Right- and Left-Handed α-Helical Structures in Poly(L-alanyl-D-alanyl-L-alanyl-L-alanyl)[1,2]

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ABSTRACT: Regular-sequence copolymers of Ala$_4$(LDLL) have been prepared by active-ester polycondensation, and fractionated to yield oligomers (LDLL)$_n$, with n = 1–6, where n is the number of tetramer units in the oligomer. Appreciable helix content was observed with $n > 4$ in both water and in 90% aqueous trifluoroethanol, being higher in the latter solvent. The helix content deduced from hypochromism measurements was higher than that obtained from optical rotatory dispersion measurements. This discrepancy can be resolved by assuming that both right- and left-handed α helices, as well as random coils, are present at the same time in a sample of (LDLL)$_n$. Indeed, conformational energy calculations showed that the D residues do not interfere sterically with the formation of a right-handed α helix, and that the right-handed α-helical form of (LDLL)$_n$ is only slightly more stable than the left-handed one.

In a previous paper,[4] it was shown by conformational energy calculations that there is no steric hindrance to α-helix formation in alternating regular-sequence copolymers of D and L residues of any of a number of representative naturally occurring amino acids. The present paper is an extension of this work, in which we consider both the experimental and theoretical aspects of the conformation of regular-sequence copolymers formed from the tetramer unit (L-alanyl-D-alanyl-L-alanyl-L-alanyl).

With these materials, we can obtain information about the conformational behavior of a guest residue (D-alanine in this case) imbedded among a large number of host residues (L-alanine in this case) in a copolymer. Since it is known that poly(L-alanine) forms right-handed α helices in water,[5,6] a study of poly(LDLL-alanine) will indicate the effect of an occasional D-alanine residue on the stability of the α-helical form of poly(L-alanine).

The copolymers were prepared from the tetramer by active-ester polycondensation, and fractionated to yield homogeneous oligomers of the general formula (L-Ala-D-Ala-L-Ala-L-Ala)$_n$, designated here as (LDLL)$_n$. Ultraviolet absorption and optical rotatory dispersion measurements, and conformational energy calculations were carried out for these oligomers to obtain information about their conformation.

I. Materials and Methods

A. Solvents and Reagents. Dioxane was purified by refluxing for 6 hr over sodium and then distilling at atmospheric pressure. Dimethylformamide was dried over anhydrous K_2CO_3 and distilled under reduced pressure. Triethylamine was dried over KOH and distilled at atmospheric pressure. Other reagents and solvents were of reagent grade, and were used without further purification.

B. Syntheses. The regular-sequence alanine peptides, having the structure (LDLL)$_n$, were prepared as described schematically in Figure 1.

Benzyloxycarbonyl-L-alanyl-D-alanyl-L-alanyl-L-alanine (III). A solution of benzyloxycarbonyl-L-alanine hydroxysuccinimide ester[7] (I) (23 mmol) in absolute dioxane (40 ml) was added to an aqueous solution (40 ml) of D-alanyl-L-alanyl-L-alanine[8] (II) (23 mmol) containing sodium bicarbonate (46 mmol). After 18 hr at room temperature, the clear solution was diluted with water (200 ml), and the protected tetrapeptide (III) was precipitated by acidification with aqueous HCl (75 mmol) to pH 1. The suspension was kept overnight at 4°, filtered on a sintered glass filter, and the product was washed with 0.02 N HCl and water, and dried under vacuum over concentrated sulfuric acid: yields 7.2 g (72%). *Anal.* Calcd for $C_{20}H_{28}N_4O_7$: C, 55.0; H, 6.4; N, 12.8; neut equiv, 436. Found: C, 54.7; H, 6.2; N, 12.8; neut equiv, 462.

No ninhydrin-positive spots were obtained in high-voltage paper electrophoresis at pH 6.5. When chlorine gas was used for detection, only one spot, migrating similarly to Z-(L-Ala)$_4$, was observed.

Benzyloxycarbonyl-L-alanyl-D-alanyl-L-alanyl-L-alanine Hydroxysuccinimide Ester (IV). The solution of III (13 mmol) and N-hydroxysuccinimide (13 mmol) in dioxane (30 ml) and dimethylformamide (40 ml) was cooled to 0°, and dicyclohexylcarbodiimide (13 mmol) was added. The mixture was stirred at 0° for 1.5 hr and then kept without stirring at 4° for 12 hr. The dicyclohexylurea formed (2.7 g, mp 242°) was filtered off and the clear filtrate was concentrated under reduced pressure to about half of its volume. After precipitation with a mixture of benzene and ether (1:2, v/v), the product was filtered and recrystallized from isopropyl alcohol (25 ml) to yield 3.6 g (yield, 52%) active ester IV.

L-Alanyl-D-alanyl-L-alanyl-L-alanine Hydroxysuccinimide Ester·HBr (V). The solution of IV (6.2 mmol) in 45% HBr in acetic acid[9] (10 ml) was kept at room temperature for 15 min. The product was precipitated with ether, recrystallized twice by dissolving it in isopropyl alcohol (8 ml) and precipitating it with ether, and dried under vacuum over concentrated sulfuric acid and KOH pellets: yield, 2.7 g (90%).

Poly(L-alanyl-D-alanyl-L-alanyl-L-alanyl) (VI). (LDLL)$_n$. The active ester V (3.1 mmol) was dissolved in dimethylformamide (3 ml), and a solution of triethylamine (3.3 mmol) in dimethylformamide (3 ml) was added at once. The reaction mixture became semisolid immediately, and was kept overnight at room temperature. The polycondensation product was precipitated with ether (100 ml), filtered on a sintered glass filter, washed with chloroform (to remove the triethylammonium bromide) and ether, and dried under vacuum: yield 0.9 g (100%). The following approximate composition (wt %) was determined by high-voltage paper electrophoresis at pH 1.4: LDLL, 3%; (LDLL)$_2$, 25%; (LDLL)$_3$, 30%; (LDLL)$_4$, 25%; and (LDLL)$_5$, 15%.

