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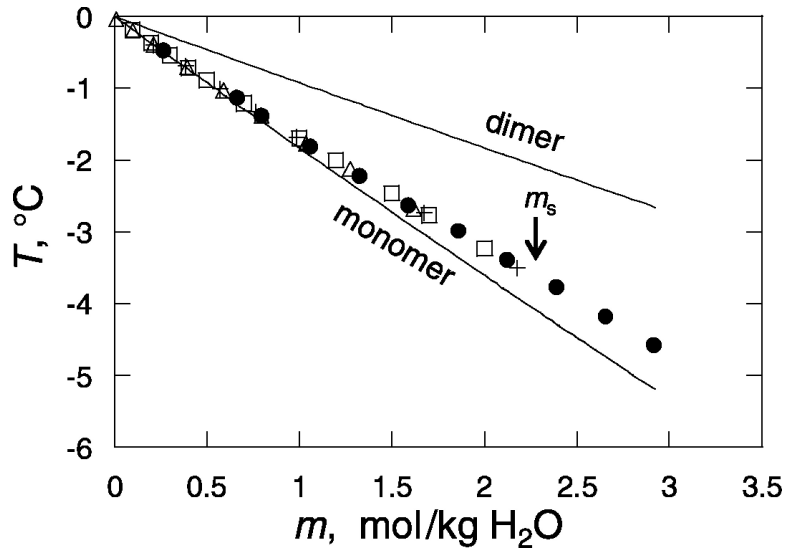

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Glycine Exists Mainly as Monomers, Not Dimers, in Supersaturated Aqueous Solutions: Implications for Understanding Its Crystallization and Polymorphism

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Abstract: Glycine, the simplest amino acid, is described as existing as hydrogen-bonded cyclic dimers in supersaturated aqueous solutions and, as a result, crystallizing in a centrosymmetric polymorph (polymorph α) for which the dimer can be viewed as the building unit, in favor of other polymorphs of polar structures. In exhibiting this relation between polymorphic selectivity and self-association in solution, glycine is thought to illustrate a general principle. We measured the freezing-point depression of glycine−water up to 30% supersaturation and found that glycine exists mainly as monomers, not dimers, and that the dimer stability constant K_D is smaller than 0.1 kg of H_2O/mol if the observed osmotic abnormality is attributed to dimerization. We also revisited a report cited as evidence for glycine dimerization: the slowdown of glycine diffusion with solution age. Pulsed gradient spin−echo NMR spectroscopy was used in place of the previous method of Gouy interferometry to avoid perturbations to sloution structure caused by the interdiffusion between two solutions of different concentrations. No aging effect was observed on glycine diffusion up to 24% supersaturation after five days. The solute size calculated from diffusivity, viscosity, and the Stokes−Einstein relation showed no increase with concentration or solution age. We conclude that glycine exists in supersaturated aqueous solutions mainly as monomers, not dimers, and remains so upon aging. This result does not invalidate the theories of how pH and additives affect glycine's polymorphic preference, because they begin with the assumption that α glycine is the preferred polymorph under normal conditions, but requires a new explanation for that assumption itself.

Introduction

From a liquid able to crystallize in multiple polymorphs (crystalline phases of the same composition but different molecular packing and/or conformation), which polymorph is favored to crystallize remains poorly understood.[1−3] It is still unpredictable whether one polymorph will nucleate or grow faster than another from the same liquid, even with the knowledge of their structures and thermodynamic relations. This lack of understanding is made clearer by experimental observations that polymorphs can grow from the same liquid at rates orders of magnitude different.[4,5] Better understanding of crystallization in polymorphic systems is essential for controlling the phenomenon in the manufacture of pharmaceuticals, pigments, explosives, and other materials.[6]

Glycine, the simplest amino acid, is a model system for studying the polymorphic selectivity of crystallization. An observation of special interest is the selective crystallization of α glycine from water in favor of two other polymorphs (β and γ) known to exist under ambient conditions.[7] Chew et al.[5] reported that α glycine grows 500 times faster than γ glycine from water at 10% supersaturation; Lee et al.[8] reported that α glycine probably nucleates many times faster than γ and β glycine in aqueous solutions. The three polymorphs differ from each other in that α glycine is centrosymmetric and the other two are polar. α Glycine contains hydrogen-bonded molecular bilayers related by inversion, γ glycine contains helical chains of molecules connected head-to-tail by hydrogen bonds, and β glycine contains hydrogen-bonded molecular sheets similar to those in α glycine but in polar organization. The three polymorphs all contain glycine molecules in the zwitterion form $^{+}H_3NCH_2COO^{-}$, the same species prevalent in aqueous solutions, and their thermodynamic stabilities follow the order γ > α > β under ambient conditions.[7,9,10] The selective crystallization of α glycine from water is further appreciated in reference to the special conditions under which other polymorphs crystallize: γ glycine crystallizes on changing the solution pH,[7] addition

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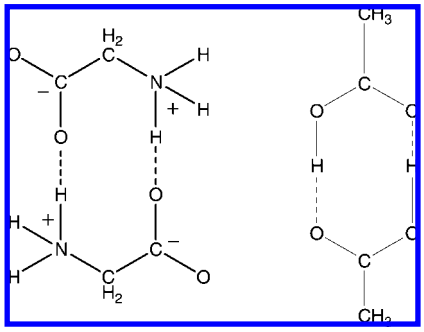

Figure 1. Hydrogen-bonded cyclic dimers of glycine and acetic acid.

of "tailor-made" additives,[11] changing the solvent from H_2O to D_2O,[12] and under other conditions;[13] β glycine crystallizes from alcohol−water.[14]

The standard explanation for the selective crystallization of α glycine from water is that in supersaturated aqueous solutions, glycine exists mainly as hydrogen-bonded cyclic dimers, similar to the structural unit of α glycine (Figure 1), but unlike the structural units of other polymorphs.[11,14−17] This view has been invoked to explain why crystallization in alcohol−water yields β glycine,[14] and how solution additives[11] and pH change[18] affect the polymorphic selectivity of glycine crystallization.

