Hydration Structure of Glycine Molecules in Aqueous Alkaline Solutions

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Time-of-flight (TOF) neutron diffraction measurements have been carried out on the alkaline aqueous 2 mol% glycine solution in heavy water. The isotopic substitution technique has been applied to both nitrogen and hydrogen atoms within glycine molecules in order to obtain information concerning the hydration structure around the amino- and methylene group in the glycine molecule under the high-pH condition. Results revealed that the nitrogen atom in the amino group forms a hydrogen bond of the N···D–O type with ca. one D_2O molecule ($r_{N\cdots D}$ = 1.97(3) Å, ∠N···D–O = 174(5)°), and simultaneously forms bonds of N–D···OD_2 type with ca. two D_2O molecules ($r_{N\cdots O}$ = 2.92(3) Å) in the present alkaline aqueous solution. This behavior is in contrast to the hydration structure around the amino group in the glycine molecule reported for the neutral aqueous solution, in which the amino group forms the hydrogen bonds of N–D···OD_2 type with ca. three D_2O molecules. The first hydration shell around the methylene group in the glycine molecule involves ca. two D_2O molecules per one glycine molecule. The orientational correlation between the methylene–hydrogen atoms and neighboring D_2O molecules is weak.

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 these 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 amino acid molecules strongly depends on the pH value of the solution. For example, glycine molecules exist as the zwitterion $N^+H_3CH_2COO^-$ in neutral solution. The hydration structure of the amino-nitrogen atom within the glycine molecule in the neutral solution has recently been investigated by neutron diffraction measurements on $^{14}N/^{15}N$ and H/D substituted aqueous 5 mol% glycine solutions.[3,4] The result has showed that three water molecules are hydrogen-bonded (N–D···OD_2 type) to the amino-nitrogen atom with intermolecular distances of $r_{N\cdots O}$ = 2.85(5) Å and $r_{N\cdots D}$ = 3.25(5) Å. On the other hand, glycine molecules in the high pH solution has been found to take the anionic form $NH_2CH_2COO^-$. Nevertheless, little information has been obtained on the structural change in hydration properties of glycine molecules in aqueous solutions, particularly in the high-pH solutions.

The present paper describes the results of TOF neutron diffraction measurements on three aqueous 2 mol% glycine solutions in heavy water under the high-pH condition, in which both amino-nitrogen and methylene–hydrogen atoms within the glycine molecule are isotopically changed. The difference function, $\Delta_N(Q)$, between scattering cross sections observed for $(^{14}ND_2CH_2COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$ and $(^{15}ND_2CH_2COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$ solutions supplies useful information on the hydration structure around the amino group in the glycine molecule in these high-pH solutions. The hydration structure around the methylene–hydrogen atoms within the glycine molecule is deduced also from the difference function, $\Delta_H(Q)$, between scattering cross sections observed for $(^{14}ND_2CD_2COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$ and $(^{14}ND_2CH_2COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$ solutions. The differences in the hydration structure of the glycine molecule in the neutral solutions and in the high-pH solutions are discussed.

Experimental

Materials. 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 deuterated aqueous NaOD solution in D_2O (40 wt% NaOD, 99.0% D, Isotec Inc.) was added to each sample solution to prepare three kinds of alkaline aqueous 2 mol% glycine solutions with different isotopic compositions of both amino-nitrogen and methylene–hydrogen atoms within the glycine molecule, i.e., I: $(^{14}ND_2CH_2COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$, II: $(^{15}ND_2CH_2COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$, and III: $(^{14}ND_2CD_2COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$, respectively. The solute concentration has been chosen considering the solubility of glycine with coexisting NaOD in the present sample solutions. The "pD" value of the present solution was determined to be 10.6, implying that 92% of solute glycine molecules are in the anionic form. The sample parameters are summarized in Table 1.

Neutron Diffraction Measurements. The sample solution was sealed into a cylindrical quartz cell (7.3 mm in inner diameter and

Table 1. Isotopic Composition and Mean Scattering Lengths, b_N and b_{H_M} of Amino-Nitrogen and Methylene-Hydrogen Atoms, Total Cross Sections and the Number Densities Scaled in the Stoichiometric Unit, $(^*ND_2C^*H_{M2}COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$, σ_t and ρ, Respectively, for the Sample Solutions

Sample	^{14}N/%	^{15}N/%	H_M/%[a]	D_M/%[a]	$b_N/10^{-12}$ cm	$b_{H_M}/10^{-12}$ cm	σ_t/barns[b]	ρ/Å^{-3}
I $(^{14}ND_2CH_2COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$	99.6	0.4	100	0	0.936	−0.374	14.362	
II $(^{15}ND_2CH_2COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$	1.0	99.0	100	0	0.647	−0.374	14.265	0.03203
III$(^{14}ND_2CD_2COOD)_{0.02}(NaOD)_{0.02}(D_2O)_{0.96}$	99.6	0.4	2.0	98.0	0.936	0.647	13.249	

a) For methylene-hydrogen atoms. b) For incident neutron wavelength of 1.0 Å.

