Polydepsipeptides. 7. Conformational Analysis of Poly(L-alanyl-L-alanyl-L-lactic acid)

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ABSTRACT: The conformational properties of poly[(L-Ala)$_2$-L-Lac] are described. Circular dichroism studies indicate this polymer is α helical in chloroform solutions but undergoes a helix-to-coil transition by solvent or temperature denaturation. The transition occurs near 50 °C. Infrared dichroism indicates that the polymer, in polyoxyethylene films, exists as a distorted α helix with the same average structure as poly[γ-ethyl L-glutamate]. The nonhydrogen-bonded ester groups are transverse to the helical axis, distorting the helix. A statistical thermodynamic theory for depsipeptide melting is developed and applied to this polymer. The theory indicates that melting for the parent peptide poly[L-Ala] should occur above 200 °C.

In this paper we extend our series of communications on polydepsipeptides, copolymers of α-amino and α-hydroxy carboxylic acids, to present the conformational properties of poly[(L-Ala)$_2$-L-Lac]. Our interest in polydepsipeptides is primarily as weakly perturbed models for the conformational properties of polypeptides and proteins. The preceding paper[1] reports the synthesis of several polydepsipeptides containing alanine and lactic acid. A previous communication from our laboratory[2] contains a comparison of the semiempirical energy diagrams of depsipeptide and peptide structural units. Differences between these diagrams result from the smaller skeletal bond angle at the ester oxygen atom and from the reduced steric interactions of the skeletal oxygen atom, compared to the amide NH group.

Calculated conformational properties of randomly coiling depsipeptide and peptide chains reflect the differences noted in the energy diagrams of their structural units. The energy calculations we have reported also reveal that the L-hydroxy acid units of depsipeptide chains can be readily incorporated into the right-handed α helix without significant loss of important, stabilizing van der Waals and dipolar forces.[2] However, substitution of the ester oxygen atoms for the amide NH groups reduces the number of hydrogen bonds stabilizing the helix. Consequently, comparison of depsipeptide and peptide conformational properties, particularly α-helical stabilities, is especially useful for the analysis of the importance of hydrogen bonding in peptide conformational stability.

We have reported results from an experimental conformational analysis of poly(L-Ala-L-Lac)[3] which, in contrast to the very stable, right-handed α helix formed by poly(L-Ala),[4] is not helical either in the solid state or in organic solvents such as chloroform and carbon tetrachloride. Apparently, the reduced hydrogen bonding capability of poly(L-Ala-L-Lac), amounting to half the number available in poly(L-Ala), does not impart sufficient stability to its α-helical conformation. We show here, however, that poly[(L-Ala)$_2$-L-Lac] adopts a helical conformation in dilute chloroform solution and in oriented polyoxyethylene films. In addition, the thermal and solvent denaturation of the helical form is described.

The observed conformational preferences of poly(L-Ala), poly[(L-Ala)$_2$-L-Lac], and poly(L-Ala-L-Lac) allow assessment of the importance of hydrogen bonding in determining α-helical stability. Because semiempirical energy calculations do not adequately account for entropy changes or for polymer–solvent interactions, effects which are undoubtedly important for order–disorder transitions,[5] they are not well suited for comparisons of helix stabilities. We believe that such comparisons are best made through a statistical thermodynamic analysis of the polypeptide α-helix-to-coil transition which explicity accounts for the presence and sequence of ester groups. Accordingly, we have modified Lifson's statistical treatment,[6] based on sequence generating functions, of the polypeptide α-helix-to-coil transition to include effects resulting from the depsipeptide ester groups. A correlation of the helical stabilities of poly(L-Ala), poly[(L-Ala)$_2$-L-Lac], and poly(L-Ala-L-Lac) is presented here to illustrate the resulting theory.

Materials and Methods

Poly[(L-Ala)$_2$-L-Lac]. The synthesis of this polydepsipeptide was based on the procedure of Nissen, Gilon, and Goodman.[7] The detailed procedure has been described in the preceding paper.[1] The trifluoroacetate salt of (L-Ala)$_2$-L-Lac pentachlorophenyl ester was heated at 118 °C for 16 h on Celite. The resulting polymer was extracted from the Celite support with chloroform and isolated by precipitation with ether. The polymer (5 g) was stirred with 100 mL of trifluoroethanol and the insoluble material was removed by filtration through a Millipore HAWP 04700 0.45 filter. An equal volume of ether was added to the filtrate and the resulting precipitate was collected by filtration. The precipitate was washed with trifluoroethanol–ether (1:1) and dried in vacuo to yield 3.3 g of poly[(L-Ala)$_2$-L-Lac]. Its intrinsic viscosity, determined in dichloroacetic acid at 25 °C, of 0.17 dL/g suggests a molecular weight of approximately 20 000 daltons.