C. Fractionation of the Crude (VI). Well-defined oligomers were obtained by gel filtration on Sephadex G-15 (particle size 40–120 μ). A preliminary attempt, using distilled water as eluent, was unsuccessful. Better resolution was obtained with 0.01 N HCl, and this solvent was used thereafter. The composition of the various fractions was established by high-voltage paper electro-

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(2) A preliminary report of this work was presented before the Division of Biological Chemistry at the 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967.

(3) (a) On leave from the Van't Hoff Laboratorium, Rijksuniversiteit Utrecht, The Netherlands, 1971–1972. (b) Deceased on Nov 1, 1972.

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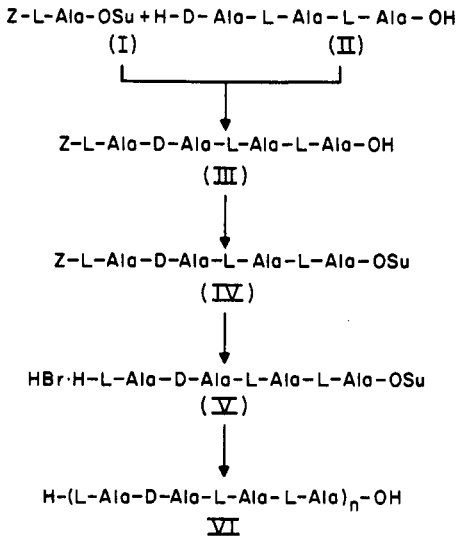

Figure 1. Schematic representation of the synthesis of $(LDLL)_n$. Z and OSu represent the benzyloxycarbonyl and hydroxysuccinimide groups, respectively.

phoresis at pH 1.4. After the first chromatography, the various oligomers were clearly separated, but not to the extent required (Figure 2). Therefore, the separation procedure was repeated twice; after each chromatographic run, the cut fractions were lyophilized and redissolved in a small volume of 0.01 *N* HCl. Finally, the purity (wt %) of the oligomers was: LDLL, 98%; $(LDLL)_2$, 96%; $(LDLL)_3$, 96%; and $(LDLL)_4$, 92%. The fraction $(LDLL)_{5-6}$ [henceforth $(LDLL)_5$] contained about equimolar amounts of the two components; the same was the case for the fraction $(LDLL)_{6-7}$ [henceforth $(LDLL)_6$].

D. Analytical Methods. The neutralization equivalent of the peptides was determined in isopropyl alcohol–dioxane (2:1, v/v) by anhydrous titration with sodium methoxide. The chloride content was determined by coulometric titration,[10] using an Automatic chloride titrator (American Instrument Co., Silver Spring, Md.). The high-voltage paper electrophoresis and the detection of peptides on paper were carried out as described previously.[8]

The chromatographic separation of the peptides on the Sephadex column was monitored by measuring the ultraviolet (uv) absorption at 220 mμ of the various fractions. The absorbances of the highly concentrated fractions were measured at higher wavelengths (where the optical density was lower), using the following ratios[11] of extinction coefficients $\epsilon_\lambda/\epsilon_{220}$: 0.49 (226 mμ), 0.27 (230), 0.135 (234), and 0.063 (238).

The optical purity of $(LDLL)_2$ was determined by enzymatic digestion with papain under the conditions described previously.[12,13] It is estimated that racemization in excess of 1% could have been detected by this technique.

The concentration of the peptides in the solutions used for uv absorption and optical rotatory dispersion (ORD) measurements was determined by nitrogen analysis.

E. Ultraviolet Absorption. Ultraviolet absorption spectra were measured in quartz cells of 1-mm light path, using a Cary Model 15 spectrophotometer, with continuous nitrogen flushing. The concentration of the solutions was about 1 mg/ml, determined precisely by nitrogen analysis, as described above. Extinction coefficients, expressed per mole of peptide bonds, ϵ_{pep}, were determined from the nitrogen analytical data after correcting for the absorption by the Cl^- and by the terminal COO^- group. If *A* is the measured absorption, then ϵ_{pep} is given by

$$\epsilon_{pep} = \frac{A - \epsilon_{Cl} l c_{Cl}}{[z/(z+1)] l c_N} - \frac{\epsilon_{COO^-}}{z} \qquad (1)$$

where *l* is the path length in centimeters, *z* is the number of peptide bonds in the molecule (equal to $4n - 1$), c_{Cl} is the concentration of Cl^- in moles per liter, and c_N is the concentration of the oligopeptide in moles of nitrogen per liter. The extinction coefficients ϵ_{Cl} and ϵ_{COO^-} of Cl^- and COO^- were determined on solutions of HCl and CH_3COONa, respectively, in water and in 90% trifluoroethanol, and their values are given in Table I.

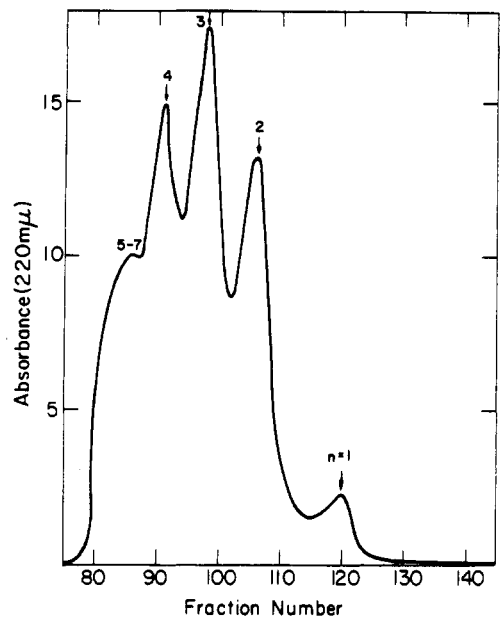

Figure 2. Chromatography of $(LDLL)_n$ on a column (2.2 × 190 cm) of Sephadex G-15 (40–120 μ). Load: 300 mg, dissolved in 5 ml of 0.01 *N* HCl. Eluent: 0.01 *N* HCl at 23 ml/hr, 3.5 ml/fraction. The composition of the eluted fractions was determined as described in the text.

