TABLE II

WEIGHT AVERAGE MOLECULAR WEIGHTS BY EQUILIBRIUM ULTRACENTRIFUGATION OF OLIGOMERIC PEPTIDES DERIVED FROM γ-METHYL-L-GLUTAMATE IN DICHLOROACETIC ACID AND DIMETHYLFORMAMIDE

Peptide	Formula weight	Dichloroacetic acid Concn. range, % w./v.	Mol. wt.	Dimethylformamide Concn. range, % w./v.	Mol. wt.
Tri-	624				
Tetra-	767	3	760 ± 50		
Penta-	910	1.5–3	930 ± 85		
Hexa-	1053				
Hepta-	1196	0.5–3	1070 ± 95	0.5–2	1240 ± 70
				.8	1142 ± 70*
Nona-	1483	0.75–1.5	1440 ± 200	.3–1.4	1480 ± 70
				.3–1.0	1460 ± 130*
Undeca-	1769			.8–1.5	1890 ± 190

equilibrium ultracentrifugation technique.[34] A model E ultracentrifuge was used, equipped with phase plate, Rayleigh interference optics and temperature control. The multichannel short column cells were machined of Kel-F, as were the single channel synthetic boundary cells of the Kegeles type.[35] With dichloroacetic acid (DCA) as solvent the term $(1 - \bar{V}\rho)$, where $\bar{V}$ is the partial specific volume of the solute and ρ is the density of the solution, was negative. Accordingly, the solutions were less dense than solvent and it was essential to layer solution on top of solvent in these synthetic boundary runs. Also, the oligomers flotated rather than sedimented; the obvious modifications in technique were made. Fluorocarbon FC-43[36] was used as base fluid since it was only sparingly soluble in the solvents. A few runs were made using long columns (~ 3 mm. column height); in these runs three-place double channel cells of Kel-F[37] were used and the interferometric techniques of Richards and Schachman[38] were employed for the measurements. All equilibrium runs were performed at room temperature and at 39,460 r.p.m.; at this speed it was found helpful in obtaining good interference records to use fine aperture masks (0.020″) and either very fine interference slits (0.007″) or light source slits (<0.001″). Equilibrium was attained within 2 hours with the short (~0.7 mm.) columns even with DCA. The longer (~3 mm.) columns were run for about 18 hours. Solvent densities were interpolated from data in the "International Critical Tables." An estimate of the partial specific volumes of the oligomers in water by the methods of Traube[39] and Cohn, *et al.*,[40,41] gave values ranging from 0.72 to 0.74. In the less polar solvents used here it is expected that the volumes would probably be somewhat higher. Accordingly, the value 0.75 was used; this value provided good agreement between the values of the molecular weights in the two solvents. The values of the molecular weights are quite sensitive to a choice of $\bar{V}$, particularly with DCA as solvent: the molecular weights using $\bar{V} = 0.72$ were approximately 38% higher in DCA and about 10% lower in dimethylformamide (DMF) than the values presented. The possible differences in compressibilities between solvents and solutes were neglected since at the middle of the short columns the pressure was less than 8 atmospheres.

No regular concentration dependence was observed; therefore the molecular weights found for the various concentrations in a given solvent were averaged. The results are given in Table II where the second column lists the formula weights, the third column the concentration ranges used in DCA and the fourth column the average values of the molecular weights found in DCA and their standard deviations. Similarly, columns five and six give the concentration ranges and molecular weights in DMF. The starred values were obtained using the longer columns of solution.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN, N. Y., AND THE ROCKEFELLER INSTITUTE, NEW YORK, N. Y.]

Conformational Aspects of Polypeptides. IV.[1] Folded and Associated Forms of Oligomeric Peptides Derived from γ-Methyl Glutamate

BY MURRAY GOODMAN, EDWARD E. SCHMITT[2,3] AND DAVID A. YPHANTIS[3]

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This report presents the results of studies on the conformational characteristics of oligomers based on γ-methyl glutamate in solution. Dichloroacetic acid solvates the peptide chain yielding a "random coil." Dimethylformamide and *m*-cresol allow intramolecular hydrogen bonds to form, beginning in the range of the pentamer through the nonamer. The results for the pentamer and higher in dioxane are interpreted as a combination of intramolecular hydrogen bonding and association. The temperature dependence of the optical activity for the entire oligomeric series in dimethylformamide is explained by a helix–random coil transition. The differences between oligomers and high polymers are discussed as are association phenomena and infrared spectra of the peptides.

Introduction

In the past decade much has been learned about the conformations[4] of native proteins and synthetic polypeptides. The conformational structures were studied *via* a number of techniques which include optical rotatory properties,[5–18] infrared spectros-

(1) Previous paper in this series, M. Goodman, E. E. Schmitt and D. A. Yphantis, *J. Am. Chem. Soc.*, **84**, 1283 (1962).

(2) Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the Polytechnic Institute of Brooklyn.

(3) This research was supported by grants from the National Association of Glue Manufacturers, National Science Foundation (G8514) and the National Institutes of Health (A2493).

(4) The terms *configuration* and *conformation* have been used interchangeably by workers in this field for the description of the secondary structures possible for peptide chains. We have adopted a convention which has received support elsewhere; namely, to reserve the use of *configuration* to its original sense, *i.e.*, the spatial relationship of the asymmetric carbon atoms, and to let the word *conformation* refer only to the secondary structure (see for example, ref. 11, p. 239).

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copy,[19-24] light scattering and viscosity measurements,[25,26] dielectric constant measurements,[27] and deuterium exchange.[28-32]

It was considered likely that some of these techniques could also be employed to study the secondary structure of oligomeric peptides. In this paper we wish to describe the conformational studies which we have carried out with oligomeric compounds derived from γ-methyl L-glutamate (dimer through undecamer). The synthesis of these compounds is described in the previous paper in this series.[1] It should be recalled that the oligomeric polyglutamates are fully esterified and blocked at the amino end with a carbobenzoxy group.

