Inter-Peptide Hydrogen Bonding in Monolayers of Oligoglycine Amphiphiles

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N-Octadecanoyl oligoglycine (monomer to pentamer) ethyl esters were synthesized. The structures of their monolayers and LB films transferred from pure water were investigated by π–*A* isotherms and FT-IR reflection absorption spectroscopy (FT-IR, RAS). All the glycine residues were involved in intermolecular hydrogen bonding as indicated by shifts of the NH stretching band and other IR features. The strength of the hydrogen bonds was increased with increasing numbers of the glycine residues in a molecule. The FT-IR characteristics of these monolayers are close to those of polyglycine II, despite the fact that these oligopeptides are not long enough for conformational fixation in bulk water.

Hydrogen bonds play a most important role in defining precise three-dimensional structures of proteins and their supramolecular complexes. The nature and strength of the hydrogen bond in amino acids and peptides have been widely studied by various spectroscopic methods as bases for understanding protein-folding mechanisms, host–guest recognition, and enzymatic catalysis. Most of these investigations were done in isotropic environments, e.g., in homogeneous nonpolar media or in aqueous solution; however, many biological interactions occur at the macromolecular interface, especially at the surface of biomembranes. Organized molecular monolayers and bilayers are especially useful as mimics of these interactions occurring at the interface because they can provide uniquely oriented surface environments. Monolayers composed of peptides or polypeptides and peptide/lipid mixtures have been investigated extensively.[1—7] Most of these papers were concerned with orientation, bioactivity, and intramolecular hydrogen bonding of the peptides and proteins involved. The problem of intermolecular hydrogen bonding among peptides and proteins in monolayers was taken up by Higashi et al.[8] and by Röthlisberger et al.[9] recently. The former group showed the presence of a β-like structure in compressed monolayers of poly(L-glutamic acid) derivatives; and the latter used molecular dynamics to study intermolecular hydrogen bonding of peptide moieties in self-assembling monolayers.

To conduct a systematic examination of inter- and intramolecular hydrogen bonding occurring in peptide monolayers, we synthesized a series of oligoglycine amphiphiles and investigated their monolayer properties and conformations at the air/water interface. The strength of intermolecular hydrogen bonds is enhanced with increasing numbers of the glycine residue. These results give us useful information on how to design organized hydrogen bonding systems at the interface.

Experimental

Materials. Glycine ethyl ester hydrochloride, glycylglycine, and triglycine were purchased from the Peptide Institute, Inc. Tetraglycine and pentaglycine were purchased from Sigma Chemical Co. They were used without further purification. The oligoglycine amphiphiles, $C_{18}Gly_nOEt$, were synthesized by esterification of oligoglycines followed by reaction with octadecanoyl chloride.

Glycylglycine Ethyl Ester *p*-Toluenesulfonate (PTS Gly_2OEt). Glycylglycine (3.00 g, 22.7 mmol) and *p*-toluenesulfonic acid monohydrate (8.64 g, 45.4 mmol) were dissolved in 300 cm^3 of EtOH and refluxed for 4 h. After EtOH was evaporated, 100 cm^3 of acetone was added to the oily residue and evaporated again. The resulting white solid was dispersed in 150 cm^3 of hot acetone, and the insoluble product, 3.58 g (10.8 mmol), was collected by filtration after cooling. Yield 47.5%; mp 125.0—126.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.20 (t, J = 7.2 Hz, 3H, $COOCH_2CH_3$), 2.29 (s, 3H, Ar-CH_3), 3.62 (s, 2H, glycine CH_2), 3.95 (t, J = 2.9 Hz, 2H, glycine CH_2), 4.11 (q, J = 7.1 Hz, 2H, $COOCH_2CH_3$), 7.11 (d, J = 7.5 Hz, 2H, Ar H), 7.47 (d, J = 8.1 Hz, 2H, Ar H), 8.04 (br, 3H, NH_3^+), 8.76 (br, 1H, CONH). Found: C, 46.82; H, 6.00; N, 8.41%. Calcd for $C_{13}H_{20}N_2O_6S$: C, 46.98; H, 6.07; N, 8.43%.

PTS Gly_3OEt, PTS Gly_4OEt, and PTS Gly_5OEt were obtained by similar procedures.

