New Insights into the Crystallization and Structural Evolution of Glycine Dihydrate by *In-Situ* Solid-State NMR Spectroscopy

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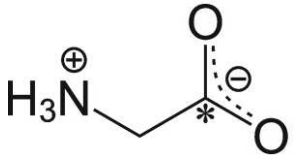
Abstract: *In-situ* solid-state NMR is exploited to monitor the structural evolution of a glycine/water glass phase formed on flash cooling an aqueous solution of glycine, with a range of modern solid-state NMR methods applied to elucidate structural properties of the solid phases present. The glycine/water glass is shown to crystallize into an intermediate phase which then transforms to the β polymorph of glycine. Our *in-situ* NMR results fully corroborate the identity of the intermediate crystalline phase as glycine dihydrate, which was first proposed only very recently.

Polymorphism in glycine was first observed, although not fully understood, by Fischer in 1905.[1] Although originally ascribed to possible isomerism, his observations are now attributed to the existence of the α and β polymorphs of glycine.[2] A third polymorph (γ polymorph) was discovered in 1954.[3] Except for three further polymorphs formed at high pressure,[4] no new solid form of glycine was reported until over 60 years later, with the very exciting discovery and structural characterization of a crystalline dihydrate of glycine, reported by Xu *et al*. in 2017.[5]

In general, crystallization of glycine from pure aqueous solution at neutral pH gives the α polymorph,[6] which transforms to the stable γ polymorph over time.[7] Crystallization from water in nano-confined environments promotes the formation of the β polymorph.[8] In spite of extensive studies, no hydrated crystalline phase of glycine was reported until the recent study by Xu *et al*.[5] In contrast, hydrate phases of several other amino acids are well known,[9] and interconversion processes between anhydrous and hydrate phases have been studied in detail.[9e,9f] For certain amino acids (e.g., L-lysine and L-arginine), the anhydrous solid form is very hygroscopic and transforms readily to a hydrate phase under normal atmospheric conditions.[10]

The recent work[5] that proved the existence of glycine dihydrate (GDH) relied on a preparation procedure reported by Pyne and Suryanarayanan[11] and later by Surovtsev *et al*.[12] In this procedure, an aqueous solution of glycine is quench frozen in liquid nitrogen to form a glycine/water glass phase (frozen solution). When this glass phase is warmed to around 209 K and held at this temperature, crystallization occurs to give a new solid phase. Xu *et al*.[5] prepared the new crystalline phase *in-situ* in a synchrotron powder X-ray diffractometer and determined the structure directly from the powder XRD data, revealing that the new phase is a dihydrate of glycine. It was also shown[5] that, on heating above 250 K, this phase transforms to the β polymorph of glycine with loss of water.

In the present work, the procedure reported previously[5,11,12] has been carried out inside an NMR spectrometer, exploiting *in-situ* solid-state NMR techniques to monitor the structural evolution, as a function of temperature and time, of the glycine/water glass formed after flash cooling an aqueous solution of glycine. In recent years, *in-situ* NMR techniques for monitoring crystallization processes have been applied to a range of systems,[13] including crystallization of glycine from aqueous solution near ambient temperature.[13a,13b] A significant advantage of solid-state NMR for *in-situ* studies of such processes is that it allows the identification and characterization of both amorphous and crystalline phases. Thus, the *in-situ* solid-state NMR study presented here offers the prospect of monitoring details of the structural evolution from the glycine/water glass phase to the dihydrate of glycine and then to the β polymorph of glycine. Furthermore, a range of modern solid-state NMR techniques are exploited to elucidate specific structural aspects of the solid phases present at different stages of the process.



Scheme 1. Molecular structure of glycine (the asterisk indicates the labelled ^{13}C site in 1-^{13}C-glycine).

Our *in-situ* solid-state ^{13}C NMR study used a sample of glycine ^{13}C-labelled in the carboxylate group (1-^{13}C-glycine; Scheme 1); thus, we focus on the carboxylate region of the ^{13}C NMR data. An aqueous solution (1.8 M) of 1-^{13}C-glycine prepared at ambient temperature was flash cooled in a few seconds to *ca*. 145 K inside the NMR spectrometer. The frozen glycine/water solution was then warmed slowly (2 K min^{-1}) and ^{1}H→^{13}C CPMAS NMR spectra were recorded (MAS frequency, 8 kHz[14]) at several (fixed) temperatures during warming (Figure 1). At lower temperatures (e.g., 165 K; Figure 1a), a broad signal centred at 173.9 ppm is observed and is attributed to the glycine/water glass phase. However, on warming to 211 K, a significantly sharper peak appears at 173.5 ppm, with gradual loss of the broad peak due to the glass phase. The position of the sharp peak <u>*does not*</u> match the ^{13}C NMR spectra of the α, β or γ polymorphs of glycine under similar operating conditions (see Figure S1 and Table S1; Supporting Information).

