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Hydration Structure of Alanine Molecule in Concentrated Aqueous Solutions

Yasuo Kameda,* Kentaro Sugawara, Takeshi Usuki, and Osamu Uemura

Department of Material and Biological Chemistry, Faculty of Science, Yamagata University, Yamagata 990-8560

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Time-of-Flight (TOF) neutron diffraction measurements have been carried out on aqueous 3 mol% D,L-alanine solutions. The isotopic substitution technique has been applied to both nitrogen and methyl-hydrogen atoms within the alanine molecule in order to obtain information concerning the hydration structure around the amino- and the methyl groups within the alanine molecule in the aqueous solution. There exist, on the average, 2.4(1) water molecules that are hydrogen bonded to the amino group with an intermolecular distance of $r_{NO} = 2.88(2)$ Å. The tilt angle between the N–D···O axis and the molecular plane of the D_2O molecule has been determined to be 60.4(5)°. The first hydration shell of the methyl group involves ca. two water molecules per one alanine molecule. The nearest neighbor H_M(methyl)–O_W(water) and H_M(methyl)–H_W(water) distances have been determined to be 2.58(1) and 2.99(2) Å, respectively.

Hydration structure of amino acid molecules has long been a matter of interest for extensive areas of chemical and biological sciences. In aqueous solution, the amino acid molecule is known to exist as a zwitterion. Each functional group within the amino acid molecule is considered to form a particular hydration environment, which should be affected by intermolecular interaction between atoms involved in the functional group and neighboring water molecules.[1] The hydration structure around the amino group of the glycine molecule in aqueous solutions of neutral,[2] alkaline,[3] and acidic[4] conditions has been investigated by means of neutron diffraction with isotopic substitution technique. The results have showed that three water molecules form hydrogen bonds of the N–D···OD_2 type in the neutral ($r_{NO} = 2.85(5)$ Å),[2] and in the acidic ($r_{NO} = 2.90(2)$ Å)[4] solutions, respectively. On the other hand, the amino group of the glycine molecule forms one hydrogen bond of the N···D–OD type ($r_{ND} = 1.97(3)$ Å) and simultaneously forms bonds of N–D···OD_2 type with ca. two water molecules ($r_{NO} = 2.92(3)$ Å) in the alkaline solution.[3] These results confirm that the hydration structure around the amino group is strongly pH dependent.

The hydration structure around the apolar methylene group within the glycine molecule has also been investigated by neutron diffraction using the H/D isotopic substitution method.[3,4] The intermolecular distribution function around the methylene-hydrogen atom observed for aqueous 2 mol% glycine solutions in D_2O with both alkaline and acidic conditions exhibit incompletely resolved first peaks located at $r = 2.63$ and 2.68 Å for the alkaline and acidic solutions, respectively. However, the orientation of neighboring water molecules has not been determined. The average coordination numbers of D_2O molecules around a methylene-hydrogen atom of the glycine molecule, are 0.81(6)[3] and 0.66(1)[4] for the alkaline and the acidic solutions, respectively. It should be of considerable interest to investigate the hydration structure of the other amino acids with more complex molecular structure. In order to obtain the structural information concerning the hydration around a particular functional group of the amino acid molecule, neutron diffraction with the isotopic substitution technique is one of the most suitable experimental methods.

In this paper, we describe the results of the TOF neutron diffraction measurements on $^{14}N/^{15}N$ and H/D isotopically substituted five 3 mol% alanine solutions. The difference function, $\Delta_N(Q)$, between scattering cross sections observed for $(^{14}ND_2CH_3CHCOOD)_{0.03}(D_2O)_{0.97}$ and $(^{15}ND_2CH_3CHCOOD)_{0.03}(D_2O)_{0.97}$ solutions provides information concerning the hydration structure around the amino group in the alanine molecule. The details of the hydration structure around the methyl-hydrogen atoms are deduced from the partial structure factors, $a_{H_MH_{ex}}(Q)$ and $a_{H_MZ}(Q)$, (H_M: methyl-hydrogen, H_{ex}: exchangeable hydrogen, and Z: O, N, H_α, H_M, and C atoms, respectively) which are deduced from the combination of the observed difference functions, $\Delta_H(Q)$, between $(^{14}ND_2CD_3CHCOOD)_{0.03}(D_2O)_{0.97}$ and $(^{14}ND_2CH_3CHCOOD)_{0.03}(D_2O)_{0.97}$, and $\Delta_{H'}(Q)$, between $(^{14}NX_2CD_3CHCOOX)_{0.03}(X_2O)_{0.97}$ and $(^{14}NX_2CH_3CHCOOX)_{0.03}(X_2O)_{0.97}$ solutions (X: H/D = 20/80), respectively. In order to obtain reliable difference functions, we adopted the inelasticity correction procedure to the observed scattering data, employing the self-scattering intensity observed for the null water, ^0H_2O, in which the average coherent scattering length of the hydrogen atom is zero.

Experimental

Materials. Isotopically enriched D,L-$^{15}NH_2CH_3CHCOOH$ (99.0% ^{15}N, Isotech Inc.), D,L-$^{14}NH_2CD_3CHCOOH$ (99.0% methyl-D (manufacturer's specification), 99.6% ^{14}N (natural abundance), Isotech Inc.), and natural D,L-$NH_2CH_3CHCOOH$ (99.6% ^{14}N (natural abundance), Nacalai Tesque, guaranteed grade) were deuterated by dissolving them into 10 times the molar quantity of D_2O (99.9% D, Aldrich Chemical Co., Inc.), followed by the dehydration under the vacuum. This procedure was repeated 4 times. The H/D isotopic composition of the methyl-hydrogen atoms in the deuterated crystalline D,L-$^{14}ND_2CD_3CHCOOD$ was evaluated from the integrated intensity of the diffuse reflectance infrared spectra of the C–H stretching vibrational band. The infrared spectra were obtained using a JASCO FT/IR-410 spectrometer with DR-81 diffuse reflection attachment. The observed value of the D content, 99.1 ± 0.5%, is in good agreement with that quoted in the manufacturer's specification. The required amounts of enriched compounds, D,L-$^{14}ND_2CH_3CHCOOD$, D,L-$^{15}ND_2CH_3CHCOOD$, and D,L-$^{14}ND_2CD_3CHCOOD$, were dissolved into D_2O and into the H_2O–D_2O mixture, X_2O, (H/D = 20/80) to prepare five kinds of aqueous 3 mol% alanine solutions with different isotopic compositions of both the amino-nitrogen and the methyl-hydrogen atoms within the alanine molecule, and also the exchangeable hydrogen atoms, i.e., Ⅰ: $(^{14}ND_2CH_3CHCOOD)_{0.03}(D_2O)_{0.97}$, Ⅱ: $(^{15}ND_2CH_3CHCOOD)_{0.03}(D_2O)_{0.97}$, Ⅲ: $(^{14}ND_2CD_3CHCOOD)_{0.03}(D_2O)_{0.97}$, Ⅳ: $(^{14}NX_2CD_3CHCOOX)_{0.03}(X_2O)_{0.97}$, and Ⅴ: $(^{14}NX_2CH_3CHCOOX)_{0.03}(X_2O)_{0.97}$, respectively. The isotopic ratio of the exchangeable hydrogen atoms in the sample solutions Ⅳ and Ⅴ (H/D = 20/80), has been chosen to avoid the large incoherent scattering from H atom and to keep a sufficient difference in the scattering length of the exchangeable hydrogen atoms within both water and alanine molecules. The H/D ratio of the exchangeable hydrogen atoms within the D_2O solutions (Ⅰ, Ⅱ, and Ⅲ), was carefully determined through measurements for the integrated absorption intensity of the O–H stretching vibrational band using the JASCO FT/IR-410 spectrometer with the ATR attachment (ATR-500/M with a Ge prism). The D content of the exchangeable hydrogen atoms was estimated to be 99.9 ± 0.1%. The sample parameters are listed in Table 1.

Neutron Diffraction Measurements. The sample solution was sealed into a cylindrical fused quartz cell (7.3 mm in inner diameter and 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 counters covering scattering angles of $10 \leq 2\theta \leq 157°$. The data accumulation time was ca. 6 h for each sample. Measurements were made in advance for an empty cell, background, and a vanadium rod of 8 mm in diameter, respectively.