Glycylglycylglycine Ethyl Ester *p*-Toluenesulfonate (PTS Gly_3OEt). Yield 72.2%; mp 142.0—142.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.18 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 2.28 (s, 3H, Ar-CH_3), 3.59 (s, 2H, glycine CH_2), 3.84 (m, 4H, 2 glycine CH_2), 4.08 (q, J = 7.1 Hz, 2H, $COOCH_2CH_3$), 7.09 (d, J = 7.9 Hz, Ar H), 7.46 (d, J = 8.1 Hz, 2H, Ar H), 7.98 (br, 3H, NH_3^+), 8.42 (br, 1H, CONH), 8.63 (br, 1H, CONH). Found: C, 46.06; H, 5.96; N, 10.79%. Calcd for $C_{15}H_{23}N_3O_7S$: C, 46.26; H, 5.95; N, 10.79%.

Glycylglycylglycylglycine Ethyl Ester *p*-Toluenesulfonate (PTS Gly_4OEt). Yield 86.0%; mp 179.0—179.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6), δ = 1.19 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 2.29 (s, 3H, Ar-CH_3), 3.57 (m, 2H, glycine CH_2), 3.74 (m, 2H,

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glycine CH_2), 3.84 (m, 4H, 2 glycine CH_2), 4.09 (q, J=7.2 Hz, 2H, $COOCH_2CH_3$), 7.11 (d, J=7.8 Hz, 2H, Ar H), 7.47 (d, J=8.1 Hz, 2H, Ar H), 7.72 (br, 1H, CONH), 8.30 (br, 4H, NH_3^+, CONH), 8.57 (br, 1H, CONH). Found: C, 45.63; H, 5.83; N, 12.45%. Calcd for $C_{17}H_{26}N_4O_8S$: C, 45.73; H, 5.87; N, 12.55%.

Glycylglycylglycylglycylglycine Ethyl Ester *p*-Toluenesulfonate (PTS Gly_5OEt). Yield 78.2%; mp 191.0—192.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.19 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 2.28 (s, 3H, Ar-CH_3), 3.61 (s, 2H, glycine CH_2), 3.76 (m, 4H, 2 glycine CH_2), 3.84 (m, 4H, 2 glycine CH_2), 4.09 (q, J=6.9 Hz, 2H, $COOCH_2CH_3$), 7.11 (d, J=7.8 Hz, 2H, Ar H), 7.47 (d, J=7.8 Hz, 2H, Ar H), 8.00 (br, 3H, NH_3^+), 8.19 (br, 1H, CONH), 8.29 (br, 2H, 2 CONH), 8.61 (br, 1H, CONH). Found: C, 44.56; H, 5.79; N, 13.59%. Calcd for $C_{19}H_{29}N_5O_9S\cdot 1/2H_2O$: C, 44.53; H, 5.90; N, 13.66%.

***N*-Octadecanoylglycylglycine Ethyl Ester ($C_{18}Gly_2OEt$).** PTS Gly_2OEt (0.20 g, 0.60 mmol) and triethylamine (0.50 cm^3, 3.59 mmol) were dissolved in 50 cm^3 of CH_2Cl_2, and 0.25 cm^3 (0.74 mmol) of stearoyl chloride was added. The reaction mixture was stirred for 21 h in a 1000 cm^3 Erlenmeyer flask with a $CaCl_2$ tube. The solution turned turbid and viscous during the reaction. After the solvent was evaporated, the resulting white solid was washed with dilute hydrochloric acid and ethyl ether. Recrystallization from 10 cm^3 of CH_2Cl_2 gave 0.15 g (0.41 mmol) of the product as colorless solid. Yield 59%; mp 129.5—130.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.86 (t, J = 7.1 Hz, 3H, $COCH_2CH_2(CH_2)_{14}CH_3$), 1.18 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 1.24 (m, 28H, $COCH_2CH_2(CH_2)_{14}CH_3$), 1.47 (br, 2H, $COCH_2CH_2(CH_2)_{14}CH_3$), 2.11 (t, J = 7.5 Hz, 2H, $COCH_2CH_2(CH_2)_{14}CH_3$), 3.70 (d, J = 4.7 Hz, 2H, glycine CH_2), 3.82 (d, J = 5.8 Hz, 2H, glycine CH_2), 4.08 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 8.05 (br, 1H, CONH), 8.21 (br, 1H, CONH). Found: C, 66.91; H, 10.69; N, 6.63%. Calcd for $C_{24}H_{46}N_2O_4\cdot 1/4H_2O$: C, 66.86; H, 10.87; N, 6.50%.

$C_{18}GlyOEt$, $C_{18}Gly_3OEt$, $C_{18}Gly_4OEt$, and $C_{18}Gly_5OEt$ were obtained by similar procedures. In the synthesis of $C_{18}GlyOEt$, commercially available glycine ethyl ester hydrochloride was used instead of PTS GlyOEt. Dry DMF was used as the solvent in the case of $C_{18}GlyOEt$, $C_{18}Gly_4OEt$, and $C_{18}Gly_5OEt$.

