Supporting Information

Glycine's pH-dependent polymorphism: A perspective from self-association in solution

Weiwei Tang,[a] Huaping Mo,[c] Mingtao Zhang,[b] Junbo Gong,*[,a] Jingkang Wang,[a] Tonglei Li,*[,b]

[a] School of Chemical Engineering and Technology, State Key Laboratory of Chemical Engineering, The Co-Innovation Center of Chemistry and Chemical Engineering of Tianjin, Tianjin University, Tianjin 300072, People's Republic of China

[b] Department of Industrial and Physical Pharmacy, College of Pharmacy, Purdue University, West Lafayette, Indiana 47907, USA

[c] Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue University, West Lafayette, Indiana 47907, USA

Experimental and Computational Methods

Materials. Glycine (≥99% purity, α form) and deuterium oxide (99.9 % D) was purchased from Sigma-Aldrich, and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS, ⩾97% purity) from Cambridge Isotope Laboratories. H_2O was double deionized freshly made in the laboratory. Hydrochloric acid (HCl, 36.5%-38.0%) was obtained from J.T. Baker, solid NaOH (⩾98%) from Macron Fine Chemicals, and solid NaCl (⩾99.0%) from EMD Millipore without further treatments. The γ form was prepared by recrystallization of 5.0 M glycine solution at pH=10.0 and at room temperature (~295 K). The harvested crystals were found to be pure γ form as determined from powder X-ray diffraction and FTIR spectra.

NMR Spectroscopy. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 800 MHz Bruker Avance-III spectrometer equipped with a 5mm QCI Z-gradient cryoprobe at 298 K. Data were processed and analyzed using TOPSPIN software (version 3.2). ^{1}H-NMR and ^{13}C-NMR spectra were acquired at 298 K with the number of scans 32 and 256, respectively. ^{1}H and ^{13}C chemical shifts were determined relative to an internal reference DSS. The FID resolutions were 0.37 and 1.5 Hz for ^{1}H-NMR and ^{13}C-NMR, respectively. Changes in chemical shifts were fitted to a dimerization or a combined ionization and dimerization isotherm model with purpose written software. The program uses a Simplex algorithm to fit experimental data and determine the optimum solutions for model parameters (e.g., dimerization constant).

NMR Dilution Experiments. The chemical shift measurements were performed in both undersaturated and supersaturated solutions. NMR samples were prepared by diluting known concentration of Gly solutions in D_2O or 95% (v/v) H_2O/D_2O. The 5% D_2O was applied to lock magnetic field during measurements.

Nuclear Overhauser Effect Spectroscopy (NOESY). 2D NOESY experiments were carried out for 1.6 and 3.0 M Gly solutions in D_2O at 298 K. 2D NOE spectra were acquired with a standard pulse over a sweep width of 4.0 ppm in D_2O for both F1 and F2 dimensions. The number of F1 increment was 256, each with 8192 data points in the F2 dimension. A mixing time of 0.5 s and a relaxation delay of 3 s were applied. Numbers of scans was set to 8 and dummy scans 16.

NMR Titration Experiments. A stock solution containing known amount (5.0×10^{-3} M or 2.7 M) of Gly was initially prepared in 95% (v/v) H_2O/D_2O without or with NaCl, and then used to prepare known concentrations of 0.1 M HCl, 2.5 M HCl, 0.1 M NaOH, and 2.6 M NaOH. The titration was conducted in an initially 25 mL HCl solution and the desired pH values were achieved by addition of NaOH solution; 1-1.5 mL of samples were then withdrawn for NMR measurements. The addition of NaCl (1.4 M and 2.7 M) in 5.0×10^{-3} M Gly aqueous solution was used to investigate the salt effect (ionic strength) on CH_2 proton chemical shift during NMR titration experiments. The impact of salt concentration on proton chemical shift in 95% (v/v) H_2O/D_2O was also examined by the addition of different known amounts of NaCl.

FTIR Spectroscopy. FTIR spectra of Gly solid samples were recorded on a Cary 600 Series FTIR spectrometer (Agilent Technologies Inc.) equipped with PIKE MIRacle ATR ZnSe accessory. The IR spectra of solution samples were recorded on the same spectrometer using Multi Reflection HATR accessory. For both solid and solution samples, at least 64 scans were collected over a spectral region from 800-4000 cm^{-1} with a resolution of 2 cm^{-1}. The IR data were processed with Agilent Resolutions Pro software (version 5.2).

pH Measurements. Measurements of pH were made using a Mettler Toledo FiveEasy Plus pH meter equipped with a combined LE 438 electrode and an ATC probe for temperature compensation. Calibration was performed using aqueous buffers before each measurement.