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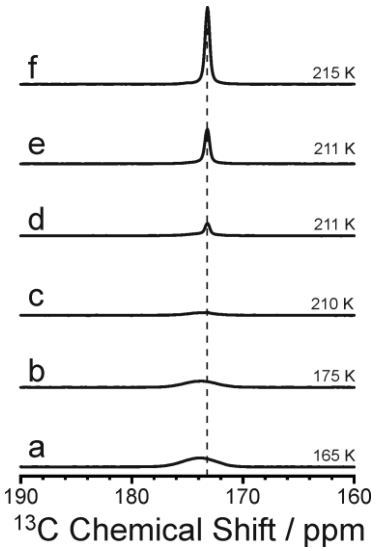

Figure 1: $^{1}H \rightarrow ^{13}C$ CPMAS NMR spectra for an aqueous solution of 1-^{13}C-glycine following flash cooling to 145 K and then slow warming to 215 K. The spectra were recorded at: (a) 165 K, (b) 175 K, (c) 210 K, (d) 211 K (recorded immediately on reaching this temperature), (e) 211 K (recorded after 3 min at this temperature) and (f) 215 K (recorded after 25 min at this temperature).

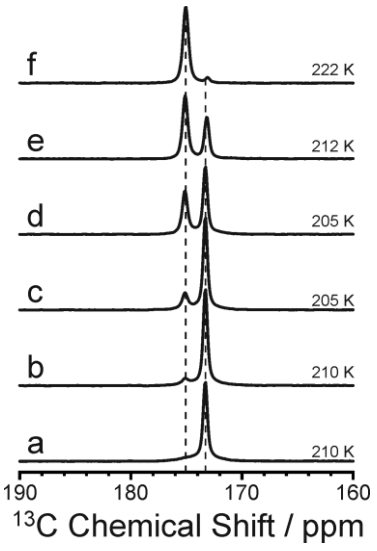

Figure 2: $^{1}H \rightarrow ^{13}C$ CPMAS NMR spectra for an aqueous solution of 1-^{13}C-glycine following flash cooling to 145 K and then slow warming to 210 K. The spectra were recorded according to the following time/temperature sequence: (a) after 150 min at 210 K, (b) after a further 225 min at 210 K, (c) after 360 min at 205 K, (d) after a further 285 min at 205 K, (e) after 50 min at 210 K and then 50 min at 212 K, and (f) after 50 min at 222 K.

Similar behaviour is observed in *in-situ* $^{1}H \rightarrow ^{15}N$ CPMAS NMR spectra for an aqueous solution (1.8 M) of ^{15}N-labelled glycine subjected to the same temperature schedule (Figure S3). The isotropic ^{15}N chemical shift (30.3 ppm) of the crystalline phase that emerges at 211 K is clearly distinct from those of the α, β and γ polymorphs of glycine (Figure S2 and Table S2).[15]

The results from another *in-situ* solid-state ^{13}C NMR study at a different set of temperatures are shown in Figure 2. After flash cooling the aqueous solution of 1-^{13}C-glycine and then warming to 210 K, the sharp peak due to the new crystalline phase again appears at 173.5 ppm (Figure 2a). However, after a total of 375 min at 210 K, a new peak emerges at 175.3 ppm, characteristic of the β polymorph (Figure 2b). The transformation to the β polymorph continues even on reducing the temperature to 205 K (Figure 2c,d) and continues more rapidly at higher temperatures (Figure 2e,f). By the end of the experiment (at 222 K), the transformation to the β polymorph is virtually complete.

The evolution of the solid crystalline phases observed in our *in-situ* solid-state NMR studies mirrors the behaviour reported in the powder XRD study of Xu *et al.*[5] In their work, using the same procedure[11,12] of flash cooling an aqueous solution of glycine below 145 K followed by slow warming to 209 K, a new powder XRD pattern was observed and the crystal structure of the new crystalline phase was determined from the powder XRD data as a dihydrate of glycine (GDH). On further warming of the sample to 250 K, the powder XRD pattern of GDH disappeared and was replaced by that of the β polymorph of glycine.[5,16] Given the similarity between our results and those of Xu *et al.*[5] concerning the evolution of solid phases starting from the glycine/water glass phase, progressing to a new crystalline phase and then to the β polymorph of glycine, we conclude that the new crystalline phase giving the ^{13}C NMR signal at 173.5 ppm and the ^{15}N NMR signal at 30.3 ppm in our work is GDH.