***N*-Octadecanoylglycine Ethyl Ester ($C_{18}GlyOEt$).** Yield 92.0%; mp 83.0—83.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.88 (t, J = 6.7 Hz, 3H, $COCH_2CH_2(CH_2)_{14}CH_3$), 1.25 (m, 28H, $COCH_2CH_2(CH_2)_{14}CH_3$), 1.29 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 1.62 (t, J = 7.5 Hz, 2H, $COCH_2CH_2(CH_2)_{14}CH_3$), 2.36 (t, J = 7.6 Hz, $COCH_2CH_2(CH_2)_{14}CH_3$), 4.04 (d, J = 5.0 Hz, 2H, glycine CH_2), 4.22 (q, J = 7.1 Hz, 2H, $COOCH_2CH_3$), 5.92 (br, 1H, CONH). Found: C, 70.94; H, 11.64; N, 3.75%. Calcd for $C_{22}H_{43}NO_3\cdot 1/4H_2O$: C, 70.64; H, 11.72; N, 3.74%.

***N*-Octadecanoylglycylglycylglycine Ethyl Ester ($C_{18}Gly_3OEt$).** Yield 35.7%; mp 200.8—201.3 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.85 (t, J = 5.9 Hz, 3H, $COCH_2CH_2(CH_2)_{14}CH_3$), 1.19 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 1.23 (m, 28H, $COCH_2CH_2(CH_2)_{14}CH_3$), 1.47 (br, 2H, $COCH_2CH_2(CH_2)_{14}CH_3$), 2.11 (t, J = 7.7 Hz, 2H, $COCH_2CH_2(CH_2)_{14}CH_3$), 3.72 (m, 4H, 2 glycine CH_2), 3.82 (d, J = 5.4 Hz, 2H, glycine CH_2), 4.09 (q, J = 7.1 Hz, 2H, $COOCH_2CH_3$), 8.05 (br, 1H, CONH), 8.12 (br, 1H, CONH), 8.24 (br, 1H, CONH). Found: C, 63.36; H, 9.96; N, 8.65%. Calcd for $C_{26}H_{49}N_3O_5\cdot 1/2H_2O$: C, 63.38; H, 10.23; N, 8.53%.

***N*-Octadecanoylglycylglycylglycylglycine Ethyl Ester ($C_{18}Gly_4OEt$).** Yield 58.8%; mp 266 °C (decomp); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.87 (t, J = 6.5 Hz, 3H, $COCH_2CH_2(CH_2)_{14}CH_3$), 1.21 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 1.27 (m, 28H, $COCH_2CH_2(CH_2)_{14}CH_3$), 1.53 (br, 2H, $COCH_2CH_2(CH_2)_{14}CH_3$), 2.15 (t, J = 7.3 Hz, 2H, $COCH_2CH_2(CH_2)_{14}CH_3$), 3.76 (m, 6H, 3 glycine CH_2), 3.83 (m, 2H, glycine CH_2), 4.12 (q, J=7.0 Hz, 2H, $COOCH_2CH_3$), 7.77 (br, 4H, 4 CONH). Found: C, 61.76; H, 9.61; N, 10.17%. Calcd for $C_{28}H_{52}N_4O_6\cdot 1/4H_2O$: C, 61.68; H, 9.71; N, 10.28%.

***N*-Octadecanoylglycylglycylglycylglycylglycine Ethyl Ester ($C_{18}Gly_5OEt$).** Yield 47.0%; mp 250 °C (decomp); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.84 (t, J = 6.6 Hz, 3H, $COCH_2CH_2(CH_2)_{14}CH_3$), 1.17 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 1.22 (m, 28H, $COCH_2CH_2(CH_2)_{14}CH_3$), 1.46 (br, 2H, $COCH_2CH_2(CH_2)_{14}CH_3$), 2.10 (t, J = 7.4 Hz, 2H, $COCH_2CH_2(CH_2)_{14}CH_3$), 3.69 (m, 2H, glycine CH_2), 3.72 (m, 6H, 3 glycine CH_2), 3.81 (m, 2H, glycine CH_2), 4.07 (q, J = 7.1 Hz, 2H, $COOCH_2CH_3$), 8.08 (m, 4H, 4 CONH), 8.23 (br, 1H, CONH). Found: C, 58.98; H, 9.04; N, 11.36%. Calcd for $C_{30}H_{55}N_5O_7\cdot 2/3H_2O$: C, 59.09; H, 9.31; N, 11.48%.