Results and Discussion

Optical Activity.—Many workers[5,7,8] have shown that dextrorotatory enhancements of the specific rotations for polyglutamic esters and acids are indicative of helical structures. It has been further established in certain cases that positive (concentration independent) optical rotation values at long visual wave lengths are due to helical conformations. We have measured the specific rotations of the oligomers in both random coil- and helix-forming solvents (see Fig. 1). In a non-helix-forming solvent such as dichloroacetic acid, the specific rotations of the oligomeric peptides become increasingly more negative as the number of residues increases. In helix-forming solvents, another curve is superimposed upon that of the random coil. This second curve results from the contribution of the secondary structure to the specific rotation. Hence the composite curves such as are

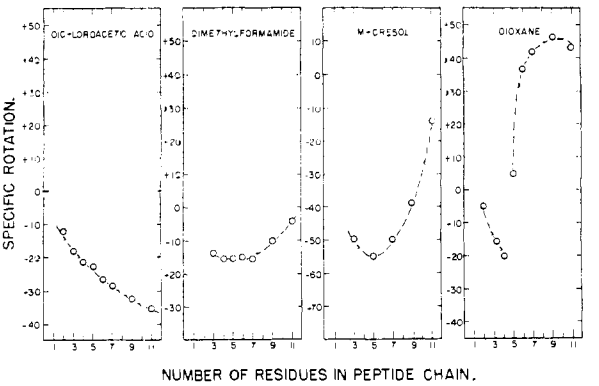

Fig. 1.—Optical activity (measured at 589 mμ) of the oligomeric peptides derived from γ-methyl-L-glutamate in various solvents at 25°.

obtained experimentally in dimethylformamide, *m*-cresol and dioxane have a similar shape to that found for the random coil solvent until sufficient residues are present to allow peptide chain folding. At this point the experimental curve departs from the dichloroacetic acid type of curve and acquires more positive specific rotations.

The magnitude of the dextrorotational-enhancing effects varies with the nature of the solvent. In dimethylformamide these optical activity changes seem to be small. In *m*-cresol each positive contribution is greater per residue. Thus, the curve is steeper than in dimethylformamide. An even more dramatic shift is observed in the plot of the specific rotation of the oligomeric peptides dissolved in the third helix-forming solvent, dioxane. Here a dextrorotatory optical activity enhancement, beginning at the pentapeptide, is so large that the resulting specific rotation actually becomes positive.[33] We interpret these changes in optical activity with increasing size of the oligomeric peptides partly as indicative of the formation of intramolecular hydrogen bonded structures.[34]

There is of course a competition at hydrogen bonding sites between amide–amide interactions and amide–solvent interactions. Certain solvents interact strongly with amide bonds and consequently do not allow intramolecular hydrogen bonding. When all hydrogen bonds are between the peptide and the solvent, the medium is termed a random coil solvent. All degrees of solvation may exist, however, in partial helix-forming solvents. The differences in behavior of the same oligomeric compounds in various helix-forming media may be attributed to the solvation characteristics of the solvents themselves. Dioxane interacts with the peptide bonds less strongly than either dimethylformamide or *m*-cresol. As a result, dimethylformamide and *m*-cresol can break more intramolecular hydrogen bonds at the critical sizes, such as a pentamer, hexamer or heptamer. For larger peptides, enough intramolecular hydrogen bonds can form to stabilize the folded structure which is resistant to the interaction with these solvents.

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TABLE I

SUMMARY OF PHYSICAL DATA OF OLIGOMERIC PEPTIDES DERIVED FROM γ-METHYL L-GLUTAMATE MEASURED AT 25°

Peptide	Configuration	M.p., °C.	Specific rotations 2% in DCA	1% in DMF	1% in *m*-cresol	2% in dioxane	Optical rotatory dispersion data Dimethylformamide b_0[e]	λc, mμ	Dioxane b_0[e]	λc, mμ	Dichloroacetic acid b_0[e]	λc, mμ
Di-	All-L	86	−12.4°	−13.4°	−29.0°	− 5.1°						
Tri-	All-L	124	−18.0	−13.9	−50	−15.9	+ 52[f]	183	− 7.7[m]	219[m]	+46.1[g]	168[s]
Tri-	DLL	101				− 9.9						
Tetra-	All-L	139	−21.3	−15.7		−20.3	+ 49[g]	181[g]	− 38.7[c]	231[c]		
Penta-	All-L	200	−22.7	−15.5	−55	+ 4.7	+ 4[f]	212[f]	+ 32.2[n,o]	249[n,o]		
Penta-	LLDLL	161				−13.9						
Hexa-	All-L	250	−26.7	−15.4		+36.3	− 18[h]	219[h]	+106.4[p,o]	241[p,o]		
Hepta-	All-L	259	−28.7	−15.5	−50[a]	+41.7[b]	− 53[i]	230[i,j]	+122.6[q,o]	244[q,o]		
Nona-	All-L	Dec.	−32.6	−10.5	−39[a]	+46.0[c]	−191[k]	286[k,j]	+ 96.1[r]	240[r]		
Undeca-	All-L	Dec.	−35.6	− 4.5	−14[a]	+43.0[d]	−251[e]	320[e]	+ 74.4[d]	248[d]		

[a] Concn. = 0.50%. [b] Concn. = 1.4%. [c] Concn. = 0.22%. [d] Concn. = 0.08%. [e] Corrected for index of refraction; a value of 212 mμ was used as λ_0. [f] Concn. = 1.02%. [g] Concn. = 1.05%. [h] Concn. = 1.86%. [i] Concn. = 1.24%. [j] Slope was not linear at higher wave lengths; approximate values were obtained from that portion of the curve which was linear. [k] Concn. = 1.33%. [l] Concn. = 0.86%. [m] Concn. = 2.70%. [n] Concn. = 2.47%. [o] Data for these values were measured by Dr. K. Norland in the laboratories of Dr. E. R. Blout, The Children's Cancer Research Foundation, The Children's Medical Center, Boston, Mass. [q] Concn. = 1.21%. [r] Concn. = 0.66%. [s] Concn. = 1.66%.