0.5 mm in thickness). TOF neutron diffraction measurements were carried out at 25 °C using the HIT-II spectrometer[5] installed at the High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. Scattered neutrons were detected by 104 ^{3}He detectors covering scattering angles of $10 \leq 2\theta \leq 157°$. 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.

Data Reduction. 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 coherent scattering lengths, and scattering and absorption cross sections for the constituent nuclei were respectively referred to those tabulated by Sears.[8] The wavelength dependence of total cross sections, σ_t, for H and D nuclei was estimated by the help of observed total cross sections for liquid H_2O and D_2O.[9] The corrected scattering intensity was converted to the absolute scattering cross section, $(d\sigma/d\Omega)^{obs}$, using scattered neutron intensities from the vanadium rod.

The first-order difference function,[10,11] $\Delta_N(Q)$, is defined as the numerical difference of normalized scattering cross sections from samples I and II, which are chemically identical in all respects except for the different isotopic composition of the amino-nitrogen atom within the glycine molecule,

$$\Delta_N(Q) = (d\sigma/d\Omega)^{obs}(\text{for sample I}) - (d\sigma/d\Omega)^{obs}(\text{for sample II}). \quad (1)$$

Since contributions from atom pairs which are independent of the nitrogen atom are completely canceled out in $\Delta_N(Q)$, the Fourier transform of $\Delta_N(Q)$ gives the distribution function around the nitrogen atom, $G_N(r)$. In addition, such a subtraction method has an advantage that the inelasticity distortion arising from the self-scattering contribution of H and D atoms can be eliminated. Since the difference functions from 66 sets of lower scattering angle detectors located at $10 \leq 2\theta \leq 51°$ in the present study agree well with each other, they were combined for the subsequent analysis.

$\Delta_N(Q)$, scaled at the stoichiometric unit, $(^*ND_2CH_{M2}COOD)_x(NaOD)_x(D_2O)_{1-2x}$, can be written as a linear combination of partial structure factors relating to the nitrogen atom as below:

$$\begin{aligned}\Delta_N(Q) = {} & A_N[a_{NN}(Q) - 1] + B_N[a_{NH_M}(Q) - 1] \\ & + C_N[a_{NC}(Q) - 1] + D_N[a_{NNa}(Q) - 1] \\ & + E_N[a_{NO}(Q) - 1] + F_N[a_{ND}(Q) - 1], \end{aligned} \quad (2)$$

where

$$\begin{aligned} A_N &= x^2(b_{^{14}N}^2 - b_{^{15}N}^2), \quad B_N = 4x^2 b_{H_M}(b_{^{14}N} - b_{^{15}N}), \\ C_N &= 4x^2 b_C(b_{^{14}N} - b_{^{15}N}), \quad D_N = 2x^2 b_{Na}(b_{^{14}N} - b_{^{15}N}), \\ E_N &= 2x(1+x) b_O(b_{^{14}N} - b_{^{15}N}), \quad F_N = 4x b_D(b_{^{14}N} - b_{^{15}N}). \end{aligned}$$

Numerical values of the weighting factors in Eq. 2, A_N—F_N, are listed in Table 2. The distribution function around the amino-nitrogen atom, $G_N(r)$, is derived by the Fourier transform of the observed $\Delta_N(Q)$:

$$\begin{aligned} G_N(r) = {} & 1 + (A_N + B_N + C_N + D_N + E_N + F_N)^{-1} \\ & \times (2\pi^2\rho r)^{-1} \int_0^{Q_{max}} Q\Delta_N(Q)\sin(Qr)dQ \\ = {} & [A_N g_{NN}(r) + B_N g_{NH_M}(r) + C_N g_{NC}(r) \\ & + D_N g_{NNa}(r) + E_N g_{NO}(r) + F_N g_{ND}(r)] \\ & \times (A_N + B_N + C_N + D_N + E_N + F_N)^{-1}. \end{aligned} \quad (3)$$

The upper limit, Q_{max}, was set to be 20 Å^{-1} in the present study.

