Impact of Molecular Speciation on Crystal Nucleation in Polymorphic Systems: The Conundrum of γ Glycine and Molecular 'Self Poisoning'

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Abstract: The polymorphism of the simple amino acid glycine has been known for almost a century. It is also known that in aqueous solutions, at the isoelectric point (p*I* 5.9), the metastable α polymorph crystallizes, while the stable γ form of glycine only nucleates at high and low pH. Despite the importance of understanding the process by which crystals nucleate, the solution and solid-state chemistry underlying this simple observation have never been explored. In this contribution, we have combined solution chemistry, crystallization, and crystallographic data to investigate the mechanisms by which this effect occurs. It is concluded that solution speciation and the consequent interactions between charged species and developing crystal nuclei determine the structural outcome of the crystallization process.

Introduction—Historical Background and the Conundrum

Of the many aspects of the crystallization of materials from solutions, it is our understanding of the molecular phenomena that surround the nucleation event that remains elusive. While the classic equations of Volmer[1,2] and Becker and Doring[3] describe the global behavior of supersaturated solutions, the precise nature of solute assembly and its relationship to the ultimate observed macroscopic crystal structure is not understood.[4] The simple amino acid, glycine, provides an excellent example.

In his compendium of chemical crystallography, Groth[5] lists and describes the phases and morphologies of many hundreds of organic molecules as measured by the goniometric and microscopic techniques that preceded X-ray diffraction studies. At that time, the phenomenon of polymorphism was well-known and yet his 1910 entry for glycine[5] indicates only one known monoclinic, centrosymmetric crystal form, which we now refer to as the α polymorph. The absence of any mention of other glycine polymorphs by Groth is rather surprising since in 1905 Fischer[6] first reported the appearance of the β form which

crystallized from alcohol−water mixtures. In 1931, Bernal[7] reported the first X-ray crystallographic studies of both α and β forms, but it was not until the work of Iitaka[8] in 1954 that the γ form was discovered. Crystals were first identified in commercial samples but then prepared 'after several trials' by recrystallization of α from aqueous solution acidified with acetic acid.[8] By 1958, Iitaka[9] had found that γ could also be crystallized from solutions made basic with ammonium hydroxide and had solved its crystal structure. It was now clear that while α is centrosymmetric, both β and γ are noncentric forms. It was also suggested by Iitaka[9] that 'γ glycine may be a stable form, at least at room temperature'. More recent work has consolidated and refined many of these observations. It turns out[10] that β can be prepared at high supersaturation but is unstable with respect to both α and γ. Recent solubility,[11] thermal[12] and calorimetric data[13] have finally confirmed that at room temperature γ is the most stable form, related enantiotropically to α.

Experimentally, the unusual feature of this system is that crystallization from aqueous solution at the natural isoelectric pH (5.97) always gives the α polymorph and it seems that under these conditions γ never appears, despite its thermodynamic stability. This result has, in the past,[14−16] led to the incorrect assumption that α is the most stable form and gives rise to the

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(1) Volmer, M. *Kinetik der Phasenbildung*; Steinkopf: Dresden, 1939.
(2) Davey, R. J.; Garside, J. *From Molecules to Crystallizers—an Introduction to Crystallisation*; Oxford University Press: Oxford, 2000.
(3) Becker, R.; Doring, W. *Ann. Phys*. **1935**, *24*, 719−752.
(4) Davey, R. J.; Allen, K.; Blagden, N.; Cross, W. I.; Lieberman, H. L.; Quayle, M. J.; Righini, S.; Seton, L.; Tiddy, G. J. T. *Cryst. Eng. Comm*. **2002**, *4*, 257−264.
(5) Groth, P. *Chemische Krystallographie Vol III*; Engelmann: Leipzig, 1910; p 98.
(6) Fischer, E. *Ber. Deut. Chem. Ges*. **1905**, *38*, 2917−2925.

