphism literature, actually holds only if the crystals are collected sufficiently quickly after formation. The results obtained here suggest that a subsequent transformation (probably a solution-mediated polymorphic transformation rather than a direct solid-state transformation) occurs on leaving the initially formed crystals within the crystallization solution for a sufficient length of time before collection and analysis.

It is relevant to note that a very recent *in situ* solid-state ^{13}C NMR study of the crystallization of glycine from aqueous solution[22] showed that, at 20 °C and under conditions of magic-angle spinning at 8 kHz, crystallization from a system corresponding to 86% deuteration led initially to the rapid formation of the α polymorph, which reached a maximum amount after *ca.* 1.5 hours. At this stage, the amount of the α polymorph decreased, while the γ polymorph emerged, and after *ca.* 12 hours the γ polymorph had almost completely replaced the α polymorph. Under the same conditions, crystallization in a natural-isotopic-abundance system was observed to yield the α polymorph, with no subsequent transformation to form the γ polymorph occurring within the time-frame of the *in situ* NMR measurement. These observations are clearly in good agreement with the results of the present study, but additionally provide direct evidence that the tendency to favour the formation of the γ polymorph in crystallization from a deuterated system is not due to preferential nucleation of the γ polymorph. Instead, crystallization from the deuterated system occurs (as in the natural-isotopic-abundance system) by the initial formation of the α polymorph, followed by a subsequent transformation to produce the γ polymorph.

The results of the present study suggest that the behaviour of the natural-isotopic-abundance system is actually qualitatively similar, such that if the initially formed crystals of the α polymorph are left for a sufficient length of time in the crystallization solution, a (solution-mediated) transformation occurs to produce the γ polymorph. However, it is clear that the rate of this transformation from the initially formed α polymorph to the γ polymorph is significantly slower in the natural-isotopic-abundance system than in deuterated systems. Furthermore, our results suggest that only very low levels of deuteration are required to promote the more rapid occurrence of this transformation.

Clearly, the immediate priority is to establish deeper insights into the fundamental factors that underlie the observed isotope effects in this system, including experiments to determine quantitative details of the rate of the (solution-mediated) transformation from the α polymorph to the γ polymorph as a function of the level of deuteration of the system.

Experimental

Crystallization experiments were carried out in 50 ml Teflon beakers (to eliminate the possibility of glycine crystals forming by thin-film evaporation on the sides of the crystallization vessel, which occurs readily in crystallization from glass beakers and is known[12] to induce formation of the γ polymorph). Solutions of molarity 6 mol dm^{-3} were prepared with a solution volume of 5 cm^3. The percentage deuteration was controlled by varying the ratio of natural-isotopic-abundance water (denoted H_2O) and deuterated water (denoted D_2O; Fluorochem, 99.9% ^{2}H) used as the solvent, and using a natural-isotopic-abundance sample of glycine.[16] In the case of the fully deuterated system, a sample of glycine deuterated in the exchangeable hydrogen sites (*i.e.* $D_3N^+\cdot CH_2\cdot CO_2^-$, prepared by prior repeated recrystallization from D_2O) was used with D_2O as the solvent.

Each crystallization beaker was sealed with a double layer of Nescofilm® and placed in an incubator, initially at ambient temperature. The temperature was then raised over 1 hour to 80 °C and held at this temperature for at least 15 hours to ensure complete dissolution (this temperature is *ca.* 20 °C higher than the temperature at which a 6 mol dm^{-3} solution becomes supersaturated). The seal on each beaker was then broken (the incubator was opened briefly for this purpose) and the temperature was then reduced to 10 °C over 4 hours with a linear temperature profile. In some experiments (as discussed in the text), the crystals were then collected immediately, whereas in other experiments, the crystallization solutions were kept at 10 °C for a period of time (1, 3 or 6 days) before collection of the crystals. After collection (at ambient temperature), the crystals were dried on filter paper. The entire sample of crystals collected from a given beaker was ground in a mortar and pestle for investigation by powder X-ray diffraction.[17] The time between collecting the crystals and recording the powder X-ray diffraction data was typically about 1–4 hours.

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16 In the present work, we consider deuteration only of the exchangeable hydrogen sites in the glycine molecule—*i.e.*, the three hydrogens of the NH_3^+ group. In aqueous solution, these three hydrogens exchange rapidly with the solvent and thus the ^1H–^2H ratio of the NH_3^+ group of glycine and the ^1H–^2H ratio of water will rapidly equilibrate to approximately the same value (assuming equal partitioning of ^1H and ^2H between glycine and water). Thus, for example, dissolution of a sample of glycine with natural isotopic abundances (*ca.* 99.99% ^1H; 0.01% ^2H) in D_2O will give rise to approximately equal ^1H–^2H ratios for the NH_3^+ group of glycine and the water molecules. The actual value of the ^1H–^2H ratio depends on the amounts of glycine and D_2O used to prepare the solution. The quoted values of percentage deuteration have been calculated to take account of the effects of such exchange. The hydrogen sites of the CH_2 group of glycine do not undergo appreciable exchange in neutral aqueous solution.

17 Powder X-ray diffraction data were recorded at ambient temperature on a Bruker D8 diffractometer operating in transmission mode ($CuK_{\alpha 1}$, Ge-monochromated; linear position-sensitive detector covering 12° in 2θ; 2θ range, 5–50°; step size, 0.017°; data collection time, 25 min; foil sample holder).

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20 In several crystallization experiments, the sample was observed to take on a faint yellow coloration, which has also been observed previously[8] and attributed to photo-induced decomposition. Given that our crystallization experiments were carried out in the dark, it seems likely that the decomposition in our experiments is caused by heating during dissolution.

21 Although much of the literature on glycine crystallization has implied that only the α polymorph is observed in crystallization from water at neutral pH (in a natural-isotopic-abundance system), our results are in agreement with observations of Boldyreva *et al.*,[7] who note that the α and γ polymorphs can be obtained as concomitant polymorphs under these conditions.

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