The intramolecular N$\cdots\alpha$ contribution within the glycine molecule, $I_N^{intra}(Q)$, described below was evaluated employing molecular structural parameters from previous single crystal X-ray and neutron diffraction studies,[12,13] and from gas-phase electron diffraction studies,[14]

$$\begin{aligned} I_N^{intra}(Q) = {} & \sum 2c_N b_\alpha (b_{^{14}N} - b_{^{15}N}) \\ & \times \exp(-l_{N\alpha}^2 Q^2/2)\sin(Qr_{N\alpha})/(Qr_{N\alpha}), \end{aligned} \quad (4)$$

where c_N is the number of the N atoms in the stoichiometric unit. Parameters $l_{N\alpha}$ and $r_{N\alpha}$ denote the root-mean-square amplitude and internuclear distance for the N$\cdots\alpha$ pair, respectively. The evaluated $I_N^{intra}(Q)$ is thereafter subtracted from the observed total $\Delta_N(Q)$ to obtain the intermolecular difference function, $\Delta_N^{inter}(Q)$,

$$\Delta_N^{inter}(Q) = \Delta_N(Q) - I_N^{intra}(Q). \quad (5)$$

The intermolecular distribution function, $G_N^{inter}(r)$, is obtained by the Fourier transform of $\Delta_N^{inter}(Q)$ using Eq. 3 with the upper limit of the integral, $Q_{max} = 20$ Å^{-1}.

The first-order difference function, $\Delta_H(Q)$, involving structural information around the methylene–hydrogen atom within the glycine molecule, is given by the numerical difference of observed scattering cross sections from sample solutions with different H/D isotopic ratios with respect to the methylene–hydrogen atom, i.e.,

$$\begin{aligned} \Delta_H(Q) = {} & (d\sigma/d\Omega)^{obs}(\text{for sample III}) - (d\sigma/d\Omega)^{obs}(\text{for sample I}) \\ & + \text{correction term}. \end{aligned} \quad (6)$$

Table 2. Values of the Coefficients of $[a_{ij}(Q) - 1]$ in Eq. 2

Difference function	$A_N/10^{-3}$ barns	$B_N/10^{-3}$ barns	$C_N/10^{-3}$ barns	$D_N/10^{-3}$ barns	$E_N/10^{-3}$ barns	$F_N/10^{-3}$ barns
$\Delta_N(Q)$	0.18	−0.17	0.31	0.08	6.85	15.40

The correction term in Eq. 6 arises from the incomplete cancellation of the inelasticity distortion effect due to the difference in the inelasticity contribution from the methylene–hydrogen atom between the two solutions. In order to estimate the correction term for the present $\Delta_H(Q)$, the following procedure was adopted.

1) The intramolecular contribution, $I_H^{intra}(Q)$, was first subtracted from the observed $\Delta_H(Q)$ to obtain the uncorrected intermolecular difference function, $\Delta_H^{inter}(Q)$, involving the inelasticity distortion.

2) The uncorrected $\Delta_H^{inter}(Q)$ was Fourier-transformed to obtain the intermolecular distribution function, $G_H^{inter}(r)$. Unphysical ripples at $r \leq 1.5$ Å are then removed.

3) The corrected $G_H^{inter}(r)$ was back Fourier-transformed, and the resultant function was subtracted from the observed uncorrected $\Delta_H^{inter}(Q)$ to obtain the correction curve, $\delta(Q)$, involving errors associated with both the inelasticity distortion and statistical uncertainties.

4) $\delta(Q)$ was extensively smoothed and thereafter subtracted from the observed $\Delta_H(Q)$ to deduce the corrected difference function, $\Delta_H(Q)$.

$\Delta_H(Q)$, scaled at the stoichiometric unit, $(ND_2C^*H_{M2}COOD)_x$-$(NaOD)_x(D_2O)_{1-2x}$, can be expressed as a linear combination of partial structure factors relating to the methylene–hydrogen atoms within the glycine molecule, i.e.,

$$\begin{aligned}\Delta_H(Q) = {} & A_H[a_{H_MH_M}(Q) - 1] + B_H[a_{H_MC}(Q) - 1] \\ & + C_H[a_{H_MNa}(Q) - 1] + D_H[a_{H_MN}(Q) - 1] \\ & + E_H[a_{H_MO}(Q) - 1] + F_H[a_{H_MD}(Q) - 1], \end{aligned} \quad (7)$$

where

$$\begin{aligned} & A_H = 4x^2(b_{H_M}^2 - b_{H_M}'^2), B_H = 8x^2 b_C(b_{H_M} - b_{H_M}'), \\ & C_H = 4x^2 b_{Na}(b_{H_M} - b_{H_M}'), D_H = 4x^2 b_N(b_{H_M} - b_{H_M}'), \\ & E_H = 4x(1+x) b_O(b_{H_M} - b_{H_M}'), F_H = 8x b_D(b_{H_M} - b_{H_M}'). \end{aligned}$$