Pressure-Area Isotherms. A computer-controlled film balance system, FSD-110 (trough size, 100×200 mm^2, USI system, Japan), was used. π–A Isotherms were taken at a compression rate of 4 mm min^{-1} and a subphase temperature of 20.0±0.3 °C. The subphase water was deionized and doubly distilled. The spreading solutions of oligoglycine amphiphiles were 0.16 mg cm^{-3} in CF_3COOH–chloroform (2 : 98 by volume).

LB Films. Monolayers were transferred onto Au-deposited glass slides or onto CaF_2 plates in the vertical mode at a surface pressure of 20 mN m^{-1} and at up-stroke and down-stroke motions of 8 and 100 mm min^{-1}, respectively, from pure water as the subphase. The transfer ratio was 1.0±0.1 in the up-stroke mode but there was no transfer in the down-stroke mode.

FT-IR Measurements. Infrared spectra were obtained on an FT-IR spectrometer (Nicolet 710) with a MCT detector (for RAS, reflection absorption spectroscopy) or with a DTGS detector (for the transmission method). All data for $C_{18}GlyOEt$, $C_{18}Gly_2OEt$, $C_{18}Gly_3OEt$, and $C_{18}Gly_5OEt$ were collected by the RAS method. The data for $C_{18}Gly_4OEt$ were collected by both of the RAS and transmission methods. The transmission absorption spectrum was similar to its RAS spectrum except for relative intensities between some peaks including amide I and amide II, and these peak intensities were much weaker than those from RAS. Peaks ascribed to CH_2 twisting and wagging vibrations of the glycine residues were more clearly seen in reflection spectra, thus we mainly used the RAS method to evaluate the LB film structure.

Results and Discussion

Monolayer Behavior. Polyglycine is not soluble in pure water because of their strong intermolecular hydrogen bonding, although commercially available oligoglycines such as diglycine to pentaglycine can be easily dissolved in water. The number of the intermolecular hydrogen bonds should increase with the increase in the glycine residues in amphiphiles $C_{18}Gly_nOEt$, (n = 1—5). These hydrogen bonds together with the hydrophobic packing force among alkyl chains make these amphiphiles high melting (see Experimental Section) and hardly soluble in water and in organic solvents including DMF, DMSO, THF, chloroform, and benzene. It was possible to spread these amphiphiles onto a water surface only by using a mixed solvent of CF_3COOH (TFA) and $CHCl_3$, (2 : 98 by volume, the amphiphiles were dissolved first in TFA and then diluted by $CHCl_3$).

π–A Isotherms of $C_{18}GlyOEt$, $C_{18}Gly_3OEt$, and $C_{18}Gly_5OEt$ monolayers are shown in Fig. 1. π–A patterns of amphiphiles $C_{18}Gly_2OEt$ and $C_{18}Gly_4OEt$ are essentially the same as that of $C_{18}GlyOEt$ monolayer. None of these isotherms show expanded phases. The limiting areas are 29.7, 29.8, 29.1, 29.0, and 30.0 Å^2, respectively, for $C_{18}GlyOEt$, $C_{18}Gly_2OEt$, $C_{18}Gly_3OEt$, $C_{18}Gly_4OEt$, and $C_{18}Gly_5OEt$ monolayers. These limiting areas are larger than that of stearic acid monolayer (20 Å^2), and it is suggested that the monolayer components are tilted more than the case of stearic acid on water. These close limiting areas imply that a common packing structure exists for these monolayers. The monolayers of $C_{18}GlyOEt$, $C_{18}Gly_2OEt$, and $C_{18}Gly_4OEt$ show collapse pressures at about 30 mN m^{-1}, and $C_{18}Gly_3OEt$ and $C_{18}Gly_5OEt$ monolayers show collapse pressures at around 40 mN m^{-1}. Destruction of the monolayers is noticed visually at surface pressures lower than the collapse pressure. LB films of $C_{18}Gly_nOEt$ (n = 1—5) monolayers transferred from pure water are deposited as Z types, i.e., the monolayers are deposited only in the upstroke motion.