Circular Dichroism Spectroscopy. The solvents, "SpectrAR" chloroform (Mallinckrodt, Inc., St. Louis, Mo.) and "Gold Label" trifluoroethanol and trifluoroacetic acid (Aldrich Chemical, Milwaukee, Ill.,), were dried over Linde 4A molecular sieves before use. Solutions of thoroughly dried polymer were prepared immediately before measurement; concentrations were determined by weight. Circular dichroism spectra were measured as previously described.[8] Measurements which extended below 210 nm used the 0.005-mm path length optical cell described by Radding et al.;[8] other measurements employed a 0.1-mm path length cell supplied by HG Helma Optical Co. The latter cell was used with the Cary thermostated cell block, whose temperature was controlled with circulating water from a Lauda K-2/R constant temperature bath. Reported temperatures were measured with a thermistor in the solution in the cell. Results were expressed as mean residue ellipticity, $[\theta]$, defined as:

$$[\theta] = \theta^0 \times M/(10lc) \text{ deg cm}^2/\text{dmol}$$

where θ^0 is the observed ellipticity, M is the average residue molecular weight and was taken as $\frac{1}{3}$ of the L-Ala-L-Ala-L-Lac repeat unit molecular weight, l is the optical path length in cm, and c is the solute concentration in g/cm^3.

Infrared Spectroscopy. Infrared dichroic spectra were determined according to the procedure of Ingwall, Gilon, and Goodman.[9] Poly[(L-Ala)$_2$-L-Lac] was incorporated in uniaxially oriented polyoxyethylene films according to the following

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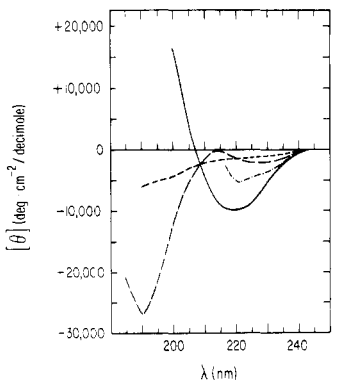

Figure 1. The circular dichroism of poly(L-alanyl-L-alanyl-L-lactic acid): (—) in chloroform at 25 °C; (– • –) in chloroform at 55 °C; (- - -) in trifluoroethanol at 25 °C; (– –) in trifluoroacetic acid at 25 °C.

procedure. The polydepsipeptide (2–3 mg) was mixed with 0.5 mL of a 10% solution of polyoxyethylene (Union Carbide Co. WSRN 750; mol wt 300 000) in either chloroform or trifluoroethanol and spread to 1.5 cm square on a silanized microscope slide. Solvent evaporation at room temperature yielded films which were cut into strips and partially oriented by uniaxial stretching to approximately seven times their original length. Infrared dichroic spectra were recorded with a Perkin-Elmer 180 spectrophotometer equipped with a Perkin-Elmer wire grid polarizer.

Results and Discussion

Circular Dichroism Spectroscopy. The circular dichroism spectra for poly[(L-Ala)$_2$-L-Lac] in chloroform at two temperatures, in trifluoroethanol and in trifluoroacetic acid, are illustrated in Figure 1. The spectrum in chloroform at 25 °C (solid line) exhibits a negative band at 220 nm with a mean residue ellipticity, $[\theta]$, of −10 000 deg cm^2/dmol, a crossover at about 207 nm, and positive ellipticity at lower wavelengths; it resembles, therefore, circular dichroism spectra observed for polypeptides in the right-handed α-helical conformation.[10]

The amide and ester chromophores are quite similar; the UV circular dichroism of each is dominated by a n–π^* and a π–π^* transition. We expect, therefore, peptide and depsipeptide circular dichroism to exhibit similar dependence on polymer conformation. This expectation is supported by our calculation of the circular dichroism for right-handed α-helical poly(L-Ala-L-Lac),[3] which closely resembles that of polypeptides. Moreover, the circular dichroism of poly[(L-Ala)$_2$-L-Lac] should bear greater resemblance to polypeptides than that of poly(L-Ala-L-Lac) because of the higher fraction of amide groups in poly[(L-Ala)$_2$-L-Lac]. Consequently, we believe that the circular dichroism spectra of Figure 1 provide strong evidence that poly[(L-Ala)$_2$-L-Lac] in chloroform solution assumes a conformation closely related to the polypeptide right-handed α helix.