Weighting factors in Eq. 7, A_H—F_H, are numerically given in Table 3. The distribution function around the methylene–hydrogen atom within the glycine molecule, $G_H(r)$, is evaluated by the following Fourier transform:

$$\begin{aligned} G_H(r) = {} & 1 + (A_H + B_H + C_H + D_H + E_H + F_H)^{-1} \\ & \times (2\pi^2\rho r)^{-1} \int_0^{Q_{max}} Q\Delta_H(Q)\sin(Qr)\mathrm{d}Q \\ = {} & [A_H g_{H_MH_M}(r) + B_H g_{H_MC}(r) + C_H g_{H_MNa}(r) + D_H g_{H_MN}(r) \\ & + E_H g_{H_MO}(r) + F_H g_{H_MD}(r)] \\ & \times (A_H + B_H + C_H + D_H + E_H + F_H)^{-1}. \end{aligned} \quad (8)$$

The intramolecular $H_M\cdots\alpha$ contribution, $I_H^{intra}(Q)$, is estimated by the following equation:

$$\begin{aligned} I_H^{intra}(Q) = {} & \sum 2c_{H_M} b_\alpha (b_{H_M} - b_{H_M}') \\ & \times \exp(-l_{H_M\alpha}^2 Q^2/2)\sin(Qr_{H_M\alpha})/(Qr_{H_M\alpha}), \end{aligned} \quad (9)$$

where, c_{H_M} is the number of the methylene–hydrogen atoms. Values, $l_{H_M\alpha}$ and $r_{H_M\alpha}$, corresponding to the r.m.s. amplitude and internuclear distance for $H_M\cdots\alpha$ pair, respectively, were referred to those reported in the literature.[10—12] $I_H^{intra}(Q)$ calculated was then subtracted from the corrected $\Delta_H(Q)$ to deduce the intermolecular difference function, $\Delta_H^{inter}(Q)$:

$$\Delta_H^{inter}(Q) = \Delta_H(Q) - I_H^{intra}(Q). \quad (10)$$

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 $\Delta_X^{inter}(Q)$ (X: N, H) employing the following model function, $\Delta_X^{model}(Q)$, involving both short- and long-range contributions:[15—17]

$$\begin{aligned} \Delta_X^{model}(Q) = {} & \sum 2c_X n_{X\alpha} b_\alpha (b_X - b_X') \\ & \times \exp(-l_{X\alpha}^2 Q^2/2)\ \sin(Qr_{X\alpha})/(Qr_{X\alpha}) \\ & + 4\pi\rho(A_X + B_X + C_X + D_X + E_X + F_X) \\ & \times \exp(-l_{0X}^2 Q^2/2) \times [Qr_{0X}\ \cos(Qr_{0X}) \\ & - \sin(Qr_{0X})]Q^{-3}, \end{aligned} \quad (11)$$

where, $n_{X\alpha}$ denotes the coordination number of the α atom around a given X atom (=N, H). 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, $n_{X\alpha}$, $l_{X\alpha}$, $r_{X\alpha}$, l_{0X}, and r_{0X} in Eq. 11 are respectively determined from the least squares fit to the observed $\Delta_X^{inter}(Q)$. The present fitting procedure was performed in the range of $1.0 \leq Q \leq 20.0$ Å^{-1} with the SALS program,[18] under the assumption that the statistical uncertainties uniformly distribute.