IR Spectroscopic Data of LB Films. FT-IR spectra of LB films of $C_{18}Gly_nOEt$ (n = 1—5) monolayers transferred from pure water are shown for the region of 1800—1000 cm^{-1} in Fig. 2 and 3400—3200 cm^{-1} in Fig. 3. The NH stretching vibrations (ν_{NH}) are located at 3325—3294 cm^{-1}. The antisymmetric and symmetric CH_2 stretching bands are located at 2918 and 2850 cm^{-1}, respectively. Their intensities in RAS are decreased with increasing numbers of the glycine residues in a molecule, which implies the lipid chains become less tilted with increasing numbers of glycine residues.[10] The ester carbonyl stretching is found commonly at 1740 cm^{-1}, with an additional shoulder at 1750 cm^{-1} in the case of $C_{18}Gly_2OEt$ monolayers. The amide I band is at 1647 cm^{-1} for $C_{18}GlyOEt$ monolayer, at 1655 and 1637 cm^{-1} for $C_{18}Gly_2OEt$ monolayer, and at 1653 cm^{-1} with a shoulder at 1637 cm^{-1} for $C_{18}Gly_nOEt$ (n = 3—5) monolayers. The amide II band is very intense compared with the amide I band, and is at 1552—1564 cm^{-1}, shifting to higher frequencies with increasing numbers of glycine residues per molecule. However, the amide I band has a much stronger intensity in the transmission spectrum (figure not shown) than that in the RAS. Since the electric field of IR radiation in the transmission measurement is parallel to the film surface and that in the RAS is perpendicular,[10] these results suggest that the oligoglycine moiety orient almost perpendicular to the film surface with their tilted lipid chains. The CH_2 bending vibration is found commonly at 1420 cm^{-1} (weak), and the corresponding wagging and twisting vibrations are at 1377 cm^{-1} (wag), ca. 1289 cm^{-1} and ca. 1250 cm^{-1} (twist), respectively. The C–O stretching vibration of the ester group

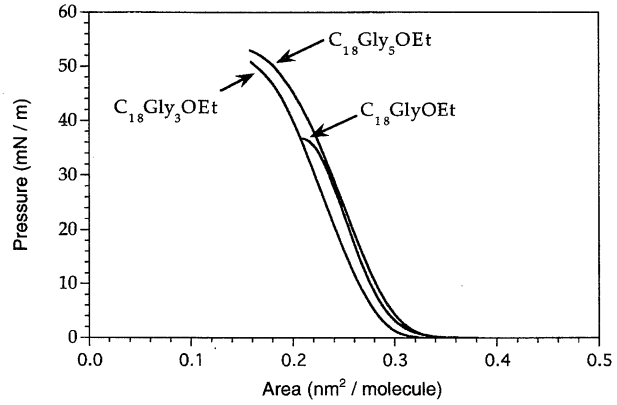

Fig. 1. π–A isotherms of $C_{18}Gly_nOEt$ monolayers on pure water, 20±0.2°C.

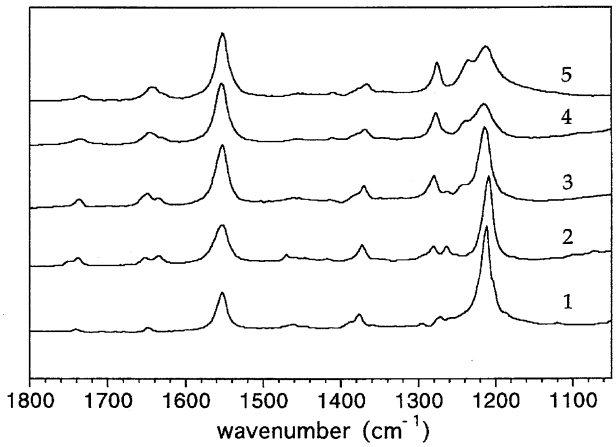

Fig. 2. FT-IR-RAS spectra of LB films of $C_{18}Gly_nOEt$ monolayers transferred from pure water in the region of 1000—1800 cm^{-1}, 12 layers. (1) $C_{18}GlyOEt$, (2) $C_{18}Gly_2OEt$, (3) $C_{18}Gly_3OEt$, (4) $C_{18}Gly_4OEt$, (5) $C_{18}Gly_5OEt$.

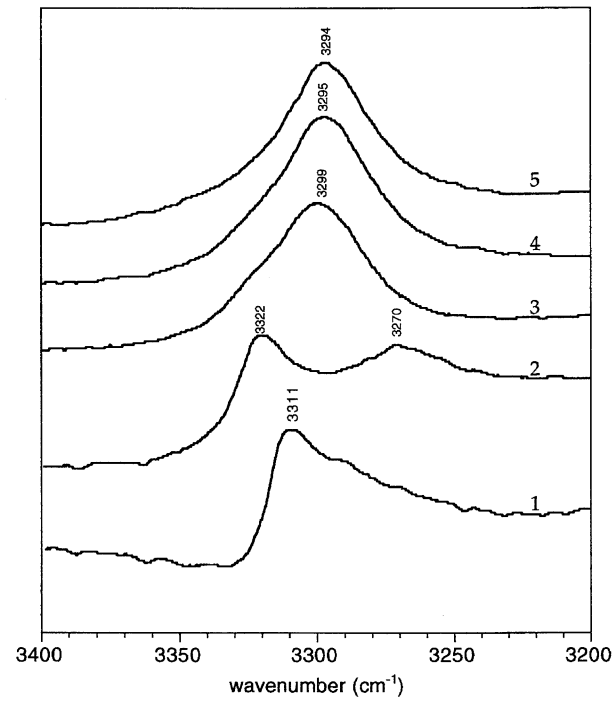

Fig. 3. FT-IR-RAS spectra of LB films of $C_{18}Gly_nOEt$ monolayers transferred from pure water in the region of 3200—3400 cm^{-1}, 12 layers. (1) $C_{18}GlyOEt$, (2) $C_{18}Gly_2OEt$, (3) $C_{18}Gly_3OEt$, (4) $C_{18}Gly_4OEt$, (5) $C_{18}Gly_5OEt$.

