(32) C. H. Bushweller, J. W. O'Neal, and H. S. Bilofsky, *Tetrahedron*, **27**, 3065 (1971).
(33) R. J. Ouellette, *J. Am. Chem. Soc.*, **96**, 2421 (1974).
(34) C. H. Bushweller and J. A. Brunelle, *J. Am. Chem. Soc.*, **95**, 5949 (1973).
(35) D. R. Lide, Jr., and D. E. Mann, *J. Chem. Phys.*, **28**, 572 (1958).
(36) J. J. Breen, J. F. Engel, D. K. Myers, and L. D. Quin, *Phosphorus*, **2**, 55 (1972).
(37) S. O. Grim, W. McFarlane, and E. F. Davidoff, *J. Org. Chem.*, **32**, 781 (1967).
(38) L. D. Quin and J. J. Breen, *Org. Magn. Reson.*, **5**, 17 (1973).
(39) This conclusion was arrived at independently by another group: M. Haemers, R. Ottinger, D. Zimmerman, and J. Reisse, *Tetrahedron*, **29**, 3539 (1973).
(40) D. H. Williams and I. Fleming, "Spectroscopic Methods in Organic Chemistry", 2nd ed, McGraw-Hill, New York, N.Y., 1973, p 82.

Conformational Analysis of Peptides in Oriented Polyoxyethylene by Infrared Dichroism

R. T. Ingwall, C. Gilon,[1] and M. Goodman*

Contribution from the Department of Chemistry, University of California at San Diego, La Jolla, California 92037. *Received January 20, 1975*

Abstract: A new procedure for the determination of infrared dichroic spectra of oligo- and polypeptides is described. The peptide is incorporated in a polyoxyethylene film and partially oriented by uniaxial stretching. The infrared characteristics of the polyoxyethylene support allow measurement of the dichroic spectra of amide N–H stretching bands between 3500 and 3000 cm^{-1}, of amide I and II bands between 1700 and 1500 cm^{-1}, and of far-infrared bands below 800 cm^{-1}. Dichroic spectra of both high molecular weight polypeptides and oligopeptides, whose low molecular weight had hindered their orientation, can be conveniently determined in polyoxyethylene. Procedures for measuring the kinetics of N–H to N–D isotopic exchange reactions of molecules oriented in polyoxyethylene are also described. The infrared dichroic spectra of gramicidin S and of several synthetic oligo- and polypeptides are presented. Gramicidin S exhibits a "cross-β" dichroic spectrum which could arise from extensive association of the β-sheet conformation of Hodgkin–Oughton and Schwyzer into ribbon-like aggregates. Polypeptides were found to be oriented in the α-helical, β-sheet, and "cross-β" conformation in polyoxyethylene films.

We describe here a new technique, based on linear infrared dichroism, for conformational analysis of oligo- and polypeptides. The molecules under investigation are incorporated into a polyoxyethylene film and partially oriented by uniaxial stretching. Infrared dichroic spectra are then recorded using common spectroscopic techniques. The relative orientation of transition dipole moments of various chromophores and their relation to the direction of molecular orientation are derived from the dichroic spectra. This type of important conformational information was previously available only for high polymeric molecules from which oriented films or fibers could be formed; it is now readily obtained for small oligopeptides and for larger molecules which do not form satisfactory films.

The infrared characteristics of the polyoxyethylene (POE) support make it particularly suitable for analysis of the amide N–H stretching band, ν(NH), located near 3300 cm^{-1} and the amide I and II bands between 1700 and 1500 cm^{-1}. Far-infrared bands below 800 cm^{-1} can also be examined. Polyoxyethylene's solubility in water as well as in organic solvents such as chloroform and trifluoroethanol and the ease with which its films can be oriented greatly enhance its usefulness.

Thulstrup, Michl, and Eggers[2a] and Mazur and coworkers[2b] have employed analogous techniques to study the uv dichroism of molecules oriented in stretched polyethylene films. Although polyethylene has infrared windows in spectral regions of important amide absorptions, its low polarity makes it incompatible with most peptides.

We also describe procedures for determining the rates of hydrogen to deuterium isotopic exchange reactions of peptides oriented in POE. The dependence of the dichroism of N–H vibration bands upon extent of N–H to N–D conversion is observed directly during exchange in POE. These experiments allow both the rate of exchange, which reflects accessibility to the solvent medium, and the orientation of each spectrally distinct N–H group to be determined simultaneously. The correlation of exchange kinetics and dichroism greatly enhances the value of the separate measurements for conformational analysis.

The dichroic spectra presented here of gramicidin S and of several synthetic oligo- and polypeptides serve to illustrate the method.

Experimental Section

Materials. Polyoxyethylene (POE), M = 300,000 was purchased from Union Carbide Co. (WSRN) 750 and further purified by repeated methanolic precipitations from chloroform. The polymer was collected and dried under vacuum.

Gramicidin S (Bacillus Brevis) was purchased from Schwarz–Mann, lot No. E V3917, and further recrystallized four times from ethanol: 1 M HCl mp 309° [lit.[3] 277–278°]; $[\alpha]^{20}D$ −290.7° (c 0.43 in 70% ethanol v/v) [lit.[3] −289 (c 0.43 in 70% ethanol v/v].

Poly(γ-benzyl L-glutamate) was purchased from Schwarz–Mann, lot No. PBG-γ-6003, M = 90,000–100,000.

Poly(γ-ethyl L-glutamate) was prepared by polymerization of γ-ethyl L-glutamate N-carboxyanhydride in dioxane with sodium methoxide catalyst (N/I = 15) according to the procedure of Goodman and Hutchison;[4] $\overline{DP}$ = 25.

Poly(L-alanine) was purchased from Pilot Chemical Co., lot No. 6911, M = 35,000.

Z-(γ-ethyl L-glutamate)$_{12}$ ethyl ester (Z, benzyloxycarbonyl) was prepared according to the procedure described by Goodman and Rosen.[5]

Solvents used for preparation of POE stock solutions were Matheson Coleman and Bell spectroquality and were used without further purification.

