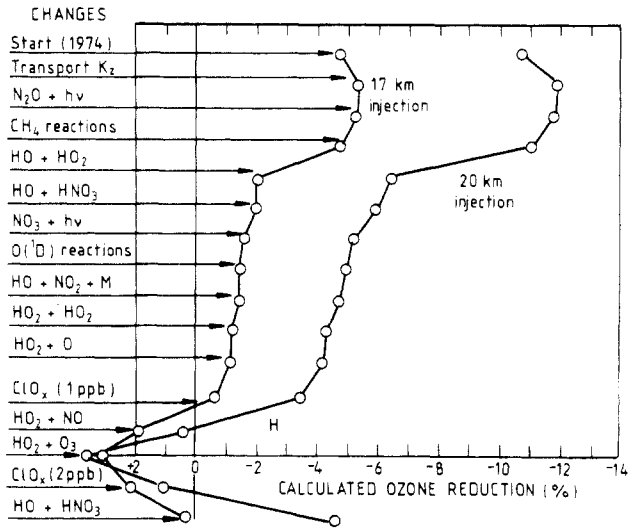

Figure 9. Predicted ozone depletions for large fleets of Concordes or proposed U.S. SST aircraft, corresponding to 17- and 20-km altitude injection of nitrogen oxides, respectively. Top point corresponds to the termination of the Climatic Impact Assessment Program in 1974; successive lower points correspond to increased understanding of atmospheric chemistry or new laboratory determination of the rate coefficient for an atmospheric reaction; lowest point corresponds to the situation in 1980.

HO_2 also undergoes a third-order combination reaction with NO_2 to form pernitric acid (HO_2NO_2),[22] a species which may have some importance in atmospheric chemistry. In contrast, HO_2 + NO undergo the bimolecular transfer reaction 8 which has a negative temperature coefficient,[23] presumably because it proceeds via the intermediary of a vibrationally excited pernitrous acid molecule (HO_2NO). Interestingly, ClO shows exactly analogous behavior to HO_2 in these reactions, bringing to mind the similarities between the reactions of Cl and HO which were noted by kineticists many years ago.

The recent observation by Radford[24] that the F + CH_3OH reaction yields both CH_3O and CH_2OH and that the latter, which is thermodynamically the more stable, reacts rapidly with O_2 to yield CH_2O + HO_2 whereas CH_3O does not shows that we may expect many interesting new applications of LMR spectroscopy in chemical kinetics.

Conclusion

Because far fewer gaseous species have identifiable UV spectra than have characteristic far-infrared or infrared spectra, the potential of these spectral regions for both kinetic and structural studies is considerable. The very narrow line widths of lasers and the possibility of intracavity detection give LMR spectroscopy a much higher sensitivity for free radicals than Fourier transform infrared spectroscopy. This is particularly important for the application to the atmospheric reactions discussed in this Account. Although tunable infrared lasers have considerable potential for studies of this sort, the problems of frequency measurements and stability need to be solved for infrared lasers to become as useful for gaseous species as laser magnetic resonance spectroscopy.

This work owes much to the colleagues whose names appear in the references and to generous support by Science Research Council, The Royal Society, Chemical Manufacturers' Association, and Federal Aviation Administration.

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Conformational Studies of Poly(oxyethylene)-Bound Peptides and Protein Sequences

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A thorough knowledge of the conformation of proteins and biologically active peptides is necessary for the understanding of their structure–activity relationships and physicochemical properties.[1,2] The conformation of a peptide or a protein is determined by the balance of the intramolecular noncovalent interactions between the various groups in the amino acid sequence as well as of interactions between these groups and the surrounding solvent medium.[3] The biological activity and functions of proteins depend mainly on the way the molecule is folded, and in many cases conformational changes appear to be the prerequisites for effective biological action.[4,5]

Experimental and theoretical investigations on model peptides have been extremely useful in the interpreta-

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tion of the conformational behavior of proteins and for the elucidation of the interaction between proteins and other systems. Since the conformation of a peptide consisting of a specified amino acid sequence under a set of conditions is spontaneously adjusted,[6] these investigations deal substantially with the molecular mechanisms responsible for the origin of the preferred conformations. Short-range interactions play a significant role in these mechanisms for the adoption of the biologically active conformations of proteins.[7-9] Experimental investigations on polypeptides, oligopeptides, and relatively short peptide fragments of proteins provide useful information about these interactions since many of the structural features occurring in proteins can be realized in these synthetic models. Thus, studies on such model peptides give valuable information about the effects of composition, sequence, chain length, and solvation on the secondary structure.