Data Reduction. Observed scattering intensities from the sample were corrected for the instrumental background, absorption of both the sample and cell,[6] and for multiple[7] and incoherent scatterings. The coherent scattering lengths, scattering and absorption cross sections for the constituent nuclei were referred to those tabulated by Sears.[8,9] The wavelength dependence of total cross sections, σ_t, for H and D nuclei was estimated from observed total cross sections for liquid H_2O and D_2O, respectively.[10] The corrected scattering intensity was converted to the absolute scattering cross section, $(d\sigma/d\Omega)^{obs}$, using corrected scattering intensities from the vanadium rod.

The first-order difference function, $\Delta_\alpha(Q)$, can be essentially derived from a numerical difference of observed scattering cross section between sample solutions which are identical in all except for the isotopic composition of the α atom.[11,12] The inelasticity effect, mainly arising from water hydrogen atoms, is generally expected to cancel out in the $\Delta_\alpha(Q)$ function. However, the inelasticity distortion remaining in the observed difference function, $\Delta_H(Q)$, obtained from the H/D isotopic substitution experiment, can frequently be a troublesome obstacle to extracting structural properties from the observed $\Delta_H(Q)$ and its Fourier transform $G_H(r)$ functions. In spite of considerable effort to elucidate theoretical estimation of the inelasticity correction for the observed neutron scattering data,[10,13–17] no successful procedure has been found that can be applied with sufficient accuracy at present for the sample containing large numbers of hydrogen atoms. Recently, empirical approaches using polynomial functions have been proposed for neutron diffraction data obtained from the reactor[18] and TOF[19] experiments. However, in the present case, it was found to be difficult to evaluate the inelasticity term by the polynomial method without any assumption of the structure factor for the sample solution itself, particularly in the lower-Q region. We therefore applied the correction procedure employing the scattering cross section observed for liquid ^0H_2O[20] in which the average scattering length of the hydrogen atom is set to be zero. Since the observed scattering cross section of the ^0H_2O involves no contribution from H–H and O–H interference terms, the self scattering term of the ^0H_2O can be derived by subtracting the O–O partial structure factor, $a_{OO}(Q)$, from the observed scattering cross section of the ^0H_2O.

$$(d\sigma/d\Omega)^{self}(\text{for } ^0H_2O) = (d\sigma/d\Omega)^{obs}(\text{for } ^0H_2O) - b_O{}^2[a_{OO}(Q) - 1]. \quad (1)$$

In the present analysis, $a_{OO}(Q)$ for the liquid water was evaluated using the short- and the long-range structure parameters determined in our previous neutron diffraction study.[21] The self-scattering term in Eq. 1 consists of the sum of the inelasticity terms mainly arising from the H atom and the elastic scattering contributions from H, D, and O atoms, which can be treated as a constant value against Q.

Table 1. Isotopic Compositions and Mean Scattering Lengths, b_N, b_{H_M}, and b_{H_W}, of Nitrogen, Methyl-Hydrogen, and Exchangeable Hydrogen Atoms, Total Cross Sections and Number Density Scaled in the Stoichiometric Unit, $(^*N^*H_2C^*H_3CHCOO^*H)_{0.03}(^*H_2O)_{0.97}$, σ_t and ρ, Respectively

Sample	^{14}N /%	^{15}N /%	H_M a) /%	D_M a) /%	H_W b) /%	D_W b) /%	b_N /10^{-12} cm	b_{H_M} a) /10^{-12} cm	b_{H_W} b) /10^{-12} cm	σ_t c) /barns	ρ/Å^{-3}
Ⅰ $(^{14}ND_2CH_3CHCOOD)_{0.03}(D_2O)_{0.97}$	99.6	0.4	100	0	0.1	99.9	0.936	−0.374	0.666	17.627	0.03075
Ⅱ $(^{15}ND_2CH_3CHCOOD)_{0.03}(D_2O)_{0.97}$	1.0	99.0	100	0	0.1	99.9	0.647	−0.374	0.666	17.434	
Ⅲ $(^{14}ND_2CD_3CHCOOD)_{0.03}(D_2O)_{0.97}$	99.6	0.4	1.0	99.0	0.1	99.9	0.936	0.657	0.666	14.546	
Ⅳ $(^{14}NX_2CD_3CHCOOX)_{0.03}(X_2O)_{0.97}$	99.6	0.4	1.0	99.0	20.0	80.0	0.936	0.657	0.459	27.034	
Ⅴ $(^{14}NX_2CH_3CHCOOX)_{0.03}(X_2O)_{0.97}$	99.6	0.4	100	0	20.0	80.0	0.936	−0.374	0.459	29.766	

a) For methyl-hydrogen atoms of alanine molecule. b) For exchangeable hydrogen atoms. c) For incident neutron wavelength of 1.0 Å.

$$\begin{aligned}(\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{self}}(\text{for } {}^{0}\mathrm{H_2O}) = {} & (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{inelasticity}}(\text{for H}) \\ & + (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{inelasticity}}(\text{for D}) \\ & + (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{inelasticity}}(\text{for O}) \\ & + \text{constant}. \qquad (2)\end{aligned}$$

Since the magnitude of the inelasticity term of both O and D atoms in Eq. 2 is expected to be much smaller than that for the H atom, the inelasticity part of the $(\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{self}}$(for ${}^{0}\mathrm{H_2O}$) can be approximately regarded as the inelasticity contribution from the H atom. Consequently, we adopted the inelasticity correction for the sample solutions by the following equation:

$$\begin{aligned}(\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{corrected}}(\text{for sample}) = {} & (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{obs}}(\text{for sample}) \\ & - N_{\mathrm{H}}{}^{\mathrm{sample}}/N_{\mathrm{H}}{}^{{}^{0}\mathrm{H_2O}} \\ & \times (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{self}}(\text{for } {}^{0}\mathrm{H_2O}). \qquad (3)\end{aligned}$$

Here, $N_{\mathrm{H}}{}^{\mathrm{sample}}$ and $N_{\mathrm{H}}{}^{{}^{0}\mathrm{H_2O}}$ denote the number of H atoms involved in the stoichiometric units, $(\mathrm{N^{*}H_2C^{*}H_3CHCOO^{*}H})_{0.03}(^{*}\mathrm{H_2O})_{0.97}$ and ${}^{0}\mathrm{H_2O}$, respectively. Figure 1 compares the scattering cross sections for aqueous 3 mol% ${}^{14}\mathrm{NX_2CD_3CHCOOX}$ solution in $\mathrm{X_2O}$ (X: H/D = 20/80) observed at the scattering angles of $2\theta = 14$, 30, and 51°, before and after the inelasticity correction. The interference feature of the corrected scattering cross sections observed for three independent scattering angles agree satisfactorily, and favorably oscillate around a horizontal line. The results indicate that the present inelasticity correction procedure can be applied successfully to the aqueous solutions containing considerable numbers of H atoms.

The first-order difference function, $\Delta_{\mathrm{N}}(Q)$,[11,12] is derived from the numerical difference between fully corrected scattering cross sections of samples I and II, which are chemically identical in all except for the different isotopic composition of the amino nitrogen atom within the alanine molecule,

$$\begin{aligned}\Delta_{\mathrm{N}}(Q) = {} & (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{corrected}}(\text{for sample I}) \\ & - (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{corrected}}(\text{for sample II}). \qquad (4)\end{aligned}$$

Since the difference functions from 64 sets of forward angle detectors located at $10 \le 2\theta \le 51°$ agree well within the statistical uncertainties, they were combined at the Q-intervals of 0.1 Å^{-1}, and used for the subsequent analysis.

