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Hydration Structure of Glycine Molecules in Aqueous Acidic Solutions

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TOF neutron diffraction measurements have been carried out on the acidic aqueous 2 mol% glycine heavy solutions. The isotopic substitution technique has been applied to both nitrogen and hydrogen atoms within glycine molecule in order to obtain information concerning the hydration structure around the amino- and methylene-group in the glycine molecule under the low-pH condition. It has been revealed that the nitrogen atom in the amino group forms hydrogen bonds of N—D$\cdots$OD$_2$ type with 3.0(2) D_2O molecules in the present acidic solutions. The value of the intermolecular distance between the nitrogen atom in the amino group and D_2O molecules in the first hydration shell is determined to be r(N$\cdots$O(water)) = 2.90(2) Å. The present hydration structure around the amino-group is very close to the hydration structure reported for the neutral aqueous solution. The hydration number around the methylene-hydrogen atom has been determined to be 0.66(1), with the average distance of 2.68(1) Å, and the orientational correlation between the methylene-hydrogen atoms and the nearest neighbor D_2O molecules is considered to be very weak.

KEYWORDS: $^{14}N/^{15}N$ and H/D isotopic substitution, TOF neutron diffraction, hydration structure, glycine

§1. Introduction

Structural properties of hydrated amino acid molecules in aqueous solutions have long been a matter of interest for extensive areas of chemistry and biology. The hydration structure of amino acid molecules in the solution as well as the network structure of solvent hydrogen bonds around those molecules has also been one of the most important subjects in recent computer simulation studies.[1,2] It is well known that the ionization state (or charge) of the amino acid strongly depends on the pH value of the solution. Glycine molecules exist as the zwitterion, $N^+H_3CH_2COO^-$, in neutral solution, as the anion, $NH_2CH_2COO^-$, in alkaline solution. The hydration structure of the amino-nitrogen atom within the glycine molecule in neutral and in alkaline solutions has recently been investigated by neutron diffraction measurements on $^{14}N/^{15}N$ and H/D substituted aqueous glycine solutions.[3,4] Glycine molecule exist as the cationic form in the low-pH condition, $N^+H_3CH_2COOH$. Nevertheless, little information has been obtained on the structural change in hydration properties of glycine molecules in aqueous solutions, particularly in the low-pH solutions.

The difference in the hydration structure of the glycine molecule in neutral, in alkaline and in acidic solutions are discussed.

§2. Experimental

Isotopically enriched $^{15}NH_2CH_2COOH$ (99.0 % ^{15}N, ISOTEC Inc.) and natural $^{14}NH_2CH_2COOH$ (99.6 % ^{14}N, Nacalai tesque, guaranteed grade) were deuterated by dissolving them repeatedly into D_2O (99.9 % D, Aldrich Chemical Co., Inc.), followed by the dehydration under vacuum. The required amounts of enriched compounds, $^{14}ND_2CH_2COOD$, $^{15}ND_2CH_2COOD$, and $^{14}ND_2CD_2COOD$ (98.0 % D, Aldrich Chemical Co., Inc.), were dissolved into D_2O. A weighed amount of the concentrated aqueous DCl solution in D_2O (37 wt% DCl, 99.5 % D, Aldrich Chemical Co., Inc.) was added to each sample solution to prepare three kinds of acidic aqueous 2 mol% glycine solutions with different isotopic compositions of both amino-nitrogen and methylene-hydrogen atoms within the glycine molecule, i.e., I: $(^{14}ND_2CH_2COOD)_{0.02}(DCl)_{0.02}(D_2O)_{0.96}$, II: $(^{15}ND_2CH_2COOD)_{0.02}(DCl)_{0.02}(D_2O)_{0.96}$ and III: $(^{14}ND_2CD_2COOD)_{0.02}(DCl)_{0.02}(D_2O)_{0.96}$, respectively. The "pD" value of the present solution was determined to be 1.14, implying that 94 % of solute glycine molecules are in the acidic form.

The sample solution was sealed into a cylindrical quartz cell (7.3 mm in inner diameter and 0.5 mm in thickness). TOF neutron diffraction measurements were carried out at 25 ℃ using the HIT–II spectrometer[5] installed at the High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. The data accumulation time was ca. 12 h for each sample. Diffraction measurements were made in advance for an empty cell, background and a vanadium rod of 8 mm in diameter, respectively. Scattered neutron intensities from the sample solution were corrected for the absorption of both the sample and cell,[6] and for multiple[7] and incoherent scatterings. The first-order difference functions, $\Delta_N(Q)$ and $\Delta_H(Q)$, were derived from the difference in the scattering cross sections between two solutions with different isotopic ratios for the amino-nitrogen ($\Delta_N(Q)$ = I − II) and for the methylene-hydrogen atoms ($\Delta_H(Q)$ = III − I). The $\Delta_X(Q)$ function, scaled at the stoichiometric unit, $(^*ND_2C^*H_2COOD)_x(DCl)_x(D_2O)_{1-2x}$, can be written as a linear combination of the partial structure factors related to the X atom.