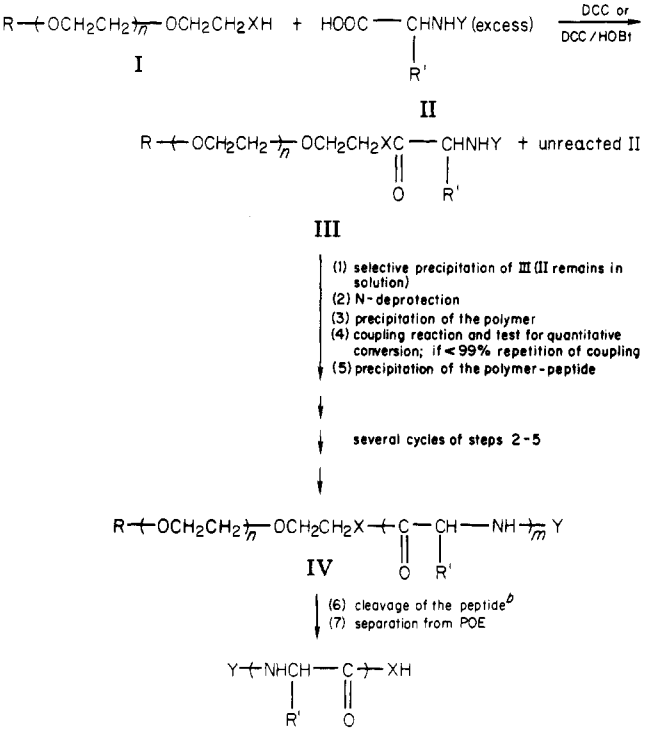
Systematic experimental investigations on the conformation of peptides and protein sequences have been limited mainly because of the tedious and time-consuming synthetic procedures and the insolubility of most of the peptide sequences in suitable solvents.[10] The problem of the low solubility of peptides is particularly critical because almost all the spectroscopic techniques employed for the conformational studies of peptides demand good solubility in organic and aqueous solvents. The investigations in aqueous solutions are of immense relevance as water plays a very important role in the process in which proteins acquire their native structure and in their interaction with other small and large molecules.[11,12] Moreover, since protein folding occurs in water, the final conformation is influenced strongly by the solvent. Experimental investigations of the structure of model peptides and protein fragments in aqueous solution are necessary to understand the nature of such interactions. The determination of the helix–coil stability constants for naturally occurring amino acids in water is particularly helpful in assessing the stability of proteins. The host–guest technique introduced by Scheraga and co-workers[13] in this connection enables one to obtain detailed information regarding the thermodynamic and conformational parameters of single guest amino acids in various positions of a host peptide chain.[14]

The solubility problem of the peptide, the conformation of which is to be investigated, was solved to some extent by copolymerizing it with a hydrophilic peptide sequence[15-18] or by using solubilizing protecting groups.[19] Gratzer and Doty first introduced the former technique to study sequences which would otherwise be insoluble.[15] They illustrated that a block copolymer, copoly(DL-glutamic acid)$_{175}$-(L-alanine)$_{325}$-(DL-glutamic acid)$_{175}$, yielded a 100% helical alanine block at pH values where the glutamates were ionized and conferred solubility. Ingwall et al.[18] investigated the helix-to-coil transition that occurs when poly(L-alanine), incorporated between two blocks of poly(DL-lysine), is subjected to changes in temperature and solvent. Here again the terminal peptide blocks are ionized in the nonhelical form and do not contribute to the optical rotation of the polymers. The method appears to be limited for cases where the specific interactions between the blocks are negligible. The method of using a low molecular solubilizing amino protecting group is suited only to special cases where the N-terminal group has no effect on the overall conformation of the peptides.[19] In addition, the solubilizing power of such groups proved not to be strong enough to overcome the severe solubility problems of many peptide sequences.

The liquid-phase method (LPM) of peptide synthesis,[20] introduced in 1971 by Mutter et al.,[21,22] makes use

Scheme I

A Generalized Scheme for the Liquid-Phase Method of Peptide Synthesis[a]



[a] In this scheme R = CH_3 or CH_2CH_2XH; X = -O- or -NH-; R' = side chain of the amino acid; Y = temporary amino-protecting group. [b] The method for the removal of the peptide from the POE support depends on the sequence in question; the use of anchoring groups of different stability between the POE and the C-terminal amino acid permits almost quantitative splitting (M. Mutter, *Tetrahedron Lett.*, 2839 (1978); V. N. R. Pillai, M. Mutter, and E. Bayer, *Tetrahedron Lett.*, 3409 (1979); V. N. R. Pillai, M. Mutter, E. Bayer, and I. Gatfield, *J. Org. Chem.*, 45, 5364 (1980)).

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of solubilizing macromolecular C-terminal protecting groups and provides a more general and facile approach to the problem. The method permits rapid stepwise synthesis of peptides in solution and is a definite simplification of the experimental procedures involved in the classical peptide synthesis. The advantage of this method over the established Merrifield solid-phase peptide synthesis is the elimination of heterogeneous reaction conditions and consequent steric problems. Through the presence of a macromolecular protecting group, polyoxyethylene (POE), at the C terminus of the peptide chain, the advantages of polymer-supported synthesis are preserved; at the same time effective coupling can be assured by working in a homogeneous solution; the POE–peptide can be purified by selective crystallization from organic solvents (Scheme I).

This relatively easy access to the desired sequences and the solubilizing effect of POE facilitate the application of the various techniques for the conformational study of peptides and protein sequences without much experimental difficulty. The POE-bound peptides can be subjected to conformational investigations in aqueous and organic solutions using CD and ORD measurements. Other commonly used methods such as IR, NMR, UV, and energy-transfer measurements can also be applied to the POE-bound peptides in solution. The crystallinity of these bound peptides allows X-ray investigations also. Detailed information regarding the critical chain length for the onset of secondary structures and dependence of the secondary structure on the sequence, solvent, temperature, etc., can be obtained by a systematic investigation of the polymer-bound peptides. This would permit the prediction of the preferred conformation of the peptides under various environments. Conversely, the influence of the conformational changes on the physicochemical properties of the growing peptide chain can be delineated from these studies. Thus, for example, the effect of the onset of any secondary structure on the stepwise coupling kinetics or on the solubility of the sequence can be systematically followed by making use of the peptide–POE esters. Such information can be exploited in planning a strategy for the stepwise or segment condensation approaches for peptide synthesis.

