$$E(\ldots t_+t_+t_+t_+t_+\ldots) = 0 = E(\ldots t_-t_-t_-t_-t_-\ldots)$$

$$E(\ldots t_+t_+tt_+t_+\ldots) = E(\ldots t_+t_+tt_-t_-\ldots) = 0.9 \text{ kcal/mol}$$

$$E(\ldots t_+t_+ttt_+\ldots) = E(\ldots t_+t_+ttt_-\ldots)$$
$$= 1.13 \text{ kcal/mol} = (0.9 + 0.23) \text{ kcal/mol}$$

$$E(\ldots t_+tttt_+\ldots) = E(\ldots t_+tttt_-\ldots)$$
$$= 1.36 \text{ kcal/mol} = (0.9 + 2 \times 0.23) \text{ kcal/mol}$$

The matrix of statistical weights then reads ($RT$ = 600 kcal/mol):

| | $t_+$ | t | $t_-$ |
|---|---|---|---|
| $t_+$ | 1 | 0.22 | 0 |
| t | 1 | 0.68 | 1 |
| $t_-$ | 0 | 0.22 | 1 |

with largest eigenvalue $\lambda$. The ratio between the conformational partition functions pertinent to the disordered and ordered states (constant volume assumed) is then

$$Z = (\alpha\lambda/\beta)^N$$

and the free energy difference is

$$G = E_{\text{pack}} + RT \ln \beta - RT \ln \lambda = 0$$

at the transition point.

The frequency of occurrence of bond rotational states is independent of the ratio $\alpha/\beta$; hence it is possible, with the methods suggested by Flory, to get the average lengths of the sequences in the three states;[13] $\langle y_t \rangle = \langle y_{t-} \rangle = 2.9$, $\langle y_t \rangle = 1.8$ with the functions of Bates; $\langle y_{t+} \rangle = \langle y_{t-} \rangle = 1.9$, $\langle y_t \rangle = 1.8$ with the functions of Giglio. The Fourier transform calculated according to the model suggested by this energetic calculation is reported in Figure 2g and appears in fairly good accordance with the experimental data.

In summary, it appears to us that a chain conformation of poly(tetrafluoroethylene) in which different senses of spiralization succeed each other frequently while the molecules are confined in a cylindrical envelope is both geometrically and energetically feasible. The x-ray diffraction data are in good agreement with the model proposed.

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## References and Notes

(1) P. De Santis, E. Giglio, A. M. Liquori, and A. Ripamonti, *J. Polym. Sci., Part*, 1383 (1963).
(2) R. G. Brown, *J. Chem. Phys.*, **40**, 2900 (1964).
(3) E. S. Clark, *J. Macromol. Sci., Phys.*, **1**, 795 (1967).
(4) T. W. Bates and W. H. Stockmayer, *Macromolecules*, **1**, 12, 17 (1968).
(5) P. Corradini, *J. Polym. Sci., Polym. Symp.*, **51**, 1 (1975).
(6) L. D'Ilario and E. Giglio, *Acta Crystallogr., Sect. B*, **30**, 372 (1974).
(7) C. W. Bunn and E. R. Howells, *Nature* **174**, 549 (1954).
(8) M. Iwasaki, *J. Polym. Sci., Part A-1*, 1099 (1963).
(9) E. S. Clark and L. T. Muus, *Z. Kristallogr., Kristallgeom., Kristallphys., Kristallchem.*, **117**, 119 (1962).
(10) T. W. Bates, *Trans. Faraday Soc.*, **63**, 1825 (1967).
(11) P. J. Flory, *Macromolecules*, **7**, 381 (1974).
(12) As shown by Flory, this term should be consistent with the intervals of rotation angles chosen for the evaluation of the summation of the partition function $Z' = \lambda^N$: P. J. Flory, "Statistical Mechanics of Chain Molecules", Wiley, New York, N.Y., 1969, p 274.
(13) P. J. Flory, Ref 12, Chapter III.

## The Influence of the Macromolecular Protecting Group in Conformational Studies on Polyoxyethylene-Bound Peptides

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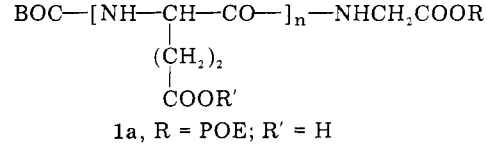
Systematic conformational studies on polypeptides in solution are mainly limited by the time-consuming synthesis and by the insolubility of many peptide sequences. The effective stepwise procedure of synthesizing peptides using solubilizing protecting groups, as realized in the liquid-phase method[1] (LPM), offers a solution to these problems. Here, the C-terminal amino acid of the peptide is attached to a soluble polymer by an ester bond; the macromolecular protecting group solubilizes the growing peptide chain and allows a quick, quantitative separation of all low molecular weight reagents. Owing to the favorable physicochemical properties in respect to the synthesis cycle, polyoxyethylene (POE) has proved to be the most suitable protecting group. The optical properties of this polymer allow investigation of the conformation of the POE-bound peptide without time-consuming deprotection and isolation steps.[2,3] Equally important is the strong solubilizing effect of the protecting group which enables conformational studies of the peptide in a great variety of solvents, including water. For the general reliability of such studies it is important to determine the extent of the influence of the POE chain on the conformational properties of the peptide.