is very intense at 1205 cm^{-1}. It is interesting that the IR spectrum of $C_{18}Gly_2OEt$ is somewhat different from those of the other oligoglycine derivatives, but is similar to those reported for other diglycine derivatives and for triglycine. For instance, triglycine has two amide groups, and its IR spectrum shows two ν_{NH} peaks at 3310—3280 cm^{-1} and two CH_2tw at 1260—1290 cm^{-1}.[11,12]

Peptide Conformation in Oligoglycine Monolayers. The ν_{NH} frequencies of the LB films of these amphiphiles are lower than 3330 cm^{-1}, and indicate that all the glycine residues in the amphiphiles are engaged in hydrogen bonding.[13] Because the amphiphile molecules in compressed monolayers are oriented in one direction at the air/water interface, the glycine residues can form only parallel β-like or helix-like structures when the number of the glycine residue is two or greater. It is useful to compare IR frequencies of these LB films with those of polyglycine in order to study the nature of hydrogen bonding and the conformation of the polar oligoglycine moiety. Polyglycines ($(Gly)_n$) can usually assume two kinds of conformations in the solid-state: $(Gly)_nI$ (β-antiparallel form) and $(Gly)_nII$ (3_1-helical form), as confirmed by X-ray structure analysis.[14—16] The glycine residues are involved in hydrogen bonding in both cases. The parallel polyglycine β-sheet has not been observed up to now, although this structure has been proposed by Bandekar and Krimm.[17] According to their calculation, IR frequencies of the parallel polyglycine rippled sheet are almost identical to those of antiparallel $(Gly)_nI$. Table 1 contains the observed and calculated IR frequencies of the antiparallel $(Gly)_nI$, the observed and calculated frequencies of the parallel $(Gly)_nII$, and the observed frequencies of $C_{18}Gly_nOEt$. By comparing these frequencies, we can see that most of IR frequencies for LB films of $C_{18}Gly_nOEt$ ($n=3$—5) are close or identical to those of $(Gly)_nII$ (parallel). Taking $C_{18}Gly_4OEt$ as an example, its ν_{NH} frequency is 3295 cm^{-1} (3293 cm^{-1} in the transmission spectrum, data not shown). Since the ν_{NH} peak of crystalline $(Gly)_nII$ is at 3281 cm^{-1}, the inter-peptide hydrogen bonding in $C_{18}Gly_4OEt$ appears weaker. The amide I band (1653 cm^{-1}, 1649 cm^{-1} in transmission spectra) with a shoulder at 1637 cm^{-1} is close to the calculated (1649 cm^{-1}) and observed (1640 cm^{-1}) values for $(Gly)_nII$ rather than those for $(Gly)_nI$ (observed at 1685 and 1636 cm^{-1}). Amide II band is at 1564 cm^{-1} for amphiphile $C_{18}Gly_4OEt$ (1552 cm^{-1} in transmission spectra.), which is close to calculated (1551 cm^{-1}) and observed (1550 cm^{-1}) values of that for $(Gly)_nII$ (1517 cm^{-1} for $(Gly)_nI$, observed). In the CH_2b, CH_2w and CH_2tw region, LB films of $C_{18}Gly_nOEt$ ($n=3$—5) monolayers give almost the same peak positions as $(Gly)_nII$. Among these peaks, the peaks at 1420 and 1378 cm^{-1} are mainly attributed to CH_2 of alkyl chains, and the peaks at 1288 and 1250 cm^{-1} are attributed to CH_2 of the glycine residues because their intensities increase with increasing numbers of the glycine residues. These monolayer peaks have counterparts at 1420, 1377, 1283, and 1249 cm^{-1} of $(Gly)_nII$. In contrast, $(Gly)_nI$ gives corresponding peaks at 1432 cm^{-1} (CH_2b), 1338 cm^{-1} (CH_2w), 1295, and 1236 cm^{-1} (CH_2tw), and these peaks are absent in the monolayer spectra.