For establishment of the above-mentioned applications of the LPM in the conformational studies of the bound peptides, the following aspects had to be considered first: Does the macromolecular C-protecting group, POE, have any influence on the conformation of the bound peptides? How are the physicochemical properties of the peptide–POE esters influenced by the chainlength of the peptide? Does POE retain its physical characteristics such as crystallinity and solubility behavior after fixation of the peptide sequences on it? Does the polymer–peptide ester behave kinetically similar to the low molecular peptide ester so as to facilitate quantitative peptide coupling during the stepwise synthesis? These considerations are of much significance also for the development of peptide synthesis on the soluble polymeric support. A detailed analysis of these problems requires the study of the conformation-dependent physicochemical characteristics of poly(oxyethylene) and poly(oxyethylene)-bound peptides.

Conformation and Physicochemical Properties of Poly(oxyethylene)

The conformation of a polymer in the solid state and in solution determines its physicochemical properties to a large extent.[23] The factors such as physical handling of the solid precipitates, inclusion of low molecular weight components, and reactivity of functional groups attached to the polymer are of relevance to the synthetic strategy, and these aspects are intimately related to the conformational characteristics of the chain molecule. Theoretical studies show that the trans,-trans,gauche conformation for the POE segment C–O–C–C has minimum energy.[24,25] This preferred conformation of POE is observed in its crystal structure, which consists of a loosely turned 7_2-helix with an identity period of 19.3 Å.[26] The linear chain molecule crystallizes in monoclinic form with 28 monomer molecules per unit cell. Theoretical calculations indicate that the trans,trans,gauche conformation is a result of short-range intramolecular interactions which dominate in POE crystals.

When POE is dissolved, the ordered crystal structure is destroyed due to rotation about the C–O and C–C bonds.[24] When the rotational isomeric state model, which takes into account the neighbor-dependent interactions along the chain molecule, is used, the physicochemical properties of POE can be adequately explained by assuming a random-coil conformation.[27,28] The conformational parameters deduced from this model indicate that the POE molecule is very flexible in solution, resulting in a high coiling of the chain in Θ-like solvents. However, in strongly polar solvents such as water, $CHCl_3$, or DMF, a significant retention of the ordered structure can be observed.[29] Consequently, the average chain dimensions of POE with respect to the average space occupied by the polar molecule are considerably increased in polar solvents, so that the chain can be considered as a freely permeable coil under these conditions.

In the kinetic investigations on POE–peptides, both functional groups behave equivalently and the reaction of POE–peptides with low molecular reagents was observed to follow linear kinetics.[30] This behavior has also its origin in the conformational characteristics of the soluble linear POE molecule. In spite of the coiling of the polymer chain in Θ-like solvents, the segmental density of POE under these conditions is only about 1%. This means that the coil provides sufficient "free space" to permit the substrate molecule to penetrate without any hindrance. The problems of diffusion and

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steric hindrance of the functional groups do not dominate here because of the much higher segmental mobility. In concentrated solutions, the situation may be different; here the molecular motion is very much suppressed due to the interpenetration of the chain in more viscous solutions.

The statistical coil structure with minimum segmental density excludes any specific interaction between the POE chain and the bound peptide. Because of this unique conformational behavior, POE is excellently suited for the use as a soluble carrier for stepwise synthesis of peptides and for their conformational analysis by using various experimental techniques. The ethylene units and the ether oxygens that alternate along the polymer chain give rise to the hydrophobic and hydrophilic character of POE. Consequently, POE is soluble in water and in a large number of organic solvents. This property is very beneficial to the concerned experimental techniques.

Properties of the POE-bound Peptides

The strategy of the LPM is based on the principle that the physical properties of the peptide are dominated by those of the C-terminal macromolecular protecting group. Specifically, the crystallization tendency and the solubility characteristics of POE should be retained throughout the stepwise incorporation of the amino acids for a successful synthesis. For quantitative coupling yields, the peptide–polymer ester must behave kinetically similar to the low molecular peptide ester; that is, a linear kinetic behavior is a necessary condition for the effective stepwise synthesis. As expected, the influence of the peptides on these properties of the polymer was found to depend on the primary sequence, side chain protection, chain length, and conformational preferences of the growing peptide chain.

X-ray studies on poly(oxyethylene)s of varying molecular weights and on differentent POE-bound peptides showed that the incorporation of the peptide does not disturb the crystal lattice of POE and its degree of crystallization is lowered only by a relatively small factor.[31,32] This high retention of crystallinity of POE even after the attachment of the amorphous peptide blocks can be understood when we consider the X-ray investigations on pure POE. These studies reveal that chain folding occurs in crystals of POE of molecular weight greater than 3000.[33-35] X-ray investigations on the block copolymer of POE and polystyrene also showed that in the crystalline state the amorphous polystyrene occupies a space between two POE blocks.[26] These results suggested a similar two-phase model for the POE–peptides in the crystal state (Figure 1); this explains the high retention of the crystallinity of POE when it is bound to the amorphous peptides. According to this model, crystalline POE layers alternate with amorphous peptide phase; due to the regular folding of the POE chains, the bulky peptide coils are arranged between the POE blocks without disturbing the crystalline structure of POE.