Optical Rotatory Dispersion.—Rotatory dispersion measurements have also been extremely valuable in the assignment of conformations for polypeptides.[7] Use may be made of the b_0 and λ_c values from an empirical standpoint. The λ_c values for poly-γ-esters of L-glutamate shift to longer wave lengths in any one solvent as the peptide chain assumes complete or partial helical conformations.[12] The b_0 values for the same polymers are always much more negative in the helical form than in the random coil structure.[11] In the latter conformation the b_0 value is generally close to zero. We determined the optical rotatory dispersion constants of the oligomeric compounds in helix and random coil solvents. In dichloroacetic acid, Moffitt plots indicate the conformation of poly-γ-methyl L-glutamate, irrespective of molecular weight, to be essentially random coil. In dimethylformamide, however, increasingly negative Moffitt slopes are observed in the range from the pentamer to the undecamer.[31] The changes in the b_0 values in dimethylformamide as a function of peptide length are illustrated in Fig. 2, where it can be seen that values common to the higher polymers are approached. Other pertinent optical rotatory data in this and other solvents are found in Table I.

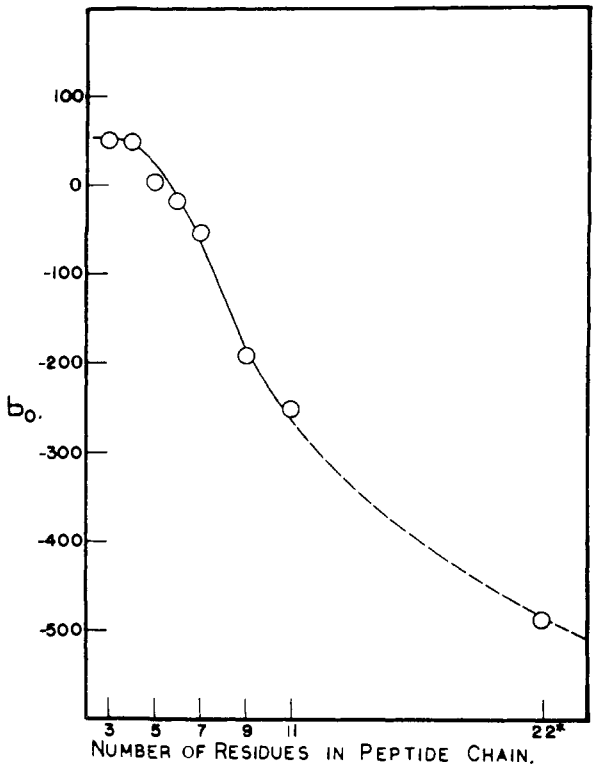

Fig. 2.—The b_0 coefficients of the oligomeric peptides derived from γ-methyl L-glutamate in dimethylformamide at 25°. *The b_0 coefficient for a lower molecular weight polymer (A/I 22) is also included in this plot.

We feel that the abnormal rotatory dispersion constants obtained in dimethylformamide are excellent indications of stable helical forms. Abnormal rotatory constants for poly-γ-benzyl L-glutamate in DMF were observed by Doty and his colleagues.[35] The shift of these values at the pentapeptide is probably caused by the first appearance of intramolecular hydrogen bonds. At the nonapeptide a more impressive shift toward values of the high polymer reflects the greater degree of stability for the intramolecular hydrogen bonding.

Infrared Spectra.—In another attempt to study the conformations of the oligomeric peptides, the infrared absorption spectra of the entire series were measured in potassium bromide pellets. Spectra were also obtained using films cast from helix-forming solvents where possible. Each compound from the dipeptide to the undecapeptide displayed simple amide I and amide II bands at 1630 and 1525 cm.$^{-1}$, respectively, which is typical of the intermolecularly hydrogen-bonded amide. There was no evidence, even with the undecapeptide, of any absorbance at 1655 and 1550 cm.$^{-1}$ which is usually, but not always,[25] associated with the intramolecularly hydrogen-bonded amide of the α-helix. Recently Miyazawa published extensive correlation of infrared bands with structure for polypeptides.[36,37] Attempts to orient

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the films of the oligopeptides were unsuccessful. Thus dichroic studies were not possible.

Association.—The 1655 and 1550 cm.$^{-1}$ bands appear predominantly in the spectra of solutions of high molecular weight polypeptides which have been dissolved in helix-forming solvents. The 1655 cm.$^{-1}$ band has been assigned to the amide stretching frequency involved in intramolecular hydrogen bonds of the α-helix.[38,39] None of our oligomers shows this band when dissolved in dioxane. A 1680 cm.$^{-1}$ band appears in tetra- and smaller peptides in dioxane, but this band is displaced by a 1635 cm.$^{-1}$ band in the penta- and larger peptides. The 1680 cm.$^{-1}$ absorption frequency is probably dependent upon the solvated amide group.[26] The 1635 cm.$^{-1}$ absorption frequency has its origin, at least in part, in an association phenomenon. The actual assignment of the 1655 cm.$^{-1}$ band probably applies only to nonterminal doubly intramolecularly hydrogen bonded amide linkages. In high molecular weight polymers, end-group effects are small while in our oligomeric peptides almost all of the amide linkages can participate in only one intramolecular hydrogen bond. This fact may well account for the observed shift from the 1655 cm.$^{-1}$ frequency to the observed 1635 cm.$^{-1}$ absorption band.

The pentamer and hexamer produce in addition to the 1680 cm.$^{-1}$ band a 1635 cm.$^{-1}$ absorption band, which increases in intensity as the dioxane solutions are made more and more concentrated. The 1680 cm.$^{-1}$ absorption frequency which is present at low concentrations of these penta- and hexapeptides disappears rapidly as the concentration is increased. Likewise, specific rotations which remain constant for the smaller peptides suddenly become concentration-dependent at the pentamer.[33] The molecular weights observed for the pentamer and hexamer in dioxane show association (Table II). The specific rotations of the heptamer and higher peptides, which seem to be less concentration-dependent than the penta- or hexapeptides (see Fig. 3), nevertheless show a large association tendency, as shown by the infrared and molecular weight data. Table II contains the molecular weight of these peptides determined by three procedures: freezing point depression measurements,[40] isothermal distillation techniques[41] and equilibrium centrifugation.[1] The results of these experiments vary depending upon the type molecular weight average obtained and the temperature but nevertheless show a tendency for association at or above the pentamer. Association of peptides in dioxane does not occur with the tri- or tetrapeptide. It may be concluded from these data that the positive enhancement of the optical activity noted in the series of oligomeric peptides in dioxane, beginning with the pentapeptide, must be caused, in part, by a type of intermolecular agglomeration.