Table 1. IR Characteristics of Polyglycines (I and II) and Monolayers of Oligoglycine Amphiphiles (cm^{-1})[a]

Assignments	$(Gly)_nI$ Obsd antiparallel β-sheet (Calcd)	$(Gly)_nII$ Obsd parallel 3_1-helix (Calcd)	LB films of $C_{18}(Gly)_nOEt$, obsd n: 1	2	3	4	5
NH	3284	3281	3311	3322	3299	3295	3294
(str)	(3272)	(3279)		3270			
CH_2	2931	2936	2918	2918	2918	2918	2918
(asym str)	(2928)	(2935)					
CH_2	2869	2850	2850	2850	2850	2850	2850
(sym str)	(2861)	(2853)					
Amide I	1685	—	—	—	—	—	—
	(1689)						
	1636	1640	1647	1655	1653	1653	1653
	(1643)	(1649)		1637	1637	1637	1637
Amide II	1517	1550	1552	1555	1561	1564	1564
	(1515)	(1551)					
CH_2	1432	1420	—	1422	1420	1420	1420
(bend)	(1439)	(—)					
CH_2	1338	1377	1376	1377	1377	1378	1377
(wag)	(1341)	(1374)					
CH_2	1295	1283	1295	1268	1289	1288	1287
(twist)	(1286)	(1290)		1285			
	1236	1249	1272	1256	1250	1250	1248
	(1242)	(1247)					

a) Observed and calculated IR data of $(Gly)_nI$ and $(Gly)_nII$ are taken from Refs. 17, 22—27.

We also compared IR characteristics of the monolayers with those of glycine homopeptides and glycine homopeptide ester hydrochlorides. The oligoglycines assume conformations identical to those of $(Gly)_nI$ and $(Gly)_nII$, although the former conformations are often interconverted between β-sheet and 3_1-helix depending on preparative conditions and the modification of the terminal group.[18—20] In these cases, the ν_{NH} and amide I frequencies of diglycine to hexaglycine are often composed of multiple peaks and their positions change irregularly. From triglycine to hexaglycine, there are two amide I peaks: a strong one is around 1635 cm^{-1}, another medium one is around 1665 cm^{-1}. Very broad amide II peaks are found for tetraglycine and pentaglycine around 1540 cm^{-1}. All of these oligoglycines have the CH_2w peaks with very weak intensities at 1350—1385 cm^{-1} (this peak is commonly assigned to the $(Gly)_nII$ structure), and the CH_2b peaks with medium intensity at 1439—1453 cm^{-1} (this peak is commonly assigned to the $(Gly)_nI$ structure). These results suggest that the glycine homopeptides may have mixed structures made of β-sheet and 3_1-helix. From these IR characteristics of the monolayers we can see that (1) all the amide NH groups in $C_{18}Gly_nOEt$ are engaged in hydrogen bonding; (2) the hydrogen-bonded glycine residues should be in a parallel form; (3) the oligoglycine moieties are not in β-sheet conformation: Amide peaks are rather close to those in $(Gly)_nII$. From these data, we conclude that $C_{18}Gly_nOEt$ ($n=3$—5) monolayers tend to form a parallel $(Gly)_nII$-like

structure. This conformational restriction is derived from parallel orientation of the glycine residues at the air/water interface and the alkyl chain packing (consequently, oligoglycine packing) in compressed monolayers.

Intermolecular Hydrogen Bonding. Figure 3 shows the expanded ν_{NH} region. It is apparent that the peak position is shifted to lower frequencies as the number of glycine residues increases. This indicates that the strength of hydrogen bonds among the glycine residues is increased with increasing numbers of the residues. This supposition is also supported by the fact that the amide II peak shifts to higher frequencies with increasing glycine residues. In addition to this general trend, there are IR characteristics specific to individual oligoglycine residues. The influence of the intermolecular hydrogen bonding appears very limited for $C_{18}GlyOEt$ as the ν_{NH} peak is located at 3311 cm^{-1}. $C_{18}Gly_2OEt$ gives two ν_{NH} peaks at 3322 and 3270 cm^{-1}. The former peak suggests weak hydrogen bonding and is probably attributed to the terminal glycine residue. The latter low frequency peak can be ascribed to the strongly hydrogen bonded, inner glycine residue. The ν_{NH} peaks for longer glycine residues, Gly_3 to Gly_5, are at almost identical positions (3299 to 3294 cm^{-1}), although slight frequency increases with increasing glycine residues are noted. There lower frequency peaks are indicative of strong hydrogen bonding. The ν_{NH} and amide II bands of $C_{18}Gly_4OEt$ and $C_{18}Gly_5OEt$ are located at identical positions. Apparently, the intermolecular hydrogen bonding at the interface becomes saturated at this glycine length, although the extent of the amide interaction is still weaker than those of polyglycines I and II (ν_{NH} at 3281—3284 cm^{-1}).