Computation Methods. Density functional theory (DFT) calculations were performed using the GAUSSIAN 09[1] software package. The geometries of monomer, 1:1 Gly-H_2O complexes (H_2O as both a donor and an acceptor), dimers were envisaged based on crystal structure of Gly polymorphs α and γ and optimized by hybrid M06-2x function and 6-311++G(d,p) basis set with Grimme's D3 dispersion correction[2] in H_2O using SMD implicit solvation model.[3] The Grimme dispersion correction allowing for better description of weak interactions including van der Waals interactions gives better geometries of monomer associates. The binding energy (ΔE_{bind}) quantifies both deformation energy of monomer and intermolecular interaction energy

$$\Delta E_{bind} = E_{AB} - E_A - E_B + BSSE \qquad (1)$$

where E_{AB} is the energy of a Gly-H_2O complex or a Gly dimer, and E_A and E_B are the energies of the isolated monomers Gly (A) and H_2O (B), respectively or both Gly monomers (A=B). All the energies have been corrected for the zero-point vibrational energies. BSSE is the basis set superposition error (BSSE) term and calculated for the correction of overestimation in binding energies due to the overlapping of basis functions.[4]

NMR chemical shifts were calculated using Gauge-independent Atomic Orbital (GIAO) method.[5] The calculations were performed on the optimized monomer and dimer structures, as well as all the dimer motifs in both α and γ forms without geometry optimization at the same M06-2x/ 6-311++G(d,p) level of theory. The reported chemical shifts were relative to those of 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) computed in the same way.

Gly Concentration Effect on pH

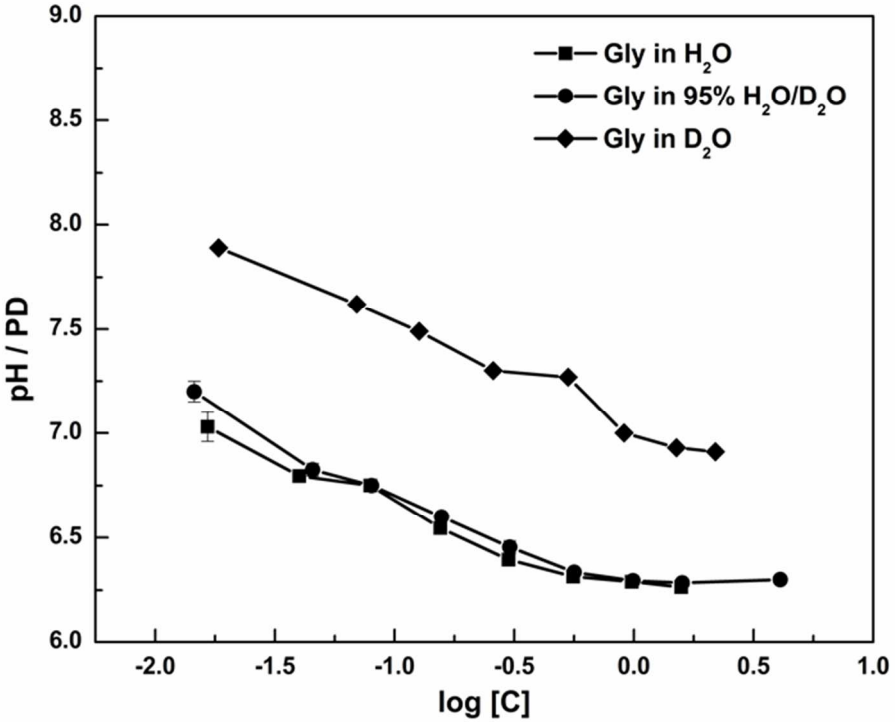

Figure S1. Solution pH in H_2O and 95% (v/v) H_2O/D_2O, and solution pD in D_2O varying at a series of Gly concentrations. pD values were corrected with the standard formula: $pD = pH^* + 0.40$, where pH^* is the observed value from pH meter.[6] The solution pH/pD increases when dilutes the concentration of Gly.

Salt (ionic strength) effect on CH_2 proton chemical shift as a function of solution pH (negative control)