(7) Bernal, J. D. *Z. Krist*. **1931**, *78*, 363−369.
(8) Iitaka, Y. *Proc. Jpn. Acad*. **1954**, *30*, 109−112.
(9) Iitaka, Y. *Acta Crystallogr*. **1961**, *14*, 1−10.
(10) Ferrari, E.; Davey, R. J.; Cross, W. I.; Gillon, A. L.; Towler, C. S. *Cryst. Growth Des*. **2003**, *3*, 53−60.
(11) Sakai, H.; Hosogai, H.; Kawakita, T.; Onuma, K.; Tsukamoto, K. *J. Cryst. Growth* **1992**, *14*, 421−423.
(12) Park, K.; Evans, J. M. B.; Myerson, A. S. *Cryst. Growth Des*. **2003**, *3*, 991−995.
(13) Perlovitch, G. L.; Hansen, L. K.; Bauer-Brandl, A. *J. Therm. Anal. Calorim*. **2001**, *66*, 699−715.
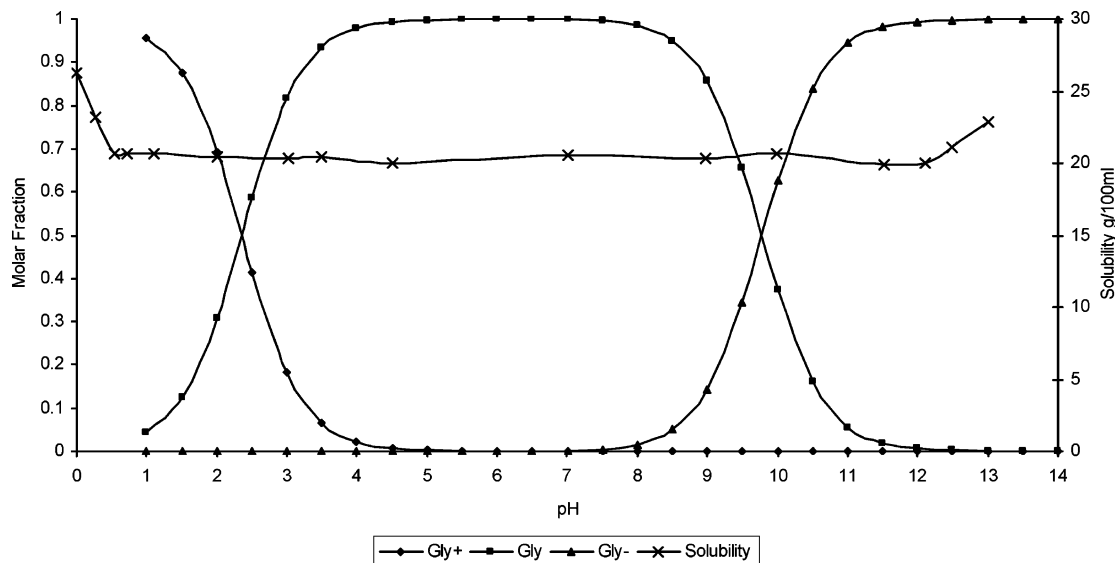
**Figure 1.** Speciation and solubility of aqueous glycine as functions of pH at 20 °C.

conundrum that we explore in this contribution: why should the metastable α form crystallize so easily and how is a change in pH able to induce the appearance of the stable γ form?

To address this issue, we look at the crystal and solution chemistry involved, particularly the way in which pH drives the molecular speciation in solution. We report new experimental data concerning the precise pH domain of the forms and ultimately test our ideas through the use of other charged species and ionic strength as potential nucleation modifiers. Ultimately, we conclude that this appearance of γ results from a 'self-poisoning' mechanism involving charged glycine species. It is important to stress that, while we deal here with the specific case of glycine, this is an example of a more general issue relating to systems in which the crystallizing molecules in solution exist in a different charge state to that in the solid.

A. Crystal and Solution Chemistry. In each of its polymorphic forms glycine molecules pack as zwitterions. In this paper, we are concerned only with α and γ, the former of which is built from centrosymmetric zwitterionic dimers[17] (space group $P2_1/n$) and the latter from polar chains[18] (space group $P3_2$). The known morphology of α[5,19] together with the kinetic data of Li and Rodriguez-Hornedo[20] confirm that the {011} faces are normally the fastest growing. γ crystals grow as trigonal prisms[9] elongated along their polar c-axes.[21,22] One end of the crystal is terminated by carboxylate-rich (00$\bar{1}$) faces and the other by NH_3^+-rich {103} facets. Previous crystal growth studies have shown the former, (00$\bar{1}$)facets to be the faster growing end of the crystal.[21]

The solution phase equilibria between the various forms of the glycine molecule are shown in Scheme 1 and Figure 1

Scheme 1. Ionic Equilibria in Glycine Solutions
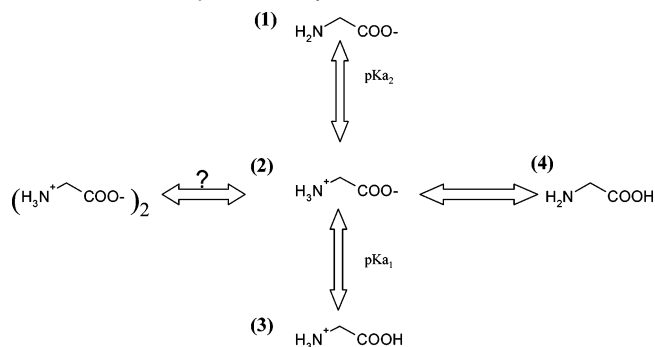
depicts the pH-speciation relationship calculated using known[23] pK values of 2.35 (carboxylic acid) and 9.78 (amine). The isoelectric point of glycine is at pH 5.97. It is expected that in aqueous solution the equilibrium between zwitterions and uncharged molecules (2 ↔ 4 in Scheme 1) lies strongly in favor of the former. Indeed, Darvey[24] has calculated that at the isoelectric point only 4 of every million glycine molecules will be uncharged.