In order to delineate the effect of POE, we synthesized homooligopeptides with a strong tendency to form secondary structures on POE. In each step of the synthesis, the POE



1a, R = POE; R′ = H
b, R = H; R′ = H
2a, R = POE; R′ = benzyl
b, R = H; R′ = benzyl

BOC = *tert*-butoxycarbonyl

group was cleaved from a sample and the conformation of the POE-bound peptide was compared with the free peptide under various conditions. Oligomers of L-Glu (**1**) and L-(γ-Bzl)-Glu (**2**) turned out to be most suitable for this purpose, because they are readily soluble.[4,5]

Oligomers of up to $n$ = 20 were synthesized according to the procedure of the LPM described elsewhere[1] using POE of $M_w$ $2 \times 10^4$ esterified by glycine; this residue served as an anchor group between peptide and POE. For the detection of any influence of the POE chain on the conformation of the peptide, the CD was measured after each step under various conditions. The CD spectra of the POE peptides **1a** in $H_2O$ are shown in Figure 1. The formation of a α-helical structure starts at $n$ = 7; at $n$ = 20 the helix content amounts to about 60%.[4] Identical CD spectra were obtained for the free oligomers **1b** for all chain lengths, including the transition region, e.g., for $n$ = 5–7 (Table I); the characteristic CD data of **1b** are in good agreement with (L-Glu) derivatives which have been obtained by polymerization of the corresponding NCA monomers.[4] The addition of POE to **1b** also had no influence upon the CD spectra (Table I). The line shape of the CD spectra was unaffected after adding 10% of POE to the solutions of the free peptides of various chain lengths. The helix–coil transition induced by the continuous neutralization of the COOH side chains did not display any detectable difference

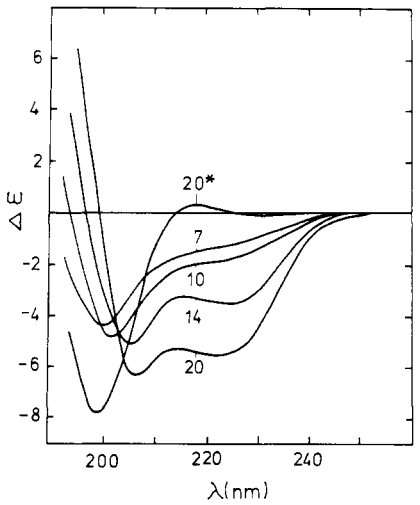



**Figure 1.** CD spectra of the oligomers **1a** and **1b** for different chain lengths $n$ in water, pH 3.9. (CD spectra were recorded on a dicrograph JASCO J-20 at 25 °C. The concentration was 0.2 mg of peptide/mL in all measurements. The concentration of the samples was determined by conductimetric titration and by amino acid analysis. The content of $\alpha$-helical structure[4] amounts to about 10% ($n$ = 7), 17% ($n$ = 10), 35% ($n$ = 14), and 60% ($n$ = 20). Curve $20^+$ corresponds to the fully neutralized oligomer **1a** with $n$ = 20 and is typical for a random-coil conformation.

in the conformational behavior between **1a** and **1b.** The fully neutralized oligomer with $n$ = 20 shows CD features characteristic of a random-coil structure (curve $20^+$ in Figure 1).

Also, the solvent-dependent properties were not changed by the POE group: Both series showed higher ellipticities in the helix-promoting solvent trifluoroethanol (TFE) compared to water, indicating stabilization of the $\alpha$ helix. The addition of increasing amounts of trifluoroacetic acid to **1a** and **1b** resulted in the expected disruption of the helical structure.

During the stepwise synthesis of the $\gamma$-benzylic-protected oligomer **2a,** a pronounced relationship between the solubility and viscosity behavior of the POE peptides and the conformation was observed. The low molecular weight oligomers with $5 < n < 10$, for which a $\beta$ conformation has been found in concentrated solutions of dioxan and dichloroethane,[5] yield highly viscous solutions in methylene chloride. Obviously the high viscosity reflects the formation of a network-like structure, arising from intermolecular hydrogen bonds of the peptide chains. With growing chain length, the viscosity of the POE-oligomer solutions decreases drastically which can only be explained by a conformational transition, e.g., by the onset of a helical structure. Similar observations were made earlier on polydisperse oligomers of this type without macromolecular protecting groups.[5] According to our results, the chain length at which the helical structure begins to predominate over the $\beta$ form in methylene chloride is approximately 10–12 residues. In the helix-promoting solvent TFE, the helical structure is the prefered conformation event at shorter chain lengths (Table I). Similar to the case of the (L-Glu) oligomers, the formation of a helical structure starts at $n$ = 7 whereas the lower oligomers have random-coil structure in this solvent. Again, identical CD spectra were obtained for the series **2a** and **2b.**