F. Optical Rotatory Dispersion. Optical rotatory dispersion (ORD) measurements were made with a Cary Model 60 spectropolarimeter equipped with a 450-W Osram xenon arc lamp, using water-jacketed quartz cells (Optical Cell Co., Brentwood, Md.) with path lengths of 1 or 10 mm. The concentration of the solutions was about 1 mg/ml, determined precisely by nitrogen analysis, as described above. The data are expressed in terms of reduced rotation per mole of peptide bonds $[m'_{pep}]$, in (deg cm^2)/dmol, by means of

$$[m'_{pep}] = [3/(n^2 + 2)](M/zlc)\alpha \qquad (2)$$

where *n* is the refractive index of the solution, *M* is the molecular weight of the peptide, *z* is the number of peptide bonds per molecule, *l* is the path length in decimeters, *c* is the concentration of the solution in g/100 ml, and α is the measured optical rotation in degrees. Obviously, for large oligopeptides (*i.e.*, for large values of *z*), $[m'_{pep}]$ becomes identical with the reduced residue rotation $[m']$. Because of the similarity of the refractive indices of trifluoroethanol and water in the visible,[14] the value of $n = 1.41$ for water in the uv region was also used for 90% trifluoroethanol.

In interpreting the ORD data in terms of conformation of the oligopeptide backbone, the following assumptions are made: (a) the reduced rotation at any wavelength is the sum of the contributions of each residue, according to its configuration (*i.e.*, L or D) and conformation [*i.e.*, random coil (RC), or right- or left-handed α helix (RH or LH, respectively)]; (b) the reduced rotations of L and D residues in the random coil conformation are equal but of opposite sign; (c) the contribution of a residue in the helical conformation depends only on the handedness of the helix (*i.e.*, RH or LH), and is independent of the configuration (*i.e.*, L or D). These assumptions are summarized in eq 3–5. The numerical

$$[m'_{pep}]_{L,RC} = -[m'_{pep}]_{D,RC} \qquad (3)$$

$$[m'_{pep}]_{D,RH} = [m'_{pep}]_{L,RH} \qquad (4)$$

$$[m'_{pep}]_{L,LH} = [m'_{pep}]_{D,LH} = -[m'_{pep}]_{L,RH} \qquad (5)$$

values used for $[m'_{pep}]_{L,RC}$ and $[m'_{pep}]_{L,RH}$ are those of $[m']^{coil}$

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Table I
Extinction Coefficients of HCl and CH_3COONa

	Solvent	Extinction coefficient[a] 187.5 mμ	189 mμ	190 mμ	192.5 mμ	195 mμ
HCl	Water	215	150	105	35	10
	90% trifluoroethanol[b]	15	5	0	0	0
CH_3COONa	Water	1525	1150	950	630	400
	90% trifluoroethanol[b]	330	230	190	130	95

[a] Expressed in l. cm^{-1} mol^{-1}. [b] Water-trifluoroethanol (1:9, v/v).

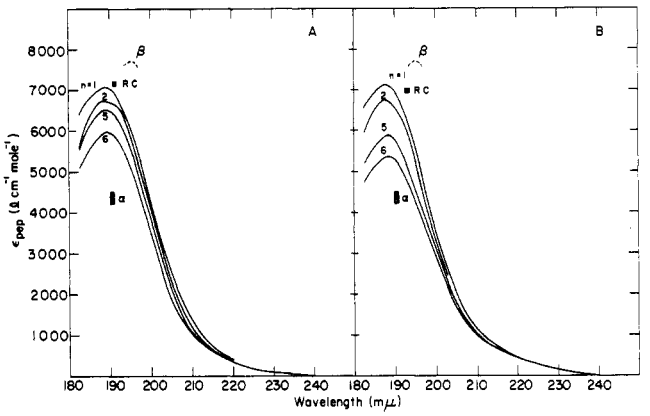

Figure 3. Ultraviolet absorption spectra of various (LDLL)$_n$ oligomers: (A) in water; (B) in 90% trifluoroethanol. The shaded rectangles marked RC and α represent the position of the absorption maxima for the random coil and for the α-helical conformations,[17] respectively, and the dashed curve marked β represents that for the β conformation.[17]

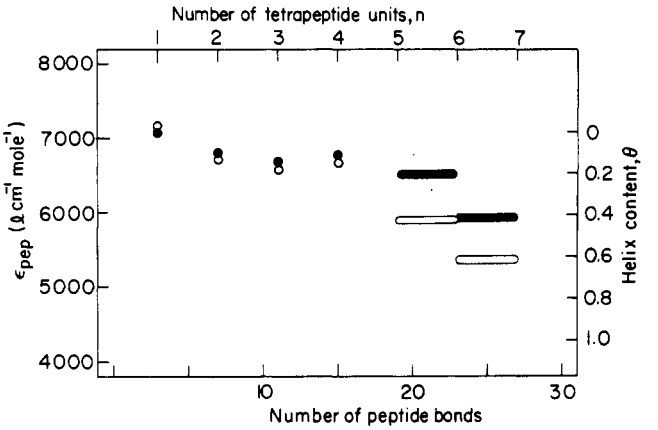

Figure 4. Dependence of ϵ_{pep} at the absorption maximum on n for (LDLL)$_n$: solid symbol, in water; open symbol, in 90% trifluoroethanol. The helix content, θ, was computed as described in the Discussion section. The horizontal bars for n = 5–6 and n = 6–7 indicate the actual composition of these fractions (see section IC).

and $[m']^{helix}$ reported previously[6] for poly(L-alanine).