The $\Delta_{\mathrm{N}}(Q)$, scaled at the stoichiometric unit, $(^{*}\mathrm{ND_2CH_3CHCOOD})_x(\mathrm{D_2O})_{1-x}$, can be written as a linear combination of partial structure factors related to the nitrogen atom:

$$\begin{aligned}\Delta_{\mathrm{N}}(Q) = {} & A_{\mathrm{N}}[a_{\mathrm{NN}}(Q)-1] + B_{\mathrm{N}}[a_{\mathrm{NC}}(Q)-1] \\ & + C_{\mathrm{N}}[a_{\mathrm{NH_\alpha}}(Q)-1] + D_{\mathrm{N}}[a_{\mathrm{NH_M}}(Q)-1] \\ & + E_{\mathrm{N}}[a_{\mathrm{NO_C}}(Q)-1] + F_{\mathrm{N}}[a_{\mathrm{NH_N}}(Q)-1] \\ & + G_{\mathrm{N}}[a_{\mathrm{NO_W}}(Q)-1] + H_{\mathrm{N}}[a_{\mathrm{NH_W}}(Q)-1], \qquad (5)\end{aligned}$$

where

$$\begin{aligned}& A_{\mathrm{N}} = x^2(b_{^{14}\mathrm{N}}{}^2 - b_{^{15}\mathrm{N}}{}^2), B_{\mathrm{N}} = 6x^2 b_{\mathrm{C}}(b_{^{14}\mathrm{N}} - b_{^{15}\mathrm{N}}), \\ & C_{\mathrm{N}} = 2x^2 b_{\mathrm{H_\alpha}}(b_{^{14}\mathrm{N}} - b_{^{15}\mathrm{N}}), D_{\mathrm{N}} = 6x^2 b_{\mathrm{H_M}}(b_{^{14}\mathrm{N}} - b_{^{15}\mathrm{N}}), \\ & E_{\mathrm{N}} = 4x^2 b_{\mathrm{O}}(b_{^{14}\mathrm{N}} - b_{^{15}\mathrm{N}}), F_{\mathrm{N}} = 6x^2 b_{\mathrm{H_N}}(b_{^{14}\mathrm{N}} - b_{^{15}\mathrm{N}}), \\ & G_{\mathrm{N}} = 2x(1-x) b_{\mathrm{O}}(b_{^{14}\mathrm{N}} - b_{^{15}\mathrm{N}}), \\ & H_{\mathrm{N}} = 4x(1-x) b_{\mathrm{H_W}}(b_{^{14}\mathrm{N}} - b_{^{15}\mathrm{N}}).\end{aligned}$$

H_α, $\mathrm{H_M}$, $\mathrm{H_N}$, and $\mathrm{H_W}$ denote the methine-, methyl-, amino-, and water-hydrogen atoms, respectively. $\mathrm{O_C}$ and $\mathrm{O_W}$ are the carboxyl- and water-oxygen atoms, respectively. Numerical values of the weighting factors in Eq. 5, A_{N} - H_{N}, are listed in Table 2. The distribution function around the amino-nitrogen atom, $\mathrm{G_N}(r)$, is deduced from the Fourier transform of the $\Delta_{\mathrm{N}}(Q)$:

$$\begin{aligned}\mathrm{G_N}(r) = 1 + (& A_{\mathrm{N}} + B_{\mathrm{N}} + C_{\mathrm{N}} \\ & + D_{\mathrm{N}} + E_{\mathrm{N}} + F_{\mathrm{N}} + G_{\mathrm{N}} + H_{\mathrm{N}})^{-1} \\ & \times (2\pi^2 \rho r)^{-1} \int_0^{Q_{\max}} Q \Delta_{\mathrm{N}}(Q) \sin(Qr)\,\mathrm{d}Q\end{aligned}$$

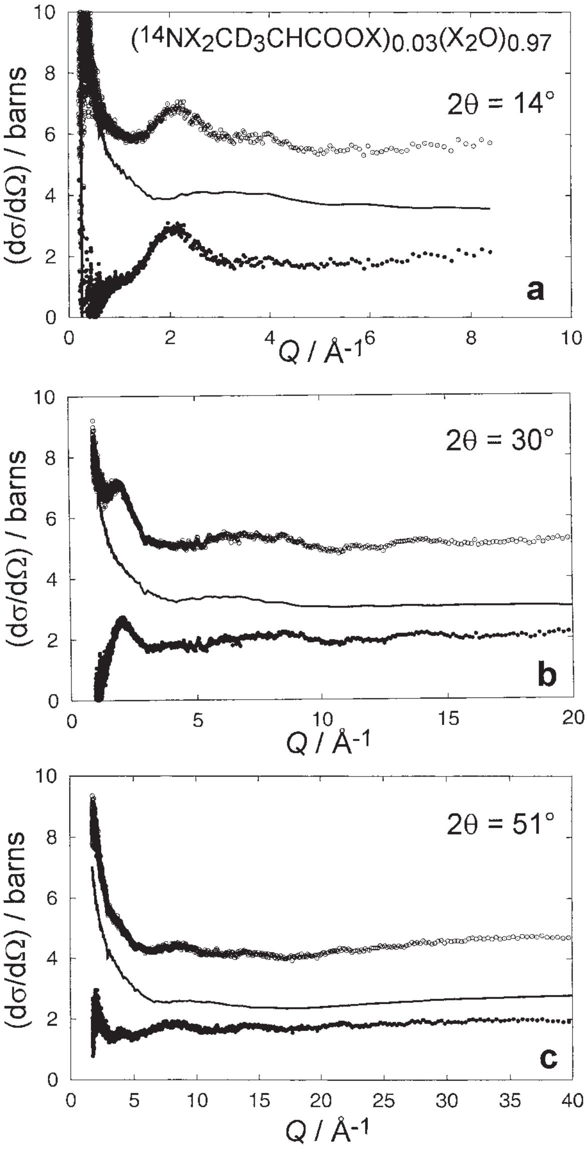

Fig. 1. a) Circles: The observed scattering cross section of the 3 mol% alanine solution with the isotopic ratio of exchangeable hydrogen atom is H/D = 20/80 (sample IV), observed at $2\theta = 14°$. Solid line: The self-scattering term of the null water, ${}^{0}\mathrm{H_2O}$, scaled by using Eq. 3. Dots: Corrected scattering cross section. b) and c) Same notations as a) except for the scattering angles of $2\theta = 30°$ and 51°, respectively.

Table 2. Values of the Coefficients of $a_{\mathrm{ij}}(Q)$ in Eqs. 5 and 12

Difference Function	A/barns	B/barns	C/barns	D/barns	E/barns	F/barns	G/barns	H/barns
$\Delta_{\mathrm{N}}(Q)$	0.00042	0.00104	−0.00020	−0.00058	0.00060	0.00104	0.00976	0.02240
$\Delta_{\mathrm{H}}(Q)$	0.00236	0.01111	−0.00208	0.00521	0.00646	0.01112	0.10451	0.23980
$\Delta_{\mathrm{H'}}(Q)$	0.00189	0.01111	−0.00208	0.00521	0.00646	0.00767	0.10451	0.16530

$$
\begin{aligned}
&= [A_{\mathrm{N}}g_{\mathrm{NN}}(r) + B_{\mathrm{N}}g_{\mathrm{NC}}(r) + C_{\mathrm{N}}g_{\mathrm{NH}_\alpha}(r)\\
&\quad + D_{\mathrm{N}}g_{\mathrm{NH_M}}(r) + E_{\mathrm{N}}g_{\mathrm{NO_C}}(r) + F_{\mathrm{N}}g_{\mathrm{NH_N}}(r)\\
&\quad + G_{\mathrm{N}}g_{\mathrm{NO_W}}(r) + H_{\mathrm{N}}g_{\mathrm{NH_W}}(r)] \times (A_{\mathrm{N}} + B_{\mathrm{N}}\\
&\quad + C_{\mathrm{N}} + D_{\mathrm{N}} + E_{\mathrm{N}} + F_{\mathrm{N}} + G_{\mathrm{N}} + H_{\mathrm{N}})^{-1}. \qquad (6)
\end{aligned}
$$

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

The intramolecular N···α contribution within the alanine molecule, $I_{\mathrm{N}}{}^{\mathrm{intra}}(Q)$, described below, was evaluated by applying molecular parameters from single crystal X-ray[22] and gas-phase electron[23] diffraction studies,

$$
\begin{aligned}
I_{\mathrm{N}}{}^{\mathrm{intra}}(Q) &= \sum 2c_{\mathrm{N}}b_\alpha(b_{^{14}\mathrm{N}} - b_{^{15}\mathrm{N}})\\
&\quad \times \exp(-l_{\mathrm{N}_\alpha}{}^2Q^2/2)\sin(Qr_{\mathrm{N}_\alpha})/(Qr_{\mathrm{N}_\alpha}), \qquad (7)
\end{aligned}
$$

where c_{N} is the number of the N atoms in the stoichiometric unit. Parameters l_{N_α} and r_{N_α} denote the root-mean-square amplitude and the internuclear distance for the N···α pair, respectively. $I_{\mathrm{N}}{}^{\mathrm{intra}}(Q)$ was then subtracted from the total $\Delta_{\mathrm{N}}(Q)$ to obtain the intermolecular difference function, $\Delta_{\mathrm{N}}{}^{\mathrm{inter}}(Q)$,

$$
\Delta_{\mathrm{N}}{}^{\mathrm{inter}}(Q) = \Delta_{\mathrm{N}}(Q) - I_{\mathrm{N}}{}^{\mathrm{intra}}(Q). \qquad (8)
$$

The intermolecular distribution function, $\mathrm{G_N}{}^{\mathrm{inter}}(r)$, is obtained by the Fourier transform of $\Delta_{\mathrm{N}}{}^{\mathrm{inter}}(Q)$ using Eq. 6 with the upper limit of the integral, $Q_{\max} = 20$ Å^{-1}.