Results and Discussion

Hydration Structure around the Amino Group. 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 alkaline 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 in $G_N(r)$ is attributed to the intramolecular N–C interaction. Partially resolved peaks at $r \approx 2.9$ and 3.2 Å, perhaps due to several intermolecular interactions, are considered to reflect the fact that water molecules in the first hydration shell of the amino group in the glycine molecule distribute with some preferred orientation. The calculated $I_N^{intra}(Q)$, 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, $\Delta_N^{inter}(Q)$, which is described in Fig. 1c. The Fourier transform of $\Delta_N^{inter}(Q)$, $G_N^{inter}(r)$, is represented in Fig. 2b. $G_N^{inter}(r)$ is characterized by the well-resolved first peak located at $r \approx 2$ Å and partially-resolved peaks appearing at $r \approx 2.9$ and 3.2 Å. The area of the first peak at $r \approx 2$ Å in the present $G_N^{inter}(r)$ was found

Table 3. Values of the Coefficients of $[a_{ij}(Q) - 1]$ in Eq. 7

Difference function	$A_H/10^{-3}$ barns	$B_H/10^{-3}$ barns	$C_H/10^{-3}$ barns	$D_H/10^{-3}$ barns	$E_H/10^{-3}$ barns	$F_H/10^{-3}$ barns
$\Delta_H(Q)$	0.44	2.17	0.59	1.53	48.35	108.78

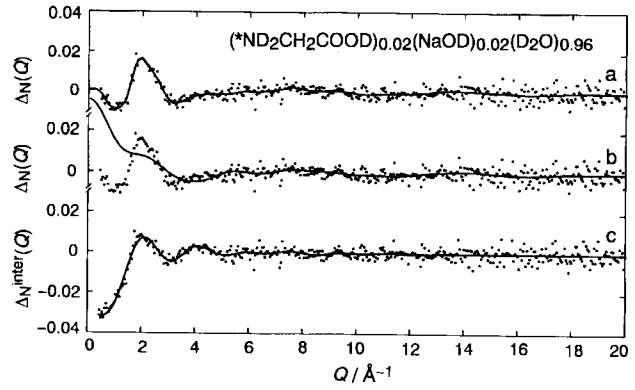

Fig. 1. a) Observed difference function, $\Delta_N(Q)$, for aqueous 2 mol% glycine heavy water solutions under high-pH condition (dots). Smoothed $\Delta_N(Q)$, which has been used for the Fourier transform (solid line). b) Observed difference function, $\Delta_N(Q)$ (dots), and the intramolecular contribution 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 $\Delta_N^{model}(Q)$ is shown by the solid line.

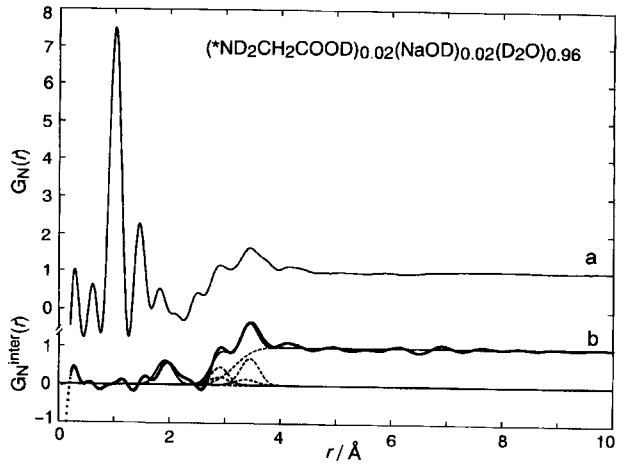

Fig. 2. a) Total distribution function around the amino-nitrogen atom, $G_N(r)$, for aqueous 2 mol% glycine heavy water solutions under high-pH condition. b) Observed intermolecular distribution function, $G_N^{inter}(r)$ (dots), and the Fourier transform of the calculated $\Delta_N^{model}(Q)$ (Fig. 1c) is shown by the solid line. Short- and long-range contributions are denoted by broken lines.