TABLE II

MOLECULAR WEIGHT DETERMINATIONS OF OLIGOMERIC PEPTIDES DERIVED FROM γ-METHYL L-GLUTAMATE IN DIOXANE

Peptide	Formula weight	Concn., % (w./v.)	Freezing pt. detmn. at 11.7°	Isothermal distln. at 25°	Equil.[42] ultracentrifugation 20-25°
Tri-	624	1.0	725	600	
		6.0	600		
Tetra-	767	1.0	720	800	
		1.2			640 ± 100
		2.0			590 ± 70
		15.0	780		
Penta-	910	0.29			1130 ± 150
		0.48			2200 ± 250
		1.0		1325	
		1.2			3800 ± 375
		2.0	1200		5300 ± 500
		8.0	1700		
Hepta-	1196	0.1			147,000 ± 20,000
		.2			191,000 ± 16,000
		.3			211,000 ± 10,000
		.5			263,000 ± 7,000

One explanation for the association phenomena has been offered by Doty and Blout, *et al.*[25,26] They have ascribed the association of polydisperse low molecular weight poly-γ-benzyl L-glutamates ($\overline{DP}_n$ 5.2) to a β-associated structure in solution. Their conclusions are based upon the evidence of concentration dependence of the optical rotations and carbonyl stretching frequency of the amide I band. It is our feeling that the data which we have obtained for our oligomeric peptides are consistent with their results, but we desire to modify their explanation. A simple transition from a random coil to a β-associated form in dioxane would not explain the suddenness of the positive enhancement to the optical rotation noted at the pentapeptide. Neither does this postulate explain the sudden onset of association at the pentapeptide as detected by the appearance of the 1635 cm.$^{-1}$ absorption band and by the molecular weight data. Nor does this hypothesis account for the complete lack of association in the same solvent for the di-, tri- and tetrapeptides up to concentrations as high as 20% while the pentapeptide exhibits association at extremely low concentration. Furthermore, when the hexa- or higher peptides are carefully warmed in dioxane solution, precipitation of a solid results which will not redissolve in dioxane. These materials during heating undergo hydrogen bond rupture, allowing the peptide chain to rearrange in a manner analogous to protein denaturation.[43] The chain is then able to associate in what we believe to be a β-extended structure. This second solid form can be reconverted to the original by dissolving it in a hydrogen bond-breaking solvent such as dimethylformamide, and precipitating the

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(41) R. P. Linstead, J. A. Elvidge and M. Whalley, "A Course in Modern Techniques of Organic Chemistry," Butterworths Scientific Publications, London, 1955, p. 150.

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peptide with ethanol. This cycle can be repeated.[33,44]

Doty, *et al.*,[45] reported that association occurs in poly-γ-benzyl L-glutamate with molecular weights between 20,000 and 50,000 in solvents with low hydrogen bonding potential (purified chloroform, dioxane, benzene). No association was found in better hydrogen bond breaking solvents (dimethylformamide, *m*-cresol, chloroform–formamide). The association arises from the formation of intermolecular hydrogen bonds. The specific rotations of our oligomeric peptides have shown positive enhancements in dimethylformamide, *m*-cresol and dioxane at, or very near, the pentapeptide. Peptides smaller than the pentamer do not exhibit any association in any of these solvent systems. The higher peptides show association properties only in dioxane.

In a more recent publication, Wada[46] has shown that the most probable association for poly-γ-benzyl L-glutamate α-helix in dimethylformamide–dioxane mixtures is a superposition of side-by-side random associations and head-to-tail oriented associations. We suggest that the sharp positive rotation enhancement in the transition from the tetrapeptide to the pentapeptide in dioxane is caused by a change from a solvated random form to an associated folded form. Even at maximum intramolecular hydrogen bonding, all these oligomeric peptides have four carbonyl and four amido groups aligned to form intermolecular hydrogen bonds. Since the tetramer does not associate, and the pentamer does, we believe that the association of these oligomeric peptides is involved with the formation and stabilization of folded forms.

In an attempt to verify this last statement, we prepared the pentapeptide derived from γ-methyl glutamate containing a D-glutamic acid residue in position three.[1] We have verified the assumption made by Applequist and Doty[47] that a helical conformation can exist for these small peptides if only L- or D-residues are involved, but not both. No positive increment to the specific rotation was noted for this L-L-D-L-L pentamer; its specific rotation in dioxane was proportionately equal to that of an all-L tripeptide.[1] Furthermore, the optical rotations were concentration independent. Lack of association was confirmed by the appearance of only the 1680 cm.$^{-1}$ absorption band in the infrared spectra, even in saturated solutions of this pentapeptide. Furthermore, the L-L-D-L-L pentamer is much less soluble in dioxane than the all-L peptide. A 2% solution of the L-L-D-L-L peptide appears to be supersaturated, since the product slowly crystallizes on standing. We suggest that the L-L-D-L-L pentamer cannot form intramolecular hydrogen bonds and as a result is unable to associate in a manner analogous to the all-L pentamer.

It was previously shown that the lack of a sharp transition of conformational properties between the tetramer and pentamer in dimethylformamide is due to the solvation of some of the amide groups. The same process which decreases the ability of the peptide chain to fold completely also inhibits intermolecular hydrogen bonding. Above the critical length a moderately solvating medium (dioxane) neither interferes with the formation of the intramolecular bonds characteristic of the α-helix, nor prevents the association that may conceivably stabilize the folded form.