Earlier work suggested, on the basis of solution,[25,26] interfacial and solid-state chemistry[27] that at and around the isoelectric point, glycine is dimerized in solution as centrosymmetric pairs of zwitterions as shown in Scheme 1 (and later in Figure 3). It is then apparent that nucleation from such solutions could lead directly and spontaneously to the metastable α structure.[28]

Experimental

Glycine, malonic acid, ethylenediamine, hydrochloric acid, and all inorganic salts were purchased from Aldrich and used without further purification. Distilled, deionized water was used throughout. Measurements of pH were made using an Accumet Basic AB15 pH meter fitted with an Accumet glass calomel pH electrode and an ATC probe for

(14) Zaccara, J.; Matic, J.; Myerson, A. S.; Garetz, B. A. *Cryst. Growth Des.* **2001**, *1*, 5−8.
(15) Weissbuch, I.; Addadi, L.; Berkovitch-Yellin, Z.; Gati, E.; Weinstein, S.; Lahav, L.; Leiserowitz, L. *J. Am. Chem. Soc.* **1983**, *105*, 6615−6621.
(16) Freeman, C. M.; Andzelm, J. W.; Ewig, C. S.; Hill, J.-R.; Delley, B. *Chem. Commun.* **1998**, 2455−2456.
(17) Marsh, R. E. *Acta Cryst.* **1959**, *11*, 654−663.
(18) Iitaka, Y. *Acta Cryst.* **1961**, *14*, 1−10.
(19) Berkovitch-Yellin, Z. *J. Am. Chem. Soc.* **1985**, *107*, 8239−8253.
(20) Li, L.; Rodriguez-Hornedo, N. *J. Cryst Growth* **1992**, *12*, 33−38.
(21) Shimon, L. J. W.; Wireko, F. C.; Wolf, J.; Addadi, L.; Berkovitch-Yellin, Z.; Lahav, M.; Leiserowitz, L. *Mol. Cryst. Liq. Cryst.* **1986**, *137*, 67−86.
(22) Weissbuch, I.; Popovitz-Biro, R.; Leiserowitz, L.; Lahav, M. *The Lock and Key Principle*; Behr, J. P., Ed.; John Wiley and Sons: Chichester, 1994, Chapter 6; pp 222−226.

(23) Christensen, J. J.; Oscarson, J. L.; Izatt, R. M. *J. Am. Chem. Soc.* **1968**, *90*, 5949−5953.
(24) Darvey, I. G. *Bio. Edu.* **1995**, *23*, 141−143.
(25) Lewis, W. C. M. *Chem. Rev.* **1931**, *8*, 81−165.
(26) Myerson, A. S.; Lo, P. Y. *J. Cryst. Growth* **1991**, *110*, 26−33.
(27) Gidalevitz, G.; Feidenhans'l, R.; Matlis, S.; Smilgies, D.-M.; Christensen, M. J.; Leiserowitz, L. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 955−959.
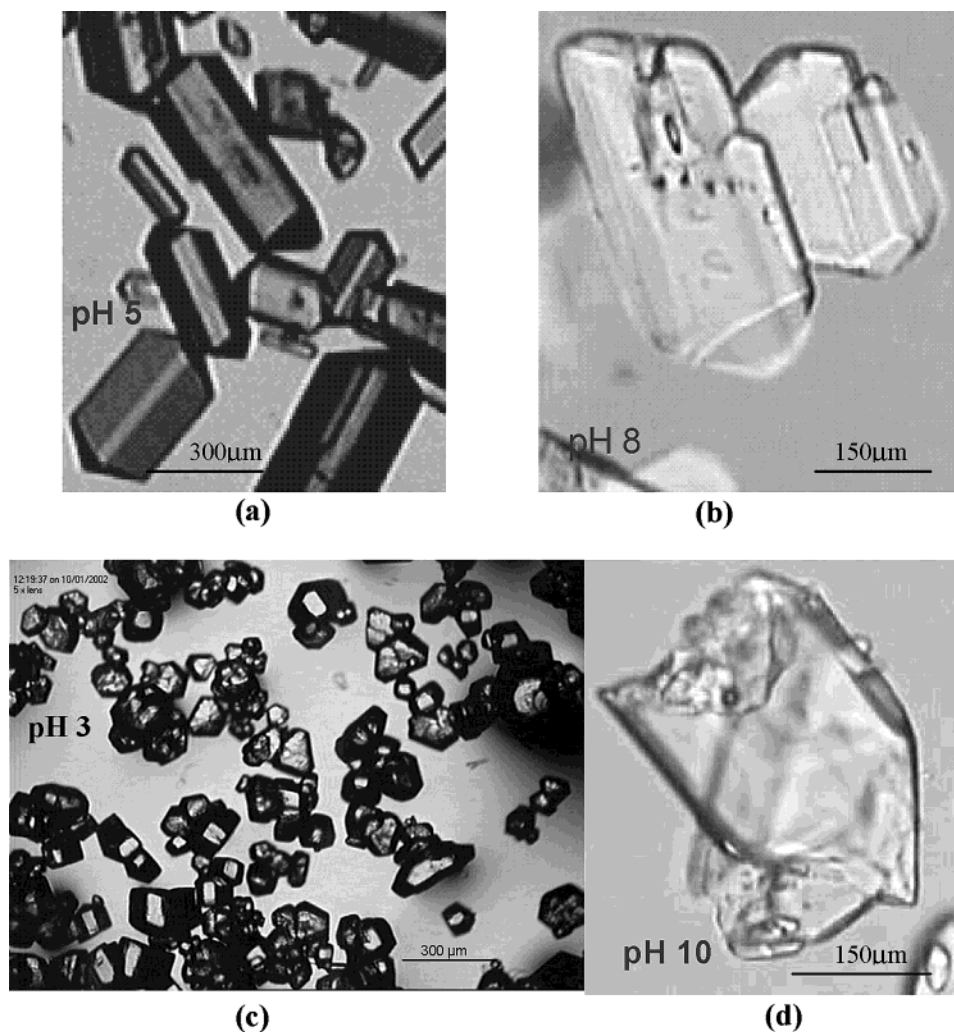(28) Weissbuch, I.; Lahav, M.; Leiserowitz, L. *Cryst. Growth Des.* **2003**, *3*, 125−150.

