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Notes

Infrared Conformational Study of Poly(ethylene glycol)-Bound Homooligoglycines in the Solid State and in Solution‡

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In the light of the recent interest in the investigation of the conformation and conformational stability of biologically active molecules covalently linked to a polymeric support,[2-4] the study of poly(ethylene glycol)-bound polypeptides appears to be of exceptional relevance in many respects. The C-terminal macromolecular protecting group poly(ethylene glycol) (PEG)[5] permits effective stepwise synthesis and allows the conformational investigation of the covalently attached peptide without the time consuming deprotection and isolation steps, due to its favorable physical and optical properties.[6,7] In particular, the strong solubilizing effect of PEG enables conformational studies of the otherwise poorly soluble peptides in a great variety of solvents. Thus, this approach permitted the conformational analysis of homooligomers of L-Ala, L-Val, L-(γ-Bzl)Glu, and L-Met by using CD and IR measurements.[8-13] These results prompted us to extend this approach to the conformational analysis of homooligoglycines. The Gly residue is of particular interest since it can explore a large conformational space, thus inducing a high flexibility to the peptide chain.[14-17] Moreover, the presence of Gly residues induces conformational characteristics in a linear peptide chain which are favorable for cyclization.[18,19]

Experimental investigations of the conformational preferences of monodispersed homooligoglycines were limited to very short chain lengths due to the low solubility of the higher oligomers. In this note we report the IR analysis in the solid state and in solution of a series of PEG-bound homooligoglycines of the general formula *t*-Boc-$(Gly)_n$-OPEG (n = 1-9).

Chromatographically and analytically pure *t*-Boc-$(Gly)_n$-OPEG's up to n = 9 have been synthesized by the liquid phase method[6] by using bifunctional PEG of molecular weight 10 000.

The results of the IR measurements in the solid state are presented in Table I. The assignments of the various bands of these poly(ethylene glycol)-bound peptides were made on the basis of the theoretical and experimental infrared data of peptides and polypeptides. The relative intensities of the bands and their changes with increasing chain length have been taken into consideration in the interpretation of the different bands. When n = 7-9 the peptides assume predominantly the antiparallel β conformation (conformation I).[20] When n = 3-5 the peptides assume a predominant conformation with hydrogen bonds, most probably of the interchain type, but quite different from the usual antiparallel β (conformation I) and ternary helix (conformation II) conformations of poly$(Gly)_n$. In the region of n = 5-7 the antiparallel β conformation adopted by the highest oligomers and the conformation adopted by the lowest oligomers coexist. Contrary to our findings, Ac-$(Gly)_{1-4}$-NHEt's were reported to assume the ternary helix conformation.[21,22] This difference in the conformational preferences of the lowest oligomers can be attributed to the effect of the bulky and bifunctional PEG. To clarify this point, we have carried out the solid-state IR measurements of the low molecular weight analogues *t*-Boc-$(Gly)_{1-3}$-OMe. The IR spectra (Table I) are in reasonable agreement with those of the corresponding Ac-

‡ Dedicated to Professor Paul J. Flory on the occasion of his 70th birthday.

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Table I
IR Absorption Frequencies (Wavenumbers in cm^{-1}) of the Various Homooligoglycines in the Solid State

peptide	3450-3250 (amide A)[a]	1770-1720 (ester)	1720-1600 (amide I)[a]	1600-1500 (amide II)	750-630 (amide V)
t-Boc-$(Gly)_n$-OPEG					
$n = 1$	*3410*,[b] *3380*	1753	**1716**		
$n = 2$	*3410*, *3330*	1750, 1736	1716, 1684, 1626	*1530*	
$n = 3$	3325[c]	1750, 1736	1715, 1678, 1627	1574, 1530	
$n = 4$	3325	1750, 1737	1715, **1674**, 1626	1573, 1530	*640*
$n = 5$	3325	1750, 1735	1715, **1675**, 1626	1574, 1532	*640*
$n = 6$	3322,[d] 3302	1750, 1736	1714, **1674**, **1632**	*1574*, 1532	*710*, *638*
$n = 7$	3300	*1750*, 1737	*1714*, 1684, 1673, **1632**	*1572*, 1528	714
$n = 8$	3300	*1750*, 1737	*1714*, 1686, 1675, **1632**	**1526**	713
$n = 9$	3300	1736	*1714*, 1686, 1674, **1631**	**1527**	715
t-Boc-$(Gly)_n$-OMe					
$n = 1$	3375	1756	1717, 1700	1520	*700*
$n = 2$	3380, 3335	1750	1714, 1680	1525	*700*, *675*
$n = 3$	3335, 3280	1757	1712, 1680, 1645	1552, 1538	688, 672, 640

[a] Of both urethane and peptide groups. [b] Boldface numbers indicate a strong band. [c] Underlined numbers indicate a medium intensity band. [c] Italic numbers indicate a very weak band. [d] Lightface numbers indicate a weak band.