The specific rotations of the higher molecular weight peptides ($\overline{DP}_n$ 12 to 60) of γ-methyl L-glutamate (prepared by the polymerization of the N-carboxyanhydride in dimethylformamide) in dioxane are less than 15°,[48] while the oligomers have rotations which are much more positive. These values are recorded in Table III. It was also noted that these polymers in dioxane do not form viscous solutions nor do they gel as readily upon cooling. Preliminary ultracentrifugation measurements in dioxane indicate that the association properties of the polymers are not as pronounced as those of the oligomeric peptides. A small amount of each of these polymers was found to contain much higher molecular weight material. A comparison between the oligomers and these polymers may not be completely valid since the oligomeric compounds are monodisperse, while the polymeric materials are polydisperse. Also, the oligomers contain the benzyloxycarbonyl blocking group, while the polymers have other amine end groups. However, the high positive rotations for the oligomers probably derive mostly from association phenomena.

TABLE III

MOLECULAR WEIGHTS AND SPECIFIC ROTATIONS (589 mμ) OF POLY-γ-METHYL L-GLUTAMATES IN DIOXANE

$\overline{DP}_n$ (calcd.)	$\overline{DP}_n$ (found)[a]	$[\alpha]^{25}D$
6.8	..	Insol.
12.3	..	9.60°
36.6	35	13.40
57.8	41	14.54
200	..	Insol.

[a] Partial molal volume assumed to be 0.74; measurements were determined in dichloroacetic acid.

Optical rotatory dispersions of the tripeptide in various solvents were examined in order to determine whether or not the b_0 values and other rotatory properties would be affected by changes in solvent. The tripeptide was chosen because no secondary structures, at least of a helical sense, are possible. Table IV shows the dependence of these values of the tripeptide on the nature of the solvent. It can be seen that the effect of solvent can also be a factor in the b_0 values of peptides capable of existing in helical conformations. Unfortunately,

(44) M. Goodman, *J. Polymer Sci.*, **49**, 150 (1961).

(45) P. Doty, J. H. Bradbury and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956).

(46) A. Wada, *J. Polymer Sci.*, **45**, 145 (1960).

(47) J. Applequist, Ph.D. Thesis, Harvard University, 1958.

(48) One sample of poly-γ-methyl L-glutamate obtained by partial extraction from the high polymer (DP_n 137) had a much higher positive rotation. The high molecular weight sample was refluxed for 16 hours in boiling dioxane in an effort to extract any lower molecular weight soluble material. A few tenths of a per cent. of the polymeric material dissolved, but formed a gel upon cooling. Successive dilutions resulted in a stable, non-gelling solution which was used for the optical activity measurements. By lyophilizing this solution, the concentration and thereby the specific rotation of +100° were obtained. The lyophilized material, however, was completely insoluble in dioxane. Unfortunately, no molecular weight data were obtained, nor could the extraction process be repeated. The possibility that this phenomenon is related to a β-association will be discussed in a later section.

TABLE IV

OPTICAL ROTATORY VALUES OF TRIPEPTIDE DERIVED FROM γ-METHYL L-GLUTAMATE AS A FUNCTION OF SOLVENT

Solvent	Concn., %	$[\alpha]^{25}D$	λc, mμ	b_0[a]
Dioxane	2.70	−14.01°	219	− 8
Ethyl acetate	2.61	− 8.14	194	+14
Chloroform	3.86	− 6.46	179	+19
Formic acid	3.89	−28.76	202	+36
Dichloroacetic acid	1.66	−18.14	168	+46
Dimethylformamide	1.02	−13.91	183	+52

[a] The value of λ_0 was set at 212 mμ.

polymers of γ-methyl L-glutamate are not soluble in all the solvents listed in Table IV. Nevertheless, a solvent dependence can be shown in Fig. 4 by plotting the b_0 values obtained from different solvent mixtures of dioxane and dimethylformamide. The variations of the rotatory dispersion constants with solvent are not clearly understood. One possible explanation involves the shift in equilibrium between helix and random coil forms yielding more highly ordered structures in dioxane. Association may also be a factor since recently Wada[46] has shown it to be important in dimethylformamide–dioxane mixtures.

Optical rotatory dispersion measurements were also carried out on the oligomeric peptides in dioxane. The values of b_0 and λ_c obtained from the dispersion of these solutions are recorded in Table V. Because of the limited solubility of some of the higher peptides, not all the solutions were of the same concentration. Consequently, interpretation is difficult in the light of the various degrees of association which could be present.

TABLE V

OPTICAL ROTATORY DISPERSION CONSTANTS OF OLIGOMERIC PEPTIDES DERIVED FROM γ-METHYL L-GLUTAMATE IN DIOXANE AT 25°

Peptide	Concentration, %	b_0[a]	λc, mμ
Tri-	2.695	− 8	219
Tetra-[b]	1.397	− 37	229
Tetra-	0.224	− 39	231
Penta-[b]	2.470	+ 32	249
Hexa-[b]	1.544	+106	241
Hepta-[b]	1.212	+123	244
Nona-	0.661	+ 96	240
Undeca-	0.078	+ 74	248

[a] The value of λ_0 was set at 212 mμ. [b] Values for these peptides were obtained by Dr. K. Norland in the laboratories of Dr. E. Blout, The Children's Cancer Research Foundation, The Children's Medical Center, Boston, Mass.

The most striking feature of these values is the positive b_0 (Moffitt constant) for the penta- and higher peptides. A maximum appears at the heptapeptide, although this may be caused by concentration differences. The higher molecular weight polymers ($\overline{DP}$'s 18 to 60), on the other hand, have negative b_0 values. The positive b_0 term may be caused, as suggested above, in part by intermolecular association. Positive b_0 values have been reported by Schellman[49] for poly-γ-benzyl L-glutamate (solvent not disclosed). These positive values have been attributed directly

(49) J. A. Schellman and C. G. Schellman, *J. Polymer Sci.*, **49**, 129 (1960).

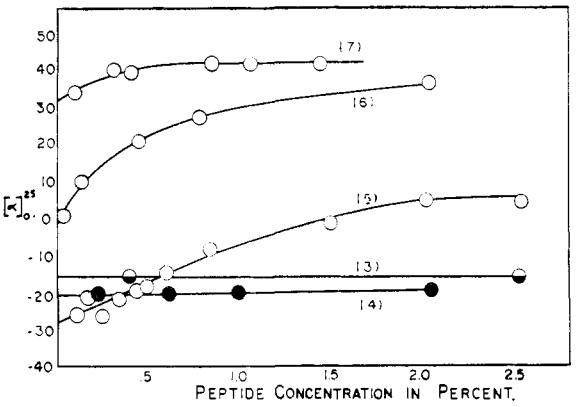

Fig. 3.—Concentration (% g./ml.) dependence in dioxane of the optical activity (measured at 589 mμ) of the oligomeric peptides derived from γ-methyl L-glutamate at 25°. Numbers in brackets represent the number of residues in each peptide.