Preparation of Films. A peptide sample (2–3 mg) was mixed with 0.5 ml of poly(ethylene oxide) stock solution (10% w/v) and the resulting solution was clarified by centrifugation. It was then spread evenly to a 2.5 × 0.7 cm strip on a silanized microscope slide and the solvent was allowed to evaporate at room temperature

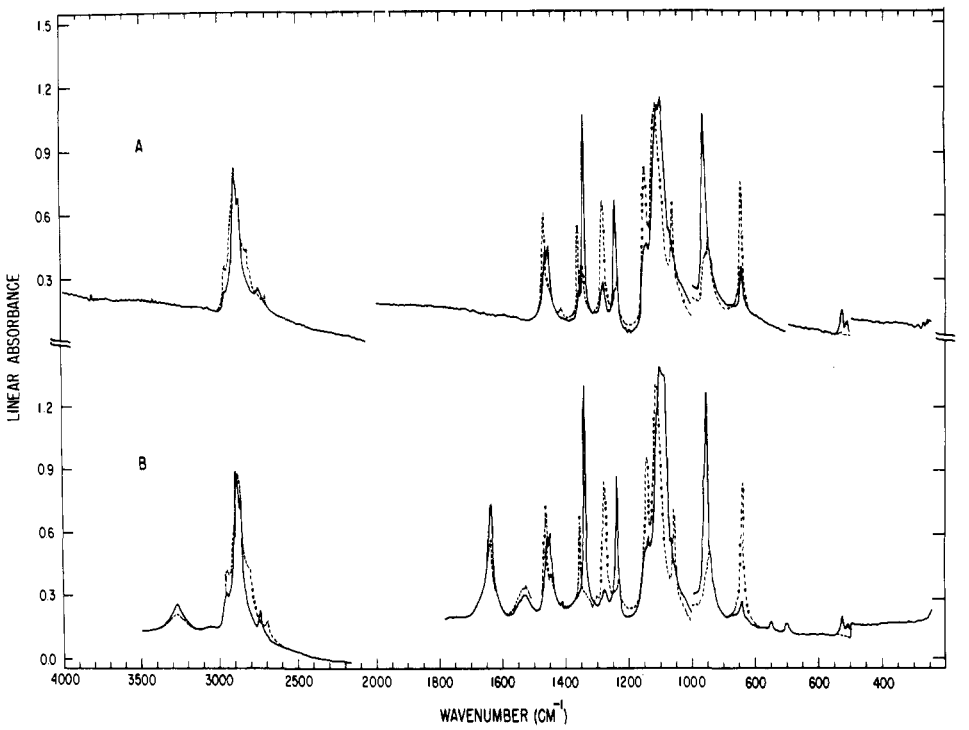

Figure 1. (A) Infrared dichroic spectrum of polyoxyethylene oriented by uniaxial stretching: polarization parallel to the stretching direction (—); polarization perpendicular to the stretching direction (- - -). (B) Infrared dichroic spectrum of gramicidin S incorporated in uniaxially oriented polyoxyethylene: polarization parallel to the orientation axis (—); polarization perpendicular to the orientation axis (- - -).

under a dust-protecting cover. The dry film was cut into 0.7 × 0.7 cm pieces which were stretched to approximately seven times their original length.

Because of the solubility characteristics of gramicidin S, it (2 mg) was first dissolved in methanol (50 μl) before mixing with aqueous stock solution. After evaporation of methanol, a film was cast and oriented as described above. Films containing poly(γ-benzyl L-glutamate) and poly(γ-ethyl L-glutamate) were cast from chloroform, the film with poly(L-alanine) was cast from hexafluoroisopropanol, and the Z-(γ-ethyl L-glutamate)$_{12}$-OEt-containing film was cast from trifluoroethanol.

Infrared Spectroscopy. Infrared spectra were recorded with a Perkin-Elmer Model 180 spectrophotometer equipped with a Perkin-Elmer gold wire grid polarizer. Spectral resolution was 2.5–3.0 cm^{-1} between 3500 and 3000 cm^{-1} and was 1.0–2.0 cm^{-1} below 1800. POE films were enclosed in a Teflon cell containing AgCl windows. Several drops of saturated solutions of selected salts[6] were placed in the cell to control the H_2O or D_2O relative humidity.

Results and Discussion

Gramicidin S. Dichroic Spectra. Gramicidin S is a cyclic decapeptide with the sequence[3] cyclo(-L-Pro-L-Val-L-Orn-L-Leu-D-Phe)$_2$. The infrared dichroic spectra of uniaxially oriented POE and of gramicidin S oriented in POE are compared in Figures 1A and 1B, respectively. Spectra recorded with light polarized parallel to the direction of stretching are drawn with a solid line; those recorded for perpendicular polarization are drawn with a dotted line. It is clear from Figure 1A that the amide NH stretching modes (3500–3000 cm^{-1}), the amide I and II vibrations (1700–1500 cm^{-1}), and the far-infrared amide IV and V bands (<800 cm^{-1}) of suspended peptides are free from interference by POE absorptions.

Expanded spectra of the ν(NH), amide I, and amide II bands of oriented gramicidin S are presented in Figure 2. Because of changes in ordinate scale, the ν(NH) absorbance of Figure 2 must be halved for correct comparison with the amide I and II bands. The single ν(NH) absorption at 3265 cm^{-1} of gramicidin in POE contrasts with observations by Ovchinnikov et al.[7] of two bands at 3426 and 3314 cm^{-1}, attributed to free and hydrogen bonded N-H groups, respectively, for gramicidin S in dilute chloroform solution. The position of the sharp N-H band at 3265 cm^{-1} clearly indicates that all N-H groups of gramicidin in POE are hydrogen bonded. The ν(NH) band, with a dichroic ratio $D_{\parallel}$ = absorbance$_{\parallel}$/absorbance$_{\perp}$ of 2.0 exhibits strong parallel dichroism.