Figure 2. Glycine crystallized over the pH range 3−10 at 20 °C: (a) α crystallized at pH 5; (b) α crystallized at pH 8; (c) γ crystallized at pH 3 (electron micrograph); (d) γ crystallized at pH 10.

temperature compensation. Crystallization experiments were performed in glass, agitated, 50 mL, thermostated vessels. Crystals were examined by optical microscopy using a Zeiss Axioplan 2 polarizing microscope and XRPD patterns of the powders were collected using a Bruker D8 Advance Diffractometer over the 2θ range 10 to 40° (step size 0.03, step time 0.6s).

To investigate the effect of pH and kinetics, solutions having supersaturations of 0.2 and 0.66 (calculated as $\ln(c/c_s)$ with concentrations, c, in g/L and with c_s the solubility of α^{29}) were prepared by dissolving glycine in water at elevated temperatures and cooling to 20 °C. The solubility of α glycine in aqueous solution at this temperature is 204.1 g/L, and the two supersaturations correspond to concentrations of 249.0 g/L and 398.2 g/L. To identify the pH values at which α and γ nucleated, crystallizations were performed at pHs in the range 1 to 11 (by adding appropriate amounts of HCl and NaOH). Additional precision was obtained in the range 3−4 and 8−9 by changing the pH in steps of approximately 0.25 units. For experiments with additives (malonic acid, ethylenediamine and ionic salts) an intermediate supersaturation of 0.53 ($c = 350$ g/L) was employed.

Results and Discussion

A. Impact of pH. Crystallization of glycine solutions in the pH range 3.8 to 8.9, always produced the α polymorph. This confirms and adds further precision to the spray drying study

of Yu and Ng.[30] Figure 2 a and b shows examples of α crystals grown in this pH range. They have the expected prismatic morphology, in which the {011} faces are clearly the fastest growing. In solutions where the pH was adjusted to below 3.8 or above 8.9, the γ polymorph always nucleated. As seen in Figure 2, parts c and d, its polar morphology is as reported previously[9,21,22] with the flat, fast growing, carboxylate-rich ends easily identifiable. Mixtures of polymorphic forms were never seen in these experiments and these results were independent of the two supersaturations chosen. It was also noted that while the α morphology remains essentially independent of pH, the c-axis growth of the γ crystals appears to be inhibited at low pH yielding more equant crystals than at high pH (Figure 2).

Overall, these data define the precise speciation conditions corresponding to the nucleation of the two forms. Using the data of Figure 1, together with the measured pH values, it is evident that this polymorph switch is associated with a mole fraction of 0.03 (of total glycine) of cationic or 0.12 of anionic glycine. We hesitate to read too much into this difference—for pHs in the range 8.5 to 8.9 the anion mole fraction depends sensitively on pH rising from 0.05 to 0.12. On average, however

(29) Carta, R.; Tola, G. J. *Chem. Eng. Data* **1996**, *41*, 414−417.
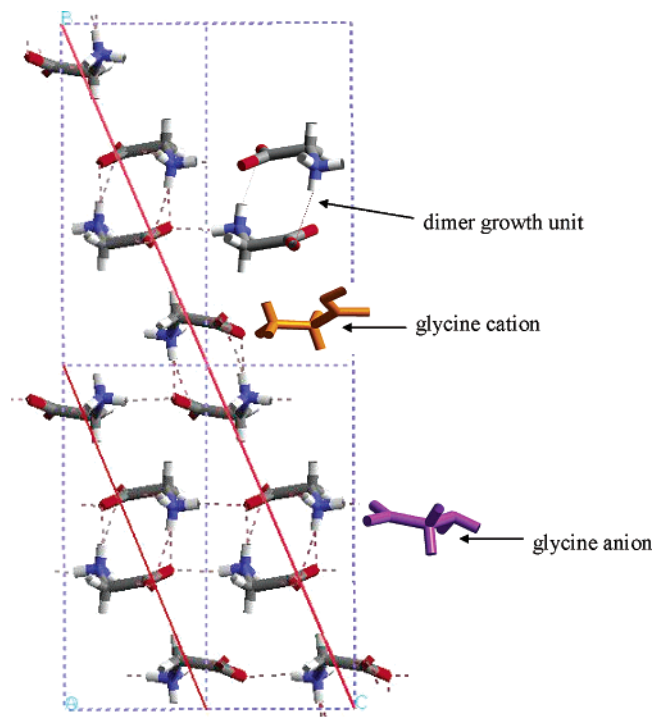(30) Yu, L.; Ng, K. J. *Pharm. Sci.* **2002**, *91*, 2367−2375.