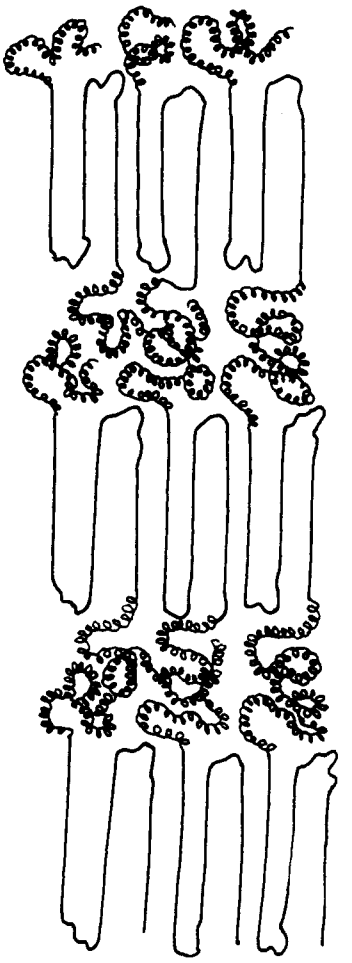

Figure 1. Model for POE–peptides in the crystal state: solid rule, POE segments; coil, peptide coils.

Poly(oxyethylene) has a strong solubilizing effect on the bound peptides. However, the solubility characteristics of the POE–peptides depend strongly on factors such as sequence, chain length, and conformation of the peptide. With increasing chain length, the properties will be dominated by those of the peptide rather than POE. However, the solubilizing power of POE proved to be strong enough for the investigation of peptides with chain lengths where conformational transitions may occur. The solubilizing effect of POE in the molecular weight range 4000–20 000 remains relatively the same. The viscosity of the solution of the POE–peptides, in general, increases starting from the sequences which contain 6–8 amino acids. This presumably is due to the association of the peptide chains. By the addition of a polar solvent such as dimethylformamide, the peptide aggregates can be destroyed and the viscosity of the solution is reduced.

POE-bound amino acids and peptides behave kinetically similar to their low molecular weight analogues. This was ascertained by a kinetic study of the aminolysis of N-protected amino acid esters by amino acid POE esters of varying molecular weights and by their low molecular weight analogues.[30,36] Within the range 2000–20 000, the reaction between the active ester of the amino acid and the amino acid POE esters strictly followed second-order kinetics from the beginning of the coupling until about 80% completion.

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The C-terminal POE group must allow the application of all commonly used methods such as CD, NMR, IR, or Raman spectroscopy, and at the same time it should not influence the conformational behavior of the peptide for a direct investigation of the conformational properties.[37] POE shows no Cotton effect and is optically transparent upto the far-UV region; consequently, the measurement of the CD in each step of the synthesis has become a routine method in the LPM for following any conformational change of the growing peptide. The solubilizing effect of POE on the peptides in solvents suitable for CD investigation, such as trifluoroethanol, trifluoroacetic acid, or water, is very strong. In the ^1H NMR spectrum, $(CH_2CH_2O)_n$ shows only one singlet for the CH_2 group at δ 3.6, and this does not interfere with the signals of the peptide which are sensitive toward conformational changes. The infrared analysis can be applied directly to the POE-peptide in the solid state and in solution; the characteristic absorption bands of POE do not interfere with the spectral regions of interest (amide A, I, II, and V regions)[38] for the conformational analysis of peptides. In particular, the O–H stretching vibration of the free alcoholic groups absorbs strongly only at frequencies higher than 3450 cm^{-1}, clearly outside the range where the urethane and amide N–H bands are observed; the strong C–O–C absorption of POE is seen at about 1100 cm^{-1}. The solubilizing effect of POE allows the IR investigations in a wider range of solvents for the bound peptides than it is possible with low molecular weight C-terminal esters.

For the general reliability of the conformational investigations of the bound peptides, it is necessary to determine the extent of influence of the POE chain. For this purpose, we synthesized homooligopeptides with a strong tendency to form secondary structures using POE (M_r 20 000) as the soluble macromolecular C-protecting support and measured the CD under various conditions after each step; these data were compared with those obtained for the unbound oligomers. The CD spectra of Boc-(L-Glu-)$_n$-OPOE in water at pH 3.9 for different values of n are shown in Figure 2. The formation of an α-helical structure starts at $n = 7$ and amounts to about 60% for $n = 20$. Identical CD spectra were obtained for the free oligomers also for all chain lengths including the transition region.[39] The addition of POE to the free oligomers had no influence on the spectra. The helix-to-coil transition induced by the neutralization of the carboxyl side chains was also the same in both the polymer-bound and free oligomers. Thus the fully neutralized oligomer with $n = 20$ showed the expected random-coil conformation (curve $20^{(-)}$, Figure 2).

The fact that POE does not influence the preferred conformation of a peptide significantly can be explained by the conformational peculiarities of POE outlined earlier in this Account. These observations suggest that the synthesis of peptides and protein sequences on the soluble macromolecular support POE can be made use of as a new technique for their conformational analysis under a variety of conditions.