Establishing whether the cyclic dimer of glycine is a predominant solution species is important not only for understanding the crystallization of glycine but also for evaluating a general principle of polymorphic selectivity, namely, the crystallization of a particular structure is linked to the existence of molecular aggregates in the liquid that resemble the crystallizing structure.[11,19] There is considerable current interest in testing this principle.[20,21] Elucidating the self-association of glycine in aqueous media is also of interest for understanding the aggregation of proteins.[22]

Two types of arguments are currently made to justify the existence of the cyclic dimers of glycine as a predominant species in supersaturated aqueous solutions. The first is based on observations of crystal growth: atomic force microscopy shows that the smallest growth step of α glycine has the height of two molecular layers;[23,24] grazing-incidence X-ray diffraction shows that the surface of a growing α glycine crystal is terminated above or below a hydrogen-bonded bilayer, exposing no "open" hydrogen bonds.[24] These studies, however, do not reveal how glycine self-associates *in solution*. The specific crystal/liquid interface observed may also stem from the fact that the alternative structures have higher energies and lower probabilities of being observed at the time scale of measurement.

The second type of argument for the dimerization of glycine builds on reports of the time dependence of certain physical properties of supersaturated (not undersaturated) glycine solutions. Myerson and co-workers reported that the diffusion coefficient of supersaturated solutions of glycine in water decreases with solution age.[25−27] They argue that this decrease occurs because "the diffusion coefficient is zero at the spinodal curve (metastable limit)" and "therefore, must decline from a finite value at saturation to zero at the spinodal".[26] Myerson and co-workers reported that concentration gradients develop in long columns of supersaturated (not undersaturated) glycine solutions, which was taken as an indication of cluster formation.[26] Recently, they reported that the radius of gyration of glycine increases in supersaturated solution before crystallization.[16,17] These observations are remarkable in that most physical properties undergo no abrupt changes on going from undersaturated to supersaturated solutions, or for pure liquids, from above to below the melting points. The report of slowing glycine diffusion in supersaturated aqueous solutions was revisited in this study with a technique deemed better suited for studying this property.

The notion of extensive dimerization of glycine in water is surprising in reference to known properties of the hydrogen-bonded dimers of acetic acid (Figure 1) and other carboxylic acids.[28,29] Though stable in organic solvents with the stability constant $K_D \approx 10^2$ to 10^3 kg of solvent/mol (K_D = [dimer]/[monomer]^2), acetic acid dimers are substantially less stable in water with $K_D \approx 0.05$ kg of H_2O/mol.[28] This decrease of dimer stability results from the strong hydrogen-bonding interactions between water and acetic acid. If the glycine dimer is the predominant species in water, its stability must be substantially higher than the stability of the acetic acid dimer.

This speculation might seem plausible given glycine's zwitterionic structure, which could lead to stronger bonding between monomers. The argument, however, fails to consider the stronger solvent−solute (water−glycine) interactions, which tend to reduce the degree of solute association. Indeed, existing data of colligative properties[22,30−35] suggest that glycine and acetic acid have comparable degrees of self-association at moderate

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concentrations (below glycine's solubility). If the observed osmotic abnormality is attributed to dimerization, the majority of glycine molecules exist as monomers in undersaturated solutions. The notion that a supersaturated glycine solution contains predominantly dimers therefore requires an abrupt increase of the dimer stability when the solution becomes supersaturated. This possibility was tested here by extending the freezing-point depression measurement beyond saturation.

The existence of the cyclic dimer of glycine as the predominant species in aqueous solutions was recently questioned on the ground of molecular dynamics simulations.[12,36] These studies observed small, short-lived clusters ("catemers") of glycine held mainly by "open" hydrogen bonds NH···OC, but observed the cyclic dimers only infrequently.

To determine the state of self-association of glycine in supersaturated aqueous solutions, we measured for the first time the freezing-point depression of water by glycine and the self-diffusion coefficient of glycine as a function of solution age in supersaturated solutions. Self (or intra) diffusion was measured with pulsed gradient spin−echo (PGSE) nuclear magnetic resonance (NMR) spectroscopy,[37] a method shown to be an accurate and precise alternative to the classic isotopic tracer method for measuring self-diffusion.[38] Myerson and Lo used Gouy interferometry to measure the mutual (or inter) diffusion between two glycine−water solutions of different concentrations.[25,26] While both diffusion coefficients (self and mutual)[39] are useful for characterizing solute association,[40,41] the PGSE NMR method has the merit of involving no solvent flow driven by concentration gradient and is better suited for detecting possible aging effects on glycine diffusion in quiescent solutions. Hughes et al. used PGSE NMR to measure the self-diffusion of glycine in water with the goal of assessing solute association.[12] Their study differs from ours in two respects: (1) they did not determine whether the diffusion of glycine slows with solution age in supersaturated solutions and (2) they measured deuterated glycine $^{+}D_3NCH_2COO^{-}$ diffusing in D_2O solutions, whereas we measured the normal isotopic species of glycine (mainly $^{+}H_3NCH_2COO^{-}$) diffusing in the normal isotopic species of water (mainly H_2O). Given their surprising observation that the selective crystallization of α glycine occurs in H_2O, but not D_2O,[12] it is unreasonable to assume the solution chemistry of glycine is identical in the two solvents and it is of interest to determine glycine's diffusion coefficient not only in D_2O, but also in H_2O.

Our freezing-point depression data indicate that glycine exists mainly as monomers, not dimers, in supersaturated solutions. Our PGSE NMR data show no evidence of slowing glycine diffusion with solution age in aqueous supersaturated solutions. This study does not support the notion that glycine exists mainly as hydrogen-bonded cyclic dimers in supersaturated solutions and suggests that new interpretations are needed for the polymorphic selectivity of glycine crystallization.