to correspond to ca. one deuterium atom through the preliminary analysis. Assuming that the hydrogen bond between the amino-nitrogen atom and one deuterium atom belonging to the nearest neighbor D_2O molecule is linear, the oxygen atom of this D_2O molecule is expected to be located at $r \approx 3$ Å. Indeed, the position of the second peak in $G_N^{inter}(r)$ roughly corresponds to this distance. The third peak located at $r \approx 3.2$ Å in $G_N^{inter}(r)$ is reasonably interpreted as the other deuterium atom within the same D_2O molecule. However, please notice that the second and third peaks in the present $G_N^{inter}(r)$ cannot be reproduced by the contribution from only one D_2O molecule, that is to say, other D_2O molecules in the first hydration shell contribute to these peaks. The position of these two peaks is in good agreement with the nearest neighbor intermolecular N···O (2.85 Å) and N···D (3.25 Å) distances found in neutral aqueous 5 mol% glycine solutions.[3,4] From this result, it may be expected that the first hydration shell of the amino-nitrogen atom within the glycine molecule is constructed by D_2O molecules with two different kinds of hydrogen bonds (or configurations) in alkaline solutions: i.e., I) One of the deuterium atoms of the D_2O molecule is directly hydrogen-bonded to the amino-nitrogen atom. II) The oxygen atom of other D_2O molecules is hydrogen-bonded through the intermediary of the amino-deuterium atom. The hydration structure around the amino group in alkaline solution is considered to be significantly different from that in the neutral solution. These interactions are involved in the theoretical intermolecular interference term (Eq. 11) employed for the present least-squares fit. The theoretical interference term was evaluated on the basis of the following assumptions. a) For the interaction between N and D_2O (by the case I), parameters, r_{ND}, l_{ND}, n_{ND}, and the bond angle, α (= ∠N···D–O), are treated as independent parameters. The molecular geometry of the D_2O molecule is fixed to that for pure heavy water ($r_{OD} = 0.983$ Å, $r_{DD} = 1.55$ Å).[19] b) Structural parameters describing the hydrogen-bonded interaction between the N and D_2O (by the case II), r_{NO}, l_{NO}, and the tilt angle between N···O axis and molecular plane of D_2O(II), β, are allowed to vary independently. c) The r.m.s. amplitudes for non-bonding interactions within the N···D_2O(I) and N···D_2O(II) units, l_{ij}^{I} and l_{ij}^{II}, are respectively approximated through the following equations:[15]

$$l_{ij}^{I} = l_{ND} \times (r_{ij}^{I}/r_{ND})^{1/2} \quad (12)$$

$$l_{ij}^{II} = l_{NO} \times (r_{ij}^{II}/r_{NO})^{1/2} \quad (13)$$

where r_{ij}^{I} and r_{ij}^{II} denote the calculated intermolecular distance within respective units. d) Structural parameters for the continuous long-range distribution of atoms, such as l_{0ij} and r_{0ij}, are taken to be identical for all N···X (X = O, D, H_M, N, C, and Na) interactions for the sake of reducing the number of independent parameters.

The best-fit result is compared with the observed $\Delta_N^{inter}(Q)$ in Fig. 1c. A satisfactory agreement is obtained between the observed and calculated $\Delta_N^{inter}(Q)$ in the range of $1.0 \leq Q \leq 20.0$ Å^{-1}. The observed and calculated $G_N^{inter}(r)$ (Fig. 2b) also agree well with each other, although periodic ripples due to the termination error are superimposed on the observed $G_N^{inter}(r)$. The final results of the least-squares fitting analysis for the present observed $\Delta_N^{inter}(Q)$ are summarized in Table 4. The present values of the bond angle α (174(5)°) and n_{ND} (1.14(3)) give a hint that the hydrogen bond of N···D–O type between the amino-nitrogen atom and ca. one D_2O(I) molecule is roughly linear. The value of r_{ND} is determined to be 1.97(3) Å. The hydration geometry found for the interaction between N and D_2O(II) is very close to that found in the neutral solution.[3] For example, it can be pointed out that some D_2O molecules in the first coordination shell of the amino-nitrogen atom are hydrogen-bonded with the N–D···OD_2 type and the molecular plane of D_2O(II) is tilted against the N···O axis. The present value of N···O distance

Table 4. Hydration Parameters around the Amino-Nitrogen Atom Observed for Aqueous Alkaline 2 mol% Glycine Solutions[a)]

Interaction	i–j	r_{ij}/Å	l_{ij}/Å	n_{ij}
N···D_2O(I)	N···D	1.97(3)	0.15(4)	1.14(3)
	α = 174(5)° [b)]			
N···D_2O(II)	N···O	2.93(3)	0.25(14)	1.93(3)
	β = 41(17)° [c)]			
		r_{0ij}/Å	l_{0ij}/Å	
Long-range	N···X	3.14(1)	0.27(2)	

a) Estimated standard deviations are given in parentheses. b) Bond angle ∠N···D–O. c) Tilt angel between N···O axis and molecular plane of D_2O.

(2.92(3) Å) is in good agreement also with the average value of the N(H)···O hydrogen-bond length found for various organic crystals (2.89 Å).[20] The tilt angle β is determined to be 41(17)°, the value of which is smaller than that found in the neutral solution (63(13)°). This suggests that there is a difference in the hydrogen-bond geometry of D_2O molecules in the second hydration shell of the amino group in alkaline and in neutral glycine solutions.