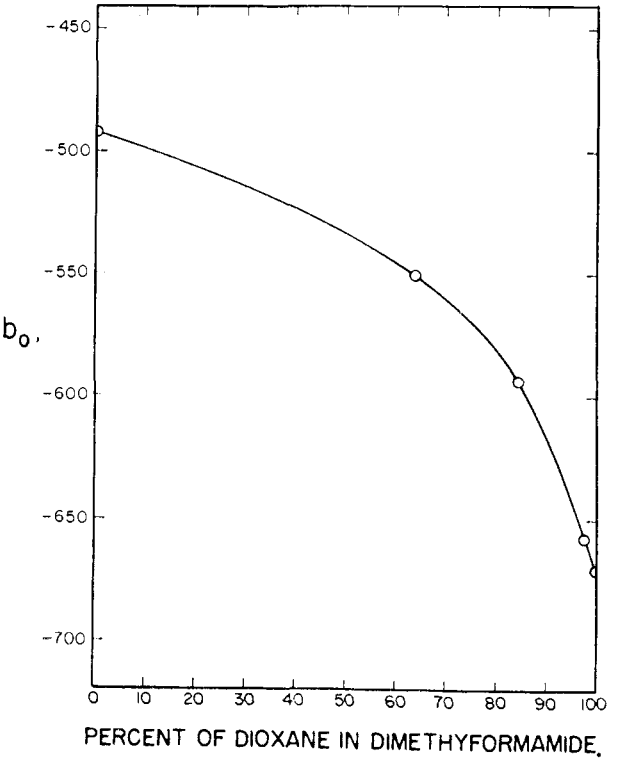

Fig. 4.—Solvent dependence of b_0 constant for poly-γ-methyl-L-glutamate (A/I 37) at 25° in dioxane dimethylformamide mixtures.

to the β-associated structure in solution. Our findings may also be explained by similar interpretations.[49]

Thermal Dependence.—Another important factor which remains to be discussed is the effect of temperature upon these folded conformations. All optical measurements described thus far have been carefully carried out at 25°. It was shown that at constant temperature the length of the peptide chain and the solvent are fundamentally important factors in the existence and stability of the folded conformation. Another aim of our work is to demonstrate that temperature deserves equally

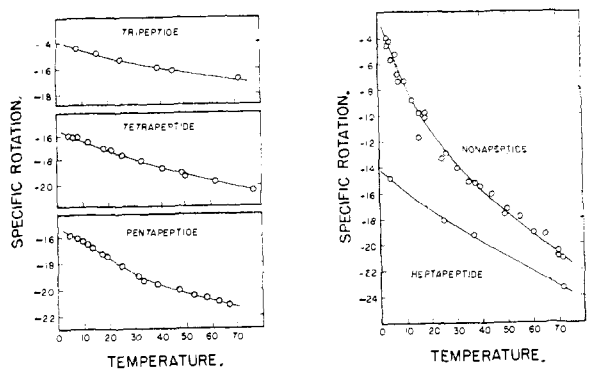

Fig. 5.—Optical activity (measured at 546 mμ) of the oligomeric peptides derived from γ-methyl L-glutamate in dimethylformamide at various temperatures.

important consideration in the development and study of these folded forms.[50,51]

Thermal energy may have many effects upon a peptide chain which is capable of existing in a helical conformation.[9,10,51,52] Only some of these effects cause the folding or unfolding of helical forms. Hence, discretion must be used in the interpretation of temperature studies. Solvents have been used which eliminate the problem of association. The problem of the extent of interaction between solvent and peptide cannot be excluded, since all helix-forming solvents that prevent association may also interact to some degree with the intramolecular hydrogen bonding of the chain.

The relationship between temperature and specific rotations for our oligomeric peptides is shown in Fig. 5. Smooth curves are observed, the higher peptides showing greater temperature dependence of the optical activity than the tri- and tetrapeptides. The plots of the pentamer and higher peptides seem to indicate a combination of effects. The pentamer, in particular, shows a temperature dependence similar to that of the tetramer above 35°, while below this temperature it possesses a dependence almost identical to that displayed by the heptamer.

The data from these relationships may be used to construct graphs relating specific rotation to the size of the peptide at various temperatures. Figure 6 contains such data for 0°, 25° and 70°. The shapes of the curves have been interpreted as unfolding of the intramolecularly hydrogen bonded forms for the penta- and higher peptides as one raises the temperature. The greater temperature dependence of the optical rotation of the higher members of the series is most probably due to the larger proportion of folded forms in these peptides. We are at present calculating thermodynamic data from these and other temperature studies and will subsequently report our findings.

Conclusions.—We have shown that certain oligomeric peptides possess folded (or helical) conformations in solution. We have found that the polypeptide need not have relatively large chain

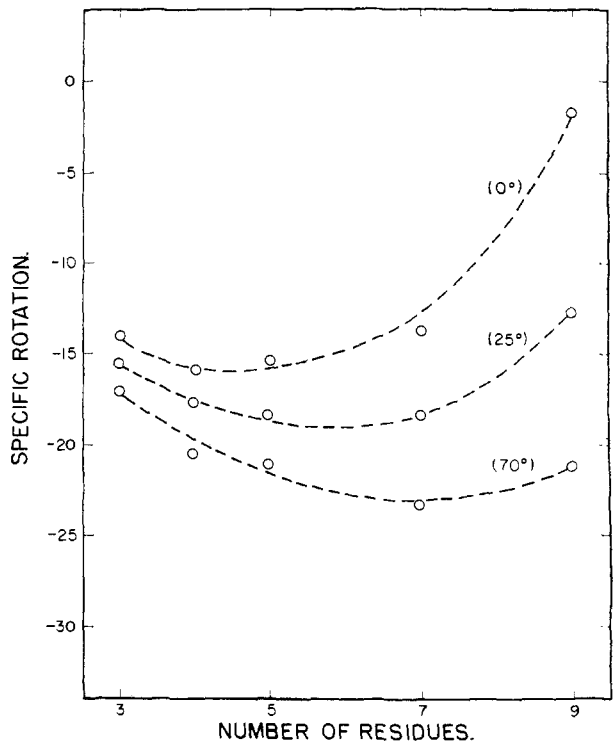

Fig. 6.—Optical activity (measured at 546 mμ) of the oligomeric peptides derived from γ-methyl L-glutamate in dimethylformamide at 0°, 25° and 70°.