Experimental Section

Materials and Solutions. Glycine (α polymorph) was obtained from Mallinckrodt. H_2O was deionized. Glycine solutions of various concentrations were made by weighing their components. Supersaturated solutions were prepared by dissolving the solute at 40 °C and cooling to room temperature. The solutions thus made were used directly for freezing-point depression measurement but filtered before being used for diffusion measurements. Filters and syringes were preheated to 40 °C for filtration of supersaturated solutions to prevent crystallization during the process.

Freezing-Point Depression. Freezing-point depression was measured with an Advanced Instruments osmometer model 3250. The instrument was checked with a Clinitrol 290 reference solution every time before use. Approximately 0.25 mL of solution was used for each measurement. Freezing-point depression was calculated from the reported osmolality as ΔT = (osmolality) (1.858 °C).

Diffusion Measurement by PGSE NMR. NMR experiments were performed on a Varian UNITY-INOVA spectrometer at 11.74 T (500 MHz proton frequency) using a 5-mm inverse-detection Varian HCX probe equipped with a z-axis gradient coil. Unless otherwise specified, measurements were obtained with the sample temperature regulated at 25 °C. Calibration of the temperature controller to determine the actual temperature followed the method of Van Geet, using methanol.[42]

The PGSE method of Stejskal and Tanner[37] was used to measure translational diffusion coefficients via the relationship

$$A(G_z) = A_0 \exp[-D(\gamma\delta G_z)^2(\Delta - \delta/3)] \quad (1)$$

In this expression, A is the resonance amplitude (measured as the integrated area for this work), A_0 is the initial amplitude, and γ is the magnetogyric ratio of the observed nuclide (^{1}H); D is the diffusion coefficient, δ is the duration of the gradient pulses, Δ is the delay between the encoding and decoding gradient pulses, and G_z is the magnitude of the applied gradient. Values of δ = 2.0 ms and Δ = 60.0 ms were used for the PGSE measurements of glycine diffusing in aqueous solutions (both glycine and water with natural isotopic abundances), and δ = 2.0 ms and Δ = 30.0 ms were used for calibration measurements using H_2O/D_2O (1/99).

The variable G_z was arrayed in equally spaced steps of either 16 (calibration) or 18 (glycine) increments ranging between approximately 2 and 62 gauss/cm (0.02−0.62 T/m). These parameter values provided (i) stable and reproducible gradient pulse amplitudes via moderate duration of δ,[43] (ii) adequate time, via Δ, for loss of initial spatial phase encoding through translational diffusion, and (iii) sufficient range of gradient strength, G_z, to ensure attenuation of the resonance to 10% or less of its initial amplitude over the extent of the acquisition array. All measurements utilized a recycle

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(39) The mutual (or inter) diffusion coefficient characterizes the diffusive mixing driven by a concentration (or chemical potential) gradient. The self (or intra) diffusion coefficient is defined by Albright, J. G.; Mills, R. *J. Phys. Chem.* **1965**, *69*, 3120–3126, as follows. "In a section of a homogeneous multicomponent system at uniform temperature, let a portion of one of the components present be replaced by an isotopically labeled form of the same component and hence by a new component whose concentration may be separately measured. Further, let the chemical differences between the new and original components be negligible with regard to diffusion in the system. Then equivalent and opposite concentration gradients will be established between these two components which will result in equivalent and opposite flows relative to the volume fixed frame of reference. . It is this mutual diffusion between the two chemically equivalent components which we define to be intradiffusion." The self diffusion coefficient of a solute need not be the same as the mutual diffusion coefficient of the solution, but the two converge at infinite dilution.
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time large enough to allow complete recovery of longitudinal relaxation between transient acquisitions.

Calibration experiments were routinely performed to determine the final instrument response to the digital-to-analog conversion (DAC) values, g, used as the primary independent variable, and thus determine the resultant gradient strengths, G_z. These experiments used a sample of H_2O/D_2O in the ratio 1/99, for which the diffusion coefficient was taken as 1.90×10^{-9} m^2/s at 25 °C.[38] For each calibration, a PGSE experiment was executed using the above-indicated sample and acquisition parameters; postacquisition processing included phase and baseline correction before integration of the HOD resonance. The peak area data were then analyzed via nonlinear fit[44] to eq 1 as a function of g to simultaneously obtain A_0 and κ ($G_z = g\kappa$) using the known diffusion coefficient. In practice, at least one calibration experiment was performed for every session of glycine PGSE experiments. Typical values thus determined for κ were 0.001875 gauss/cm per DAC unit; standard deviations were on the order of 10^{-5}, and a variation of ±0.000020 represents a 95% confidence range for κ, which propagates to an estimate of approximately 10% determinate error in D related to calibration uncertainty.

For diffusion measurements of glycine in aqueous solutions, acquisition parameters and data processing were as described above; the methylene ^1H resonance was integrated for subsequent analysis of the $A(G_z)$ data, which were similarly fit to eq 1 to obtain both A_0 and D as best-fit parameters. Diffusion coefficients could be determined to within approximately 5% error in practice, as determined from replicate measurements (cf. the data and error bars shown in Figure 3).

Results

Freezing-Point Depression. Figure 2a shows the freezing-point depression of water by glycine measured in this study up to 2.92 mol/kg of H_2O, along with data from previous studies measured up to 2.1 mol/kg of H_2O.[22,30,31] Our data agree well with the previous data where comparable. Some of our solutions had concentrations higher than the eutectic composition of α glycine and ice (2.25 mol/kg of H_2O), and the observed freezing points were below the eutectic temperature (−3.6 °C).[9] These solutions were supersaturated with respect to glycine at the (depressed) freezing point of water. These measurements were possible because glycine did not crystallize during the time required for measurement (3−5 min). The absence of glycine crystallization was always verified by inspecting the solution at the end of the experiment. Relative to the eutectic composition (2.25 mol/kg of H_2O), the maximal concentration of our measurement (2.92 mol/kg of H_2O) corresponds to 30% supersaturation. The degree of supersaturation is even higher relative to the solubility of α glycine at the actual freezing point of this solution (−4.6 °C).