Hydration parameters concerning the amino-nitrogen atom within the alanine molecule were determined through the least squares fitting procedure for observed intermolecular $\Delta_{\mathrm{N}}{}^{\mathrm{inter}}(Q)$ employing the following model function, $\Delta_{\mathrm{N}}{}^{\mathrm{model}}(Q)$, involving both short- and long-range contributions:[24–26]

$$
\begin{aligned}
\Delta_{\mathrm{N}}{}^{\mathrm{model}}(Q) &= \sum 2c_{\mathrm{N}}n_{\mathrm{N}_\alpha}b_\alpha(b_{^{14}\mathrm{N}} - b_{^{15}\mathrm{N}})\\
&\quad \times \exp(-l_{\mathrm{N}_\alpha}{}^2Q^2/2)\ \sin(Qr_{\mathrm{N}_\alpha})/(Qr_{\mathrm{N}_\alpha})\\
&\quad + 4\pi\rho(A_{\mathrm{N}} + B_{\mathrm{N}} + C_{\mathrm{N}} + D_{\mathrm{N}} + E_{\mathrm{N}} + F_{\mathrm{N}} + G_{\mathrm{N}} + H_{\mathrm{N}})\\
&\quad \times \exp(-l_0{}^2Q^2/2)[Qr_0\cos(Qr_0) - \sin(Qr_0)]Q^{-3}, \qquad (9)
\end{aligned}
$$

where n_{N_α} denotes the coordination number of α atoms around the N atom. The long-range parameter, r_0, means the distance beyond which the continuous distribution of atoms around the N atom can be assumed. The parameter, l_0, describes the sharpness of the boundary at r_0. Structural parameters, n_{N_α}, l_{N_α}, r_{N_α}, l_0, and r_0 are respectively determined from the least squares fit to the observed $\Delta_{\mathrm{N}}{}^{\mathrm{inter}}(Q)$. The fitting procedure was performed in the range of $0.8 \le Q \le 12$ Å^{-1} with the SALS program,[27] assuming that the statistical uncertainties distribute uniformly.

The first-order difference functions, $\Delta_{\mathrm{H}}(Q)$ and $\Delta_{\mathrm{H'}}(Q)$, involving structural information around the methyl-hydrogen atoms within the alanine molecule, are given by numerical differences between corrected scattering cross sections observed for sample solutions Ⅲ and Ⅰ, and between those for sample solutions Ⅳ and Ⅴ, respectively.

$$
\begin{aligned}
\Delta_{\mathrm{H}}(Q) &= (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{corrected}}(\text{for sample Ⅲ})\\
&\quad - (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{corrected}}(\text{for sample Ⅰ}), \qquad (10)\\
\Delta_{\mathrm{H'}}(Q) &= (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{corrected}}(\text{for sample Ⅳ})\\
&\quad - (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{corrected}}(\text{for sample Ⅴ}). \qquad (11)
\end{aligned}
$$

The difference function, $\Delta_{\mathrm{H}}(Q)$, can be expressed as a linear combination of partial structure factors relating to the methyl-hydrogen atoms within the alanine molecule, i.e.,

$$
\begin{aligned}
\Delta_{\mathrm{H}}(Q) &= A_{\mathrm{H}}[a_{\mathrm{H_MH_M}}(Q) - 1] + B_{\mathrm{H}}[a_{\mathrm{H_MC}}(Q) - 1]\\
&\quad + C_{\mathrm{H}}[a_{\mathrm{H_MH_\alpha}}(Q) - 1] + D_{\mathrm{H}}[a_{\mathrm{H_MN}}(Q) - 1]\\
&\quad + E_{\mathrm{H}}[a_{\mathrm{H_MO_C}}(Q) - 1] + F_{\mathrm{H}}[a_{\mathrm{H_MH_N}}(Q) - 1]\\
&\quad + G_{\mathrm{H}}[a_{\mathrm{H_MO_W}}(Q) - 1] + H_{\mathrm{H}}[a_{\mathrm{H_MH_W}}(Q) - 1], \qquad (12)
\end{aligned}
$$

where

$$
\begin{aligned}
&A_{\mathrm{H}} = 9x^2(b_{\mathrm{H_M}}{}^2 - b_{\mathrm{H_{M'}}}{}^2), B_{\mathrm{H}} = 18x^2b_{\mathrm{C}}(b_{\mathrm{H_M}} - b_{\mathrm{H_{M'}}}),\\
&C_{\mathrm{H}} = 6x^2b_{\mathrm{H_\alpha}}(b_{\mathrm{H_M}} - b_{\mathrm{H_{M'}}}), D_{\mathrm{H}} = 6x^2b_{\mathrm{H_M}}(b_{\mathrm{H_M}} - b_{\mathrm{H_{M'}}}),\\
&E_{\mathrm{H}} = 12x^2b_{\mathrm{O}}(b_{\mathrm{H_M}} - b_{\mathrm{H_{M'}}}), F_{\mathrm{H}} = 18x^2b_{\mathrm{H_N}}(b_{\mathrm{H_M}} - b_{\mathrm{H_{M'}}}),\\
&G_{\mathrm{H}} = 6x(1 - x)b_{\mathrm{O}}(b_{\mathrm{H_M}} - b_{\mathrm{H_{M'}}}),\\
&H_{\mathrm{H}} = 12x(1 - x)b_{\mathrm{H_W}}(b_{\mathrm{H_M}} - b_{\mathrm{H_{M'}}}).
\end{aligned}
$$

Numerical values of the weighting factors in Eq. 12, A_{H}–H_{H}, for difference functions, $\Delta_{\mathrm{H}}(Q)$ and $\Delta_{\mathrm{H'}}(Q)$, are given in Table 2. The distribution function around the methyl-hydrogen atom, $\mathrm{G_H}(r)$, is evaluated by the following Fourier transform:

$$
\begin{aligned}
\mathrm{G_H}(r) &= 1 + (A_{\mathrm{H}} + B_{\mathrm{H}} + C_{\mathrm{H}}\\
&\quad + D_{\mathrm{H}} + E_{\mathrm{H}} + F_{\mathrm{H}} + G_{\mathrm{H}} + H_{\mathrm{H}})^{-1}\\
&\quad \times (2\pi^2\rho r)^{-1}\int_0^{Q_{\max}} Q\Delta_{\mathrm{H}}(Q)\sin(Qr)\,\mathrm{d}Q\\
&= [A_{\mathrm{H}}g_{\mathrm{H_MH_M}}(r) + B_{\mathrm{H}}g_{\mathrm{H_MC}}(r) + C_{\mathrm{H}}g_{\mathrm{H_MH_\alpha}}(r)\\
&\quad + D_{\mathrm{H}}g_{\mathrm{H_MN}}(r) + E_{\mathrm{H}}g_{\mathrm{H_MO_C}}(r) + F_{\mathrm{H}}g_{\mathrm{H_MH_N}}(r)\\
&\quad + G_{\mathrm{H}}g_{\mathrm{H_MO_W}}(r) + H_{\mathrm{H}}g_{\mathrm{H_MH_W}}(r)] \times (A_{\mathrm{H}} + B_{\mathrm{H}}\\
&\quad + C_{\mathrm{H}} + D_{\mathrm{H}} + E_{\mathrm{H}} + F_{\mathrm{H}} + G_{\mathrm{H}} + H_{\mathrm{H}})^{-1}. \qquad (13)
\end{aligned}
$$