Figure 3. Visualization of the fast growing {011} faces of α glycine: showing growth via dimers and inhibition via charged species.

it would appear that a relatively minor change in speciation of ca. 7 wt % charged species (on glycine) is needed to induce crystallization of the γ form. Further, the independence of these results on supersaturation confirms that they are indeed related to speciation and not the result of other factors (e.g., interfacial tension, solubility) influencing the relative nucleation kinetics of the forms.

B. Resolving the Conundrum. Three possible explanations for these observations are considered. In the first, we examine the possibility that the change in pH increases the barrier to nucleation of the α polymorph thereby allowing γ to form. The second explanation examines the possibility that the charged glycine species are capable of self-inhibition of the growth of the α form since this would also permit γ to crystallize. Finally, it may be that rather than the crystallization of α being disrupted that the nucleation of the γ form is catalyzed.

1. Disrupting the Nucleation of the α Form. As discussed above, it has been previously concluded that the facile nucleation and apparent 'stability' of the metastable α form at pHs close to the isoelectric point is a reflection of the presence of centrosymmetric dimer 'growth units' in solutions. The simple corollary of this is that, because singly charged glycine molecules will not form cyclic dimers, the effect of moving the pH away from the isoelectric point is to reduce the proportion of this α form 'growth unit'. This would then increase the proportion of monomeric zwitterions available to form the polar chain structure of the γ polymorph. Our new data allow us to see that this is a highly unlikely scenario since at pHs associated with the nucleation of γ there is a relatively small concentration of charged glycine in solution. Indeed, the associated decrease (assuming all zwitterions to exist as dimers) in the number of moles of dimer pairs is very modest ~7%. Since this represents a constant proportion in both saturated and supersaturated solutions it has no impact on the driving force
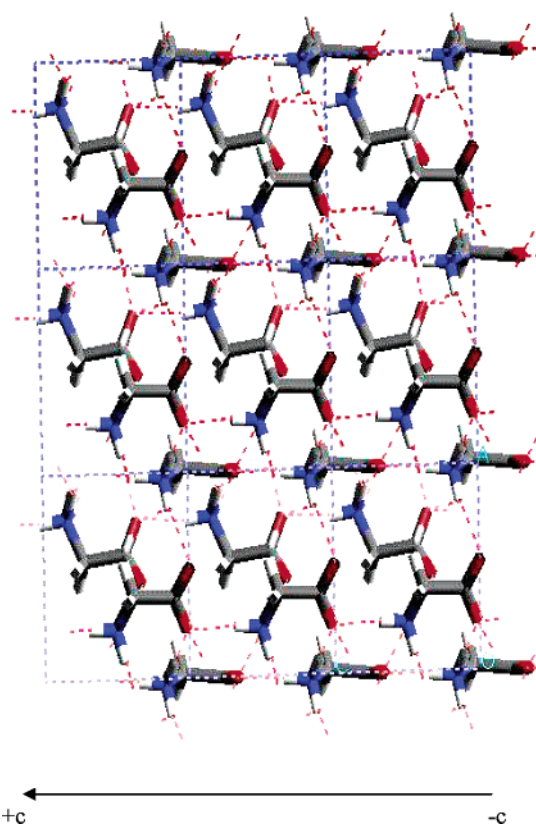
Figure 4. Visualization of the γ glycine structure showing the polar *c*-axis, the slow growing NH_3^+ rich, pointed, end ($+c$) and the fast growing COO^- end ($-c$).

for nucleation. Overall then, these quantitative data do not support this mechanism and do not explain the observations.