Figure 2a also shows the calculated freezing-point depression assuming that glycine exists as monomers or dimers. The equation used was $\ln x_0 = (\Delta H_m/R)(1/T_m - 1/T)$, where x_0 is the effective mole fraction of water (the subscript "0" signifies the solvent), R is the gas constant, $T_m = 273.15$ K is the freezing point of pure water, $\Delta H_m = 6010$ J/mol is the heat of fusion of ice, and T is the depressed freezing point. In the absence of glycine self-association (monomers only), $x_0 = 55.5/(55.5 + m)$, where m is the stoichiometric molality of glycine (mol/kg of H_2O); if all glycine molecules exist as dimers, $x_0 = 55.5/(55.5 + m/2)$. This calculation assumes that the monomers and the dimers form ideal solutions with water.[41] At lower concentrations, the observed T is well described by the monomer line; at higher concentrations, the observed T deviates from the monomer line, but still agrees better with the monomer line than with the dimer line.

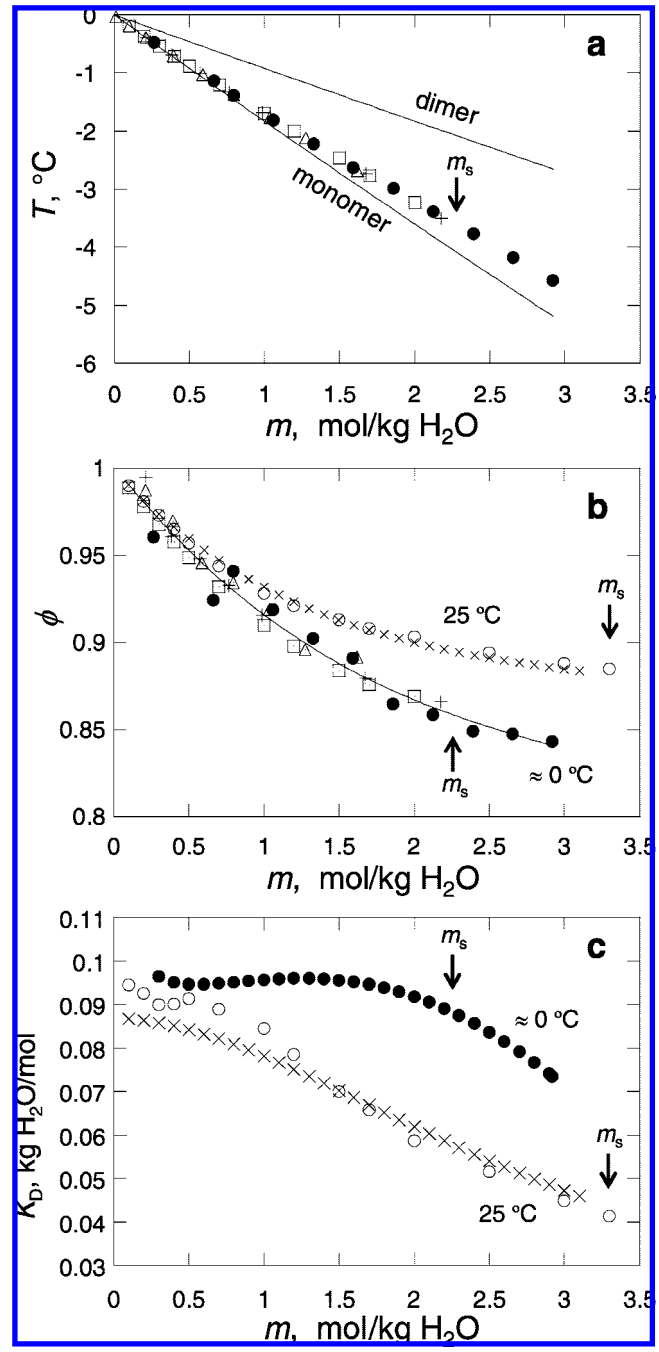

Figure 2. (a) Freezing-point depression of glycine−water solutions. (●) This work; (+) ref 22; (Δ) ref 30; (□) ref 31. The two lines are expected melting points assuming glycine exists as monomers and dimers in ideal solutions. (b) Osmotic coefficients ϕ of glycine−water calculated from freezing-point depression (same key as in (a)) and vapor pressure at 25 °C (○: ref 32; ×: ref 33). The solid curve is the fit to all freezing-point depression data. (c) Dimer stability constant K_D calculated from data in (b). (●) Near 0 °C (based on the fit to all freezing-point depression data); (○) 25 °C (ref 32); (×) 25 °C (ref 33). m_s is the solubility of α glycine (2.25 mol/kg of H_2O at −3.6 °C, the eutectic temperature of α glycine/ice; 3.33 mol/kg of H_2O at 25 °C).

Figure 2b compares the osmotic coefficient calculated from the freezing-point depression data from this and previous studies:[22,30,31] $\phi = (T_m - T)/(Km)$, where $K = 1.858$ kg of H_2O/mol is the cryoscopic constant. This parameter is more sensitive

(44) *Scientist*, version 2.01; MicroMath, Inc.: St. Louis, MO. http://www.micromath.com/.