The intramolecular $\mathrm{H_M}$···α contribution, $I_{\mathrm{H}}{}^{\mathrm{intra}}(Q)$, is estimated by the following equation:

$$
\begin{aligned}
I_{\mathrm{H}}{}^{\mathrm{intra}}(Q) &= \sum 2c_{\mathrm{H}}b_\alpha(b_{\mathrm{H_M}} - b_{\mathrm{H_{M'}}})\\
&\quad \times \exp(-l_{\mathrm{H_M\alpha}}{}^2Q^2/2)\sin(Qr_{\mathrm{H_M\alpha}})/(Qr_{\mathrm{H_M\alpha}}), \qquad (14)
\end{aligned}
$$

where c_{H} is the number of the methyl-hydrogen atoms in the stoichiometric unit. Values $l_{\mathrm{H_M\alpha}}$ and $r_{\mathrm{H_M\alpha}}$ were referred to those reported in the literature.[22,23] Calculated $I_{\mathrm{H}}{}^{\mathrm{intra}}(Q)$ was then subtracted from the corrected $\Delta_{\mathrm{H}}(Q)$ to deduce the intermolecular difference function, $\Delta_{\mathrm{H}}{}^{\mathrm{inter}}(Q)$:

$$\Delta_H{}^{inter}(Q) = \Delta_H(Q) - I_H{}^{intra}(Q). \tag{15}$$

The intermolecular H_M–H_{ex} (H_{ex}: exchangeable hydrogen atom) partial structure factor, $a_{H_MH_{ex}}(Q)$, is derived by the second-order difference between observed $\Delta_H{}^{inter}(Q)$ and $\Delta_{H'}{}^{inter}(Q)$, in which the isotopic composition of the exchangeable hydrogen atoms is changed,

$$\begin{aligned}\Delta_H{}^{inter}(Q) - \Delta_{H'}{}^{inter}(Q) &= 18x^2(b_{H_N} - b_{H_{N'}}) \\ &\quad \times (b_{H_M} - b_{H_{M'}})[a_{H_MH_N}(Q) - 1] \\ &\quad + 12x(1-x)(b_{H_W} - b_{H_{W'}}) \\ &\quad \times (b_{H_M} - b_{H_{M'}})[a_{H_MH_W}(Q) - 1] \\ &= 6x(2+x)(b_{H_W} - b_{H_{W'}}) \\ &\quad \times (b_{H_M} - b_{H_{M'}})[a_{H_MH_{ex}}(Q) - 1],\end{aligned} \tag{16}$$

where, the condition that $b_{H_N} - b_{H_{N'}} = b_{H_W} - b_{H_{W'}}$ has been adopted. Since the magnitude of the coefficient for the $a_{H_MH_W}(Q)$ is ca. 22 times larger than that for the $a_{H_MH_N}(Q)$ in the present experimental condition, the derived $a_{H_MH_{ex}}(Q)$ can be regarded as the structure function which expresses correlation between the methyl-hydrogen and the water-hydrogen atoms. The intermolecular partial structure factor, $a_{H_MZ}(Q)$ (Z: O, N, H_α, H_M, and C), is obtained by subtracting the $a_{H_MH_{ex}}(Q)$ from the $\Delta_H{}^{inter}(Q)$,

$$\begin{aligned}&\Delta_H{}^{inter}(Q) - 6x(2+x)(b_{H_M} - b_{H_{M'}})b_{H_W}/(b_{H_W} - b_{H_{W'}})[a_{H_MH_{ex}}(Q) - 1] \\ &= A_H[a_{H_MH_M}(Q) - 1] + B_H[a_{H_MC}(Q) - 1] \\ &\quad + C_H[a_{H_MH_\alpha}(Q) - 1] + D_H[a_{H_MN}(Q) - 1] \\ &\quad + E_H[a_{H_MO_C}(Q) - 1] + G_H[a_{H_MO_W}(Q) - 1] \\ &= (A_H + B_H + C_H + D_H + E_H + G_H)[a_{H_MZ}(Q) - 1].\end{aligned} \tag{17}$$

Since the contribution from the H_M–O_W partial structure factor occupies 82% of the $a_{H_MZ}(Q)$ in the present experimental condition, the $a_{H_MZ}(Q)$ is well approximated by the interaction between the water-oxygen and the methyl-hydrogen atoms within the alanine molecule. Intermolecular partial distribution function, $g_{H_Mj}(r)$ (j: H_{ex}, Z), is obtained from the Fourier transform as follows:

$$g_{H_Mj}(r) = 1 + (2\pi^2\rho r)^{-1}\int_0^{Q_{max}} Q[a_{H_Mj}(Q) - 1]\sin(Qr)\,dQ. \tag{18}$$

The upper limit of the integral is set to be $Q_{max} = 10$ Å^{-1} in the present analysis.

Structural parameters concerning hydration around the methyl-hydrogen atoms were determined through the least squares fitting analysis for the observed $a_{H_MH_{ex}}(Q)$ and $a_{H_MZ}(Q)$ by employing the following model function, $a_{H_Mj}{}^{model}(Q)$, which involves both short- and long-range contributions:[24–26]

$$\begin{aligned}a_{H_Mj}{}^{model}(Q) &= \sum \beta_j n_{H_Mj}\exp(-l_{H_Mj}Q^2/2)\sin(Qr_{H_Mj})/(Qr_{H_Mj}) \\ &\quad + 4\pi\rho\exp(-l_{0j}^2Q^2/2)[Qr_{0j}\cos(Qr_{0j}) \\ &\quad - \sin(Qr_{0j})]Q^{-3},\end{aligned} \tag{19}$$

where, $\beta_{H_{ex}} = 2(1-x)/(2+x)$ and $\beta_Z = E_H/(A_H + B_H + C_H + D_H + E_H + G_H)$, were applied in order to obtain parameters for H_M–H_W and H_M–O_W interactions, respectively. The fitting procedure was performed in the range of $0.1 \le Q \le 10$ Å^{-1} with the SALS program,[27] assuming that the statistical uncertainties distribute uniformly.

Results and Discussion

Hydration Structure around the Amino Group. The observed difference function, $\Delta_N(Q)$, and the corresponding distribution function around the amino-nitrogen atom within the alanine molecule, $G_N(r)$, are shown in Figs. 2a and 3a, respectively. In Fig. 2a, an evident first peak at $Q \approx 2$ Å^{-1} and oscillational feature of the $\Delta_N(Q)$ extending to the higher-Q region, are clearly observed. The calculated intramolecular interference term, $I_N{}^{intra}(Q)$ (Fig. 2b), was then subtracted

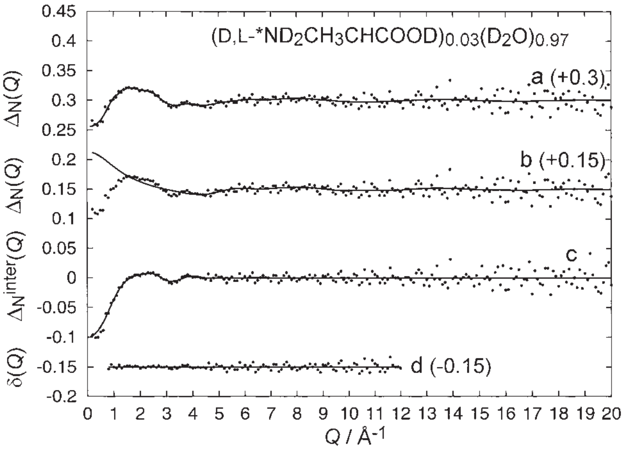

Fig. 2. a) Observed difference function, $\Delta_N(Q)$, for aqueous 3 mol% alanine heavy water solutions (dots). Smoothed $\Delta_N(Q)$, which has been used for the Fourier transform (solid line). b) Observed difference function, $\Delta_N(Q)$ (dots), and the intramolecular contribution within the alanine 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. d) The residual function, $\delta(Q)$ (dots).