Examination of Figure 2 reveals that the amide I band comprises a weak, perpendicularly polarized shoulder at 1680 cm^{-1} and a strong peak with parallel polarization at 1640 cm^{-1}. This pattern is characteristic of polypeptides in the "cross-β" conformation.[8,9] The dichroic asymmetry of the 1640-cm^{-1} peak apparently results from an unresolved absorption near 1660 cm^{-1} with weak or moderate perpendicular polarization. Absorption near 1660 cm^{-1} is expected for the two tertiary Phe-Pro amide groups. The perpendicularly polarized amide II band is observed at 1530 cm^{-1}. Spectral parameters of gramicidin in oriented POE are collected in Table I.

Deuterium Exchange. Since POE strongly absorbs large amounts of water vapor,[10] hydrogen to deuterium exchange reactions of incorporated molecules are readily effected by exposure to D_2O vapors. It is expected that H → D exchange rates will distinguish classes of amide N-H groups according to their accessibility to interaction with absorbed D_2O. In particular, amide N-H groups involved in either intramolecular or interpeptide hydrogen bonds should exhibit characteristically slow rates of exchange. Kinetics of hydrogen to deuterium exchange reactions for molecules incorporated in oriented POE can be determined from decay rates of either the ν(NH) or amide II absorption bands. The

Table I. Infrared Spectral Parameters of Gramicidin S Oriented in POE

Band	Position, cm^{-1}	Dichroic ratio $D_{\parallel}$
ν(NH)	3265	2.0
Amide I	1680 (w)	<1.0
	1640 (s)	1.4
Amide II	1530	0.7

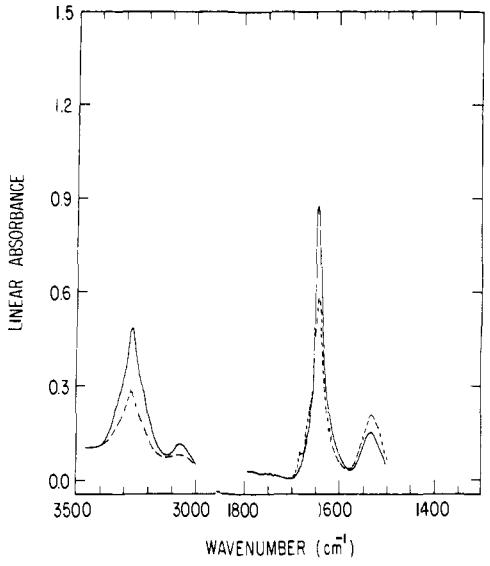

Figure 2. Infrared dichroic spectrum of the ν(NH), amide I, and amide II regions of gramicidin S incorporated in uniaxially oriented polyoxyethylene: polarization parallel to the orientation axis (—); polarization perpendicular to the orientation axis (- - -).

usual procedure for distinguishing classes of exchangeable N-H groups from nonlinear first-order rate plots can be supplemented in the case of molecules oriented in POE by observation of the time dependence of the dichroic ratio for N-H vibrational bands undergoing isotopic exchange. For first-order exchange, the dependence of the dichroic ratio $D_\parallel(t)$ on time t is given by eq 1, where $a_{i,\parallel}{}^0$ and $a_{i,\perp}{}^0$ are the initial parallel and perpendicularly polarized absorbancies, respectively, of class i amide N-H groups and k_i is the corresponding exchange rate constant. At least two classes of exchangeable N-H groups are required to produce a time dependence for $D_\parallel$. An increase in $D_\parallel$ with extent of exchange would indicate that the less accessible N-H groups have transition moment vectors aligned approximately parallel to the stretching direction. A decrease in $D_\parallel$ would result from their perpendicular alignment.

$$D_\parallel(t) = \sum_i a_{i,\parallel}{}^0 e^{-k_i t} \Big/ \sum_i a_{i,\perp}{}^0 e^{-k_i t} \qquad (1)$$

Results of exchange reactions can be conveniently represented as $\theta_H(t)$, the fraction of the initial absorbance remaining after exchange time t. Values of log (θ_H) determined from the amide II absorption of oriented gramicidin S are plotted in Figure 3 against time of exposure to vapors of D_2O saturated with DCl. For purposes of comparison, results of H → D exchange kinetics for the model compound *N*-acetyl-L-Leu-*N'*-methylamide are also shown. The exchange kinetics for each compound are accountable by a single, first-order rate process. This contrasts with observations made in solution of two or more kinetic classes of exchangeable hydrogens for gramicidin S.[11,12] In addition, the dichroic ratios of the gramicidin S ν(NH) and amide II bands were found to be independent of time during the first 60% of the exchange reaction. Because of the small absorbancies in the latter stages of the exchange, it was difficult to extend accurate measurements beyond 60% reaction. It is apparent from a comparison of the exchange half-lives of 20 and 80 min for the model compound and for gramicidin S, respectively, that none of the amide groups of gramicidin S are readily accessible to solvation by the absorbed water.

These observations suggest that the amide groups of gramicidin S comprise a single class characterized by partial protection from interaction with solvent. Such protection from solvation probably results from participation of

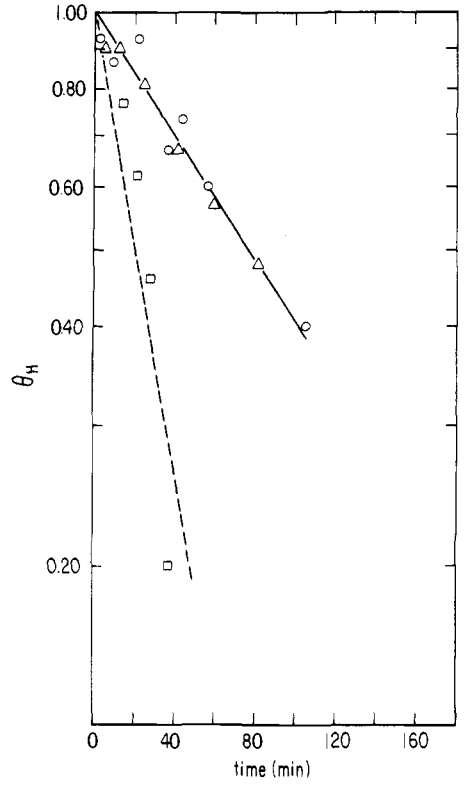

Figure 3. A log plot of θ_H, the fraction of N-H bonds that have not exchanged, against time of exposure to vapors of D_2O saturated with DCl: *N*-acetyl-L-leucine-*N'*-methylamide in uniaxially oriented polyoxyethylene, θ_H determined from the parallel polarized amide II absorption (□); gramicidin S in uniaxially oriented polyoxyethylene, θ_H determined from the parallel polarized amide II absorption (○), θ_H determined from the perpendicularly polarized amide II absorption (△).

the secondary amide groups in strong hydrogen bonding interactions.