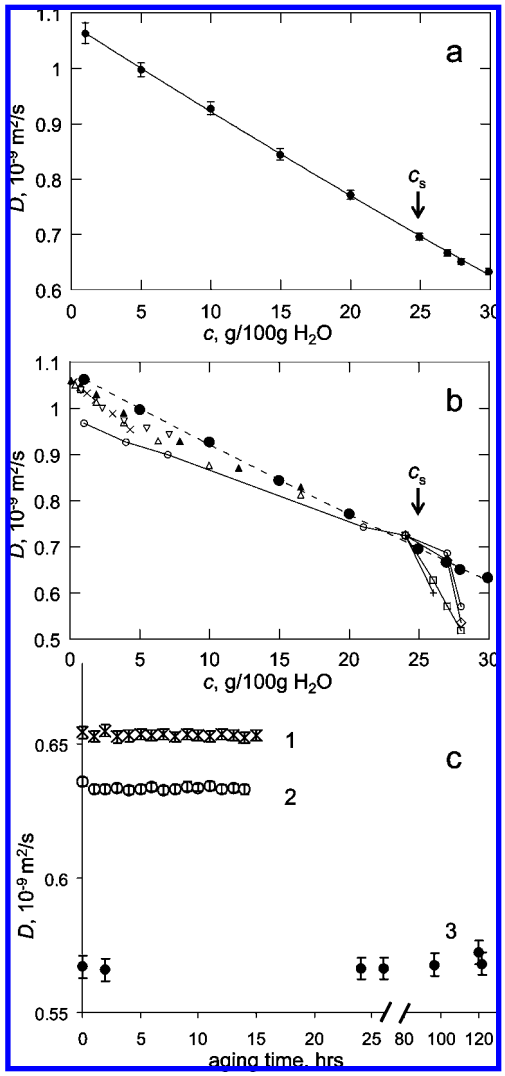

Figure 3. (a) Self-diffusion coefficient D of glycine in H_2O at 25 °C vs concentration. Glycine and H_2O are of natural isotopic abundances. Error bar is one standard deviation from fitting the PGSE data to eq 1. c_s is the solubility of α glycine (25.0 g/100 g of H_2O). (b) Comparison of the self-diffusion coefficients from this work (●) and Wang's radioactive tracer study (▲).[47] Also compared are mutual diffusion coefficients from interferometry studies of Lyons and Thomas (×),[49] Ellerton et al. (Δ),[50] Myerson and co-workers (○, 1 h of aging; ◇, 7 h; □, 53 h; +, 102 h),[25,26] and Ma et al. (▽).[51] (c) Effect of solution age on the self-diffusion coefficient of glycine in three supersaturated solutions: (1) 27.9 g/100 g of H_2O at 25 °C ($S = 11.6\%$); (2) 29.9 g/100 g of H_2O at 25 °C ($S = 19.6\%$); (3) 27.9 g gly/100 g of H_2O at 20 °C ($S = 23.9\%$). Supersaturation S is relative to the solubility of α glycine (25.0 g/100 g of H_2O at 25 °C; 22.5 g/100 g of H_2O at 20 °C). The data show no decrease of D with solution age as reported in refs 25 and 26 (solid lines in (b)).

for testing the consistency between various sets of data and allows for comparison in the same graph of the osmotic coefficients near 0 °C obtained by freezing-point depression and at 25 °C obtained by isopiestic measurements of water vapor pressure.[32,33] Assuming dimerization is the sole reason for the deviation of ϕ from unity, the dimer stability constant K_D can be calculated.[41] To calculate K_D near 0 °C, we used the best fit to all the osmotic coefficients derived from freezing-point depression data (solid curve in Figure 2b). This calculation began with determining the water activity from the observed freezing point: $\ln a_0 = (\Delta H_m/R)(1/T_m - 1/T)$. The water activity was then equated with the effective mole fraction of water in the presence of dimers, $x_0 = a_0 = 55.5/(55.5 + m_1 + m_2)$, where m_1 and m_2 are the molalities of the monomer and the dimer. Solving the last equation for $(m_1 + m_2)$ and recalling $m = m_1 + 2m_2$ (stoichiometric molality of glycine) enabled the calculation of m_1 and m_2 and, in turn, the molality-scale dimer stability constant K_D $(= m_2/m_1^2)$. The freezing-point depression data were used in this way to determine the K_D of acetic acid dimers,[35] and the result ($K_D = 0.05$ kg of H_2O/mol) agrees with those obtained with other methods.[28] The K_D obtained from this analysis is shown in Figure 2c (solid circles). By this analysis, at 2.92 mol/kg of H_2O (the highest concentration of our measurement), K_D is 0.07 kg of H_2O/mol and 25% of the glycine molecules form dimers and the remaining 75% are monomers.

To calculate the K_D at 25 °C, the dimerization was again assumed to be the cause for the deviation of ϕ from unity. The activity of water was calculated from the definition of ϕ that is appropriate for the isopiestic determination of water activity:[41] $\phi = -1000 \ln a_0/(Mm)$, where M is the molecular weight of water. The subsequent steps to obtain m_1, m_2, and K_D were the same as those described above for processing the freezing-point depression data. This analysis yielded $K_D = 0.04$ kg of H_2O/mol at 25 °C and 3.33 mol/kg of H_2O (solubility of α glycine);[45] in this solution, 18% of glycine molecules are estimated to exist as dimers. The last value is in approximate agreement with the result of the molecular dynamics simulations of Hamad et al. at 20 °C.[36]

Glycine Diffusion in Aqueous Solutions. Our initial PGSE NMR measurements were made using glycine with natural isotopic abundances dissolved in D_2O in order to use D_2O for locking the magnetic field of the spectrometer. This locking proved unnecessary, however, because the instrument's magnetic field was sufficiently stable. As a result, we were able to measure the diffusion of glycine (natural isotopic abundances) dissolved in H_2O (also natural isotopic abundances). Given the report that changing the solvent from H_2O to D_2O alters the polymorphic selectivity of glycine crystallization,[12] it is of interest to study the diffusion of glycine in both solvents and the data relevant for understanding the selective crystallization of α glycine from H_2O is the diffusion coefficient of glycine in H_2O. Figure 3a shows glycine's self-diffusion coefficient measured at 25 °C up to 30 g/100 g of H_2O (4.0 mol/kg of H_2O), exceeding the glycine solubility 25 g/100 g of H_2O at 25 °C (3.33 mol/kg of H_2O). For easier comparison with refs 25 and 26, the concentration unit used is grams of glycine/100 g of H_2O. The chemical shift of the methylene proton resonance, used for the diffusion measurement, was approximately constant in the concentration range of this study ($\delta_{CH_2} = 3.556$ ppm at 0.022 g/100 g of D_2O and 3.577 ppm at 24.7 g/100 g of D_2O, both referenced to internal 2,2-dimethyl-2-silapentane-5-sulfonic acid[46]).