2. Inhibiting the Growth of the α Form. The potential for charged species to inhibit the growth of the α polymorph can be explored by considering its crystal morphology. The fast growing {011} faces, shown in Figure 3, expose both protonated amine and carboxylate functionality at this surface and their fast growth is driven by the strong coulombic interactions within the (010) layer. When either deprotonated amino or protonated acid species are present in solution they may join the surface and in doing so modify the charged nature of the interface preventing the further addition of zwitterions as seen in Figure 3. (In the construction of Figure 3 the conformation of these charged species have been minimized in Cerius2 using MOPAC). These ions would thus act as selective 'tailor-made' additives to inhibit the crystallization of α. At the same time they could not totally inhibit the appearance of the more stable γ form since, as seen in Figure 4, its major growth (*c*) axis accepts NH_3^+ functionality at its faster growing, carboxylate rich, end and carboxylate functionality at its slower growing, amino rich, end. Thus, the fast growing $-c$ direction will be inhibited at low pH and the slow growing $+c$ at high pH as is indeed observed in Figure 2. Overall however, this means that at least one end of the *c*-axis is always available for growth and hence that the crystallization of γ cannot be completely inhibited by these charged species. This impact of crystallographic and stereochemical features on crystallization of centrosymmetric versus noncentric structures has been success-
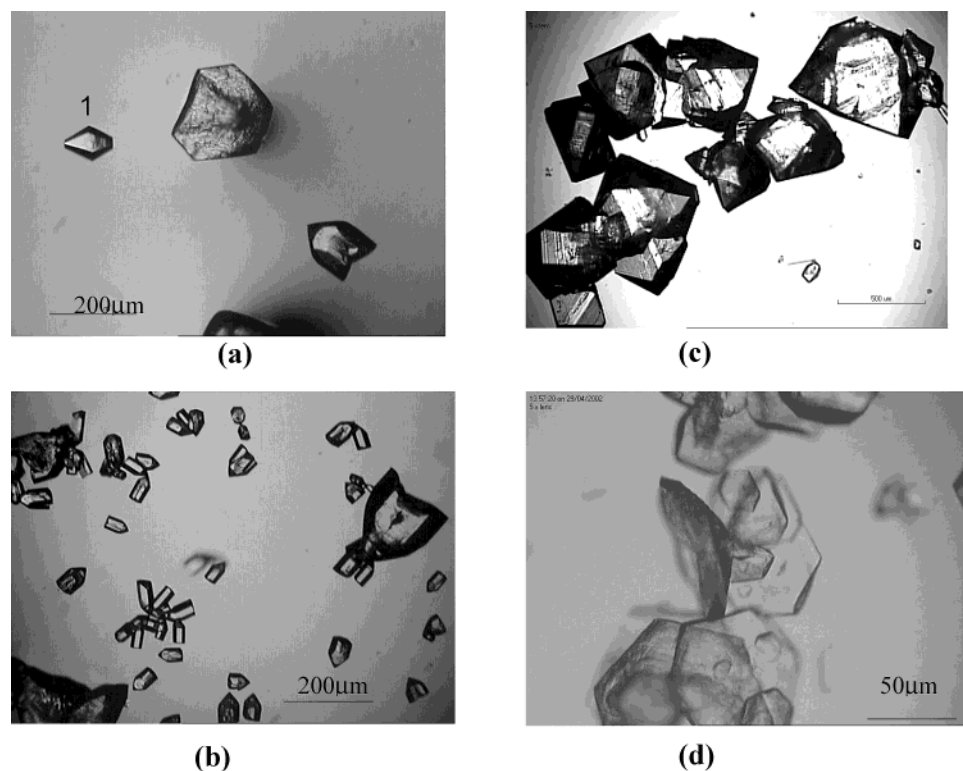
Figure 5. Impact of malonic acid and ethylenediamine on the appearance and morphology of α and γ glycine: (a)1 wt % malonic acid pH 5.02; (b) 2 wt % malonic acid pH 4.06; (c) 0.1% ethylenediamine pH 8; (d) 10% ethylenediamine.

fully used previously for the selection of additives to induce the nucleation of a specific polymorphic form.[22,31]

A consequence, and hence a test of this mechanism, is that as the concentration of charged species increases so the growth rate of the {011} faces should decrease and the morphology of the α crystals should change, with the {011} faces becoming of increasing morphological importance.[2] Unfortunately, it was not possible to obtain α crystals under conditions of high concentrations of charged species to see their effect on the morphology of α—at pHs 4 and 8, the practical limit of the α domain, no morphological change was seen. This is not too surprising since at these pHs only 2wt % (on glycine) of the charged species are actually present. To circumvent this problem we performed some additional crystallization experiments but with added malonic acid (HOOC−CH₂−COOH). This molecule was chosen for two reasons: first due to its molecular similarity to glycine it is likely to act as a tailor-made growth inhibitor, second, its pK_a values are 2.85 and 5.7.[32] This means that in the pH region 4−5, where glycine is zwitterionic in solution, malonic acid exists predominantly as a negatively charged, monodeprotonated, species. In this way we had hoped to use malonic acid as a mimic for anionic glycine species. The results of these experiments are shown in Figure 5. At malonic acid levels as low as 0.1 and 1.0 wt % on glycine (pHs 5.02 and 4.38 respectively) γ and α glycine appear concomitantly (Figure 5a). In addition there is some indication from these micrographs that the morphology of α glycine is modified to give more isometric crystals with large {011} facets (see eg Figure 5a, crystal 1 and compare to Figure 2a). When 2 wt % malonic