Orientation by POE. Consideration of the structure of POE film should aid comprehension of its orientation effect on incorporated molecules. Takahashi and Tadokoro[13] have concluded from X-ray analysis that the polymer chains of the crystalline regions of POE adopt a (7/2) helical conformation having 7 repeat units per 2 turns of the helix. Both C-O bonds of the repeat unit [-O-CH_2-CH_2-] are in the trans conformation; the single C-C bond assumes a gauche conformation. The trans-trans-gauche conformation is also favored by chains in the amorphous phase of POE.[14] Methylene groups coat the outside of the (7/2) POE helix while oxygen atoms line its large helical groove. Additional conformations of POE are known. Takahashi, Sumita, and Tadokoro[15] observed a planar zigzag modification of POE fixed under tension. Various other chain conformations have been noted in crystalline complexes with urea,[16] thiourea,[17] and $HgCl_2$.[18] Each crystalline modification of POE has a characteristic infrared dichroic spectrum.

The spectral bands of POE in both Figures 1A and 1B are identical with those reported[19] for the (7/2) helical modification. It is apparent from this observation that gramicidin S has not altered or disrupted the structure of the crystalline regions of the POE film. The suspended molecules must therefore be confined to the film's amorphous areas. POE chains in the amorphous phase will be partially aligned in the direction of stretching. Orientation of molecules incorporated in stretched POE can result from: (i) specific nonbonded interactions, such as hydrogen bonding, with the polar POE chains; or (ii) nonspecific interactions, such as steric repulsions, which influence molecular packing and act to align the long axes of suspended molecules paral-

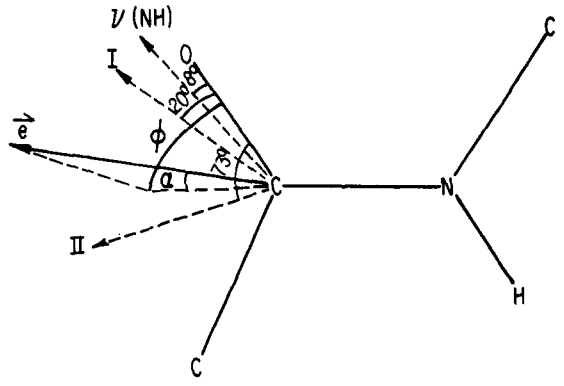

Figure 4. Schematic diagram of the amide group illustrating the molecular orientation axis e, the orientation angles α and ϕ, and the ν(NH), amide I and amide II transition moment directions.

lel to the direction of stretching. Neither the structural features of amorphous POE, nor the modes of polar interactions between POE chains and suspended molecules are likely to be understood in sufficient detail to allow prediction of preferred directions of orientation by mechanism i. On the other hand, orientation by ii, which aligns the longest molecular axis parallel to the stretching direction, can be predicted for sufficiently asymmetric molecules from a knowledge of their conformation.[1,2]

Since POE films are uniaxially oriented by stretching,[19] orientation by either i or ii is symmetric about the stretching axis. Beer has shown[20] for such symmetric orientation that the dichroic ratio $D_{\parallel}$ of an absorption is related to Θ, the angle between its transition moment vector and the molecular orientation axis, according to eq 2. For a homogeneous sample, the orientation factor f will assume the same value for each chromophore and transition. It is convenient to consider f as the fraction of oriented molecules in a hypothetical, equivalently dichroic sample comprised of perfectly oriented and randomly oriented molecules.[20]

$$D_{\parallel} = \frac{2\cos^2\Theta + \frac{2}{3}(1-f)/f}{\sin^2\Theta + \frac{2}{3}(1-f)/f} \qquad (2)$$

Since θ is related to molecular conformation, its evaluation is usually of considerable interest. It can be readily calculated from the experimental dichroic ratio and the orientation factor according to eq 2. Unfortunately, the uncertain orientational effects of stretched POE rarely allow prediction of values for f. We have therefore restricted our analysis to determination of upper and lower limits to Θ after the procedure of Fraser.[21] He presents an equation for calculation of f_m, the minimum value of f consistent with the observed dichroic ratio, and shows how f_m can be used to derive bounds for Θ.

Values of Θ for the ν(NH), amide I, and amide II bands, thus derived, allow determination of orientation modes for constituent amide groups with respect to helical or other molecular axes, provided directions for the associated transition moment vectors are known. Pursuant to such determinations, we first describe amide group orientation according to the two angles α and ϕ shown in Figure 4. The molecular axis of orientation e is inclined at an angle of α to the amide plane; its projection onto the plane makes an angle ϕ with the C=O bond. The ν(NH), amide I, and amide II transition moment vectors lie in the amide plane. Their directions as determined by Bradbury and Elliott[22] are also shown in Figure 4. The angle Θ_i between e and each transition i = ν(NH), amide I, or amide II is readily related to α and ϕ

$$\cos\Theta_i = \cos\mu_i\cos\alpha\cos\phi + \sin\mu_i\cos\alpha\sin\phi \qquad (3)$$