The self-diffusion coefficient of glycine from this study agrees well (within 3%) with the result of Wang's radioactive tracer study (Figure 3b).[47] This agreement shows that the magnetic field of NMR measurements has minimal effect on the observed diffusion coefficient, a conclusion already reached for other systems.[38] Further evidence for the minor influence of the magnetic field on the solution chemistry of glycine is that crystallization in the presence of a magnetic field (8 T) yields

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α glycine, the same polymorph obtained in the absence of the magnetic field.[48]

Figure 3b also shows the mutual diffusion coefficients of glycine−water solutions measured by Lyons and Thomas (using Gouy interferometry),[49] Ellerton et al. (Gouy interferometry),[50] Myerson and Lo (Gouy interferometry),[25,26] and Ma et al. (holographic interferometry).[51] The mutual diffusion coefficient of glycine−water is expected to agree with the self-diffusion coefficient of glycine at infinite dilution.[39] This is the case for every set of mutual diffusion coefficients except for Myerson and Lo's. The D of this study extrapolates to $D_0 = 1.08 \times 10^{-9}$ m^2/s at infinite dilution, which agrees with the literature value, $D_0 = 1.06 \times 10^{-9}$ m^2/s; the latter value is the average of six values obtained by Taylor dispersion[52] and by Gouy, Rayleigh, and laser holographic interferometry, all summarized in ref 52. With increasing concentration, the mutual diffusion coefficient of glycine−water decreases slightly faster than the self-diffusion coefficient of glycine, a feature already pointed out by Wang.[47]

The D_0 for glycine diffusing in H_2O is 25% higher than the D_0 for deuterated glycine $^+D_3NCH_2COO^-$ diffusing in D_2O (0.85×10^{-9} m^2/s), which was obtained by extrapolating the data of Hughes et al. to zero concentration.[12] The latter value agrees well with that expected if D_0 scales with solvent viscosity:[53] $D_0(D_2O) = D_0(H_2O)\eta(H_2O)/\eta(D_2O) = (1.06 \times 10^{-9}$ $m^2/s)(0.8903$ cP$)/(1.100$ cP$) = 0.86 \times 10^{-9}$ m^2/s.

Two sets of data exist above 25 g/100 g of H_2O (solubility of α glycine): one from Myerson and Lo[25,26] and the other from this study. The two sets of data are inconsistent in this concentration range in that our data decrease smoothly with increasing concentration, whereas their data decrease abruptly as the concentration exceeds the solubility of α glycine (Figure 3b).

Effect of Solution Age on Glycine Diffusion in Supersaturated Aqueous Solutions. Myerson and Lo reported that the diffusion coefficient of glycine in supersaturated aqueous solutions decreases with solution age.[25,26] Their technique, Gouy interferometry, measures interdiffusion driven by concentration gradients. In this study, we measured the self-diffusion of glycine by PGSE NMR, which required no interdiffusion that might erase possible effects of aging on solution structure. Figure 3c shows three sets of measurements. Measurements 1 and 2 were performed with glycine solutions that remained stationary in the NMR probe (no spinning) throughout the experiment; D was measured every hour for up to 15 h. Measurement 3 was performed with a sample aged both inside and outside the NMR probe for a total of five days; when outside the NMR probe, the sample was placed on a bench in the NMR laboratory, whose temperature was 20.0 ± 0.5 °C; the sample was periodically returned to the NMR probe for measurement at the same temperature (the temperature control of the probe was turned off). At some time points, the sample remained in the NMR probe for hours to allow several measurements. The values of D obtained after the sample was just returned to the NMR probe and after it had remained in the NMR probe for several hours were consistent, again indicating the magnetic field had no significant effect on the diffusion of glycine. The supersaturation for each solution was calculated relative to the solubility of α glycine (25.0 g glycine/100 g of H_2O at 25 °C; 22.5 g glycine/100 g of H_2O at 20 °C).[45] The highest supersaturation tested was 24% in the case of the 27.9 g/100 g of H_2O sample aged at 20 °C. In none of the aging experiment was any significant change of D observed; the largest fluctuation of D was 0.74%.

Discussion

Does Glycine Dimerize in Supersaturated Aqueous Solutions? The freezing-point depression data (Figure 2a) show that glycine exists mainly as monomers, not dimers, in supersaturated aqueous solutions near 0 °C. The conclusion agrees with that reached by Hamad et al. on the basis of molecular dynamics simulations performed between 20 and 50 °C.[36] If the observed osmotic abnormality is attributed to dimerization, the dimer stability constant K_D is estimated to be 0.07 kg of H_2O/mol at 2.92 mol/kg of H_2O (30% supersaturation); in this solution, approximately 25% of glycine molecules is estimated to exist as dimers. This study provides no information on whether these dimers are cyclic or open chain, a pertinent question for testing the connection between polymorphic precipitation and solution chemistry. Hamad et al. found by molecular dynamic simulation that the main type of association between glycine molecules is the single N−H···O−C hydrogen bonds.[36] The studies of acetic acid self-association suggest cyclic and open-chain dimers are both possible.[54,55]

This study observed no slowdown of glycine diffusion in supersaturated solutions with solution age,[25−27] a result cited as evidence for glycine dimerization. Because PGSE NMR, the technique of this study, requires no liquid flow driven by concentration gradients and Gouy interferometry, the previous technique, does, PGSE NMR is deemed better suited for revealing possible effects of solution age on glycine diffusion. If any aging effect existed on the solution structure, the effect could be erased by perturbations from the interdiffusion of two solutions of different concentrations during measurement by Gouy interferometry.