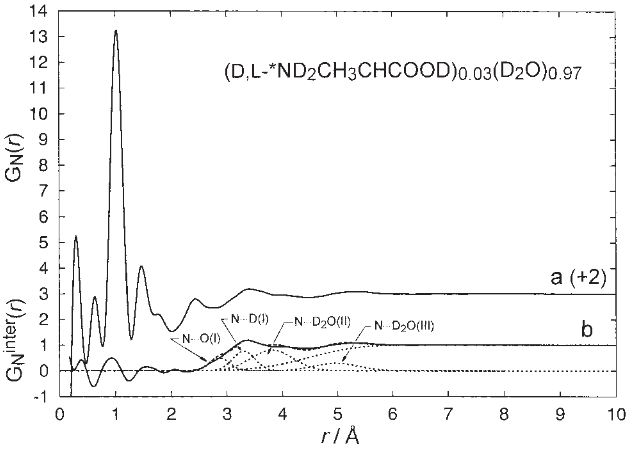

Fig. 3. a) Total distribution function around the amino-nitrogen atom, $G_N(r)$, observed for aqueous 3 mol% alanine heavy water solutions. b) Observed intermolecular distribution function, $G_N{}^{inter}(r)$ (solid line), and the Fourier transform of the calculated $\Delta_N{}^{model}(Q)$ (Fig. 2c) is shown by the broken line. Short- and long-range contributions are denoted by dotted lines.

from the observed $\Delta_N(Q)$. The normalization factor, γ, defined by $I_N^{\text{intra}}(Q) = \gamma \times \Delta_N(Q)$ (in the sufficiently high-Q region), was obtained to be $\gamma = 0.98 \pm 0.05$ from the least squares fit in the range of $6 \le Q \le 20$ Å^{-1}. The result implies that the present data correction and normalization procedures have been adequately carried out; the overall normalization error in the present $\Delta_N(Q)$ is roughly estimated to be within 5%. The intermolecular difference function, $\Delta_N^{\text{inter}}(Q)$ (Fig. 2c), is characterized by the first and second peaks located at $Q \approx 2$ and 4 Å^{-1}, respectively.

The total and intermolecular distribution functions, $G_N(r)$ and $G_N^{\text{inter}}(r)$, are represented in Figs. 3a and 3b, respectively. A dominant first peak at $r = 1.03$ Å in the $G_N(r)$ is assigned to the intramolecular N–D interaction within the alanine molecule. The second peak at $r = 1.47$ Å is attributable to the intramolecular N–C interaction. A sum of contributions from intramolecular N$\cdots$C$_\alpha$ and N$\cdots$C$_M$ (C$_\alpha$: α-carbon, C$_M$: methyl-carbon atoms, respectively) pairs can be observed as the third peak in the $G_N(r)$ located at $r = 2.4$ Å. The calculated $I_N^{\text{intra}}(Q)$, involving contributions from all possible intramolecular N$\cdots\alpha$ pairs, was subtracted from the observed $\Delta_N(Q)$ to deduce the intermolecular difference function, $\Delta_N^{\text{inter}}(Q)$, which is shown in Fig. 2c. The Fourier transform of the $\Delta_N^{\text{inter}}(Q)$, $G_N^{\text{inter}}(r)$, is represented in Fig. 3b. The first peak at $r = 3.4$ Å in the $G_N^{\text{inter}}(r)$ may involve the correlation between the amino-nitrogen atom and D_2O molecules in the first hydration shell of the amino group. An indication of further structure can be recognized as a more broadened second peak found at $r = 5.3$ Å. In order to obtain structural parameters concerning the hydration around the amino-nitrogen atom, the least squares fitting analysis was applied to the observed $\Delta_N^{\text{inter}}(Q)$. The following assumptions were adopted in evaluating the theoretical interference term. a) Parameters for the first nearest neighbor N$\cdots$D$_2$O interaction, r_{NO}, l_{NO}, l_{ND}, and n_{NO}, were independently refined. The tilt angle, θ, between the N$\cdots$O axis and the molecular plane of D_2O was treated as an independent parameter. The geometry of the D_2O molecule was fixed to that reported for pure heavy water ($r_{OD} = 0.983$ Å, $r_{DD} = 1.55$ Å).[29] b) The second and the third nearest neighbor N$\cdots$D$_2$O interactions were also taken into account in the present model function, in which each contribution was treated as a single interaction with the coherent scattering length in Eq. 9, b_α, being $2b_D + b_O$. c) Structural parameters for the continuous long-range random distribution of atoms, l_0 and r_0, were treated as independent parameters.

The best-fit result is compared with the observed $\Delta_N^{\text{inter}}(Q)$ in Fig. 2c. A satisfactory agreement is obtained in the range of $0.8 \le Q \le 12$ Å^{-1}. The observed and calculated $G_N^{\text{inter}}(r)$ (Fig. 3b) also agree well with each other. The final results of the least squares fit are summarized in Table 3. The present value of the N(–D)$\cdots$O distance, $r_{NO} = 2.88(2)$ Å, is in good agreement with those reported for aqueous glycine solutions in neutral ($r_{NO} = 2.85(5)$ Å),[2] and acidic ($r_{NO} = 2.90(2)$ Å)[4] conditions. The present N$\cdots$O distance agrees well with the average value of the N(–H)$\cdots$O hydrogen bond length found in various organic crystals ($r_{NO} = 2.89$ Å).[28] The present coordination number for the first nearest neighbor N$\cdots$O interaction, $n_{NO} = 2.4(1)$, is 20% smaller than the values, $n_{NO} = 3.0(6)$[2] and 3.0(2),[4] observed for aqueous glycine solutions with neutral and acidic conditions, respectively. This may indicate that the methyl group within the alanine molecule perturbs the first hydration shell around the amino group. The present value of the tilt angle, $\theta = 60.4(5)°$, is in good agreement with that observed for aqueous glycine solution with neutral condition ($\theta = 63(13)°$).[2]

Table 3. Results of the Least Squares Rifinement for the $\Delta_N^{\text{inter}}(Q)$ Observed for 3 mol% Aqueous Alanine Solutions in D_2O[a)]

Interaction	i$\cdots$j	r_{ij}/Å	l_{ij}/Å	n_{ij}	θ/°[b)]
N$\cdots$D$_2$O(I)[c)]	N$\cdots$O	2.88(2)	0.18(2)	2.4(1)	60.4(5)
	N$\cdots$D	(3.31)[d)]	0.20(2)	(4.8)[e)]	
N$\cdots$D$_2$O(II)[f)]	N$\cdots$D$_2$O	3.85(5)	0.38(1)	4.5(1)	
N$\cdots$D$_2$O(III)[g)]	N$\cdots$D$_2$O	5.18(2)	0.40(1)	2.9(1)	
		r_0/Å	l_0/Å		
Long-range	N$\cdots$X[h)]	4.58(2)	0.69(1)		

a) Estimated standard deviations are given in parentheses. b) Tilt angle between N$\cdots$O axis and molecular plane of D_2O. c) The first nearest neighbor N$\cdots$D$_2$O interaction. d) Calculated from values, $r_{NO} = 2.88$ Å and $\theta = 60.4°$. e) Fixed at the value $2n_{NO}$. f) The second nearest neighbor N$\cdots$D$_2$O interaction. g) The third nearest neighbor N$\cdots$D$_2$O interaction. h) X: N, O, C, H, and D.