where μ_i is the angle between the ith transition moment and

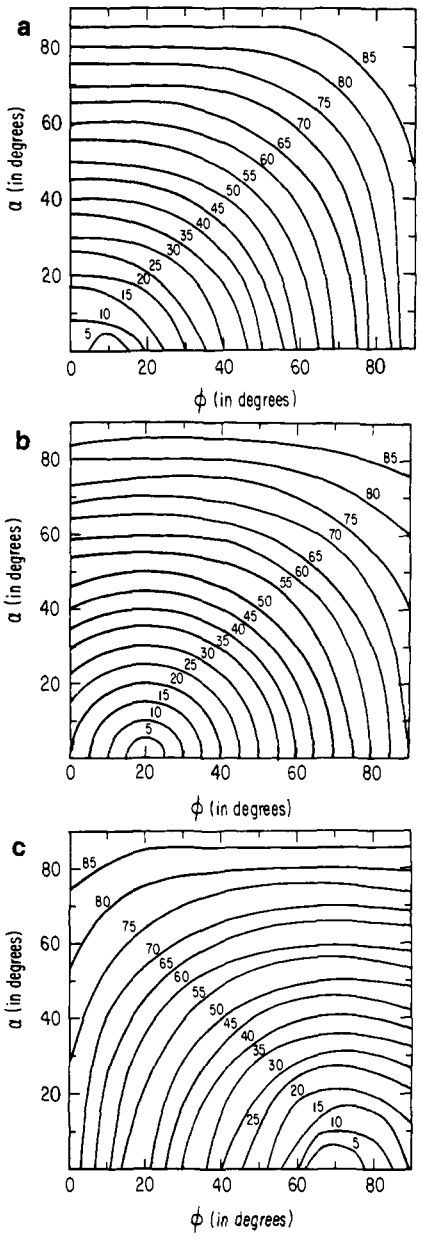

Figure 5. The relation of the orientation angles α and ϕ calculated according to eq 3 for the indicated values of Θ: (a) calculated for the ν(NH) transition; (b) calculated for the amide I transition; (c) calculated for the amide II transition.

the C=O bond. Equation 3 is represented in graphical form for the ν(NH), amide I, and amide II transitions, in Figures 5a, 5b, and 5c, respectively. Values for the orientation angles α and ϕ that are consistent with the range derived for each Θ_i are revealed by examination of Figure 5; orientation vectors e related by reflection through the amide plane or by inversion through the C′ carbon atom are obviously indistinguishable by infrared dichroism. Although only a range of acceptable values for α and ϕ can be determined by this procedure, it is anticipated that under favorable circumstances the derived orientation of the amide group can provide important conformational information.

Orientation of Gramicidin S in POE. In order to apply these considerations to the dichroism of gramicidin S in stretched POE, we assume that each of its eight secondary amide groups is oriented equivalently. Our observation of single, sharp, and symmetric peaks for the ν(NH) and amide II transitions suggests that the secondary amide groups have equivalent environments. Also, this assumption should be roughly correct for the β-sheet conformation of

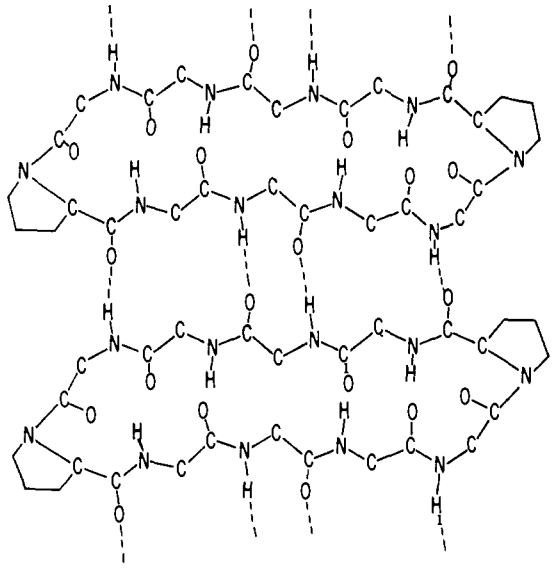

Figure 6. Illustration of the proposed "cross-β" type aggregate of gramicidin S in oriented polyoxyethylene.

gramicidin S proposed by Hodgkin and Oughton[23] and by Schwyzer[24] and supported by the subsequent investigations of Ovchinnikov et al.[7] The two Val-Orn-Leu chain segments are connected by hydrogen bonds to form an antiparallel β-sheet structure in the Hodgkin-Oughton-Schwyzer (HOS) model. The extended chain ends are linked by two D-Phe-Pro bends.

Application of the procedure of Fraser[21] to the dichroic ratios of gramicidin S listed in Table I yields a minimum value for the orientation factor of $f_m = 0.25$ as well as the ranges $0° < \Theta_{\nu(NH)} < 45°$, $35° < \Theta_I < 50°$, and $60° < \Theta_{II} < 80°$ for angles between the molecular orientation axis and the ν(NH), amide I, and amide II transition moments, respectively. According to the graphs of Figure 5, only the orientation angles $35° < \alpha < 45°$ and $0° < \phi < 28°$ are simultaneously consistent with each of the three ranges quoted above. These results suggest that the secondary amide planes of gramicidin S are tilted at an angle of $\alpha \approx 40°$ with respect to the molecular orientation axis; their C=O bonds make an acute angle with the alignment axis.

Conformation of Gramicidin S in POE. The X-ray analysis of Schmidt, Hodgkin, and Oughton[25] revealed a twofold axis of symmetry for gramicidin S in its crystalline state. It is known, primarily from NMR experiments, that this crystal symmetry is maintained in dilute solutions of a variety of solvents.[26] Because spectroscopic analysis has shown that the conformation of gramicidin S is only slightly affected by changes in solvent and temperature, it is reasonable to assume gramicidin S retains a twofold symmetry axis in POE. Its molecular axis of orientation in POE must either be coincident with or transverse to its symmetry axis. The observed infrared dichroism reported here is consistent with the HOS model with its orientation axis perpendicular to both its twofold axis and the extended chains of its β-sheet structure. The long axis of the HOS model, which connects the two D-Phe-L-Pro bends, must in this case be aligned transverse to the direction of stretching. Thus, in accordance with the dichroism analysis described above, the secondary amide N—H and C=O bonds are approximately parallel to the stretching direction and the extended chain direction, perpendicular. A similar chain arrangement is assumed by proteins and polypeptides in the "cross-β" conformation. "Cross-β" polypeptides exhibit infrared dichroism patterns[8] that are nearly identical with that observed here for gramicidin S oriented in POE.