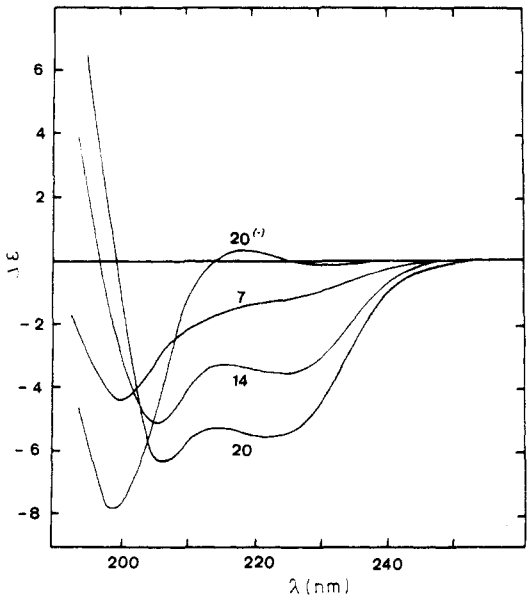

Figure 2. CD spectra of POE-bound (L-Glu)$_n$ for different values of n in water at pH 3.9; curve $20^{(-)}$ corresponds to the fully neutralized oligomer; $\Delta\epsilon$ in mol^{-1} L cm^{-1}.

Conformational Analysis of Homooligopeptides by Use of CD Studies

The stepwise synthesis on the soluble polymeric carrier POE permitted the systematic analysis of the strong dependence of the preferred conformations of a number of homooligopeptides on the chain length, solvent polarity, N-protection, side chains, pH, concentration, temperature, and ionic interactions.[37,39,40-43] Most notably, the attachment to POE enabled for the first time the CD spectral measurements of the β-forming hydrophobic oligomers of Ala, Val, and Ile up to medium chain lengths.[41] In the case of POE-bound oligoalanine, a transition from statistical coil to β conformation occurred at a chain length of 7–8 residues; in the case of $(Ala)_7$, a complex CD pattern, attributable to a mixture of unordered and β forms, is visible. The N-protected, POE-bound $(Ala)_8$ is the first fully protected monodispersed alanine oligopeptide which exhibits a significant amount of ordered secondary structure in water. This observation confirms that the ordered secondary structure preferred by fully protected homooligoalanines in solvents of low solvating ability is the β structure.[10,19,41,44-46]

High concentration, temperature, and ionic strength enhance the β-structure formation in POE-bound $(Ala)_n$ and $(Val)_n$. The influence of the concentration emphasizes the importance of interpeptide hydrogen bonds in the stabilization of the β forms in these peptides. The enhancement of β-structure formation by heating or by the addition of salts points to the role of

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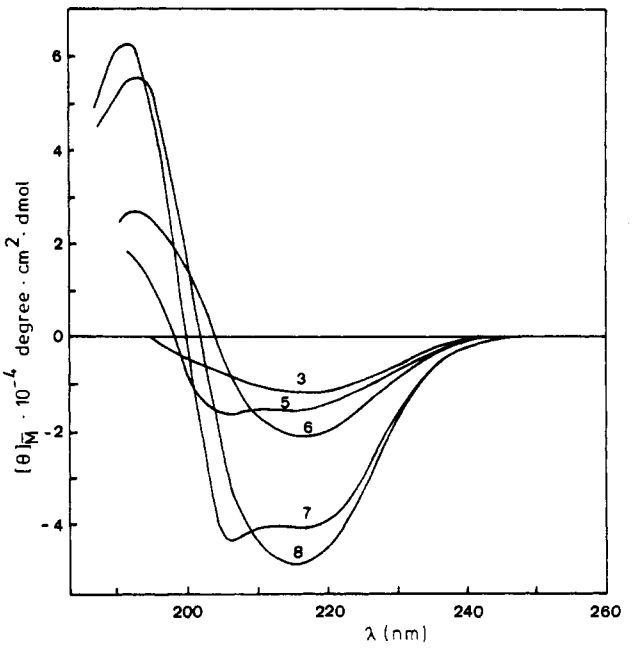

Figure 3. CD spectra of N-*t*-Boc-(L-Ile)$_n$-OPOE for different values of n in trifluoroethanol; Φ in molar ellipticities per residue.

hydrophobic interactions on the stability of the aggregated species.[47-52]

The characteristic CD data of POE-bound homooligoisoleucines points to the onset of a partial β structure at the 6-peptide level (Figure 3). As in the case of (Ala)$_n$ and (Val)$_n$, the spectra of the 7-peptide and 8-peptide are completely different from those of the lower oligomers. In contrast to the Ala series, here the random-coil → β-structure transition starts at shorter chain lengths. This indicates a much higher stability for the β structure in the oligoisoleucines. Even concentrations as low as 0.02 mg of peptide/mL did not result in a significant disruption of the β sheets for (Ile)$_8$. This unusual stability of the 8-peptide may be explained by the possibility of the formation of an intramolecular β structure (cross-β structure). Such a model should result in the bending (β bends) of the peptide to form intramolecular hydrogen bridges which are not destroyed by dilution. From these observations, it can be seen that the tendency to adopt associated β structures in solution at chain lengths larger than 6–8 residues increases in the order Ala < Val < Ile.

The CD investigations on the conformational properties of homooligomethionine POE esters to the 15-peptide level under different conditions revealed that the methionine peptides exist in the right-handed α-helical, β-, and statistical-coil conformations depending upon the chain length, N-protection, solvent polarity, temperature, concentration, pH, and ionic strength.[43] In the N-protected series, the peptides up to $n = 6$ exist predominantly as statistically unordered forms, characterized by a relatively intense negative maximum near 200 nm (amide $\pi \rightarrow \pi^*$ transition) accompanied by a weak negative maximum located at about 220 nm (amide n → π* transition). The CD spectrum indicative of the onset of the right-handed α-helical conformation is clearly seen at the 7-peptide level (intense negative maxima at 220–221 nm and at 207–208 nm);[53-56] these characteristic features remain substantially unaltered further throughout the series.