Table II
IR Absorption Frequencies and A_H/A_F Values of the Various Homooligoglycines in Deuterated Chloroform Solution[a]

	at low dilution ($\simeq 10^{-2}$ M)		at high dilution ($\simeq 10^{-4}$ M)	
peptide	3500-3200 cm^{-1}	1800-1500 cm^{-1}	3500-3200 cm^{-1}	A_H/A_F value
t-Boc-$(Gly)_n$-OPEG				
$n = 1$	3465	1748, 1714, 1642, 1508	3462	0
$n = 2$	3450, *3350*	1741, 1716, 1686, 1530	3450, *3340*	0.11
$n = 3$	3450, 3360	1740, 1710, 1672, *1634*, 1524	3440, 3345	0.43
$n = 4$	3445, 3350	1738, 1672, *1634*, *1606*, 1530	3440, 3340	0.92
$n = 5$	3440, 3345	1738, **1666**, *1634*, 1605, 1528	3438, 3335	1.31
$n = 6$	*3440*, 3310	1740, **1660**, *1634*, *1604*, 1530	*3440*, 3330	3.78
t-Boc-$(Gly)_n$-OMe				
$n = 1$	3450	1748, 1716, 1508	3455	0
$n = 2$	3440	1746, 1716, 1690, 1524	3445, *3350*	0.04
$n = 3$	3440, 3360	1748, 1720, 1680, 1516	3440, 3360	0.19

[a] See footnote *b*, Table I.

$(Gly)_n$-NHEt,[21,22] but different from those of the PEG-bound analogues.

The above mentioned effect of PEG on the solid-state conformations of homooligoglycines is noteworthy in view of the observed absence of a significant influence of PEG on the conformations of homooligovalines,[11] homooligoalanines,[11] and homooligomethionines.[13] One possible explanation may be that for Val, Ala, and Met peptides (in the latter case for $n < 13$) the interchain interactions of the β structures are so dominant that the influence of the macromolecular C-protecting group is almost leveled off.

The IR measurements of the series of N-protected homooligoglycines with -OPEG and -OMe as the C-protecting groups have also been carried out in different solvents such as $CDCl_3$, CD_2Cl_2, D_2O, and TFE. Considering a typical analysis of the IR spectra in $CDCl_3$ at high dilution ($\simeq 10^{-4}$ M) (Table II), it can be seen that the ratio of the N–H---O = C hydrogen-bonded N–H absorption to free (solvated) absorption (A_H/A_F), which is directly proportional to the amount of intrapeptide-chain hydrogen-bonded folded forms, rapidly increases with increasing chain length. This observation of the increasing folding with increasing chain length, as delineated by the A_H/A_F values, in a solvent with a relatively low solvating power for –CONH– groups, is quite similar to that reported for the Ala series.[11] At high concentrations ($\simeq 10^{-2}$ M), as the chain length increases, aggregation tends to increase also (Table II); a partially developed β structure is present at $n = 6$ (particularly in CD_2Cl_2), characterized by bands at about 3305, 1635, and 1530 cm^{-1}. These observations are in close agreement to those in the solid state.

To summarize, we have shown a dependence of the chain length, concentration, and nature of the C-terminal protecting group upon the conformation of oligoglycines. For the observation of a well-developed antiparallel β structure in the solid state a critical chain length of about $n = 7$ is necessary; for very short chain lengths, a significant influence of the polymeric C-terminal protecting group has been delineated. In solvents of low polarity both intrapeptide-chain hydrogen-bonded folded forms and interpeptide-chain hydrogen-bonded associated forms, most probably of the extended type, occur, the relative amounts of which depend on peptide concentration.

The observed structural multiplicity of homooligoglycines is an indication of the important role of short-range interactions for the overall conformation of small peptides in the solid state and in solution. As expected from theory,[16] the stability of the ordered structures for oligoglycines is low. Thus, in harmony with the experimental findings, minor changes in the local environment of the peptide chain may affect the distribution of the various conformational species.