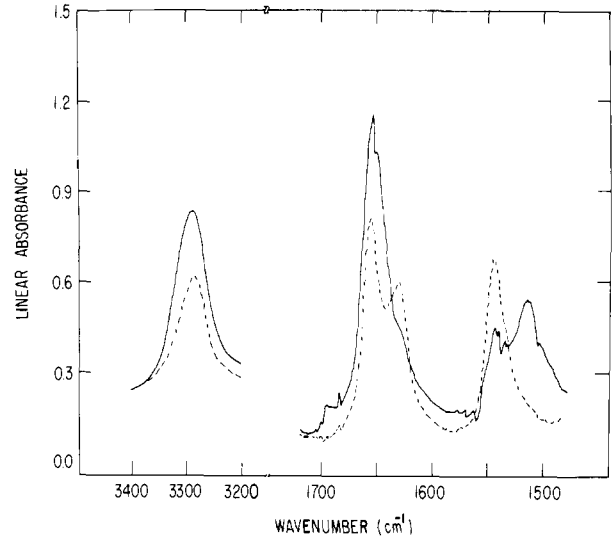

Figure 7. Infrared dichroic spectrum of poly(L-alanine), M = 35,000, incorporated in uniaxially oriented polyoxyethylene: polarization parallel to the orientation axis (—); polarization perpendicular to the orientation axis (- - -).

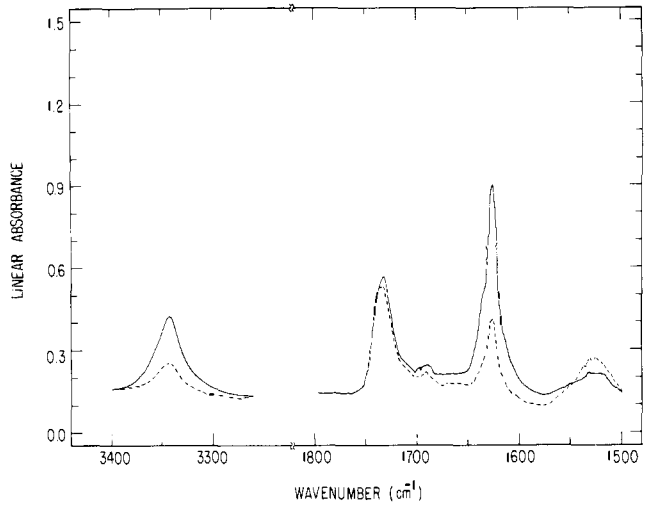

Figure 8. Infrared dichroic spectrum of Z-(γ-ethyl L-glutamate)$_{12}$OEt incorporated in uniaxially oriented polyoxyethylene: polarization parallel to the orientation axis (—); polarization perpendicular to the orientation axis (- - -).

It is readily apparent from examination of a molecular model of the HOS conformation of gramicidin S that it can aggregate to form the "cross-β" structure shown in Figure 6. Alignment of adjacent molecules with their twofold axes parallel permits formation of antiparallel β-sheet hydrogen bonds between them. Such aggregates form ribbon-like structures with the polar ornithine side chains on one side and the nonpolar side chains on the other side of the ribbon. A potential for formation of bilayers by association of two ribbons with opposed hydrophobic sides is also evident from the molecular models.

"Cross-β" aggregates comprised of five or more gramicidin S molecules would have their long axes parallel to the β-sheet hydrogen bonds and, in accordance with orientation by mechanism ii, parallel to the stretching direction. Since deuterium exchange kinetics indicate that most amide N-H groups are protected from solvation, it is unlikely that orientation of gramicidin S results from specific polar interactions with POE according to mechanism i.

Synthetic Polypeptides. Poly(L-alanine) (PLA), poly(γ-benzyl L-glutamate) (PBLG), Z-(γ-ethyl L-glutamate)$_{12}$OEt (ELG$_{12}$), and poly(γ-ethyl L-glutamate)

Table II. Infrared Spectral Parameters of Selected Synthetic Peptides Oriented in Polyoxyethylene

	PLA[a]		PBLG[b]		Z-[Glu(OEt)]$_{12}$OEt[c]		PELG[d]	
Transition	Position, cm^{-1}	$D_{\parallel}$	Position, cm^{-1}	$D_{\parallel}$	Position, cm^{-1}	$D_{\parallel}$	Position, cm^{-1}	$D_{\parallel}$
ν(NH)	3290	1.6	3290	2.3	3285	2.7	3285	1.8
ν(C=O) ester			1725	~1	1735	~1	1734	~1
Amide I	1690 (w)	>1	1647	2.0	1626	3.0	1693 (w)	<1
	1650 (s)	1.5					1650 (m)	1.9
	1630 (m)	<1					1624 (s)	1.5
Amide II	1544 (m)	0.5	1545	0.3	1530	0.7	1547 (m)	0.5
	1515 (m)	13					1524 (m)	0.6

[a] Poly(L-alanine); molecular weight = 35,000. [b] Poly(γ-benzyl L-glutamate); molecular weight = 90,000. [c] Z-(γ-ethyl L-glutamate)$_{12}$OEt. [d] Poly(γ-ethyl L-glutamate); molecular weight = 3,900.

(PELG) were each readily oriented in POE. Examples of dichroic spectra, obtained for PLA and ELG$_{12}$, are presented in Figures 7 and 8, respectively. Infrared spectral parameters obtained for all four peptides are collected in Table II.

Poly(L-Alanine). It is apparent from the results shown in Figure 7 and Table II that our PLA sample, M = 35,000, exists as a mixture of α-helical and β-sheet conformations in POE. The parallel dichroic amide I peak at 1650 cm^{-1} and the perpendicularly dichroic amide II peak at 1544 cm^{-1} are attributable to the α-helix;[27] the remaining peaks in the amide I region, the weak parallel dichroic band at 1690 cm^{-1} and the perpendicularly dichroic band at 1630 cm^{-1}, and in the amide II region, the parallel dichroic band at 1515 cm^{-1}, arise from absorptions by the β-sheet conformation.[27] Accordingly, the PLA α-helices and the extended chains of the β-sheets are aligned parallel to the stretching direction. Since there are no possibilities for hydrogen bonding between the two ordered structures observed for PLA and the POE chains, orientation of both conformations likely results from alignment of their long axes parallel to the stretching direction according to mechanism ii. Thus the long axis of the β-sheet conformation is parallel to the extended chain direction.