We now consider specific structural aspects of GDH inferred from our *in-situ* NMR studies and results from additional NMR experiments, which further support this assignment.

First, the reported crystal structure of GDH[5] has one molecule of glycine in the asymmetric unit ($Z' = 1$). Our solid-state NMR results are fully consistent with this assignment, as the $^{1}H \rightarrow ^{13}C$ and $^{1}H \rightarrow ^{15}N$ CPMAS NMR spectra of GDH contain only one isotropic peak for each of the CO_2^-, CH_2 and NH_3^+ environments.

Second, a significant feature of the reported crystal structure of GDH[5] is that glycine molecules are hydrogen-bonded only to water molecules, with no direct glycine-glycine hydrogen-bonding. To investigate whether the material assigned as GDH in our *in-situ* NMR study is consistent with this feature, we have carried out 2D ^{1}H-^{13}C and ^{1}H-^{15}N HETCOR experiments at 212 K (Figures 3 and S4, respectively) on this material and the material (assigned as the β polymorph) to which it transforms over time. In the ^{1}H-^{13}C HETCOR spectrum (Figure 3), a strong correlation peak is observed between the CO_2^- group of glycine (^{13}C) and water molecules (^{1}H), consistent with glycine and water molecules in intimate proximity in a crystalline phase. In contrast, for the phase (β polymorph) into which GDH transforms over time, no correlation peak is observed between the carboxylate group of glycine (^{13}C) and water molecules (^{1}H), fully consistent with the assignment that this phase is *not* a hydrate phase of glycine. Together with the other evidence presented above, these observations are fully consistent with the assignment that the initially formed crystalline phase is GDH, which transforms over time to the (anhydrous) β polymorph of glycine. We note that, for the β polymorph, no correlation peak is observed between ^{1}H in the NH_3^+ group and ^{13}C in the CO_2^- group as a consequence of rotational motion of the NH_3^+ group. As discussed in SI, three resonances are observed for the NH_3^+ group in the ^{1}H NMR spectrum of the β polymorph at 200 K, but coalescence occurs in

the temperature regime around 212 K due to rotation of the NH_3^+ group, producing a very broad peak that is not seen in the contour plot in Figure 3. We note that rotational dynamics of NH_3^+ groups has been observed for several amino acids in the solid state,[17] including the α and γ polymorphs of glycine.[17a,17d]

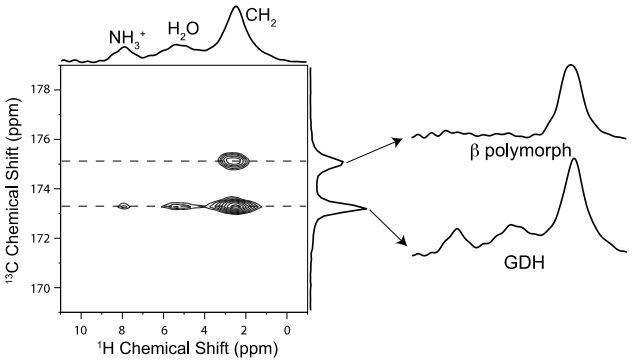

Figure 3: ^{1}H-^{13}C HETCOR spectrum recorded at 212 K after flash cooling an aqueous solution of 1-^{13}C-glycine to 145 K and then slow warming to 212 K. Homonuclear ^{1}H decoupling was applied during t_1 and a mixing time of 0.5 ms was used under Lee-Goldburg conditions. This spectrum was recorded during the *in-situ* study shown in Figure 2 (corresponding to the period of 50 min at which the sample was at 212 K; see caption of Figure 2e). Clearly some transformation of GDH into the β polymorph occurred during measurement of this spectrum (as confirmed from ^{1}H→^{13}C CPMAS NMR spectra recorded before and after the ^{1}H-^{13}C HETCOR experiment). The extracted ^{1}H NMR spectra of GDH and the β polymorph are shown at the right hand side (^{1}H chemical shifts of GDH: 2.5 ppm, CH_2; 8 ppm, NH_3^+; 5 ppm, H_2O).

The corresponding ^{1}H-^{15}N HETCOR spectrum (Figure S4) also shows a correlation peak between the NH_3^+ group of glycine (^{15}N) and water molecules (^{1}H), further supporting our assignment that the initially formed crystalline phase contains glycine and water molecules in intimate proximity and consistent with the assignment of this phase as GDH.