Hydration Structure around the Methylene Group. The observed $\Delta_H(Q)$ shown in Fig. 3a is characterized by the relatively smeared first peak at $Q \approx 2.8$ 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 (H_M), $G_H(r)$, 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 at $r \approx 2.2$ Å in $G_H(r)$ is attributable to various intramolecular non-bonding interactions in the

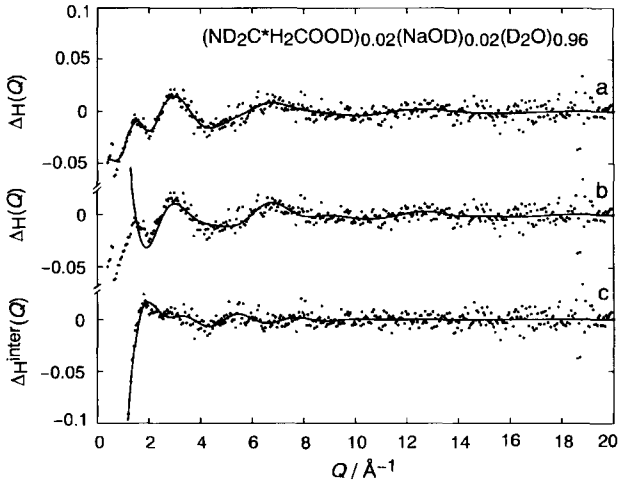

Fig. 3. a) Observed difference function, $\Delta_H(Q)$, for aqueous 2 mol% glycine heavy water solutions under high-pH condition (dots). Smoothed $\Delta_H(Q)$, which has been used for the Fourier transform (solid line). b) Observed difference function, $\Delta_H(Q)$ (dots), and the intramolecular contribution 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 $\Delta_H^{model}(Q)$ is shown by the solid line.

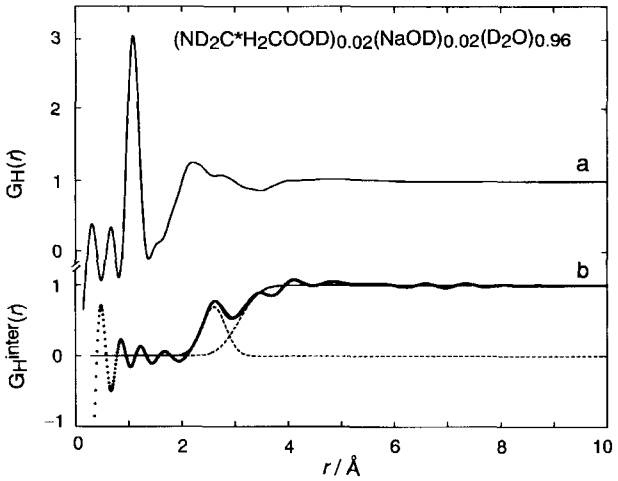

Fig. 4. a) Total distribution function around the methylene-hydrogen atom, $G_H(r)$, for aqueous 2 mol% glycine heavy water solutions under high-pH condition. b) Observed intermolecular distribution function, $G_H^{inter}(r)$ (dots), and the Fourier transform of the calculated $\Delta_H^{model}(Q)$ (Fig. 3c) is shown by the solid line. Short- and long-range contributions are denoted by broken lines.

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 next attempt to subtract the calculated $I_H^{intra}(Q)$ (Fig. 3b) from the observed $\Delta_H(Q)$ to deduce the intermolecular difference function, $\Delta_H^{inter}(Q)$, the result of which is given in Fig. 3c.

As seen in Fig. 4b, the partially-resolved first peak at $r \approx 2.6$ Å in $G_H^{inter}(r)$ obtained by the Fourier transform of $\Delta_H^{inter}(Q)$ clearly suggests that the weak hydration shell is present around the methylene group, although $G_H^{inter}(r)$ is rather structureless beyond this peak position. It is generally difficult to make an unambiguous assignment of the peak observed in $G_H^{inter}(r)$, because $G_H^{inter}(r)$ involves both contributions from H_M···O and H_M···D pairs. As the preliminary assignment, the peak at $r \approx 2.6$ Å in $G_H^{inter}(r)$ may be expected to correspond to the H_M···O contribution alone. However, the estimated coordination number of the O atom around the H_M atom is found to exceed 3, which is hardly acceptable from the viewpoint of the steric hindrance. Then, it can be interpreted that this peak involves the H_M···D contribution besides the H_M···O one. In the present least-squares fitting procedure, the contribution to the first hydration shell around the H_M atom was treated as a single kind of interaction, in which the sum of coherent scattering lengths of deuterium and oxygen atoms within a single D_2O molecule; $2b_D+b_O$, was taken as the coherent scattering length in Eq. 11, b_α. The fitting range of Q was $1.0 \leq Q \leq 20.0$ Å^{-1} using the SALS program.[18]

The result of the least-squares fit for the observed $\Delta_H^{inter}(Q)$ is represented in Fig. 3c. A satisfactory agreement is established between the observed and calculated $\Delta_H^{inter}(Q)$ in the whole Q-range covered. Final values of all independent parameters are summarized in Table 5. The coordination number, $n_{H_MD_2O} = 0.81(6)$, indicates that on the average ca.