CD studies on hydrophobic oligomers with a preference for extended $\beta$ conformations indicate that also in this case the POE group has no influence upon the formation of intermolecular $\beta$ sheets. For example, the same CD data, characteristic for a $\beta$ structure, were obtained for the oligo-(L-Ala) series with high and low molecular weight ester groups.[2] Preliminary results of POE-bound oligo-(L-Val) and oligo-(L-Ile) confirm this observation.[6] A direct comparison between the POE peptides and the free oligomers is difficult in these series because of solubility problems.[7]

**Table I**
**Characteristic CD Data of the POE-Bound 1a and 2a and the Free Oligomers 1b for $n$ = 20 [a]**

| Oligomer | $\Delta\epsilon_{207}$ [b] | $\Delta\epsilon_{222}$ [b] | Solvent |
|---|---|---|---|
| **1a** | −6.3 | −5.5 | $H_2O$, pH 3.9 |
| **1b** | −6.2 | −5.4 | $H_2O$, pH 3.9 |
| **1b** + 10% (W) POE | −6.2 | −5.4 | $H_2O$, pH 3.9 |
| **2a** | −7.1 | −6.8 | TFE |

[a] The minima at λ 207 nm ($\pi \rightarrow \pi^+$) and 222 nm ($n \rightarrow \pi^+$) are typical for an $\alpha$-helical structure. [b] The ellipticity per amino acid residue is calculated by $\theta = \Delta\epsilon \times 3300$ (deg cm$^2$)/dmol.

The present investigations show that the conformational behavior of the POE-bound and free peptides is identical. In no case could a disturbance of the conformation of the peptide by the C-terminal blocking group be detected. This important finding can be explained by the conformational properties of the polyoxyethylene chain: Under the experimental conditions, POE has a random-coil structure with extended chain dimensions compared to the ideal state (theta point) due to the effect of the excluded volume.[3,8] Because of the low density and the high flexibility of the POE chain, a specific interaction between the peptide and the POE coil is very unlikely; this was also confirmed by kinetic studies on POE peptides, whereby identical kinetic behavior of both high and low molecular weight peptide esters was found.[9]

We conclude that conformational studies on POE-bound peptides are also relevant for the corresponding free peptides. Preliminary investigations show that beside the measurement of the CD, all other currently used methods such as NMR, IR, or UV can be applied directly to the polymer peptide. Thus, the LPM for peptide synthesis also offers a powerful tool in the field of conformational studies of peptides.

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## References and Notes

(1) M. Mutter, H. Hagenmaier, and E. Bayer, *Angew. Chem.*, **83**, 883 (1971); *Angew. Chem., Int. Ed. Engl.*, **10**, 811 (1971); M. Mutter and E. Bayer, *Angew. Chem.*, **86**, 101 (1974); *Angew. Chem., Int. Ed. Engl.*, **13**, 88 (1974); M. Mutter, R. Uhmann, and E. Bayer, *Justus Liebigs Ann. Chem.*, 901 (1975).
(2) M. Mutter, H. Mutter, R. Uhmann, and E. Bayer, *Biopolymers*, **15**, 917 (1976).
(3) M. Mutter, "Habilitationsschrift", University of Tübingen, D-7400 Tübingen, W. Germany, 1976.
(4) M. Rinaudo and A. Domard, *J. Am. Chem. Soc.*, **98**, 6360 (1976); M. Goodman, A. S. Verdini, C. Toniolo, W. D. Phillips, and F. A. Bovey, *Proc. Natl. Acad. Sci. U.S.A.*, **64**, 444 (1969).
(5) T. Imae and S. Ikeda, *Biopolymers*, **11**, 509 (1972).
(6) H. Mutter, Dissertation, University of Tübingen, work in progress.
(7) M. Goodman, C. Toniolo, and F. Naider, "Peptides, Polypeptides and Proteins", Wiley, London, 1974, p 308.
(8) J. E. Mark, and P. J. Flory, *J. Am. Chem. Soc.*, **87**, 1415 (1965); K. Liu and J. L. Parsons, *Macromolecules*, **2**, 529 (1969); P. J. Flory, "Statistical Mechanics of Chain Molecules", Interscience, New York, N.Y., 1969, p 165.
(9) E. Bayer, M. Mutter, R. Uhmann, J. Polster, and H. Mauser, *J. Am. Chem. Soc.*, **96**, 7333 (1974).

## Hypersonic Relaxation in Poly(ethylene oxide)

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The observation and interpretation of the glass–rubber relaxation in semicrystalline polymers is subject to consid-