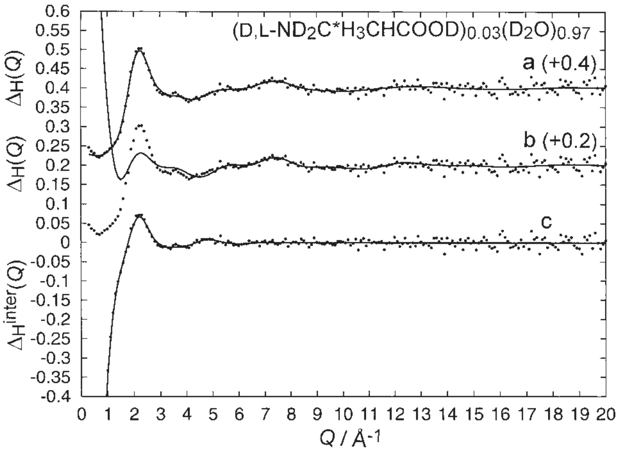

Fig. 4. a) Observed difference function, $\Delta_H(Q)$, for aqueous alanine heavy water solutions (dots). Smoothed $\Delta_H(Q)$, which has been used for the Fourier transform (solid line). b) Observed difference function, $\Delta_H(Q)$ (dots), and the intramolecular contribution within the alanine molecule, $I_H^{\text{intra}}(Q)$ (solid line). c) Intermolecular difference function, $\Delta_H^{\text{inter}}(Q)$ (dots). The inverse Fourier transform of $G_H^{\text{inter}}(r)$ shown in the solid line in Fig. 5b.

Hydration Structure around the Methyl Group. The observed $\Delta_H(Q)$ shown in Fig. 4a is characterized by a well defined first diffraction peak at $Q = 2.2$ Å^{-1}, and an oscillatory feature extending to the higher-Q region. The calculated intramolecular contribution represented as a solid line in Fig. 4b, well coincides in its amplitude with the observed $\Delta_H(Q)$ in the range of $Q \ge 6$ Å^{-1}. This implies that contribution from the intramolecular interference term is dominant in this Q region. In order to confirm the reliability of the present

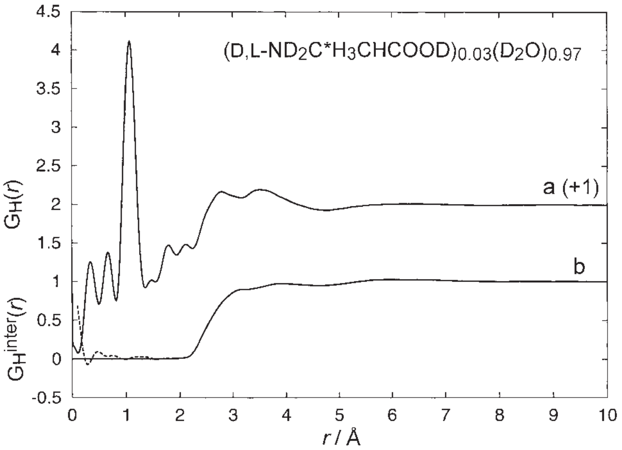

Fig. 5. a) Total distribution function around the methyl-hydrogen atom, $G_H(r)$, observed for aqueous 3 mol% alanine heavy water solutions. b) Observed intermolecular distribution function, $G_H^{inter}(r)$.

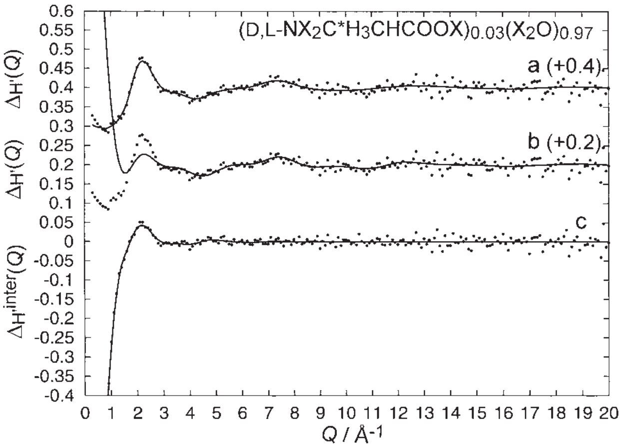

Fig. 6. Same notations as in Fig. 4 except for the 3 mol% alanine solution in X_2O (X: H/D = 20/80).

$\Delta_H(Q)$, the normalization factor, γ, was estimated by applying the same procedure described in the previous section. The value obtained ($\gamma = 1.01 \pm 0.01$) indicates that the overall normalization error in the present $\Delta_H(Q)$ is estimated within 1%. The calculated intramolecular contribution, $I_H^{intra}(Q)$ (Fig. 4b), was then subtracted from the observed $\Delta_H(Q)$ to obtain the intermolecular difference function, $\Delta_H^{inter}(Q)$, as shown in Fig. 4c.

The distribution function around the methyl-hydrogen atom, $G_H(r)$, is described in Fig. 5a. The dominant first peak located at $r = 1.07$ Å is assigned to the intramolecular C–H_M bond of the methyl group. Intramolecular non-bonding contributions may be involved in the partially resolved peak at $r = 2.8$ Å and 3.5 Å in the present $G_H(r)$. The intermolecular distribution function, $G_H^{inter}(r)$ (Fig. 5b), seems rather featureless, suggesting the weak hydration nature of the methyl group. However, an indication of the first hydration shell around the methyl group can be seen as the slightly resolved peak at $r \approx 3$ Å.

The difference function, $\Delta_{H'}(Q)$, between solutions IV and V, in which the isotopic composition of exchangeable hydrogen atoms, H/D = 20/80, is shown in Fig. 6a. The overall feature of the $\Delta_{H'}(Q)$ seems very similar to that for the $\Delta_H(Q)$. However, the height of the first diffraction peak in the $\Delta_{H'}(Q)$ is obviously lower than that observed in the $\Delta_H(Q)$, which may reflect the difference in the coherent scattering length of the exchangeable hydrogen atoms. The normalization factor, γ, was obtained to be $\gamma = 0.99 \pm 0.01$ from the least squares fit in the range of $6.0 \le Q \le 20.0$ Å^{-1}. The result indicates that the overall normalization error in the present $\Delta_{H'}(Q)$ is ca. 1%. The observed intermolecular difference function, $\Delta_{H'}{}^{inter}(Q)$, is represented in Fig. 6c. The height of the first diffraction peak at $Q \approx 2$ Å^{-1} in the present $\Delta_{H'}{}^{inter}(Q)$, is obviously lower than that for the $\Delta_H^{inter}(Q)$, which should reflect the difference in contribution of the $H_M\cdots H_{ex}$ term involved in the observed difference functions $\Delta_H^{inter}(Q)$ and $\Delta_{H'}{}^{inter}(Q)$.

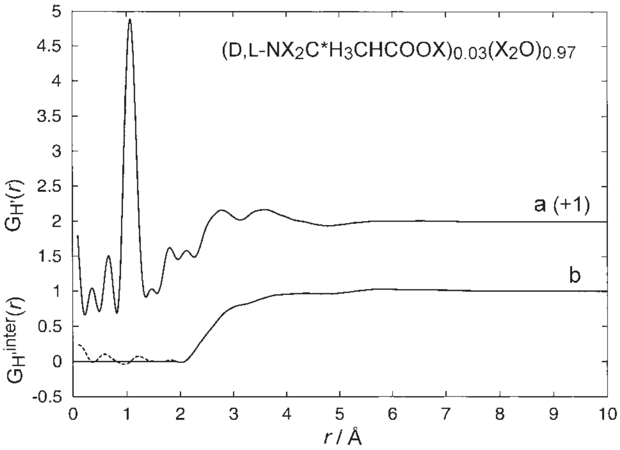

Fig. 7. Same notations as in Fig. 5 except for the 3 mol% alanine solution in X_2O (X: H/D = 20/80).

The total and intermolecular distribution functions around the methyl-hydrogen atom, $G_{H'}(r)$ and $G_{H'}{}^{inter}(r)$, are shown in Figs. 7a and 7b, respectively. The dominant first peak at $r = 1.07$ Å in the $G_{H'}(r)$ is assigned to the intramolecular C–H_M interaction within the methyl group. Partially resolved peaks at $r = 2.8$ Å and 3.6 Å should involve contributions from intramolecular non-bonding interactions within the alanine molecule. Although the intermolecular distribution function $G_{H'}{}^{inter}(r)$ (Fig. 7b) looks very similar to the $G_H^{inter}(r)$, the peak height in the $G_{H'}{}^{inter}(r)$ at $r \approx 3$ Å is lower than that observed for the $G_H^{inter}(r)$. The result implies that the intermolecular $H_M\cdots H_{ex}$ interaction falls at this radial distance.