Table 5. Hydration Parameters around the Methylene-Hydrogen Atom Observed for Aqueous Alkaline 2 mol% Glycine Solutions[a)]

Interaction	i–j	r_{ij}/Å	l_{ij}/Å	n_{ij}
$H_M \cdots D_2O$	$H_M \cdots D_2O$	2.63(1)	0.22(1)	0.81(6)
		r_{0ij}/Å	l_{0ij}/Å	
Long-range	$H_M \cdots X$	3.10(2)	0.30(3)	

a) Estimated standard deviations are given in parentheses.

two D_2O molecules are present in the first hydration shell around the H_M atom with the average distance of 2.63(1) Å. In order to obtain more detailed information containing the orientational correlation of the water molecule around the methylene group in the glycine molecule, the knowledge of partial distribution functions, $g_{H_MO}(r)$ and $g_{H_MD}(r)$, is indispensable. Such information may be supplied through further neutron diffraction experiments including the H/D isotopic substitution for solvent molecules in the aqueous glycine solution.

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References

1 M. Mezei, P. K. Mehrotra, and D. L. Biveridge, *J. Biomol. Struct. Dyn.*, **2**, 1 (1984).

2 J. M. Goodfellow, *Int. J. Biol. Macromol.*, **9**, 273 (1987).

3 Y. Kameda, H. Ebata, T. Usuki, O. Uemura, and M. Misawa, *Bull. Chem. Soc. Jpn.*, **67**, 3159 (1994).

4 Y. Kameda, H. Ebata, T. Usuki, O. Uemura, and M. Misawa, "KENS REPORT-X," (1995), p. 89.

5 T. Fukunaga, M. Misawa, I. Fujikura, and S. Satoh, "KENS REPORT-IX," (1993), p. 16.

6 H. H. Paalman and C. J. Pings, *J. Appl. Phys.*, **33**, 2635 (1962).

7 I. A. Bach and B. L. Averbach, *Phys. Rev.*, **137**, A1113 (1965).

8 V. F. Sears, "Thermal-Neutron Scattering Lengths and Cross Sections for Codensed Matter Research," Atomic Energy of Canada Ltd., AECL-8490 (1984).

9 J. R. Granada, V. H. Gillete, and R. E. Mayer, *Phys. Rev. A*, **36**, 5594 (1987).

10 A. K. Soper, G. W. Neilson, J. E. Enderby, and R. A. Howe, *J. Phys. C: Solid State Phys.*, **10**, 1793 (1977).

11 J. E. Enderby and G. W. Neilson, "Water, A Comprehensive Treatise," ed by F. Franks, Plenum Press, New York (1979), Vol. 6, p. 1.

12 P. -G. Jönsson and Å. Kvick, *Acta Crystallogr., Sect. B*, **B28**, 1827 (1972).

13 J. Almlöf, Å. Kvick, and J. O. Thomas, *J. Chem. Phys.*, **59**, 3901 (1973).

14 K. Iijima, K. Tanaka, and S. Onuma, *J. Mol. Struct.*, **246**, 257 (1991).

15 A. H. Narten, M. D. Danford, and H. A. Levy, *Discuss. Faraday Soc.*, **43**, 97 (1967).

16 R. Caminiti, P. Cucca, M. Monduzzi, G. Saba, and G. Crisponi, *J. Chem. Phys.*, **81**, 543 (1984).

17 H. Ohtaki and N. Fukushima, *J. Solution Chem.*, **21**, 23 (1992).

18 T. Nakagawa and Y. Oyanagi, "Recent Developments in Statistical Inference and Data Analysis," ed by K. Matushita, North-Holland (1980), p. 221.

19 Y. Kameda and O. Uemura, *Bull. Chem. Soc. Jpn.*, **65**, 2021 (1992).

20 L. N. Kuleshova and P. M. Zorkii, *Acta Crystallogr., Sect. B*, **B37**, 1363 (1981).