acid has been added (pH = 4) only γ nucleates (Figure 5b), mirroring exactly the behavior in pure solutions at pH 3.8 where the singly protonated glycine species has the same effect. In a related set of experiments, ethylenediamine (pKs 6.86 and 9.92[32]) was used as a mimic for the cationic form of glycine. At a concentration of 0.1% ethylenediamine on glycine (pH = 8) the proportion of monoprotonated amine is close to 100% and α crystallized. The resulting crystals had very large {011} facets (Figure 5c), an even clearer indication that charged species can inhibit their growth than was seen with malonic acid. At a higher concentration of 2 wt % (pH 9.14) the proportion of the monoprotonated species is about 80% and only γ glycine crystallized. At higher levels of ethylenediamine (10 wt %), as indeed at low pHs, the expected inhibition of the (00$\bar{1}$)face of the γ crystals occurred yielding the plates seen in Figure 5d. It is noted that these results are completely consistent with the experimental data of Weissbuch et al.[33] who showed that 1% of racemic hexafluorovaline gives modified α while 3% gives γ.

Thus, these experiments confirm the impact of pH on the relative nucleation of α and γ glycine and in addition they provide strong evidence for selective inhibition of the crystallization of α by charged species. Of course, while the effects of malonic acid and ethylenediamine may be attributed to their differing molecular structure when compared to glycine this is not the case with charged glycine species. These differ from the crystallizing zwitterions only by the presence or absence of a proton. Indeed, it seems possible that the surface exposed −NH₂ or −COOH groups of individual molecules could be transformed, by proton transfer, to zwitterions (the enthalpy

(31) Weissbuch, I.; Addadi, L.; Lahav, M.; Leiserowitz, L. *Science* **1991**, *253*, 637−645.
(32) *CRC Handbook of Chemistry and Physics*, 81st ed.; CRC Press LLC, 2000.
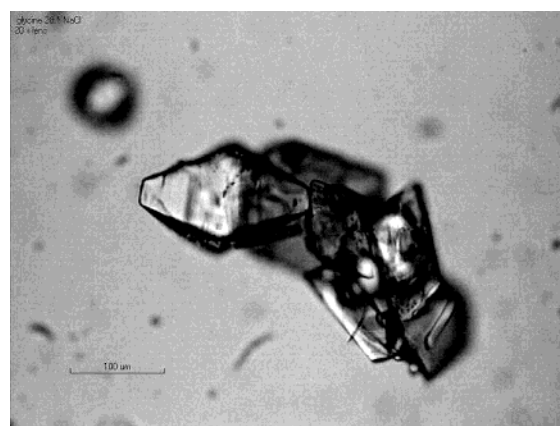(33) Weissbuch, I.; Leiserowitz, L.; Lahav, M. *Adv. Mat.* **1994**, *6*, 953−966.

changes for these processes have been estimated to lie between 1 and 10 kcal/mol[34]) and hence built into the crystal. However, it is also true that the dynamic equilibrium between solution and surface will maintain a fixed proportion of surface molecules that are protonated or deprotonated, depending on pH.
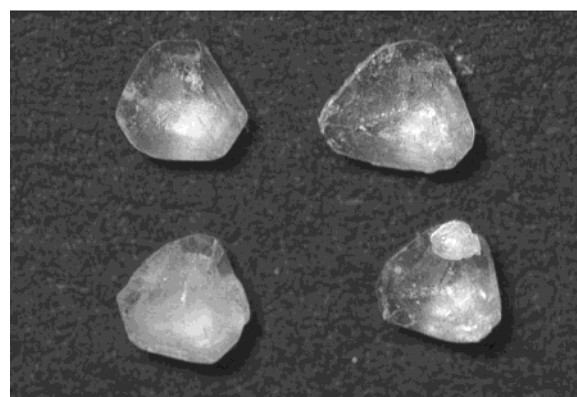
3. Induced Nucleation of the γ Form. Finally, we consider the possibility that charged glycine species stabilize or induce γ nucleation. There are two ways in which this might occur. First, the charged species may form molecular aggregates which induce the necessary polar packing of glycine molecules and stabilize the γ form. In previous work,[35] we have shown how interfaces rich in the cationic surfactant, AOT (sodium bis(2-ethyl-hexly)sulfosuccinate) can induce crystallization of the γ structure. For AOT, this is a reasonable result, since it is a surfactant known to adsorb strongly at the oil or air/water interface. For the small charged, soluble glycine molecule in aqueous solution, however, the necessary formation and segregation of stable molecular aggregates of this type seems unlikely and we dismiss this mechanism.