lengths in order to assume a helical type conformation.[35,53] We have demonstrated this with peptides derived from γ-methyl esters of glutamic acid, blocked at the amino end by the benzyloxycarbonyl group. Peptide chains with as few as five residues exhibit properties that lead us to believe that they are present partly in a folded conformation. Since the amine blocking group was not removed when studying these peptides, the pentapeptide studied actually contained six amide linkages. The possibility that this last amide linkage may have some type of stabilizing effect on the folded form must also be considered when evaluating the critical size for intramolecular hydrogen bonding.

The stability of the helix once formed is governed both by the solvent in which the peptide is dissolved and by the temperature. The solvent dependence of the helix-random coil equilibrium has its origins in the degree of solvation of the amide linkages. Depending upon the degree of interaction, random coils, folded forms, or associated folded forms may exist. Complete solvation results in a random coil analog while partial solvation will lead to helical structures. Even with a maximum of intramolecular hydrogen bonding, there still remain large end group effects (four sites at either end of the chain which can form hydrogen bonds with another peptide or solvent). Hence in solvents with very low hydrogen bonding potential, such as dioxane, oligomeric peptides may form both intra- and intermolecular hydrogen bonds accounting for the associated folded form. In

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(52) J. G. Foss and J. A. Schellman, *J. Phys. Chem.*, **63**, 2007 (1959).
(53) M. Idelson and E. R. Blout, *J. Am. Chem. Soc.*, **79**, 3948 (1957).

solvents which break up easily accessible hydrogen bonds, such as dimethylformamide and *m*-cresol, these amide sites are solvated to such an extent that no intermolecular hydrogen bonding between peptides occurs. There is also quite probably some destructive solvation at the intramolecular hydrogen bonding sites, especially in the critical range. This type of solvent interaction permits random coil, helical and partially helical species to co-exist in solution simultaneously. The equilibrium among these forms is gradually shifted to more ordered structures as more residues are added to the peptide chain. Hence there is no sharp discontinuity of properties between peptides that can and cannot form helical structures. Perhaps mixed solvent systems such as dioxane and dimethylformamide would allow for a maximum of intramolecular hydrogen bonding without intermolecular hydrogen bonding.

An enhancement of helical properties has been observed at the nonapeptide level. The fifth residue in the chain of the nonapeptide can be intramolecularly hydrogen bonded simultaneously through its carbonyl and amide groups to the first and ninth residues. Below this size all intramolecular bonding for any particular residue involves either a carbonyl or an amide group of a given residue, but not both. We feel that the onset of a bifunctionally hydrogen bonded residue imparts an added stability to the folded form since it ties up both ends of the molecule.

For high molecular weight polypeptides, essentially all residues, other than the four terminal residues at each end of the chain, are intramolecularly hydrogen bonded through both their carbonyl and NH functions. This difference between high polymers and oligomers must be kept clearly in mind in any interpretation of experimental measurements since, in actual fact, the oligomeric compounds have essentially the helical structure of the ends of any protein or high molecular weight synthetic polypeptide. The use of the same criteria to detect helix formation of polymeric peptides is no assurance that folded forms in oligomeric species will be uncovered. Such problems probably influence the position of the amide I absorption band, the positive optical activity increment, and the absolute values of the optical rotatory dispersion constants.

Neither the thermodynamics of the helix–random coil transition nor the energetics of intramolecular hydrogen bond formation are strictly comparable with the higher molecular weight analogs. The results of our temperature studies are in accord with theories of helix–random coil equilibrium.[9,10,54–61] It is apparent that, at lower temperatures, the peptides possess folded conformations and properties consistent with a helical nature. A prediction is borne out by our work that the sharpness of the helix-random coil transformation would be broadened as the chain length is decreased.[61] As one views the thermal dependence of the penta- through nonapeptide, it is apparent that the dependence becomes sharper the larger the peptide.

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Experimental

Synthesis of Peptides.—The preparation of the oligomeric peptides has already been described in a previous paper in this series.[1] For analytical details, see Table I in the previous paper.[1]

Interconversion of Dioxane-soluble and -insoluble Forms.—Benzyloxycarbonyl-octa-(γ-methyl-L-glutamyl)-diethyl L-glutamate (0.76 g.) was dissolved in 50 ml. of dried purified dioxane by heating quickly to reflux temperature. Upon further heating a precipitate formed gradually and the solution became gradually less viscous. After approximately 1 hour of heating the suspension was cooled and filtered. The filtrate was lyophilized and found to contain less than 0.01 g. of original product. The precipitate gave the identical infrared spectrum in a potassium bromide pellet as the original material, but was insoluble, even in hot dioxane.

The reverse conversion was carried out as follows. An appreciable amount (approximately 90%) of the precipitated material dissolved after standing in dimethylformamide for a few days at room temperature. Addition of ethanol to the dimethylformamide solution caused precipitation of a substance, which was subsequently filtered, triturated with both petroleum ether and ethanol, washed with ether, and dried at 56° *in vacuo* for 16 hours. The product (0.60 g.) which was reclaimed in 80% yield, was redissolved in dioxane with mild heating to give an infrared spectrum and optical rotation which was identical with the original material.