G. Conformational Energy Calculations. The conformational energies of the right- and left-handed α-helical forms of (LDLL)$_n$ (running from N to C terminus) and of (L-alanine)$_{4n}$, designated L$_{4n}$, for n = 2, 3, 4, were computed with the same method and energy functions and parameters used by Ooi *et al.*[15] The conformational energy functions include contributions from internal rotational, nonbonded, electrostatic, and hydrogen-bond energies. No explicit account was taken of the solvent except for the choice of a dielectric constant D of 4. A rapid energy-minimization technique, described elsewhere,[16] was used for locating the energy minima. The polypeptide chain was taken as $(4n + 1)$ backbone residues, beginning and ending with a C^α atom, and $4n$ side chains. As before,[15] the energy values reported are expressed in kilocalories per mole of residue, and were computed by dividing the total energy by the number of backbone residues; also, χ_1 (the side-chain dihedral angle) and r_H (the radius of the hydrogen atom) were taken as 180° and 1.2 Å, respectively. Further details are provided in the earlier paper on regular-sequence D,L copolymers.[4]

II. Results

A. Ultraviolet Absorption Spectra. Representative uv spectra of various alanine oligopeptides (LDLL)$_n$ in water and in 90% trifluoroethanol are shown in Figure 3 for various values of n. The positions of the absorption maxima for the random coil, α-helical, and β conformations[17] are also indicated in Figure 3. For large n, the absorption in aqueous trifluoroethanol is lower than that in water. In all cases, the maxima are observed at 188–190 mμ. The decrease in ϵ_{max} with increasing n is illustrated in Figure 4.

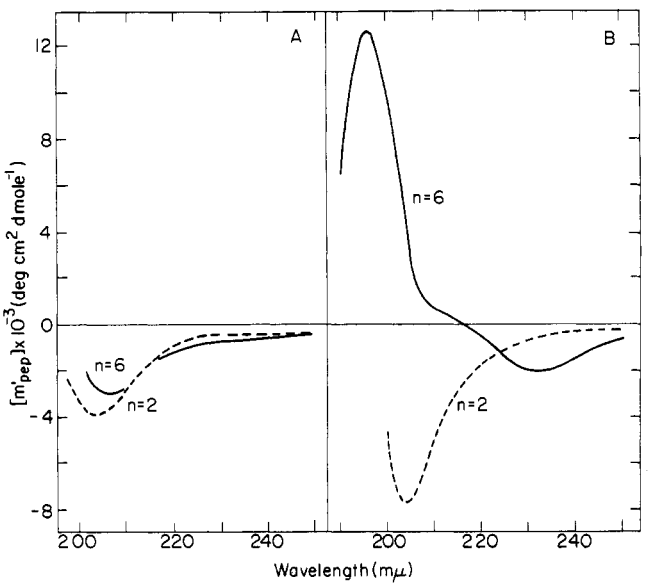

Figure 5. ORD spectra of (LDLL)$_2$ and (LDLL)$_6$: (A) in water; (B) in 90% trifluoroethanol.

B. Optical Rotatory Dispersion. The far-uv ORD curves of (LDLL)$_2$ and (LDLL)$_6$ in water and in 90% trifluoroethanol are shown in Figure 5. In water, the values of $[m'_{pep}]$ are negative at all wavelengths shown, with minima in the spectra near 205 mμ. In 90% trifluoroethanol, the spectrum of the octapeptide (n = 2) also exhibits a minimum at 205 mμ; however, for the larger oligopeptide (n = 6), the minimum occurs at 232 mμ and, moreover, the spectrum shows a maximum at 197 mμ and a crossover point at 216 mμ. The ORD spectra of the various alanine oligopeptides in water, in the wavelength range of

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Table II
Dihedral Angles for the Minimum-Energy Conformation[a] of (LDLL)$_2$

Helix Sense	Dihedral Angles[b] (deg)							
	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ψ_1	ψ_2	ψ_3	ψ_4
RH	−50.9	−49.2	−52.0	−47.8	−54.7	−57.0	−55.8	−56.9
LH	+47.4	+49.9	+48.2	+51.0	+58.2	+55.6	+57.1	+55.8

[a] The starting points, and the regularity condition assumed in the energy minimization, are defined in the text. [b] The same values (within 0.2°) were obtained for n = 3 and 4.

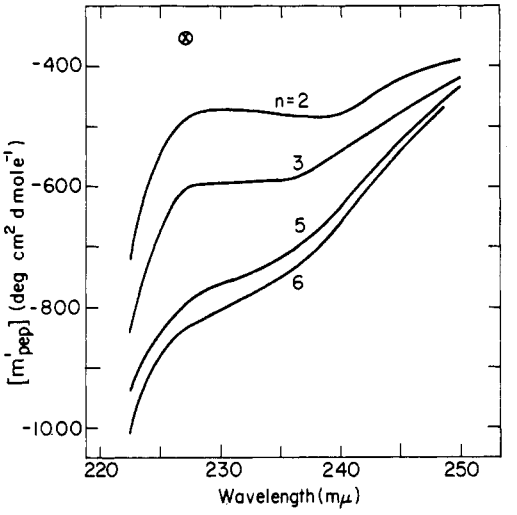

Figure 6. ORD spectra of various (LDLL)$_n$ oligopeptides in water. The symbol ⊗ is the value[18] for n = 1.