The intermolecular $H_M\cdots H_{ex}$ structure factor, $a_{H_MH_{ex}}(Q)$, (Fig. 8a) was derived from the second-order difference between observed $\Delta_H^{inter}(Q)$ and $\Delta_{H'}{}^{inter}(Q)$ as described in Eq. 16. The intermolecular $H_M\cdots Z$ (Z: O, N, H_α, H_M, and C) structure factor, $a_{H_MZ}(Q)$, (Fig. 8b) was obtained by subtracting the $a_{H_MH_{ex}}(Q)$ from the observed $\Delta_H^{inter}(Q)$. The partial distribution functions, $g_{H_MH_{ex}}(r)$ and $g_{H_MZ}(r)$, are represented in Figs. 9a and 9b, respectively. Since the weighting of the $H_M\cdots O_W$ interaction is estimated to be 82% as mentioned above, the first peak located at $r \approx 2.6$ Å in the present $g_{H_MZ}(r)$ is attributable to the nearest neighbor $H_M\cdots O_W$ interaction. The partially resolved first peak at $r \approx 3$ Å in the present $g_{H_MH_{ex}}(r)$ can be assigned to the nearest neighbor

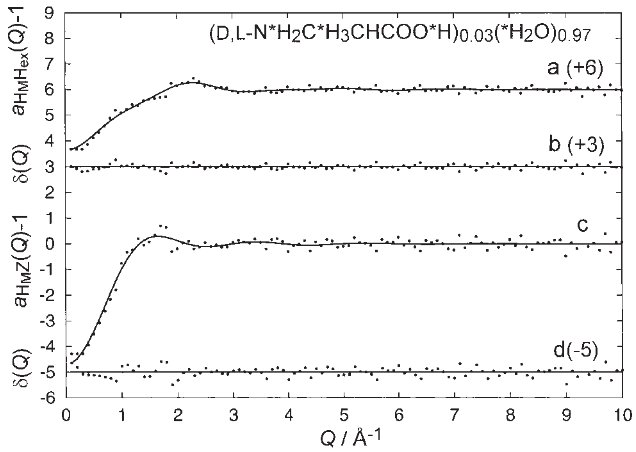

Fig. 8. a) Observed $H_M{\cdots}H_{ex}$ partial structure factor, $a_{H_MH_{ex}}(Q)$ (dots). The best-fit of the calculated $a_{H_MH_{ex}}{}^{model}(Q)$ (dots) is shown by the solid line. b) The residual functions, $\delta(Q)$, for the $a_{H_MH_{ex}}(Q)$ (dots). c) Observed $H_M{\cdots}Z$ partial structure factor, $a_{H_MZ}(Q)$ (dots). The best-fit of the calculated $a_{H_MZ}{}^{model}(Q)$ (dots) is shown by the solid line. d) The residual functions, $\delta(Q)$, for the $a_{H_MZ}(Q)$ (dots).

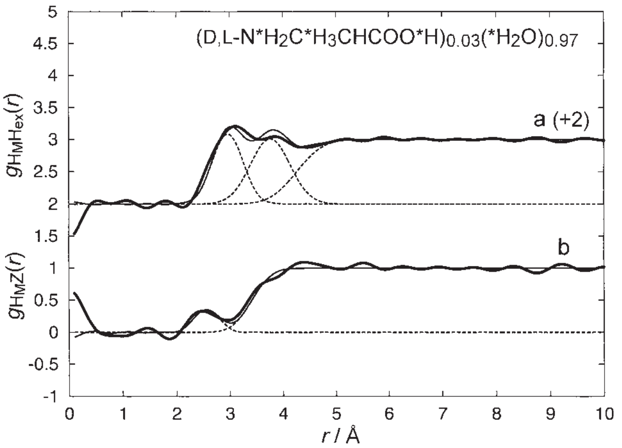

Fig. 9. Partial distribution functions, a) $g_{H_MH_{ex}}(r)$ and b) $g_{H_MZ}(r)$, observed for the aqueous 3 mol% alanine solution (thick solid lines), and the Fourier transforms of calculated $a_{H_Mj}{}^{model}(Q)$ functions (solid lines in Figs. 8a and 8c) are shown by thin solid lines. Short- and long-range contributions are denoted by broken lines.

$H_M{\cdots}H_W$ interaction. These results indicate that the water molecules within the first hydration shell of the methyl group distribute with some preferred orientation. Structure parameters concerning the nearest neighbor $H_M{\cdots}H_W$ and $H_M{\cdots}O_W$ interactions were respectively determined by the least squares fitting analysis of the observed $a_{H_MH_{ex}}(Q)$ and $a_{H_MZ}(Q)$ using the model function involving the short- and long-range interactions as indicated in Eq. 19. The number of the short-range interactions adopted for the analysis of $a_{H_MH_{ex}}(Q)$ and $a_{H_MZ}(Q)$, was assumed to be two and one, respectively.

The results of the least squares fit for the observed $a_{H_MH_{ex}}(Q)$ and $a_{H_MZ}(Q)$ are represented in Figs. 8a and 8b, respectively. A satisfactory agreement is obtained between the observed and calculated one over the whole Q-range employed. Final values of all independent parameters are summarized in Tables 4 and 5. The present value of the nearest neighbor $H_M{\cdots}O_W$ distance, $r_{H_MO_W} = 2.58(1)$ Å, is very close to the sum of the van der Waals radii of the hydrogen and oxygen atoms ($1.2 + 1.4 = 2.6$ Å), suggesting very weak interaction between the methyl-hydrogen atom and the water molecule within the first hydration shell of the methyl group. The coordination number, $n_{H_MO_W} = 0.62(1)$, indicates, on the average, ca. two water molecules are involved in the first hydration shell. The tilt angle θ between the $H_M{\cdots}O_W$ axis and the molecular plane of the water molecule, is estimated to be ca. 65°, employing the nearest neighbor $H_M{\cdots}H_W$ distance determined by the present study and the molecular structure of D_2O in the liquid state.[29] These results indicate that water molecules in the first hydration shell take a preferred orientation in which the oxygen atom of each water molecule is facing towards the methyl-hydrogen atom. According to recent neutron diffraction measurements on aqueous tertiary butanol solutions, the orientation of water molecules within the first hydration shell around the methyl group of the tertiary butanol molecule is almost random.[30,31] This may suggest that the hydration structure of the methyl group is significantly affected by the structure of the solute molecule. If we assume that the hydration number around the carboxyl group within the alanine molecule is similar to that found for the formate (4.4–4.8)[32] and for the acetate (4.0(1))[33] ions, the total number of water molecules within the first hydration sphere of the alanine molecule, can be roughly estimated to be 8–9. This implies that ca. 25% of the total water molecules are mobilized to form the first hydration shell of the alanine molecule in the aqueous 3 mol%

Table 4. Results of the Least Squares Rifinement for the Partial Structure Factor, $a_{H_MH_{ex}}(Q)$, Observed for Aqueous 3 mol% Alanine Solutions[a)]

Interaction	i···j	r_{ij}/Å	l_{ij}/Å	n_{ij}
Short-range	$H_M{\cdots}H(I)$[b)]	2.99(2)	0.285(1)	1.7(1)
	$H_M{\cdots}H(II)$[c)]	3.8(1)	0.37(3)	3.3(7)
		r_0/Å	l_0/Å	
Long-range	$H_M{\cdots}H$	4.3(2)	0.4(2)	

a) Estimated standard deviations are given in parentheses. b) The first nearest neighbor $H_M{\cdots}H_W$ interaction. c) The second nearest neighbor $H_M{\cdots}H_W$ interaction.

Table 5. Results of the Least Squares Rifinement for the Partial Structure Factor, $a_{H_MZ}(Q)$, Observed for Aqueous 3 mol% Alanine Solutions[a)]

Interaction	i···j	r_{ij}/Å	l_{ij}/Å	n_{ij}
Short-range	$H_M{\cdots}O_W$[b)]	2.58(1)	0.234(2)	0.62(1)
		r_0/Å	l_0/Å	
Long-range	$H_M{\cdots}Z$[c)]	4.3(2)	0.4(2)	

a) Estimated standard deviations are given in parentheses. b) The nearest neighbor interaction between methyl-hydrogen and water-oxygen atoms. c) Z: O, C, N, and H.

alanine solution.

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