Apparatus and Measurements. Optical Rotations.—All rotations were observed with a Rudolph No. 70 polarimeter. Rotatory dispersion measurements were taken with the aid of a Rudolph photoelectric attachment. The instrument was also equipped with a sodium and mercury lamp source and the appropriate filters so that the following wave lengths could be employed: 589 mμ, 578 mμ, 546 mμ, 435 mμ, 405 mμ, 365 mμ. Additional lines were obtained by incorporation of cadmium and thallium lamps (purchased from Gates and Co., Franklin Square, L. I., N. Y.) and the appropriate interference filters (supplied by Bausch and Lomb, Rochester, N. Y.). The supplementary wave lengths were: cadmium, 644 mμ, 509 mμ, 480 mμ, 468 mμ; thallium, 535 mμ, 378 mμ, 352 mμ.

The polarimeter tubes used for these optical rotation measurements were usually 2 dm. in length, although on occasion 1- and 4-dm. tubes were also utilized. The bore of the tube was never less than 3 mm. in diameter. The temperature of the tube was maintained at 25.0° to ±0.1° unless otherwise stated, by circulating constant temperature water either through a jacketed tube or through the polarimeter's constant temperature bath.

Calculation of Rotatory Data.—The dispersion constant, λ_c, was obtained by the modified Lowry plot proposed by Yang and Doty[12] from the square root of the slope of $[\alpha]\lambda^2$-*vs.* $[\alpha]$.

The coefficient b_0, the second term in the Moffitt equation,[13] was derived from the slope of the plot $[\alpha](\lambda^2 - \lambda_0^2)$ *vs.* $(\lambda^2 - \lambda_0^2)^{-1}$ as suggested by Blout and co-workers.[11,62] Thus b_0 is the slope of the linear plot divided by $\lambda_0^4(100\ M)\left(\frac{n^2+2}{3}\right)$, where λ_0 is 212 mμ, M is the molecular weight of the peptide and n is the refractive index of the solvent, since $[\alpha]$ was used initially instead of the mean residue rotation.[63] By this means of representing the Moffitt equations the refractive index of the solvent is assumed constant over all wave lengths (352–644 mμ). The value of the solvent refractive index used was that found at the sodium D line (589 mμ).

Infrared Spectra.—All infrared spectra were recorded on a Perkin-Elmer model 21 spectrophotometer using NaCl optics. Spectra of the solid peptides were taken in potas-

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sium bromide pellets prepared by grinding 1 mg. of sample in 350 mg. of potassium bromide and subjecting the mixture to pressure. Solution spectra were obtained in matched cells ranging in thickness from 0.01 to 0.10 mm. by running the various dioxane solutions against the pure solvent.

Molecular Weight Determinations.—Molecular weight determinations were performed on several of the oligomeric peptides derived from γ-methyl L-glutamate in order to check the molecularity and association of the compounds prepared. Both cryoscopic and isothermal distillation techniques were used for the pentamer and lower homologs. These procedures have been described by Daniels, *et al.*,[40] and Linstead, *et al.*,[41] respectively. The experimental values are listed in Table II.

Details of the equilibrium ultracentrifugation techniques are given in the preceding paper.[1] The results are contained in Table II of this paper and in the previous paper of this series.[1]

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN, N. Y.]

Conformational Aspects of Polypeptides. V. Molar Rotational Model Compounds for Poly-γ-methyl L-Glutamate[1]

BY MURRAY GOODMAN, IRVING LISTOWSKY[2] AND EDWARD E. SCHMITT[3]

RECEIVED JULY 17, 1961

This report summarizes the synthesis and use of model compounds which can be employed to explain the optical rotatory properties of oligomeric peptides and polymers of the γ-methyl L-glutamate series. The model compounds serve as a basis for determining the onset of secondary structure in the series of oligomeric peptides. Effects of residue position and neighboring group interactions on optical activity of peptides are elucidated and discussed. The model compound rotations are solvent dependent, and can be used to calculate a "random coil" optical rotation in a variety of solvents. Significant deviations of the optical activity of the oligomeric peptides from the calculated "random coil" rotation are attributed to intramolecular hydrogen bonding in dimethylformamide and *m*-cresol. In dioxane the deviations result from a combination of intra- and intermolecular hydrogen bonding. There are no large differences between calculated and experimental rotations through the undecamer in dichloroacetic acid, a "random coil" solvent. Calculated "random coil" rotations at various temperatures in dimethylformamide were obtained to show that helical effects decrease with increasing temperature.

Introduction

The optical rotatory power of a polypeptide is dependent on many factors.[4-11] To a first approximation, it is composed of the sum of the rotations for the individual asymmetric centers (configurational optical activity).[4] To this must be added the secondary (helical forms) and tertiary structural arrangements of the polymer chain as a whole (conformational optical activity).[4-11] Additional factors include specific interactions between solvent and peptide chain and, where they exist, side chain–main chain interactions[12] and also intermolecular chain–chain interactions (associational optical activity).[13,14]

The contributions of the configurational and conformational aspects are dependent upon the structure of the polypeptide in solution, which in turn is determined by the nature of solvation.[14-16] The type of solvation is related to the strength of the interactions between solvent and the peptide chain, a "random coil" resulting when all chain–chain and intra-chain hydrogen bonds are broken in favor of solvent–chain hydrogen bonds. A mildly interacting solvent does not disrupt the inter- and intramolecular hydrogen bonds, permitting association and/or helix formation.[17]

Optical rotatory properties have been employed in detecting the type of structure existing in solution.[18,19] It has been shown that there is a significant difference in the optical rotation and rotatory dispersion of a polypeptide "random coil" form as compared to the same polymer in a helical form.[20-22]

Results and Discussion

Model Compounds.—As suggested by Brand[23] and Doty[24] and developed by us in this paper, the end groups of a peptide solvate differently from internal residues. Thus their contribution to the optical activity is different from internal residues. As the molecular weight of a polymer decreases, increasing consideration must be given to these end group effects.

(1) (a) Previous paper in this series, M. Goodman, E. E. Schmitt and D. A. Yphantis, *J. Am. Chem. Soc.*, **84**, 1288 (1962). (b) This research was supported by grants from the National Science Foundation (G8514) and the National Institutes of Health (A3868).

(2) Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the Polytechnic Institute of Brooklyn.

(3) American Cyanamid Co., Stamford, Conn.

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