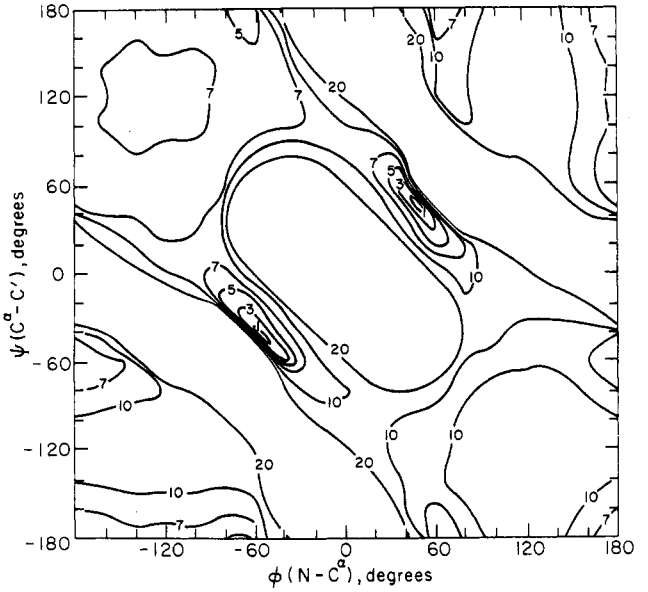

Figure 7. Energy contours for (LDLL)$_2$ helices, calculated with the regularity condition that the angles ϕ, ψ, and χ_1 are the same in all residues. The contours (interpolated from calculations at 6-20° intervals) are drawn at energies of 1, 3, 5, 7, 10, and 20 kcal per mol residue above the global minimum of −7.91 kcal/mol of residue (Table III).

220–250 mμ, are shown in more detail in Figure 6, together with a single value[18] for n = 1.

C. Conformational Energy. For an initial survey, a (ϕ,ψ) energy contour map was computed for (LDLL)$_2$ (see Figure 7) using the regularity restriction (*viz.*, that the values of ϕ, ψ, and χ_1 are the same in *every* residue, for both the D and L configurations). Comparing this map to that[15] of poly(L-alanine), it can be seen that the low-energy regions (for the right- and left-handed α helices) appear in essentially the same positions for both polymers. As in the case of the all-L polymer, the right-handed α-helical form of (LDLL)$_2$ has the lowest energy (−7.91 kcal/mol of residue compared to −7.70 kcal/mol of residue for the left-handed α helix).

The relatively low-energy region in the upper left-hand corner (β region) found in the (ϕ,ψ) map of poly(L-alanine) is not present in Figure 7 because of unfavorable (nonbonded) interactions of the side-chain methyl group of D residues with the backbone atoms of neighboring units (or, more explicitly, with the carbonyl group of the residue previous to the D residue and with the NH group of the next residue.[19]

The regularity restriction was then removed and the energy of (LDLL)$_3$, with 24 independent (ϕ,ψ) variables, was minimized, starting with a regular right-handed α-helical conformation. The resulting minimum-energy conformation (−8.9601 kcal/mol of residue) shows fluctuations in the values of ϕ and ψ (solid lines of Figure 8) within 2° around the values characteristic of the right-handed α helix. Moreover, there appears to be a fourfold periodicity of ϕ and ψ along the chain, corresponding to the tetramer character of the LDLL unit. Consequently, additional calculations were carried out, imposing the regularity condition that each LDLL unit has the same set of (ϕ,ψ) values. Thus, ϕ and ψ were allowed to vary within the tetrameric unit, but not from one LDLL unit to another. This procedure is justified by the fourfold periodicity mentioned above; it has the advantage of reducing the number of variables for (LDLL)$_n$ from 8n to 8, and therefore consumes less computer time. For (LDLL)$_3$, the minimized energy (−8.9596 kcal/mol of residue) of the conformation thus obtained with this regularity condition was only 0.0005 kcal/mol of residue higher than that obtained in the 24-variable energy minimization. The (ϕ,ψ) values in the 8-variable calculation (shown as dashed lines in Figure 8) are very nearly the same as those found in the 24-variable one, especially in the middle of the chain where end effects are negligible. Therefore, further calculations were limited to an 8-variable minimization, *i.e.*, taking the LDLL unit as a regular repeating one.

Using this regularity condition, the energies of (LDLL)$_n$ with n = 2, 3, 4 (corresponding to a 9-mer, a 13-mer, and a 17-mer, respectively) were minimized. For n = 2, the starting points for the minimization were the regular right- and left-handed α-helical conformations (*i.e.*, ϕ =

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(19) If the regularity restriction is removed, and the D residues are permitted to take on ϕ,ψ values from the lower right-hand corner (which would be the local-minimum β region for the all-D polymer), while the L residues retain the ϕ,ψ values from the upper left-hand corner, then (LDLL)$_2$ can take on low-energy structures, comparable to the β structure of L$_{4n}$. For example, at (ϕ,ψ) = (−120°, +140°), the energy is E = −4.31 kcal/mol of residue for L$_8$ and E = −0.47 kcal/mol of residue for (LDLL)$_2$; however, at (ϕ,ψ) = (−120°, +140°) for the L residues and (ϕ,ψ) = (+120°, −140°) for the D residues, E = −4.28 kcal/mol of residue for (LDLL)$_2$. Nevertheless, as shown in Table III, the lowest energies are still those of the two α-helical regions.