The Stokes−Einstein relation, $D = kT/6\pi\eta r$, suggests that the size r of the diffusing species can be evaluated from D and η (viscosity). This relation is valid for spheres moving in a continuous medium. By this relation, r is inversely proportional to $D\eta$ at constant T. The viscosity of undersaturated glycine−water solutions at 25 °C is available from several sources (Figure 4a).[27,56−60] The average of these data is well represented by the equation given in ref 56 (solid curve in Figure 4a):

$$\eta = 0.8903(1 + 0.14193m + 0.013048m^2), \quad 0 < m < 2.4 \text{ mol/kg of } H_2O \quad (2)$$

These data and our diffusion data yield an approximately constant $D\eta$ (Figure 4a), showing no increase of solute size

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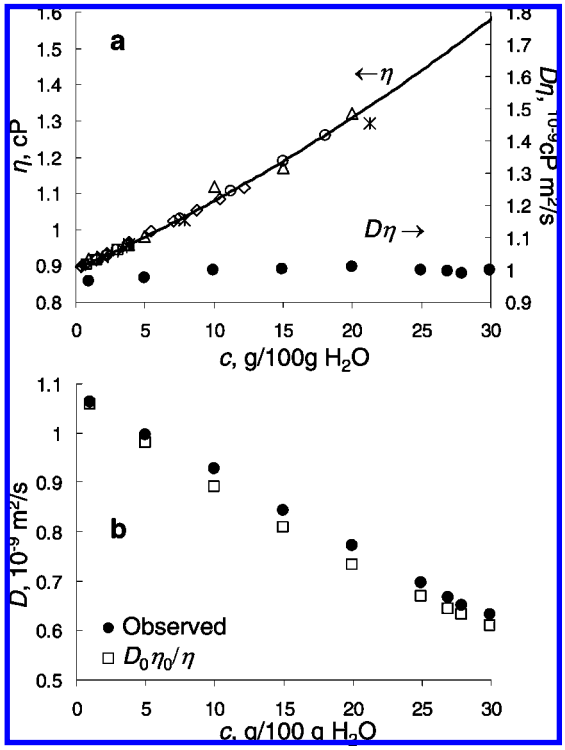

Figure 4. (a) Viscosity of glycine−water at 25 °C below 25 g/100 g of H_2O (glycine solubility). (Δ) ref 27; (○) ref 56; (*) ref 57, (◇) ref 58; (+) ref 59; and (□) ref 60. Solid line: eq 2 (extended to higher concentrations). (b) Diffusion coefficient of glycine. (●) Observed (this work); (□) D_0 (η_0/η). Parameters used: $D_0 = 1.08 \times 10^{-9}$ m^2/s, $\eta_0 = 0.890$ cP, and η is from eq 2 (extended to higher concentrations).

with concentration. Only Myerson and co-workers reported the viscosities of supersaturated glycine solutions.[27,61] Their data are somewhat inconsistent between the plot and table forms in ref 27 and between refs 27 and 61. The average of their data is approximately described by eq 2 extended to higher concentrations. If the average of their viscosity data and our diffusion data are used to calculate $D\eta$, the result is again approximately constant.

Dunn and Stokes suggested that the concentration dependence of the diffusion coefficient of acetic acid can be used to estimate the degree of dimerization.[40] This model involves comparing the observed D with $D_0(\eta_0/\eta)$, where D_0 is the diffusion coefficient at infinite dilution, η_0 is the viscosity of H_2O, and η is the viscosity of the solution in which D is measured. With D_0 assumed to represent monomer diffusion, D_0 (η_0/η) gives the monomer diffusion coefficient expected from the Stokes−Einstein relation. If the observed D is smaller than D_0 (η_0/η), dimerization might be the cause, assuming dimers diffuse more slowly than monomers. Plotted in Figure 4b are the observed D and $D_0(\eta_0/\eta)$ for glycine diffusing in H_2O at 25 °C. This analysis shows that the observed D is approximately the same as (perhaps slightly larger than) $D_0(\eta_0/\eta)$. The diffusion data therefore provide no evidence of glycine association according to this model. Hughes et al. reported the diffusion coefficient of deuterated glycine $^+D_3NCH_2COO^-$ in D_2O and interpreted its decrease with increasing concentration as evidence of solute aggregation.[12] The present analysis shows that the slowing diffusion of glycine with increasing concentration can also result from the increasing solution viscosity.

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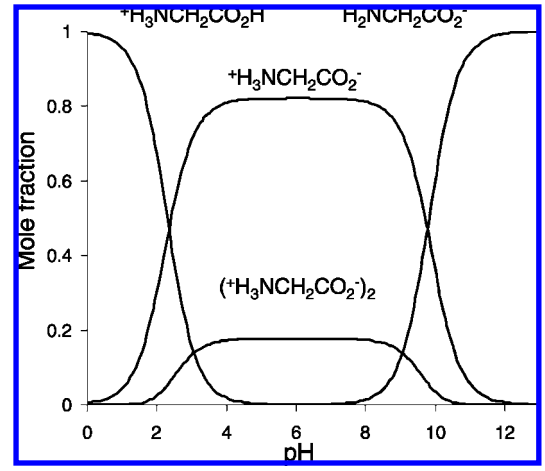

Figure 5. pH dependence of different species of glycine in a 3.33 mol/kg of H_2O solution (solubility of α glycine) at 25 °C. $pK_1 = 2.35$ (carboxylic acid); $pK_2 = 9.78$ (amine). $K_D = 0.04$ kg of H_2O/mol (Figure 2c) or 0.05 L of solution/mol.