The circular dichroism of this polydepsipeptide is very sensitive to solvent (Figure 1). The spectrum in chloroform is substantially different from those in trifluoroethanol and trifluoroacetic acid solutions. In trifluoroethanol, a featureless negative dichroism gradually increases in intensity with decreasing wavelength while in trifluoroacetic acid an intense minimum is observed at 190 nm. Circular dichroism changes as pronounced as these most likely result from conformational transitions. In this case poly[(L-Ala)$_2$-L-Lac] undergoes a solvent-induced transition from an α-helical form, in chloroform at low temperature, to disordered or partially disordered forms, in trifluoroethanol and trifluoroacetic acid.

The circular dichroism spectra of poly[(L-Ala)$_2$-L-Lac] in chloroform is also temperature sensitive (Figure 1). The ellipticity minimum at 220 nm decreases from −10 000 deg cm^2/dmol at low temperature (0–30 °C) to −5000 deg cm^2/dmol at 55 °C. If the chloroform solution is extremely dry, the change in the circular dichroism with temperature is reversible. This temperature dependence most likely reflects a thermally induced conformational transition from the low temperature α helix to a high-temperature disordered conformation; a transition temperature, for half-maximal helix content, of approximately 50 °C is suggested by the results of Figure 1.

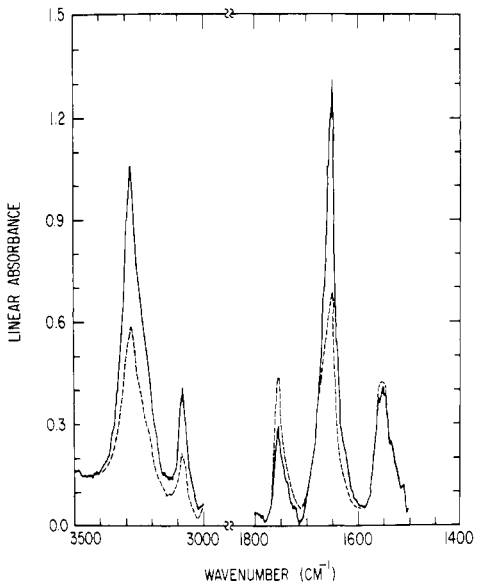

Figure 2. Infrared dichroic spectrum of poly(L-alanyl-L-alanyl-L-lactic acid) in oriented polyoxyethylene: (—) spectrum for light polarized parallel to the orientation direction, (- - -) spectrum for perpendicularly polarized light.

Infrared Spectroscopy. The infrared dichroism of poly[(L-Ala)$_2$-L-Lac] oriented in a polyoxyethylene film is shown in Figure 2. The film was cast from chloroform solution according to the procedure described above. The spectrum drawn with the solid line was recorded for light polarized parallel to the direction of orientation of polyoxyethylene; the dashed spectrum was recorded for light polarized perpendicular to the direction of orientation.

Strong parallel dichroism is exhibited by the single, sharp amide A and B peaks (N–H stretching) at 3280 and 3080 cm^{-1}, respectively, and the amide I peak (C=O stretching) at 1648 cm^{-1}. Weak perpendicular dichroism is noted for the 1550-cm^{-1} amide II band (N–H in-plane deformation). The dichroic ester carbonyl stretch with perpendicular dichroism occurs at 1755 cm^{-1}.

It has been well established that the amide A vibration shifts to lower wave numbers from above 3300 cm^{-1} upon hydrogen bond formation.[11] The position of the amide A peak of poly[(L-Ala)$_2$-L-Lac] at 3280 cm^{-1} clearly indicates therefore that all of the NH groups are hydrogen bonded. It is also evident from the sharpness and symmetry of the amide A and B peaks of Figure 2 that all of the amide groups in this polydepsipeptide experience similar hydrogen bonding environments (an observation consistent with an ordered polymer conformation). Parallel amide A dichroism is characteristic of α-helical polypeptides in the solid state[11] or in uniaxially oriented polyoxyethylene films;[9] such dichroism is indicative of an approximately parallel alignment of the NH bonds, the helix axis, and the orientation direction.

The polypeptide amide I peak position is diagnostic of each polypeptide chain conformation. It occurs near 1650 cm^{-1} for random and α-helical conformations and near 1630 cm^{-1} for the antiparallel β-sheet conformation.[11] Since the strong di-

Table I
Infrared Spectral Parameters of Poly[(L-Ala)$_2$-L-Lac] in Polyoxyethylene[a]

Transition	Position, cm^{-1}	$D_{\parallel}$[b]	Orientation angle[c] θ, deg
Amide A	3280	2.0	<45
Amide B	3080	2.1	<45
Amide I	1650	2.0	<45
Amide II	1550	0.9	52 ≪ 58
Ester	1755	0.7	60 ≪ 75

[a] For a film freshly cast from chloroform. [b] The dichroic ratio of the parallel-to-perpendicular absorbance. [c] The angle between the transition moment and the helix axis

chroism observed for poly[(L-Ala)$_2$-L-Lac] effectively precludes consideration of a random coil, its amide I peak at 1650 cm^{-1} is convincing evidence for an α-helical conformation. The parallel dichroism of the amide I band indicates that the C=O bonds and helix axis of the polymer align approximately parallel to the polyoxyethylene orientation direction.