Table III
Energy of the Right-Handed (α_{RH}) and Left-Handed (α_{LH}) Helical Conformations of (LDLL)$_n$ and L$_{4n}$

	Energy (kcal/mol of Residue)					
	(LDLL)$_n$[a]			L$_{4n}$[b]		
n	α_{RH}	α_{LH}	Δ(RH-LH)	α_{RH}	α_{LH}	Δ(RH-LH)
2	−7.91	−7.70	−0.21	−8.03	−7.66	−0.37
3	−8.96	−8.72	−0.24	−9.01	−8.57	−0.44
4	−9.46	−9.22	−0.24	−9.54	−9.07	−0.47

[a] For the (ϕ,ψ) values of Table II, and χ_1 = 180°. [b] (ϕ,ψ) = −50.5, −56.1 and +49.8, +56.2 in the minimum-energy regular right- and left-handed α helices, respectively.[15]

−50°, ψ = −56° and ϕ = 50°, ψ = 56°, respectively), and the side-chain dihedral angle χ_1 was kept constant at 180°. The minimum-energy values of (ϕ,ψ) thus obtained for the LDLL unit are given in Table II, and the corresponding energy in Table III. For n = 3 and 4, the starting points used were the values of (ϕ,ψ) obtained for n = 2; the minimum-energy conformations obtained for the LDLL unit in these polymers were essentially identical (*i.e.*, ϕ and ψ within 0.2°) with the one obtained for n = 2, and the corresponding energies[20] are listed in Table III. For comparison, the minimized energies and conformations for regular L$_{4n}$ are also given in Table III. From this Table, it can be seen that the difference in energy between the right- and left-handed α helices, Δ(RH-LH), for (LDLL)$_n$ is about half that for L$_{4n}$ for the same value of n.

III. Discussion

A. Synthesis. There are two steps in the synthesis of (LDLL)$_n$ where racemization may occur, *viz.*, in the activation of the carboxyl group of the amino-blocked tetrapeptide (Figure 1, III) and in the polymerization of the amino-free active ester (Figure 1, V) in the presence of triethylamine. While no racemization was found upon activation of benzyloxycarbonyl- or butyloxycarbonylamino acids,[21] activation of acyl peptides can result in appreciable racemization,[22] depending on the conditions employed. Formation of a peptide bond using hydroxysuccinimide and dicyclohexylcarbodiimide also resulted in some racemization.[23] In the present work, the chiral purity of the compounds synthesized has been checked by taking advantage of the stereospecificity of papain and, particularly, of the fact that neither an L–D nor a D–L bond is split by the enzyme in alanine oligopeptides containing L and D residues.[12,13] As a test compound, we used the (LDLL)$_2$ fraction. If racemization had occurred, during the synthesis described (Figure 1), the isomer LDLD–LDLL (which is resistant to digestion by papain) would have been formed. However, the octapeptide (LDLL)$_2$ was completely digested by papain, yielding only the expected[12,13] products. This finding indicates that no racemization had taken place during the synthesis.

B. Ultraviolet Absorption. Theoretical and experimental studies[17] have shown that the optical properties of the amide chromophore in poly(amino acids) are related to the conformation of the backbone of the polymer. Thus, an absorption maximum at 191 mμ (ϵ_{max} 7100 l. cm^{-1} mol^{-1}) is characteristic of an unordered (random coil) polymer structure, and similar spectra are found for simple amides[17] and for a large number of di-, tri-, tetra-, and pentapeptides containing L- and D-alanine.[24] The uv spectra of poly(amino acids) in the α-helical conformation are characterized[17] by a maximum at about 190 mμ, with a lower absorption (ϵ_{max} 4300 l. cm^{-1} mol^{-1}). Thus, the coil-to-helix transition is accompanied by a large hypochromic effect (Δϵ 2800 l. cm^{-1} mol^{-1}). Poly(amino acids) in the β conformation exhibit maximum absorption[17] at 195 mμ (ϵ_{max} 7800 l. cm^{-1} mol^{-1}). For the alanine oligopeptides investigated here, the uv spectra (Figure 3) have maxima at 188–190 mμ. Therefore, we assume that, under the experimental conditions used, β structures are not present in appreciable amounts, and that the conformation of these compounds can be described in terms of mixtures of α-helical and random coil structures. Consequently, a scale of helix content θ (based on the reported values[17] for helix and coil) was set up, and is also shown in Figure 4. It can be seen that the largest peptide investigated [*i.e.*, (LDLL)$_6$] also has the highest helix content (θ = 0.4 in water and 0.6 in 90% trifluoroethanol).

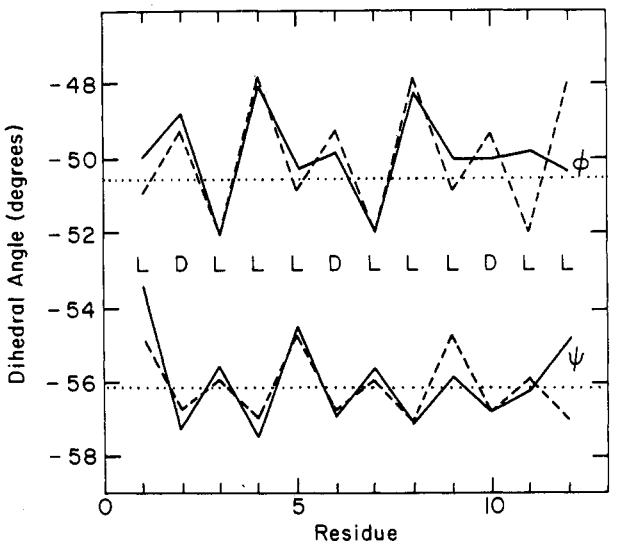

Figure 8. The values of ϕ and ψ for the minimum-energy conformation of (LDLL)$_3$, minimized with the ϕ and ψ of each of the 12 residues treated as independent variables (solid line). The dashed line corresponds to the conformation found by minimization with respect to the four ϕ's and four ψ's of an LDLL unit, each LDLL unit having the same set of (ϕ,ψ) values. The position of the L and D residues is indicated. The horizontal dotted lines at ϕ = −50.5° and ψ = −56.1° correspond to the values[15] for the right-handed α-helix of poly(L-alanine).