$$\Delta_X(Q) = A_X[a_{XN}(Q) - 1] + B_X[a_{XH_M}(Q) - 1]$$

$$+C_X[a_{XC}(Q)-1]+D_X[a_{XCl}(Q)-1]$$
$$+E_X[a_{XO}(Q)-1]+F_X[a_{XD}(Q)-1] \quad (2.1)$$

where

$$A_N = x^2(b^2_{^{14}N} - b^2_{^{15}N}), B_N = 4x^2 b_{H_M}(b_{^{14}N} - b_{^{15}N}),$$
$$C_N = 4x^2 b_C(b_{^{14}N} - b_{^{15}N}), D_N = 2x^2 b_{Cl}(b_{^{14}N} - b_{^{15}N}),$$
$$E_N = 2x b_O(b_{^{14}N} - b_{^{15}N}), F_N = 4x b_D(b_{^{14}N} - b_{^{15}N}),$$

and

$$A_H = 4x^2 b_N(b_{D_M} - b_{H_M}), B_H = 4x^2(b^2_{D_M} - b^2_{H_M}),$$
$$C_H = 8x^2 b_C(b_{D_M} - b_{H_M}), D_H = 4x^2 b_{Cl}(b_{D_M} - b_{H_M}),$$
$$E_H = 4x b_O(b_{D_M} - b_{H_M}), F_H = 8x b_D(b_{D_M} - b_{H_M})$$

The intramolecular X$\cdots\alpha$ contribution within the glycine molecule, $I_X^{intra}(Q)$, was evaluated using structural parameters in the crystalline state,[8]

$$I_X^{intra}(Q) = \sum 2c_X b_\alpha (b_X - b_{X'}) \times \exp\left(-\frac{1}{2}l^2_{X\alpha}Q^2\right)\frac{\sin(Qr_{X\alpha})}{Qr_{X\alpha}}, \quad (2.2)$$

where c_X is the number of X atom. $l_{X\alpha}$ and $r_{X\alpha}$ denote the root mean square amplitude and the internuclear distance, respectively. Calculated $I_X^{intra}(Q)$ was then subtracted from the observed $\Delta_X(Q)$ to deduce the intermolecular difference function, $\Delta_X^{inter}(Q)$.

$$\Delta_X^{inter} = \Delta_X(Q) - I_X^{intra}(Q) \quad (2.3)$$

The intermolecular distribution function around the amino-nitrogen and methylene-hydrogen atoms can be obtained by the Fourier transform of $\Delta_X^{inter}(Q)$.

$$G_X^{inter}(r) = 1 + (A_X + B_X + C_X + D_X + E_X + F_X)^{-1} \times (2\pi^2\rho r)^{-1}\int_0^{Q_{max}} \Delta_X^{inter}(Q)\sin(Qr)dQ$$
$$= [A_X g_{XN}(r) + B_X g_{XH_M}(r) + C_X g_{XC}(r) + D_X g_{XCl}(r) + E_X g_{XO}(r) + F_X g_{XD}(r)] \times (A_X + B_X + C_X + D_X + E_X + F_X)^{-1} \quad (2.4)$$

§3. Results and Discussion

The observed function, $\Delta_N(Q)$, and the corresponding distribution function around the amino-nitrogen atom within the glycine molecule, $G_N(r)$, in the acidic aqueous 2 mol% glycine solution are shown in Figs. 1 and 2, respectively. In Fig. 1a, an evident peak in $\Delta_N(Q)$ is observed at $Q \approx 2$ Å^{-1}. The oscillational feature of $\Delta_N(Q)$ extends to the higher-Q region. The dominant first peak at $r \approx 1$ Å in $G_N(r)$ in Fig. 2a can be assigned to the intramolecular N—D interaction within the glycine molecule. The second peak at $r \approx 1.5$ Å is attributed to the intramolecular N—C interaction. Broad peak at $r \approx 3$ Å is considered to reflect water molecules in the first hydration shell of the amino group in the glycine molecule. The calculated $I_N^{intra}(Q)$ (Fig. 1b), consisting of contributions from all possible N$\cdots\alpha$ pairs within the glycine molecule, was subtracted from the observed $\Delta_N(Q)$ to deduce the intermolecular difference function, $\Delta_N^{inter}(Q)$ (Fig. 1c), which was used for subsequent least squares refinement procedures. The Fourier transform of $\Delta_N^{inter}(Q)$, $G_N^{inter}(r)$, is represented in Fig. 2b.