Poly(γ-benzyl L-glutamate). Observations of single, sharp amide I and II bands at 1647 and 1545 cm^{-1}, respectively, reveal that PBLG adopts the α-helical conformation exclusively when oriented in POE. The strong parallel dichroism of the ν(NH) and amide I bands and the perpendicular dichroism of the amide II band indicates a parallel alignment of the PBLG helices along the stretching direction. The benzyl ester absorption at 1725 cm^{-1} is not dichroic. Thus, either the transition moment of this band is oriented at ~54° to the helix axis (see eq 2) or the benzyl glutamate side chains are flexible and disordered. The former view is favored by Tsuboi.[28]

Z-(γ-ethyl L-glutamate)$_{12}$OEt. It is very difficult to form oriented films of pure ELG$_{12}$ because of its low molecular weight. However, it is easily oriented in POE films. The dichroic spectrum obtained in this manner is shown in Figure 8. The positions of the amide I and II transitions at 1626 and 1530 cm^{-1}, respectively, are characteristic of a cross-β conformation. A cross-β structure in which the extended chains align transverse to the stretching direction is suggested by the dichroic ratios presented in Table II. Apparently ELG$_{12}$ oligomers aggregate to form β-sheets that have their largest dimension perpendicular to the constituent chains. Such aggregates must be comprised of 12 or more chains. As in the case of PBLG, the side chain ester absorption at 1735 cm^{-1} of ELG$_{12}$ shows little dichroism.

Poly(γ-ethyl L-glutamate). According to the results of Table II, PELG, $\overline{DP}$ = 25, assumes both the α-helical and cross-β conformations in oriented POE. Peaks at 1650 and 1547 cm^{-1} result from absorptions by the α-helix while those at 1693, 1624, and 1524 cm^{-1} arise from β-sheet transitions. A single, nondichroic peak at 1734 cm^{-1} was observed for the side chain ester group. Evidently, this absorption is not sensitive to peptide backbone conformation. Probably the smaller chains of this heterogeneous sample adopt the "cross-β" conformation in accord with the results obtained for ELG$_{12}$; likewise the α-helix is assumed only by chains with more than 12 glutamate units. These results can be compared with those obtained in trifluoroethanol and trimethyl phosphate solutions indicating that oligomeric γ-ethyl glutamates with nine or more units assume the α-helical conformation.[29] The high peptide concentration in POE, which favors associated forms such as the "cross-β", may be partly responsible for the conformational differences observed between glutamate oligomers in dilute solution and in POE films.

Orientation in POE. Orientation of the amide structural units with respect to the molecular axis can be derived from the dichroic ratios in Table II for the two compounds PBLG and ELG$_{12}$ which exhibit conformational homogeneity. We find, following the procedure of Fraser,[21] that the dichroic ratio $D_{\parallel}$ = 0.3 for the amide II transition of PBLG requires a minimum value for the orientation factor of f_m = 0.6. In addition, ranges of 37-43°, 40-45°, and 68-90° were established for the angles between the helix axis and the transition moment vectors of the ν(NH), amide I, and amide II transitions, respectively. These values can be compared with the corresponding angles 28, 39, and 75° determined by Tsuboi[28] for oriented PBLG films. Good agreement is seen for orientation of the amide I and II transitions; a discrepancy of $\approx$10° is noted for the ν(N-H) transition. Both our results and those of Tsuboi[28] are strictly valid only for homogeneously oriented samples, a situation which is certain to be more closely realized for the highly oriented PBLG films used by Tsuboi[28] than for partially oriented PBLG in POE. In the former samples almost all of the molecules are completely oriented, while in the latter we expect preferential orientation of the largest molecules. In light of these considerations, the agreement between our results and Tsuboi's[28] is quite satisfactory.

Values of the orientation angles were found, by reference to Figure 5, to be $35° < \alpha < 40°$ and $\phi < 15°$. The helix axis is, therefore, inclined approximately 40° to the amide plane and its projection onto the plane is nearly parallel to the C=O bond.

A similar analysis applied to the dichroic ratios of ELG$_{12}$ yields a minimum orientation factor f_m = 0.4 and the angles 15-42°, 0-37°, and 60-70° between the helix axis and the ν(NH), amide I, and amide II transition moment vectors, respectively. It is apparent from the small value of f_m = 0.4 that oligoethyl glutamate is not oriented as completely in POE as is PBLG. Precise determination of the transition moment directions is prevented by the imperfect orientation. Likewise, only relatively large ranges for the orientation angles α and ϕ of 4-37° and 3-21°, respec-

tively, could be established. It is clear, however, that the dichroic spectrum of ELG_{12} is characteristic of the "cross-β" conformation.

Conclusions

The experiments described here demonstrate that both oligopeptides and polypeptides are partially oriented upon incorporation in stretched films of polyoxyethylene. Furthermore, the infrared characteristics of the POE support allow determination of dichroic spectra for the ν(NH), amide I, and II transitions as well as for infrared bands below ~800 cm^{-1}. Since POE films rapidly absorb substantial amounts of water vapor, hydrogen to deuterium exchange reactions of suspended molecules can readily be studied. Important new conformational information is available from the combination of infrared dichroism and hydrogen exchange kinetics.

Incorporation of peptides has no measurable effect on the infrared transitions of the POE matrix. Apparently the peptides are confined to the amorphous phase of the POE film and do not alter its crystalline structure. Alignment of the long axes of suspended molecules parallel to the stretching directions appears to be the preferential mode or orientation of the peptides employed here. Only partial orientation is achieved; values for the minimum orientation factor f_m ranged from 0.4 for gramicidin S to 0.6 for PBLG. In spite of imperfect orientation, useful conformational information could usually be derived from the dichroic spectra.