C. Optical Rotatory Dispersion. The conclusions derived from uv measurements are supported by the ORD properties of these compounds since the ORD spectra of Figures 5 and 6 are qualitatively similar to those computed[25] for poly(amino acids) containing various proportions of α-helical and random coil structures. However, when θ is computed[25] from [m'_{pep}] (eq 2) and the values of [m']^{coil} and [m']^{helix} for poly(L-alanine),[6] the values obtained are much lower than those deduced above from the hypochromicity. For example, an (LDLL)$_n$ copolymer containing 60% right-handed α-helix and 40% random coil will have[25] [m'_{pep}]$_{230\,m\mu}$ = −10,000 (deg cm^2)/dmol, [m'_{pep}]$_{200\,m\mu}$

(20) The dependence of the energy per residue on the chain length arises from the particular way in which we selected the polypeptide chain, *i.e.*, as one with 4n side chains and (4n + 1) backbone residues, as explained above, and also because of the fact that the number of hydrogen bonds per residue in an α helix reaches unity only at infinite chain length. See Figure 1 of ref 4 for further clarification of this point.
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(22) H. R. Bosshard, private communication.
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Table IV
Interactions Contributing to the Total Conformational Energy of the Right- and Left-Handed α Helix of a 13-mer with LDLL- and L-Alanyl Repeating Units

Helix Sense	Repeating Unit	Interaction Energies (kcal/mol)						
		Nonbonded[a]			Electrostatic	Hydrogen Bond	Internal Rotation	Total[b] Energy
		sc–sc	sc–bb	bb–bb				
α_{RH}	LDLL	−2.65	−40.16	−40.18	−17.58	−22.42	+6.51	−116.48
	L	−1.68	−42.55	−40.27	−17.90	−21.32	+6.56	−117.16
α_{LH}	LDLL	−2.64	−35.45	−40.78	−17.45	−23.40	+6.42	−113.30
	L	−1.24	−34.88	−41.39	−17.48	−23.20	+6.50	−111.68

[a] The abbreviations sc and bb refer to side chain and backbone, respectively. [b] Total energy in this table = 13 × energy/residue given in Table III.

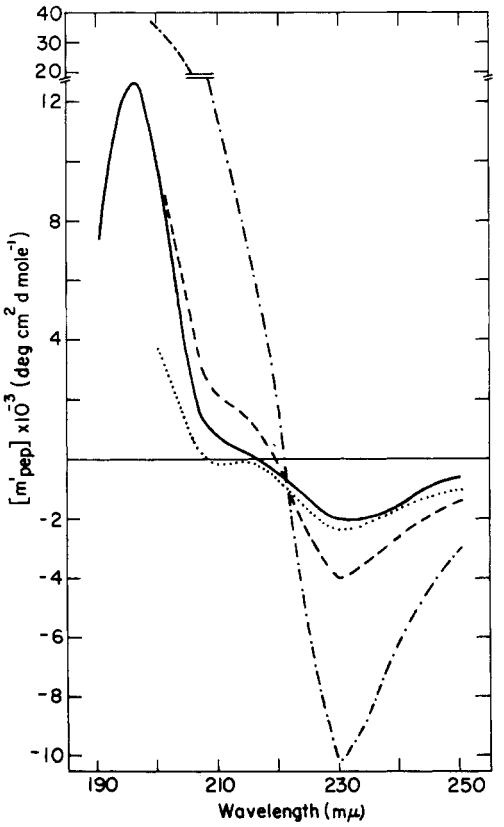

Figure 9. Comparison of experimental and theoretical ORD curves: (—) experimental data for $(LDLL)_6$ in 90% trifluoroethanol (taken from Figure 5); (···) calculated for 35% α_{RH} + 25% α_{LH} + 40% random coil; (- - -) calculated for 40% α_{RH} + 20% α_{LH} + 40% random coil); (- · - ·) calculated for 60% α_{RH} + 40% random coil. See text for the calculation procedure.

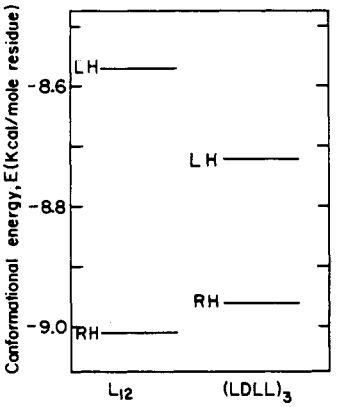

Figure 10. Schematic energy level diagram (based on data of Table III) for L_{12} and $(LDLL)_3$, indicating the energies of the right- and left-handed α-helical conformations.

= +35,000 (deg cm²)/dmol, and a crossover point at 221 mμ, whereas the corresponding values for $(LDLL)_6$ in 90% trifluoroethanol are −2000, +12,000 and 216, respectively (see Figure 9).

D. Reconciliation of Ultraviolet and Optical Rotatory Dispersion Data. Consequently, the uv and ORD data have been reinterpreted, assuming that the observed hypochromicity is independent of helix sense and is therefore a measure of total helix content (RH + LH), whereas the ORD properties reflect the excess of one helical form over the other. The latter assumption is expressed in eq 3–5. For relatively large peptides, the optical rotation per residue and per peptide bond are essentially identical, and therefore no end effects have to be taken into account. In the absence of ordered conformations (*i.e.*, in the random coil), the observed rotation can be attributed solely to the optical activity of the amino acid residues (eq 3). However, in an ordered (helical) conformation, the optical rotation of a peptide unit in the absorption region of the peptide chromophore is much larger than the rotation of the optically active residue; therefore, the latter has been neglected in eq 4 and 5. Hence, theoretical ORD curves were calculated with the assumptions summarized in eq 3–5, and are shown in Figure 9, for several combinations of RH and LH [with the total helix content (RH + LH) maintained at 60%, in accordance with the hypochromicity observations for $(LDLL)_6$ in 90% trifluoroethanol (Figure 4)]. It can be seen that the agreement achieved between the experimental and calculated ORD curves is much better than that obtained by assuming that the helix sense is all of one kind, *viz.*, RH.