Second, it is important to recognize that γ crystals express a polar morphology (see Figure 4) in which the pointed end of the *c*-axis exposes protonated amino groups and is hence, positively charged while the flat end exposes carboxylate functionality and is hence negatively charged. Such charged surfaces do not exist for α—all surfaces are neutral combinations of carboxylate and protonated amino- and it may be this additional surface energy penalty that makes the γ structure so reluctant to nucleate. The role of charged species in the solution might then be to enable an electrical double layer to form hence neutralizing the surface charge and stabilizing the γ nuclei. In this case, it is anticipated that the nature of the added charged species should be immaterial, and it should only be necessary to increase the ionic strength in order to stabilize γ. Indeed, it has recently been reported that the γ form can be crystallized from concentrated sodium chloride solutions.[36,37] Accordingly, Mg(NO$_3$)$_2$, MgSO$_4$, Ca(NO$_3$)$_2$, Na$_2$SO$_4$, Na$_2$CO$_3$, and NaCl were added individually to supersaturated glycine solutions to give in each case an ionic strength of 5.17. It is known that addition of NaCl[38] has no effect on the solubility of glycine, and the value 5.17 was taken as this was the ionic strength which, according to Bhat and Dharmaprakash,[37] gave the best conditions for producing γ glycine.

It was found that when calcium or magnesium salts were used to provide the counterions only α crystallized. Those solutions containing sodium ions, on the other hand, gave either all γ (NaCl and Na$_2$CO$_3$) or mixtures of γ and α (Na$_2$SO$_4$). This result leads us to conclude that ionic strength and consequent double layer formation alone are not sufficient for the nucleation of γ. Thus, the stabilization of charged surfaces of γ appears not to be an issue in its nucleation versus that of a. This may well be due to the screening effect of bound water molecules at the −*c* end of the γ crystals (in a manner similar to that described for (R,S)-alanine[22]) and the precise high index termination at the

(34) Takagi, S.; Chihara, H.; Seki, S. *Bull. Chem. Soc. Jap.* **1959**, *32*, 84−88.
(35) Allen, K.; Davey, R. J.; Ferrari, E.; Towler, C.; Tiddy, G. J.; Jones, M. O.; Pritchard, R. G. *Cryst. Growth Des.* **2002**, *2*, 523−527.
(36) Bhat, M. N.; Dharmaprakash, S. M. *J. Cryst. Growth* **2002**, *242*, 245−252.
(37) Bhat, M. N.; Dharmaprakash, S. M. *J. Cryst. Growth* **2002**, *236*, 376−380.
(38) Carta, R. *J. Chem. Thermodyn.* **1998**, *30*, 379−387.

(a)

(b)

Figure 6. Glycine crystals grown in the presence of NaCl: (a) α in 5% NaCl, pH 6 and (b) γ in 14% NaCl (crystals ca. 1 mm in size).

+*c* end which may enable carboxlyate groups to be exposed thereby reducing the overall surface energy.

These data do however, indicate a specific interaction of Na$^+$ with the α form which, like the charged molecular species, evidently inhibits its crystallization. At low NaCl levels, (Gly: Na greater than 10:1), mixtures of the α and γ polymorphs were crystallized and as seen in Figure 6a these α crystals are of modified morphology with large {011} facets similar to those grown in the presence of malonic acid and ethylenediamine. This is again consistent with crystallization inhibition which presumably occurs as a result of association of the sodium ion with the carboxylate group of the glycine zwitterions which are exposed on all four fast growing {011} faces of the α polymorph. In the case of γ crystals, however, Na$^+$ can only interact with the carboxylate rich −*c* direction and hence not fully inhibit its growth. Again, as with ethylenediamine, at higher levels of sodium chloride (glycine: NaCl 2:1) this inhibition of the (00$\bar{1}$) faces by binding of Na$^+$ is revealed by the platelike morphology of the γ crystals seen in Figure 6b.

This observation raises the question of the use of NaOH to control pH—are the observed effects due to pH or Na$^+$? In fact, to achieve a pH of 8.9, at which γ appears, needs 3.4 wt % NaOH, while to induce γ with NaCl at pH 6 requires 14 wt % NaCl. Thus, the impact of Na$^+$ and pH are separate phenomena, a conclusion supported by the impact of the added molecular

species, malonic acid and ethylenediamine and the previous studies[8,9] that used ammonium hydroxide and acetic acid as pH modifiers.

Conclusions

Of the three mechanisms discussed above, it is clear that the only one which gives a consistent interpretation of the data, and hence explanation of the conundrum, involves the action of charged glycine species as self-poisoning 'impurities' which selectively inhibit the nucleation and crystal growth of α. We thus have an overall picture in which around the isoelectric point the highly dimerized, zwitterionic glycine solution enables facile nucleation of the α structure. However, when the pH is changed, increasing concentrations of charged species are present which adopt the role of 'self-poisoning' impurities and inhibit the crystallization of this form. Under these conditions, the solutions are free to desupersaturate and equilibrate via the nucleation and growth of the more stable, but kinetically less favorable γ

polymorph. Two general observations can then be made. First, the above confrontation of data with theory assumes that the critical-sized molecular clusters present in solution are themselves polymorphic, having packing characteristics that mirror those of mature crystals. This assumption has proved to be useful in understanding nucleation in a range of other systems[4,31] and while this in itself does not justify the assumption its success here does add further weight to validity of the hypothesis. Second, in other systems, particularly those involving the crystallization of organic salt forms, similar issues are likely to be important with, for example, the pH and solvent choice being key variables in determining the polymorphic outcome of a form screen.