References and Notes

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Small-Angle Neutron Scattering from Stretched Polystyrene Networks

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Several papers discuss small-angle neutron-scattering (SANS) experiments on the response of polymer chains to elongation of the specimen. Picot et al.[1] studied uncross-linked polystyrene by using samples containing 1% deuterated polystyrene (PS-d_8) which had been subjected to rapid stretching above the glass transition temperature and then frozen at room temperature. The deformation of the radius of gyration, R_g, of the tagged molecules in both the longitudinal and transverse directions did not quite follow affine behavior. Benoit et al.[2] measured the separation of chain ends (crosslink points) for a swollen polystyrene network uniaxially stretched to low elongations. Hinkley et al.[3] measured the change in R_g for polybutadiene networks, where the tagged chains connected two network junctions. Scatter in the data prevented the making of a firm statement as to whether the predictions of the junction affine theory were closely followed.

In the study reported here, SANS measurements were made on stretched, tagged polystyrene networks cross-linked by irradiation. The samples were elongated above T_g, held while the stress relaxed, and then cooled to room temperature. Determination of R_g was made in directions both parallel and perpendicular to the stretch direction by using data analysis techniques developed especially for anisotropic scattering.[4] Since the time required to collect sufficient data on anisotropic samples is long compared to that of the isotropic samples,[4] measurements in the transverse direction were also made by cutting films perpendicular to the stretch direction. Thus, the neutron beam was effectively parallel to the elongation direction, and isotropic scattering resulted.

Experimental Section

The PS-d_8 was prepared by anionic polymerization: $\bar{M}_w = 1.63 \times 10^5$, $\bar{M}_n = 1.23 \times 10^5$. This was mixed in toluene with normal protonated polystyrene characterized by $\bar{M}_w = 2.91 \times 10^5$ and $\bar{M}_n = 0.74 \times 10^5$ to give 1.95% tagged molecules. After precipitation and drying, bars were compression molded with cross-sectional dimensions of 2.0 cm by 1.2–2.0 cm. The bars were sealed under vacuum in tubes and cross-linked by ^{60}Co γ radiation. Two samples were irradiated to slightly different total doses. After irradiation the samples had swell ratios in toluene of 11.0 and 14.0, giving average molecular weights between cross-links,[9] $\bar{M}_c$, of 2.3×10^4 and 2.7×10^4, respectively.

The bars were elongated at 145 °C on an Instron tensile tester and held at the final elongation α for 10 min to allow some stress relaxation. $\alpha = l/l_0$ where l is the final length of the sample after elongation and l_0 is the initial length. After cooling, 1.5 mm thick specimens were cut in both the longitudinal and transverse directions. Identical samples without the tagged molecules were prepared for background data. A range of elongations from $\alpha = 1.44$ to 2.34 was investigated.

The SANS experiments were performed on the small-angle diffractometer D11[5] at the Institut Laue-Langevin, Grenoble, France. Two sets of experimental conditions were used: (1) for the "end-on" experiments, where the scattering is isotropic, the normal velocity selector with $\Delta\lambda/\lambda$ of 10% was used and the sample to detector distance was 10 m (λ_0 was 8.0 Å); and (2) for the anisotropic measurements a velocity selector with $\Delta\lambda/\lambda$ of 50% was used thereby increasing the neutron flux by a factor of about 3 compared with the 10% selector. Because of the very broad wavelength distribution the value of λ_0 used in the analysis was calculated by using the expression[6]

$$\lambda_0 = (\overline{\lambda^{-2}})^{-1/2}$$

The value of λ_0 used in the analysis was 7.0 Å. The sample to detector distance was 20 m.

Scattering Theory

The scattering law, $S(\mathbf{Q})$, for a polymer coil is

$$S(\mathbf{Q}) = N^{-2}\sum_{ij}^{N} e^{i\mathbf{Q}\cdot\mathbf{r}_{ij}} \quad (1)$$

where $\mathbf{r}_{ij}$ is the vector joining the segments i and j in a coil with N segments, $\mathbf{Q}$ is the momentum transfer equal to $(4\pi/\lambda)\sin\theta/2$, λ is the neutron wavelength, and θ is the scattering angle. For a coil embedded in a matrix the total scattered intensity is a combination of coherent and incoherent scattering of the form

$$I(\mathbf{Q}) = A + BS(\mathbf{Q}) \quad (2)$$

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