The remaining discrepancies in Figure 9 are inherent in the crudeness of the model. First of all, the computed ORD spectra take no account of the fact that, in *short* oligopeptides, the average rotatory strengths of the $n\pi^*$ and $\pi\pi^*$ transitions in the peptide chromophore depend on the size of the helix, and may be up to 30% lower than the corresponding values for infinitely long polymers.[26,27] Secondly, the α helix of $(LDLL)_n$ has a somewhat different geometry from that of poly(L-alanine) (see Figure 8), and such differences, though small (up to 2° in ϕ and ψ), may affect the ORD properties.[27]

E. Conformational Energy Calculations. The above interpretation of the experimental data is supported by the results of the conformational energy calculations. Table IV gives the various contributions to the total energy of the right- and left-handed α-helical conformations for two 13-mer alanine peptides, *viz.*, $(LDLL)_3$ and L_{12}. A

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first interesting point is that there is no steric overlap of L and D side chains in $(LDLL)_n$; on the contrary, the nonbonded side-chain–side-chain interactions in $(LDLL)_n$ are more favorable than in $(L)_{4n}$ because of the proximity of the methyl group of a D residue to the methyl group of the third next L residue. In fact, calculations show that there is no steric hindrance even in the regular-sequence alternating D,L copolymer.[4]

Regarding the question as to which helical sense, the right- or the left-handed one, has the lowest energy, Ooi *et al.*[15] showed that the polypeptide backbone of an α helix formed from L residues does not show much preference for helix sense, *i.e.*, α_{RH} or α_{LH}. Because of the fact that, in general, L side chains are somewhat nearer to the backbone in the right-handed conformation than in the left-handed one, the resulting favorable nonbonded side-chain–backbone interactions (see Table IV) bring about a preference for the right-handed α-helical conformation for L_{4n} and *to a lesser degree* also for $(LDLL)_n$. The uniform side-chain–backbone conformation for L_{4n} is disturbed in $(LDLL)_n$ because D side chains are not as near to the right-handed α-helical backbone as L side chains are, and this unfavorable effect is greater than the energy gain from side-chain–side-chain interactions. Therefore, the energy of the right-handed α helix is slightly less favorable for $(LDLL)_n$ than for L_{4n} (see Figure 10). On the other hand, the left-handed α helix has a lower energy for $(LDLL)_n$ than for L_{4n} (see Figure 10) because of both more favorable (compared to all-L) side-chain–side-chain and side-chain–backbone interactions.

In conclusion, comparing the energies of the two helical conformations of $(LDLL)_n$, we find that the right-handed α helix has a lower energy than the left-handed α helix; however, the difference in energy is only about half of the difference found for L_{4n} (see Figure 10). Because of limitations in the accuracy of the calculations, we do not wish to place too much reliance on the absolute values of Δ(RH–LH). Instead, we would conclude that, whereas Δ(RH–LH) indicates that the right-handed α-helical form of poly(L-alanine) is more stable than the left-hand one, there is less preference for the right-handed one in poly(LDLL-alanine). Therefore, the latter polymer would be more likely to consist of a mixture of right- and left-handed helices, with a predominance of the right-handed form, as also concluded from the experimental data.

F. Concluding Remarks. The nature of this mixture of helices and random coils can be deduced from the cooperative nature of the helix-coil equilibrium in poly(α-amino acids). For an infinitely long polymer at the midpoint of the transition ($\theta = 0.5$), the average length of a helical sequence is $\sigma^{-1/2}$, where σ is the Zimm–Bragg nucleation parameter.[28] For most poly(α-amino acids), this length is about 50 residues.[29] Thus, we may conclude that, for the short oligopeptides considered here, the transition is of the "all-or-none" type; *i.e.*, a given molecule is either a random coil or a right- or left-handed α helix.

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Sequence Distribution–Glass Transition Effects. III. α-Methylstyrene–Acrylonitrile Copolymers

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ABSTRACT: To predict the glass transition temperature (T_g) of many copolymers it is often necessary to take into consideration the sequence distribution of the polymer. Homopolymer T_g values usually hold for AA or BB dyads in the AB copolymers because the A or B units experience much the same interactions as in A or B homopolymers. The formation of AB dyads results in new interactions and in many cases changes the T_g contribution of the A unit. Therefore, to obtain accurate T_g predictions, it is sometimes necessary to assign AB dyads and other sequence distributions their own T_g values. This work reports the effect of sequence distribution on the T_g of α-methylstyrene–acrylonitrile copolymers. This system exhibits a strong T_g depression effect. This T_g depression effect, the use of alternating polymers in predicting T_g's and a group additive approach to polymer T_g's are discussed in this paper.

The glass transition temperatures of copolymers are usually predicted by additive relations such as the Fox equation[1]

$$(1/T_{gP}) = (W_A/T_{gA}) + (W_B/T_{gB}) \quad (1)$$

where T_{gp} is the T_g of a copolymer containing weight fraction W_A and W_B of the two monomer units A and B for which the homopolymers have glass transitions of T_{gA} and T_{gB}. The Fox and other similar relationships do not take into consideration the effect of adjacent dissimilar monomer units on steric and energetic relations in the copolymer backbone and assume that the freedom of rotation and free volume contributed to a copolymer by a given monomer will be the same as it contributes to the homopolymer. As pointed out in previous papers, the Fox type of relationship does not hold for all copolymers.[2,3]

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