Results presented here are consistent with the Hodgkin-Oughton-Schwyzer[23,24] conformation of gramicidin S. We speculate that its "cross-β" type dichroic spectrum results from extensive aggregation to form the ribbon-like β structures shown in Figure 6. Dichroic spectra of synthetic oligo- and polypeptides reveal that both α-helical and β conformations are oriented in POE films. The latter can be oriented either with the peptide chains parallel or perpendicular to the stretching direction of the POE film.

Acknowledgment. The authors gratefully acknowledge financial support for this work by a grant from the National Institutes of Health (GM 18694).

References and Notes

(1) Postdoctoral Fellow 1971; on leave from the Hebrew University, Jerusalem, Israel.
(2) (a) E. W. Thulstrup, J. Michl, and J. H. Eggers, *J. Phys. Chem.*, **74**, 3868 (1970); (b) A. Yogev, L. Margulies, D. Amar, and Y. Mazur, *J. Am. Chem. Soc.*, **91**, 4558 (1969).
(3) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **40**, 625 (1957).
(4) M. Goodman and J. Hutchison, *J. Am. Chem. Soc.*, **88**, 3677 (1966).
(5) M. Goodman and I. G. Rosen, *Biopolymers*, **2**, 503 (1964).
(6) F. E. M. O'Brien, *J. Sci. Instrum.*, **25**, 73 (1948).
(7) Y. A. Ovchinnikov, V. T. Ivanov, V. F. Bystrov, A. I. Miroshnikov, E. N. Shepel, N. D. Abdullaev, E. S. Efremov, and L. B. Senyavina, *Biochem. Biophys. Res. Commun.*, **39**, 217 (1970).
(8) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, R. D. B. Fraser, W. E. Hanby, and T. R. R. McDonald, *J. Mol. Biol.*, **2**, 276 (1960).
(9) A. J. Geddes, K. D. Parker, E. D. T. Atkins, and E. Beighton, *J. Mol. Biol.*, **32**, 343 (1968).
(10) R. L. Davidson and M. Sittig, Ed., "Water-Soluble Resins", Reinhold Boak Corporation, New York, N.Y., 1968.
(11) A. Stern, W. A. Gibbons, and L. C. Craig, *Proc. Natl. Acad. Sci. U.S.*, **61**, 734 (1968).
(12) D. W. Urry and M. Ohnishi in "Spectroscopic Approaches to Biomolecular Conformation", D. W. Urry, Ed., American Medical Association, Chicago, Ill., 1970.
(13) Y. Takahashi and H. Tadokoro, *Macromolecules*, **6**, 672 (1973).
(14) P. J. Flory, "Statistical Mechanics of Chain Molecules", Interscience, New York, N.Y., 1969, p 165.
(15) Y. Takahashi, I. Sumita, and H. Tadokoro, *J. Polym. Sci., Polym. Phys. Ed.*, **2**, 2113 (1973).
(16) H. Tadokoro, T. Yoshihara, Y. Chatani, and S. Murahashi, *Polym. Lett.*, **2**, 363 (1964).
(17) H. Tadokoro, *Macromol. Rev.*, **1**, 119 (1966).
(18) R. Iwamoto, Y. Saito, H. Ishihara, and H. Tadokoro, *J. Polym. Sci., Part A-2*, **6**, 1509 (1968).
(19) H. Tadokoro, Y. Chatani, T. Yoshihara, S. Tahara, and S. Murahashi, *Makromol. Chem.*, **73**, 109 (1964).
(20) M. Beer, *Proc. R. Soc. London, Ser. A*, **236**, 136 (1956).
(21) R. D. B. Fraser, *J. Chem. Phys.*, **21**, 1511 (1953).
(22) E. M. Bradbury and A. Elliott, *Spectrochim. Acta*, **19**, 995 (1963).
(23) D. C. Hodgkin and B. M. Oughton, *Biochem. J.*, **65**, 752 (1957).
(24) R. Schwyzer and V. Ludescher, *Biochemistry*, **7**, 2519 (1968).
(25) G. M. J. Schmidt, D. C. Hodgkin, and B. M. Oughton, *Biochem. J.*, **65**, 744 (1957).
(26) D. Balasubramanian, *J. Am. Chem. Soc.*, **89**, 5445 (1967).
(27) T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961).
(28) M. Tsuboi, *J. Polym. Sci.*, **59**, 139 (1962).
(29) M. Goodman, A. S. Verdini, C. Toniolo, W. D. Phillips, and F. A. Bovey, *Proc. Natl. Acad. Sci. U.S.*, **64**, 444 (1969).

An Approach to the Tertiary Structure of Globular Proteins

I. D. Kuntz

Contribution from the Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143. *Received February 26, 1975*

Abstract: The "distance plots" described by Rossman and Liljas are graphs of α-carbon distances derived from the known X-ray structures of globular proteins. These plots have been shown to be useful in locating "folding domains". The surprisingly regular patterns contain much additional information about protein tertiary structure. In this paper we show that repeating square and trapezoidal patterns can correspond to distorted three-dimensional "superhelical" structures that are principal constituents of folding domains.

Characterization of tertiary structure in globular proteins is not well developed. Kauzmann's suggestion that proteins contain a hydrophobic core remains the only major generalization.[1] With the availability of X-ray structural data for many globular proteins there have been some initial efforts to locate specific features such as calcium binding sites,[2] dinucleotide binding sites,[3] and hydrophobic regions.[4] A more general approach that has considerable promise has been the identification of "folding domains" within the larger proteins.[5-7] One of the methods used for the location of domains employs "distance plots", described first by Phillips[8] and used in modified form by Rossman and Liljas.[6,7] "Distance plots" are graphs of C_α-C_α distances plotted against residue number, with contour lines drawn at fixed interatomic distances (see Figure 1 and Figures 1 and 2 of ref 7). These plots provide a concise summary of the structural information available from X-ray diffraction experiments. Rossman and Liljas have shown that