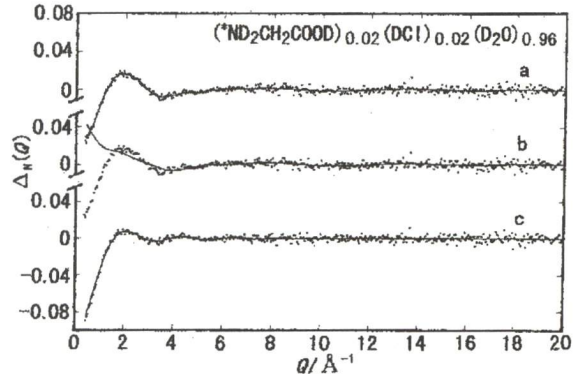

Fig. 1. a) Observed difference function, $\Delta_N(Q)$ (dots), and smoothed $\Delta_N(Q)$, which has been used for the Fourier transform (solid line). b) Observed difference function, $\Delta_N(Q)$ (dots), and the intramolecular contribution within the glycine molecule, $I_N^{intra}(Q)$ (solid line). c) Intermolecular difference function, $\Delta_N^{inter}(Q)$ (dots). The best-fit of the calculated $\Delta_N^{model}(Q)$ (solid line).

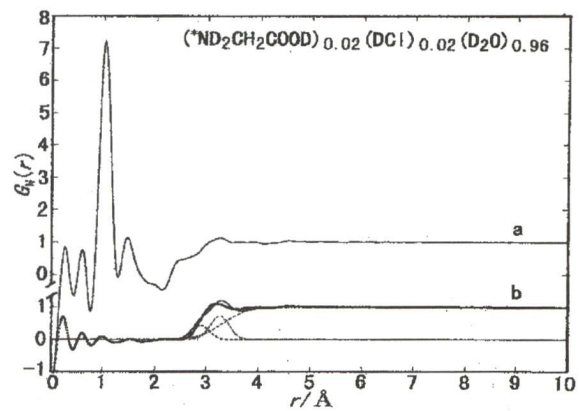

Fig. 2. a) Total distribution function around the amino-nitrogen atom, $G_N(r)$. b) Observed intermolecular distribution function, $G_N^{inter}(r)$ (dots), and the Fourier transform of the calculated $\Delta_N^{model}(Q)$ (Solid line). Short- and long-range contributions (broken lines).

The observed $\Delta_H(Q)$ shown in Fig. 3a is characterized by the relatively smeared first peak at $Q \approx 3$ Å^{-1} followed by a smaller pre-peak at $Q \approx 1.5$ Å^{-1}, and an oscillatory feature extending to the higher-Q region. The distribution function around the methylene-hydrogen atom, $G_H(r)$, is described in Fig. 4a. The dominant first peak at $r \approx 1.1$ Å in $G_H(r)$ is assigned to the intramolecular C—D bond of the methylene-group in the glycine molecule. The partially-resolved second peak $r \approx 2.2$ Å in $G_H(r)$ is attributable to various intramolecular nonbonding interactions in the glycine molecule. In addition, intermolecular contributions from D_2O molecules in the first hydration shell of the H_M atom should be involved in this r-region. Therefore, we derived $\Delta_H^{inter}(Q)$ and $G_H^{inter}(r)$, using the similar procedure employed for the $\Delta_N^{inter}(Q)$ and $G_N^{inter}(r)$ functions as described above.

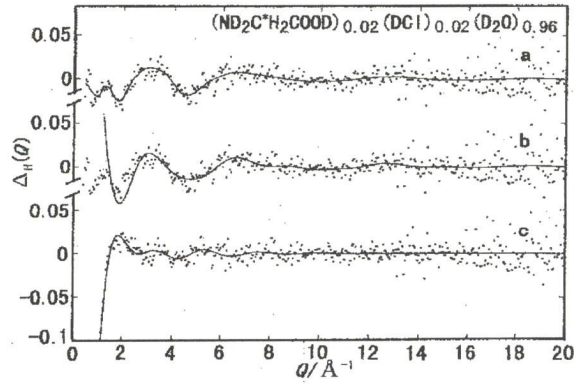

Fig. 3. a) Observed difference function, $\Delta_H(Q)$ (dots), and smoothed $\Delta_H(Q)$, which has been used for the Fourier transform (solid line). b) Observed difference function, $\Delta_H(Q)$ (dots), and the intramolecular contribution within the glycine molecule, $I_H^{intra}(Q)$ (solid line). c) Intermolecular difference function, $\Delta_H^{inter}(Q)$ (dots). The best-fit of the calculated $\Delta_H^{model}(Q)$ (solid line).