Finally, we show that studies of ^{13}C-^{13}C dipolar coupling[18] allow a more direct structural assignment of the new crystalline phase as GDH. In this work, experimental ^{13}C-^{13}C dipolar build-up curves were recorded using the POST-C7 recoupling pulse sequence,[19] with phase cycling of the reconversion block adjusted to filter zero quantum (ZQF) or double quantum (DQF) signals. The DQF/ZQF intensity ratio provides an experimental dipolar build-up curve that may be compared to the theoretical ^{13}C-^{13}C dipolar build-up curve calculated for a crystal structure using well-established methods.[18,20] Figure 4 shows the experimental ^{13}C-^{13}C dipolar build-up curve for GDH (containing 1-^{13}C-glycine). The dependence of the ^{13}C-^{13}C dipolar build-up curve on the recoupling time τ_{DQ} depends only on the ^{13}C-^{13}C couplings between labelled $^{13}CO_2^-$ sites in this material, which depend on the intermolecular ^{13}C···^{13}C distances between carboxylate groups. The theoretical ^{13}C-^{13}C dipolar build-up curve for GDH, calculated from the intermolecular ^{13}C···^{13}C distances in the reported crystal structure of GDH,[5] is also shown in Figure 4. The agreement between experimental and calculated dipolar build-up curves is excellent, giving strong corroboration that the hydrated crystalline phase of glycine observed in our *in-situ* NMR study is indeed GDH.

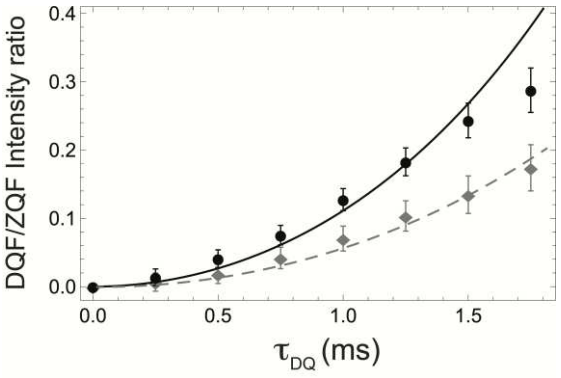

Figure 4: Experimental ^{13}C-^{13}C DQF/ZQF dipolar build-up curves plotted against recoupling time τ_{DQ} for GDH (◆) and the α polymorph (●), in each case containing 1-^{13}C-glycine. Theoretical DQF/ZQF dipolar build-up curves calculated for the crystal structures of GDH (grey dashed line) and the α polymorph (black line) are also shown.

To illustrate that the ^{13}C-^{13}C dipolar build-up curve is sensitive to structural properties, Figure 4 also shows experimental and calculated ^{13}C-^{13}C dipolar build-up curves for 1-^{13}C-glycine in a different material (the α polymorph). Again, good agreement is observed between experimental and calculated data. The fact that the DQF/ZQF curve builds up more slowly for GDH than for the α polymorph is consistent with the intermolecular ^{13}C···^{13}C distances being higher in GDH, as the glycine molecules are separated by intervening water molecules (shortest internuclear ^{13}C···^{13}C distance: α polymorph, 3.10 Å; GDH, 3.81 Å).

Our results demonstrate the advantages of exploiting *in-situ* solid-state NMR techniques to elucidate details of structural evolution during crystallization processes, reporting the first application of such techniques to explore crystallization of an organic material within a glass phase ("frozen solution") formed on flash cooling a liquid solution. Importantly, our results fully corroborate the existence of GDH as a crystalline intermediate on the transformation pathway from the glycine/water glass phase to the β polymorph, and highlight the complementarity between our *in-situ* solid-state NMR strategy[21] and the *in-situ* powder XRD approach that was first used to identify this new solid phase of glycine very recently.[5] Clearly, a major objective is now to establish whether GDH serves any role in mechanistic pathways for the crystallization of glycine from aqueous solution under ambient conditions.

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Keywords: glycine • crystallization • *in-situ* solid-state NMR • polymorphism • structural transformation

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[21] We note that *in-situ* solid-state NMR allows direct observation (in an isotope-specific manner) of the crystalline and amorphous phases present in the system as a function of time, yielding information on specific structural and dynamic aspects of these phases. By focusing on *in-situ* ^{13}C and ^{15}N NMR, our measurements selectively detect only the phases that contain glycine. Thus, our results are not complicated by the fact that pure ice phases may be present in significant amounts during the processes studied here.