Under the same experimental conditions, significantly different conformational preferences are shown by the higher molecular weight oligomethionines in the N-deblocked series.[43] Already at the 10-peptide level a CD spectrum suggesting partial onset of the β-conformation is observed. This shows that in trifluoroethanol the absence of the bulky *N*-*t*-Boc group markedly favors the tendency of the peptides to form a β structure. When the formation of the β structure is prevented, the tendency of the higher oligomers to adopt the right-handed α-helical structure becomes evident. It is interesting to note that this α-helix → β-structure transition is not observed in hexafluoro-2-propanol. An investigation of the nature of the solvent on the conformations of homooligomethionines in this connection showed that in solvents of high tendency to solvate the peptide chain the α-helical conformation is the preferred one for the N-protected higher oligomers, whereas in the less polar solvents β structure is favored.

CD Studies of POE-Bound Biologically Active Peptides and Protein Sequences

The stepwise synthesis on the soluble polymeric support POE has enabled the systematic conformational analysis of a number of biologically active peptides and protein sequences by CD studies. In addition to providing a better knowledge of the conformational transitions occurring in the peptides, these studies correlate with the recent investigations devoted to clarify the conformation and conformational stability of proteins when covalently linked to a water-soluble polymeric matrix.[57-59]

POE-Bound ACTH Sequences. The segments [Val^4]ACTH(1–10), [Pro^1,Ala^2,Ala^3,Val^4]ACTH(1–10), and ACTH(11–23) were synthesized by the LPM and the dependence of the conformational preferences on the chain length, sequence, side chain protection, and solvent was investigated by a CD study in water and in trifluoroethanol.[60] These experimental data were compared with the results from the calculation of secondary structures deduced from the empirical prediction scheme of Chou and Fasman[61,62] and the more elaborate statistical mechanical treatment of peptide conformations proposed by Tanaka and Scheraga.[63,64]

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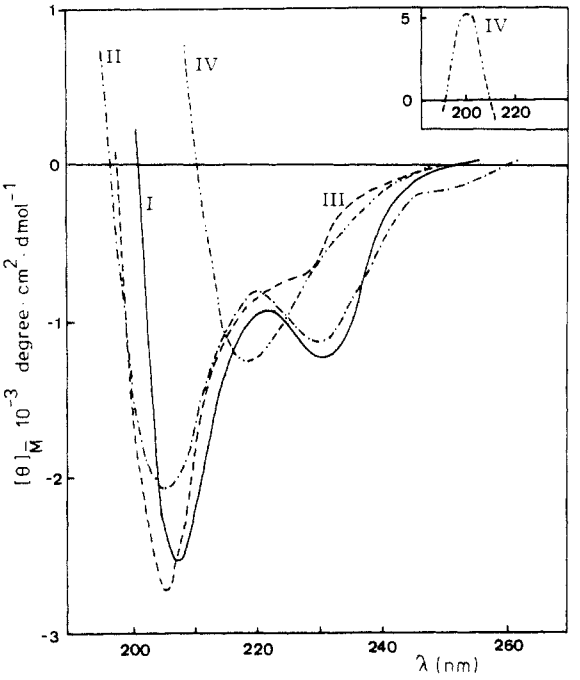

Figure 4. CD spectra of POE-bound protected (—) and deprotected (·····) [Val^4]ACTH(1–10) peptide and of protected (2–10) peptide (---) in trifluoroethanol; (·····) POE-bound protected (1–10)-peptide in water; Φ in molar ellipticities per residue.

The POE-bound 10-peptide, [Val^4]ACTH(1–10), in the protected and unprotected forms, exists in helix-promoting solvents like trifluoroethanol as a partially helical structure (Figure 4, curves I and II). From the ellipticity values and the characteristic CD data the helix content of the 10-peptide can be calculated as 5–10%. The 9-peptide exists mainly (>90%) in the random-coil conformation (curve III, Figure 4). By changing the solvent to water a sharp transition occurs: all the three peptides show a CD spectrum typical for a β structure. The drastic conformational change from unordered ones (in trifluoroethanol) to β structure (in water) can be attributed exclusively to the influence of the solvent (curve IV, Figure 4). Similar solvent effects have been observed for the homooligopeptides also.[40,43]

The influence of the N-terminal amino acid on the overall conformation of the peptide can be observed from the CD analysis of the POE-bound, modified ACTH(1–10) sequence, [Pro^1,Ala^2,Ala^3,Val^4]ACTH(1–10).[60] No transition to β structure was observed on changing the solvent, indicating a helix-inducing effect of Pro at the N-terminus of a peptide. However, a destabilization of the helical structures was observed in aqueous solution and the ellipticity values were also significantly reduced compared to the organic medium. In the case of the POE-bound ACTH(11–23) sequence, the CD spectra in trifluoroethanol and water are characteristic of partially helical structures. By changing the solvent from trifluoroethanol to water, the helix content decreases from ~20% to 10%.

The agreement between the above experimental results on POE-bound ACTH sequences and the theoretical predictions of their secondary structures by the Chou–Fasman and Tanaka–Scheraga treatments is reasonable in the case of the two 10-peptides. In the case of the 13-peptide, it is expected to adopt a partially developed extended conformation rather than helical structures. Most notably, the predicted asymmetric helix nucleation effect of Pro could be verified experimentally for the first time. On the basis of these data, a model for the preferred conformation of ACTH(11–23) was proposed,[60] which is compatible with the biological function of this peptide hormone.