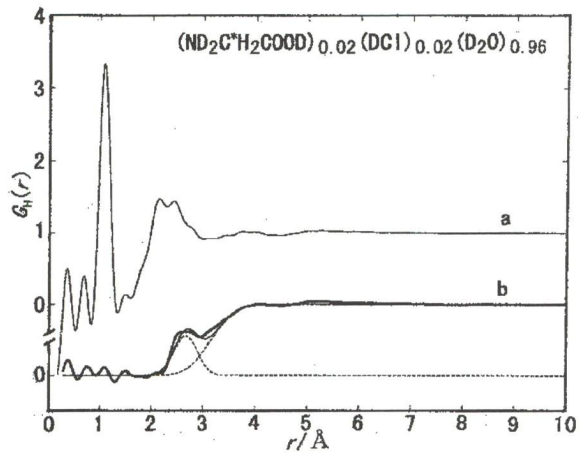

Fig. 4. a) Total distribution function around the amino-nitrogen atom, $G_H(r)$. b) Observed intermolecular distribution function, $G_H^{inter}(r)$ (dots), and the Fourier transform of the calculated $\Delta_H^{model}(Q)$ (Solid line). Short- and long-range contributions (broken lines).

Hydration parameters around the amino-nitrogen and methylene-hydrogen atoms within the glycine molecule were determined through the least-squares fitting analysis for the observed intermolecular function, $\Delta_X^{inter}(Q)$ (X: N, H), employing the following model function, $\Delta_X^{model}(Q)$, involving both short- and long-range contributions:

$$\Delta_X^{model}(Q) = \sum 2c_X n_{X\alpha} b_\alpha (b_X - b_{X'}) \times \exp\left(-\frac{1}{2} l_{X\alpha}^2 Q^2\right) \frac{\sin(Q r_{X\alpha})}{Q r_{X\alpha}} + 4\pi\rho(A + B + C + D + E + F) \times \exp\left(-\frac{1}{2} l_{0X}^2 Q^2\right) \times \frac{Q r_{0X}\cos(Q r_{0X}) - \sin(Q r_{0X})}{Q^3}, \quad (3.1)$$

where $n_{X\alpha}$ denotes the coordination number of α atom around a given X atom (= N, H). $l_{X\alpha}$ and $r_{X\alpha}$ are the rms amplitude and internuclear distance for X$\cdots\alpha$ pair, respectively. The long-range structure parameter, r_{0X}, means the distance beyond which the continuous distribution of atoms around the X atom can be assumed. The parameter, l_{0X}, describes the sharpness of the boundary at r_{0X}. Structural parameters, $n_{X\alpha}$, $l_{X\alpha}$, $r_{X\alpha}$, r_{0X} and l_{0X} in Eq. (3.1) are respectively determined from the least squares fit to the observed $\Delta_X^{inter}(Q)$. The tilt angle between N$\cdots$O axis and molecular plane of D_2O, α, was refined independently. The internuclear distance between the amino-nitrogen atom and the water-deuterium atom is calculated from the parameters, r_{NO} and α. The results are indicated in Table I. It has been revealed that the nitrogen atom within the amino-group forms a hydrogen bond of N—OD_2 type with ca. 3 D_2O molecules in the present acidic solutions, which is in contrast to the hydration structure around the amino group reported for the aqueous alkaline solutions in which the amino group forms ca. 2 hydrogen bonds of N—D$\cdots$$OD_2$ type and ca. 1 hydrogen bond of N$\cdots$D—OD type with neighboring D_2O molucules.

Table I. Hydration parameters for aqueous acidic 2 mol% glycine solutions in D_2O.

interaction	i—j	r_{ij}/Å	l_{ij}/Å	n
N$\cdots$$D_2O$	N$\cdots$O $\alpha = 68(6)°$	2.90(2)	0.17(5)	3.0(2)
long-range	N$\cdots\alpha$	3.37(9)	0.37(10)	
$H_M\cdots D_2O$	$H_M\cdots D_2O$	2.68(1)	0.23(1)	0.66(1)
long-range	$H_M\cdots\alpha$	3.20(1)	0.36(3)	

The partially-resolved first peak at $r \approx 2.7$ Å in $G_H^{inter}(r)$ (Fig. 4b) clearly suggests that the weak hydration shell is present around the methylene group, although $G_H^{inter}(r)$ is rather strucutreless beyond this peak position. Since it is difficult to divide the first peak in $G_H^{inter}(r)$ into two contributions from $H_M\cdots O$ and $H_M\cdots D$ pairs, the first hydration shell around the H_M atom was treated as a single kind of interaction, $H_M\cdots D_2O$, in the present analysis. The present hydration structure around the amino-group is very close to the hydration structure reported for the neutral aqueous solution.[3] The hydration number of the methylene-hydrogen was determined to be 0.66(1) with the average distance of 2.68(1) Å. The orientational correlation between the methylene-hydrogen atoms and the nearest neighbor D_2O molecules is considered to be very weak.

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