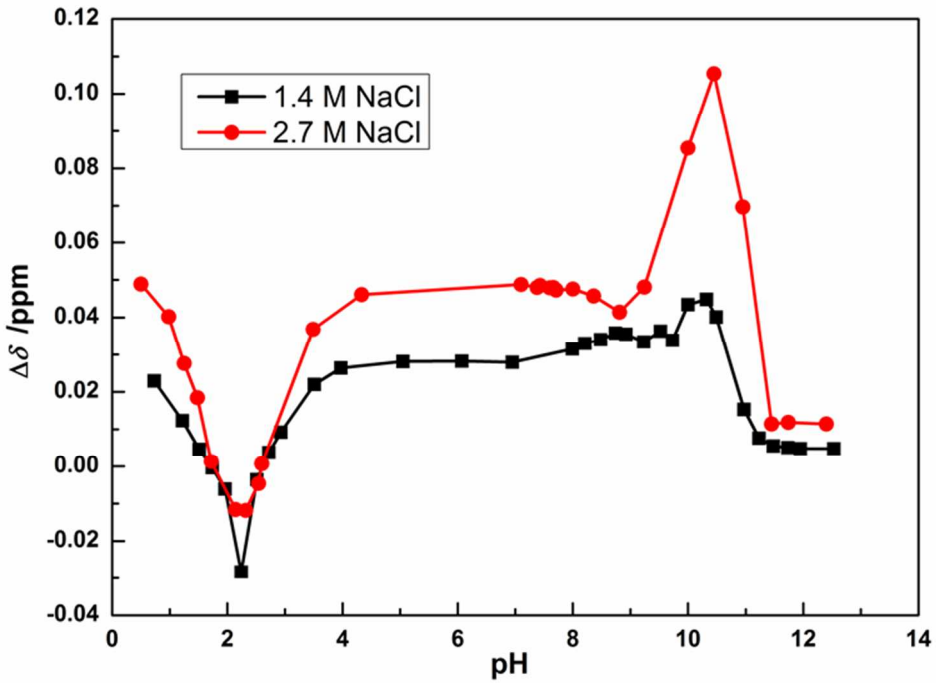

Figure S2. The effect of NaCl concentration on CH_2 ^1H chemical shift difference at the concentration of 5.0 x 10^{-3} M Glycine in aqueous solution with 1.4 M and 2.7 M NaCl relative to that of neglectable NaCl (0-0.1 M) during NMR titration. The appearance of initial decrease and then increase at nearly first and second isoelectric points at 5.0 x 10^{-3} M resembles the changes at 2.7 M Gly (Figure 6 in main text), suggesting that those changes are due to salt effect. The salt effect on chemical shift changes was further corroborated by the enhanced changes with the increase in NaCl concentration.

Salt (ionic strength) concentration effect on CH_2 proton chemical shift at a constant pH

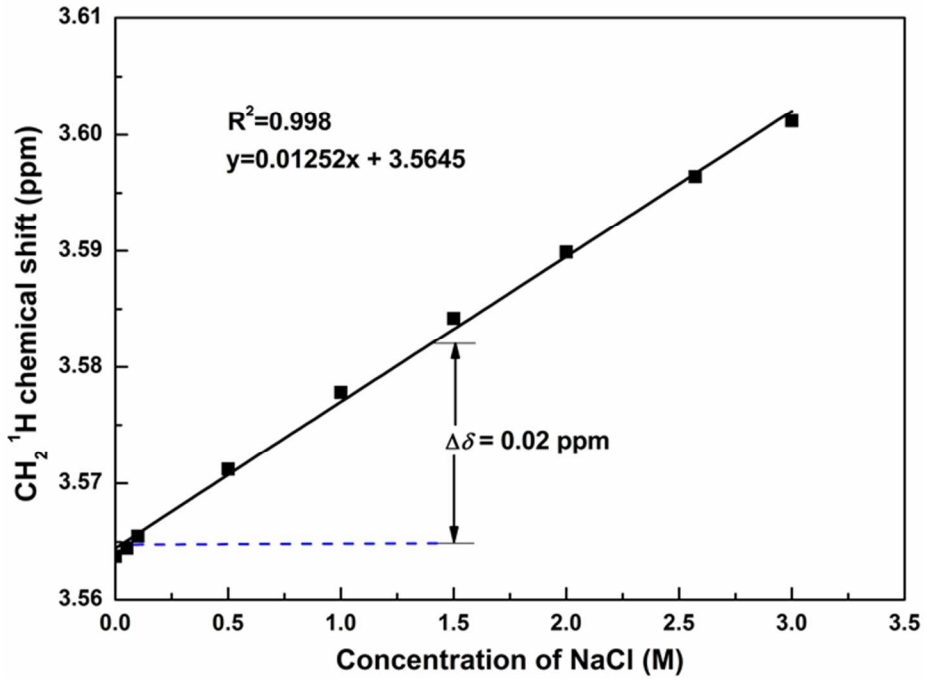

Figure S3. The effect of NaCl concentration on methylene ^1H chemical shift of 2.7 M Gly in 95% H_2O/D_2O. The blue dash line represents the concentration range of NaCl in the pH domains 4-8, and the chemical shift variation due to the salt concentration is around 0.02 ppm during the titration experiments.

Computed COO ^{13}C chemical shifts of dimer motifs in both α and γ forms

Table S1. Carboxylate ^{13}C chemical shift of all types of dimer motifs in both α and γ forms computed by M06-2x/ 6-311++G(d,p) level of theory without geometry optimization.

δ_{13C} /ppm	α Form				
	I	II	III	IV	
Open dimer	183.56	182.73			
Cyclic dimer			185.84	189.77	
Average			185.5		
δ_{13C} /ppm	γ Form				
	V	VI	VII	VIII	IX
Open dimer	181.18	181.57	182.43	183.32	185.24
Average			182.7		

REFERENCES

(1) *Gaussian 09*, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc. Wallingford, CT, USA, 2009.

(2) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. *J. Chem. Phys.* **2010,** 132, 154104.

(3) Pratt, L. M.; Truhlar, D. G.; Cramer, C. J.; Kass, S. R.; Thompson, J. D.; Xidos, J. D. *J. Org. Chem.* **2007,** 72, 2962.

(4) Boys, S. F.; Bernardi, F. d. *Mol. Phys.* **1970,** 19, 553.

(5) Cheeseman, J. R.; Trucks, G. W.; Keith, T. A.; Frisch, M. J. *J. Chem. Phys.* **1996,** 104, 5497.

(6) Stephen Reid, R.; Podányi, B. *J. Inorg. Biochem.* **1988,** 32, 183.