POE-Bound Alamethicin Sequences. The investigations on the POE-bound alamethicin *t*-Boc-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-OH and Ac-Aib-Pro-Aib-Ala-Aib-Gln-Aib-Val-Aib-Aib-Gly-OH, indicate α-helix formation in the N-protected series beginning with the 9-peptide and with the 10-peptide in the N-protonated sequences.[65] These results suggest that in lipophilic media, the alamethicin helix can extend the full length of the partial sequence between the two proline residues and that aqueous media favor an increase of the random-coil conformation. The comparison of the temperature and solvent dependences of helices of the model peptides with those of the membrane-modifying peptides reveal similar conformational behavior of the sequences.[66–69]

IR Studies of POE-Bound Peptides

The results of the IR studies of the three series of monodisperse POE-bound linear oligopeptides having the general formulas *t*-Boc-(L-Ala)$_{1-8}$-Gly-OPOE, *t*-Boc-(Val)$_{1-7}$-Gly-OPOE, and *t*-Boc-(L-Val)$_{2-8}$-Gly-OPOE-M (POE-M = monofunctional POE) in the solid state indicate that in all three series the ordered secondary structure which is developed is the β structure, characterized by a medium-intensity band at 3280–3260 cm^{-1}, a strong band at 1635–1624 cm^{-1}, and a weak but distinct band at 720–705 cm^{-1}.[70] This conformation, which is partially formed at the n = 4 level, is fully developed at the n = 5 level. In the solid state, the effects of the side chain, the C-blocking group, and the number of POE functionalities, if any, are leveled off by the dominant interpeptide chain interactions.[71,72] The IR study of these peptides in $CDCl_3$ solution at high dilution shows that a much higher content of intramolecularly hydrogen-bonded forms exist in (Ala)$_4$ than in (Val)$_4$ peptides. At higher concentrations, chain length, solvent, and side chain effects are all operative in determining the extent of peptide association in these series.[70]

IR studies of the polymer-bound homooligomethionines having the general formulas *t*-Boc-(L-Met)$_n$-NH-POE (n = 1–15) and H_2^+-(L-Met)$_n$-NH-POE (n = 1–14) indicate that the oligopeptides exhibit an essentially unordered conformation upto n = 3 and a partly developed antiparallel β-structure when n = 4–6.[73] The oligopeptides with n = 7–12 adopt essen-

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tially a β structure and when n = 13–15 the peptides have a significant percentage of α-helical conformation.

In the case of POE-bound homooligoglycines, for the observation of a well-developed antiparallel β structure a critical chain length of 7 residues is necessary;[74,75] for very short chain lengths, a significant influence of the macromolecular C-protecting group is observable. Thus, when n = 3–5, the peptides assume a predominant conformation with hydrogen bonds of the interchain type, which is quite different from the usual antiparallel β-helix and ternary helix conformations of polyglycine. Contrary to this finding, Ac-$(Gly)_{1-4}$-NHEt's assume the ternary helix conformation.[76] In solvents of low polarity, both intrapeptide chain H-bonded folded forms and interpeptide chain H-bonded associated forms, most probably of the extended type, occur.[74,75]

NMR Studies

The $^{13}C\{^{13}C-^{1}H\}$ triple resonance technique has been used for the conformational studies of polypeptides bound to POE supports.[77] In the ^{13}C NMR spectra, POE exhibits only one signal at 71.5 ppm for the inner chain carbons, and this peak does not interfere with other common signals of the bound peptides. The ratio of the number of POE carbons to the signal carbons of the amino acid residue is about 140:1 in the case of POE-6000. Therefore, the concentration of the POE–peptide solution for ^{13}C measurements should be 0.05 mol/L or higher with respect to the bound peptide in order to obtain reasonable signal-to-noise ratios. High resolution shows distinct differences between random-coil and α-helical conformations. The secondary structures of the partial sequences of the polypeptide antibiotic alamethicin have been investigated by using the triple resonance technique.[77] The results are in agreement with those obtained from CD measurements and theoretical calculations.[65–68] ^{1}H NMR measurements have also been applied to POE-bound peptides.[37]

Relationship between Conformation and Physicochemical Properties of the Peptides

The stepwise synthesis and conformational analysis of peptides on POE reveal a drastic influence of the conformational transitions of the growing peptide on its physicochemical properties. The solubility of the peptides and the reactivity of the terminal amino groups are significantly reduced in oligopeptides exhibiting β structures such as the POE-bound oligomers of Val and Ile.[78,79] Oligopeptides with tendencies to adopt α-helical or unordered structures showed no pronounced change in the solubility or coupling kinetics during chain elongation in the liquid-phase synthesis, as exemplified in the case of the oligomethionines.[78]

In the case of the POE-bound $[Glu(OBzl)]_n$, the development of α-helical conformation starts at n = 7 in helix-promoting solvents. During the stepwise synthesis, a peculiar dependence of the physicochemical properties on the chain length was observed. The solubility of the oligomers in CH_2Cl_2 decreased considerably with the growing peptide chain and showed minimum solubility in the range n = 6–10. With further elongation, a continuous increase in solubility was observed, and for n > 12, the POE–peptides exhibited almost the same solubility as the lowest oligomers (n < 5). This change in solubility with increasing chain length is paralleled by conformational transitions of the type random-coil ($n \leq 5$) → aggregated β-like structures ($5 < n \leq 8$) → α-helical conformation ($n > 8$) in this solvent.[39,80] The occurrence of minimum solubility of medium-sized peptides appears to be a general feature and seems to reflect the tendency of certain peptides to form intermolecularly H-bonded structures in this range. Similar correlation of the solubility behavior of a number of other oligopeptides with their conformations indicates that the dependence of the solubility on the chain length and conformation can be represented as in Figure 5.