Dimers of carboxylic acids are known to be stable in nonpolar solvents ($K_D \approx 10^2$ to 10^3 kg of solvent/mol) and substantially less stable in water ($K_D < 0.1$ kg of H_2O/mol).[28] The reduced stability of the dimers in water results from the strong hydrogen bonding between water and carboxylic acids. The same pattern likely exists for glycine. Studying glycine in nonpolar solvents is difficult, however, because of its low solubility.

It is of interest to learn how the concentration of the glycine dimer changes with pH. We consider a solution at 25 °C and 3.33 mol/kg of H_2O (solubility of α glycine), for which K_D is estimated to be 0.04 kg of H_2O/mol (Figure 2c). For this analysis, we assume K_D is independent of pH and dimerization occurs only between glycine zwitterions (not involving protonated or deprotonated species). Both assumptions are likely approximations only. The fractions of the various solution species of glycine can be calculated from the relevant acid−base and monomer−dimer equilibria. The result of this analysis (Figure 5) shows that the dimer fraction is approximately constant between pH 4 and 8 but decreases sharply below pH 4 and above pH 8. At pH 6, 18% of glycine molecules exist as dimers; at pH 3 and 9, the percentage decreases to 13 and 14%. This decrease results from both the ionization of glycine and the dissociation of weakly bound dimers by dilution. The decrease is therefore greater than that expected for strongly bound dimers ($K_D \rightarrow \infty$),[18] which are more resistant to dissociation by dilution.

Connection between Dimerization and Crystallization of Glycine. The standard explanation for the kinetically favored crystallization of α glycine from aqueous solutions is the existence of the hydrogen-bonded cyclic dimers of glycine as the predominant solution species. This study showed that glycine exists mainly as monomers, not dimers, in supersaturated aqueous solutions. Given this observation, it is unreasonable to believe that the cyclic dimers are the main cause for the selective crystallization of α glycine. If glycine exists mainly as monomers in solution, why is neither γ nor β glycine the favored polymorph to crystallize?

The notion that dimerization in solution causes the crystallization of α glycine seems inconsistent with other experimental results of glycine crystallization: (1) crystallizing an aqueous solution after first freezing the water (as in freeze-drying) yields

β glycine,[62] (2) crystallizing an alcohol−water solution yields β glycine,[7] and (3) crystallization by sublimation yields α glycine.[63] By the present model, lower temperature and nonaqueous solvent are expected to increase the dimer fraction and favor the crystallization of α glycine. But these conditions actually yield β glycine, a polar structure ($P2_1$) with molecules hydrogen-bonded head-to-tail into sheets.[7] Weissbuch et al. argue that the dimer fraction should *decrease* on changing the solvent from water to water−alcohol because glycine's solubility decreases.[14] This argument, however, does not consider the substantially greater stability (higher K_D) of the dimer in water−organic solvent than in pure water, as is documented for carboxylic acid dimers.[28] In the gas phase, glycine exists in the neutral form (H_2NCH_2COOH), not zwitterions ($^+H_3NCH_2COO^-$), as shown by studies of vapor pressure and heat of sublimation,[64] computation,[65] and IR spectrum of Ar matrix-isolated glycine.[66] The self-association of glycine in the gas phase is likely different from that in aqueous solutions. But crystallization from both media yields α glycine.

The selective crystallization of α glycine from water is thought to illustrate the general principle that the crystallization of a particular structure results from the existence of the building unit of that structure in the liquid.[11,19] The results of this study argue that glycine does not serve such an example. For the same reason, we suggest that the crystallization of benzoic acid does not exemplify this principle either. Only one crystal structure ($P2_1/c$)[67] is known experimentally for benzoic acid (no polymorphs), and this structure contains hydrogen-bonded dimers. Beyer and Price found by computation that a higher-energy polymorph could exist based on hydrogen-bonded chains.[68] Because the dimers of benzoic acid are stable in organic solvents,[28] it might be concluded that benzoic acid conforms to the principle of crystal−liquid structural similarity. One notes, however, that benzoic acid dimers are not strongly bound in water ($K_D < 0.1$ kg of H_2O/mol),[69] and yet crystallization from water yields the same crystal structure containing hydrogen-bonded dimers. It follows that the knowledge of solution species is insufficient for predicting the crystallizing structure, and the knowledge of the crystallizing structure is insufficient for predicting how molecules associate in solution.

The existence of glycine mainly as monomers, not dimers, does not invalidate the explanations for the effect of solution additives[11] and pH change[18] on the polymorphic selectivity of glycine crystallization. Weissbuch et al. attribute the preferred crystallization of γ glycine in the presence of racemic hexafluorovaline to the adsorption of the additive on fast-growing faces of α glycine, which otherwise dominates the crystallization product.[11] Towler et al. attribute the preferred crystallization of γ glycine upon pH change to the adsorption of charged species on all fast-growing faces of α glycine, but only some fast-growing faces of γ glycine.[18] These models begin with the preferred crystallization of α glycine, whether it be promoted by dimerization or not, and inquire how the growth of α glycine is affected by charged species (in the case of pH change) and additive adsorption (in the case of additives).

Conclusions

We studied the existence of molecular dimers in supersaturated aqueous solutions of glycine by measuring freezing-point depression and diffusion with the goal of better understanding the polymorphic selectivity of glycine crystallization. The freezing-point depression data indicate that glycine exists in supersaturated solutions mainly as monomers, not dimers. PGSE NMR data show that the diffusion of glycine does not slow with solution age, in contradiction to a previous report often cited as evidence for glycine dimerization. We conclude that glycine exists mainly as monomers, not dimers, in supersaturated aqueous solutions and remains as such upon aging. This conclusion does not rule out weaker associations between glycine molecules, but brings into question the idea of long-lived hydrogen-bonded cyclic dimers as the predominant solution species serving as units of crystal growth. The substantially different rates of nucleation and crystal growth of polymorphs in the same liquid remain a deserving problem for understanding crystallization in polymorphic systems.

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