Concluding Remarks

Figure 4 displays a conceivable model for the monolayer of oligoglycine amphiphiles by using $C_{18}Gly_3OEt$ as a typical example. The packed alkyl chains on the water surface help their Gly_3 moieties form hydrogen bonds with each other, and especially, the strengths of these hydrogen bonds can be increased with increasing numbers of the glycine residue in an amphiphile. The limiting area of a molecule is larger than either the standard cross section of the alkyl chain (20 $Å^2$) or the cross section (18.1 $Å^2$) of the glycyl chain in the $(Gly)_nII$ crystal. These area differences imply that the alkyl chains in the monolayer are packed in a tilted arrangement and that the intermolecular hydrogen bonding is longer and weaker at the interface than those in crystal.

The arrangement and conformation of peptide chains at the air–water interface provide an important research problem in relation to the behavior of peptide chains on the surface of biomembranes and other biological supramolecular systems. However, very few papers have been published on monolayers of well-characterized peptide amphiphiles. As a rare exception, Higashi et al.[8] studied the structure of the poly(L-glutamic acid) moiety appended to double alkyl chains at the air/water interface, and found that a stable β-structure was formed in the condensed phase when the peptide moiety (degree of polymerization ca. 40) was aligned in monolayers. Conventional poly(L-glutamic acid) displays a conformational transition between α-helix and random coil, depending on pH, solvent composition, and temperature.[28] It is clear that spatial confinement and chain alignment at the interface induced the β-structure rather than the conventional α-helix. Our study provides an additional example of a pep-

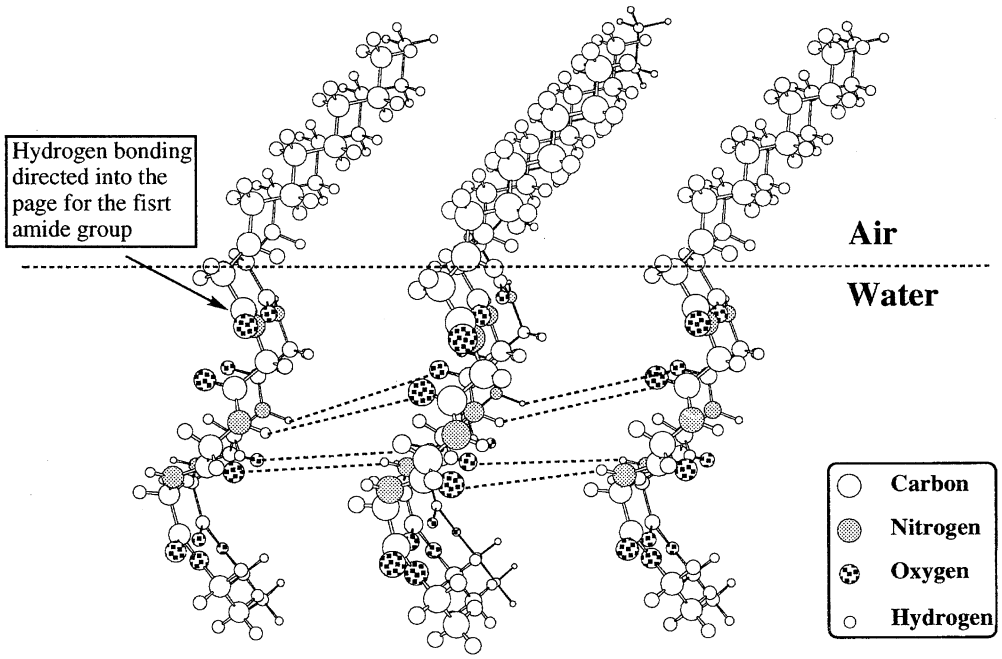

Fig. 4. A schematic representation of molecular packing and the pattern of hydrogen bonding for the $C_{18}Gly_3OEt$ monolayer. Dotted lines show hydrogen bonds.

tide conformation at an interface. The oligoglycine moiety in the monolayer forms packed hydrogen bonding structures similar to that of polyglycine II as evidenced by the common IR features. The maintenance of a specific conformation for short peptides (Gly_3 to Gly_5) is interesting, since it is not usually probable in bulk solution. This must be caused by conformational fixation through intermolecular hydrogen bonding. On the other hand, the conformational fixation may reflect the unique nature of the interface. If this contribution is significant, short peptide chains on the biological molecular surface may assume specific (functionally important) conformations that are not probable at non-interfacial sites.

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