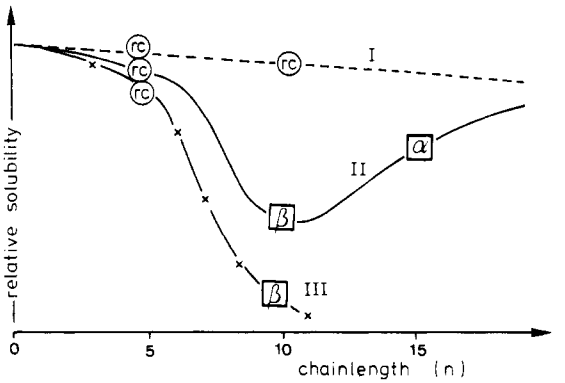

Figure 5. Dependence of the solubility of the oligopeptides with chain length: (I) peptides with random coil conformation; (II) peptides with tendency for conformational transitions; (III) β-structure-forming peptides.

Detailed information regarding the conformational effects upon the physicochemical properties could be obtained from the investigation of POE-bound co-oligopeptides.[78,80,81] Such studies based on the host–guest technique[13,14] enable the elucidation of the conformational preferences of single amino acids as well as the effect of the guest amino acids on the stability of the ordered structures in various positions of a peptide chain. Considering conformational energies, the insertion of a Gly or Pro should result in substantial disruption of ordered conformations. Due to the steric interactions with the neighboring residues, Pro is predicted to interrupt the growth of an α helix; moreover, Pro cannot be part of a regular β structure. Gly is expected to destabilize α or β conformation because of its high flexibility.

The conformational studies of a number of host–guest peptides bound to POE support the above suggestions. CD investigations show that the α-helix content in trifluoroethanol of POE-bound Boc-$(Met)_5$-Pro-$(Met)_5$ (28%) approaches that of Boc-$(Met)_7$ (30%), and it is much lower than that of Boc-$(Met)_{11}$ (80%). Similarly

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the CD spectrum of Boc-$(Ala)_{10}$-OPOE is indicative of a pure β structure whereas Boc-$(Ala)_5$-Pro-$(Ala)_4$-OPOE gives a spectrum characteristic of mostly unordered conformations.

The drastic effect of the Pro residue on the ordered conformations is also demonstrated for the case of POE-bound Boc-[L-Glu(OBzl)]$_n$ cooligopeptides in the solid state and in solution.[80,81] Similar destabilizing effects were observed for the case of a glycine residue as guest amino acid.[82]

The observed effects of Pro and Gly on the conformations of host oligopeptides are paralleled by drastic changes in the solubility and coupling rates of the peptides.[78] The destabilization or disruption of the otherwise β-structure-forming homooligopeptides by a Pro or Gly residue results in a substantial increase in solubility and coupling rates. A systematic study on a quantitative level on the interdependence of conformation and physicochemical properties of peptides using the host–guest technique will provide valuable information on the strategic considerations in planning the synthesis of longer peptides.

Summary and Conclusions

The physicochemical properties of POE's permit their use as solubilizing macromolecular C-protecting groups for peptides and biologically active protein sequences in connection with conformational studies. The investigation of the conformational behavior of peptides in solution have been limited by the low solubility, even of relatively short peptides. This limitation can be overcome by the attachment of the peptide on POE, and the resulting peptide POE esters can be subjected to the usual techniques for the conformational analysis in a variety of solvents including water. Most notably, the preferred conformations of hydrophobic peptide sequences could be delineated by this new technique.

The investigations on POE-bound peptides outlined in this Account establish that their physicochemical properties such as solubility and coupling rates depend on the conformational preferences of the peptide chain. These effects must be considered in the optimization of the reaction conditions during the peptide synthesis, if the peptide undergoes conformational transitions with increasing chain length. The stepwise synthesis on the POE support is possible as long as the physicochemical properties of the polymer-bound peptide are dominated by the polymer. In the case of helix-forming and randomly coiling peptides, the accessibility of oligomers with chain lengths upto 20 resdiues proved to be possible. However, in the case of β-forming oligomers, the limit seems to be attained at much shorter chain lengths. The problem of aggregation resulting in the low solvation of the peptide chains appears to be a less severe problem in the stepwise synthesis on an insoluble polymeric matrix.[83]

The above findings may have some practical implications in peptide synthesis. For example, segment condensation of medium-sized segments with low solubility to larger peptide chains may result in increased solubility of the reaction product due to conformational transitions. Further, whenever it is possible, amino acid residues with low tendencies for β-structure formation (i.e., with low P_β potentials[84]) should be inserted in the middle of the segments. Consequently, conformational aspects must be considered as an additional parameter in the tactics of the synthesis of larger peptides. Here the prediction of secondary structures[84,85] can be a useful tool in establishing the most efficient path of peptide synthesis. To this end, the conformational preferences of side-chain-protected trifunctional amino acid residues have to be elucidated experimentally.[86] The consideration of these phenomena in planning a synthesis strategy may help to overcome one of the most serious obstacles in peptide synthesis, the low solubility of many segments. The synthesis of peptides on the soluble polymeric support, poly(oxyethylene), and subsequent conformational analysis can be exploited for this purpose.

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