Observation of an amide II band with perpendicular dichroism at 1550 cm^{-1} supports the conclusion derived from analysis of the amide A, B, and I transitions that poly[(L-Ala)$_2$-L-Lac] adopts an α-helix-like conformation in polyoxyethylene. The ester carbonyl stretching vibration is observed at 1775 cm^{-1}. Its perpendicular dichroism contrasts markedly with the parallel dichroism of the carbonyl stretching (amide I). Evidently the ester and amide C=O bonds are not similarly aligned.

Infrared peak positions and their dichroic ratios, $D_{\parallel}$, calculated as the ratio of the parallel to perpendicular absorbance, are listed in Table I. The dichroic ratios of the amide A, I, and II bands of 2.0, 2.0, and 0.9 for poly-[(L-Ala)$_2$-L-Lac] can be compared with the corresponding dichroic ratios of 2.3, 2.0, and 0.3 determined for α-helical poly(γ-benzyl L-glutamate) oriented in polyoxyethylene.[9] It is apparent that there is qualitative agreement between the polydepsipeptide and the α-helical polypeptide. However, the perpendicular amide II dichroism of the polydepsipeptide is significantly less than that of the polypeptide.

The dichroic ratios of Table I were used to determine the angle θ between the corresponding transition moment vector and the helix axis. Because the extent of polydepsipeptide orientation in polyoxyethylene is unknown, it is only possible to determine a range of acceptable values of θ for each transition. The procedure outlined by Zbinden,[12] which accounts for incomplete and unknown polymer conformation, was used with the experimental dichroic ratios to derive the values of the θ angle in the last column of Table I.

The transition moments of the amide A, I, and II bands lie in the amide plane.[11] The amide A and I transitions are approximately aligned with the NH and C=O bonds, respectively, while the amide II transition moment is nearly perpendicular to the NH bond.[11] We assume the amide transition moments are unaffected by inclusion of α-hydroxy acids into the peptide chain. The results of Table I reveal, therefore, that the amide C=O bonds and the helix axis of poly[(L-Ala)$_2$-L-Lac] are approximately parallel, in accordance with the conformational requirements of the polypeptide α helix. The ester C=O bond and the helix axis are, however, roughly transverse.

Semiempirical energy calculations indicate that α-hydroxy acids can be readily accommodated into the polypeptide α helix, without significant conformational change, by adopting the conformational characteristics of the replaced α-amino acid. It is clear from the infrared dichroic results described here that the lactic acid units of poly[(L-Ala)$_2$-L-Lac] in polyoxyethylene have not assumed the complete α-helical conformation.

Quantitative consideration of the amide and ester orientations should aid comprehension of the unusual conformational features of poly[(L-Ala)$_2$-L-Lac]. We follow the procedure described by Ingwall, Gilon, and Goodman[9] for the infrared dichroic analysis of peptides oriented in polyoxyethylene. Accordingly, we define amide group orientation with the angles α and ϕ that represent respectively the angle between the amide plane and the helix axis and the angle between the projection of the helix axis on the amide plane and the C=O bond (see Figure 4 of ref 9). A range of values for α and ϕ consistent with the known amide vibrational transition moment directions and the θ angles reported in Table I were derived by the graphical procedure outlined in ref 9. We find that the amide plane forms an angle less than 45° with the helix axis, $0° < \alpha < 45°$, while the amide C=O bond is slightly tilted from perfect helical alignment, $14° < \phi < 37°$. A similar analysis of the dichroic spectrum of α-helical poly(γ-benzyl L-glutamate) oriented in polyoxyethylene yielded ranges of $35° < \alpha < 45°$ and $0° < \phi < 25°$.[9] The apparent lack of correspondence of the amide plane orientation for poly-[(L-Ala)$_2$-L-Lac] and poly(γ-benzyl L-glutamate) in polyoxyethylene at a draw ratio of 7 arises from incomplete orientation of the former as compared to the latter. When the films are stretched as much as 13× their initial length the values for α and ϕ for both polymers agree very well with each other: poly[(L-Ala)$_2$-L-Lac]5[d] $< \alpha < 11°$, $10° < \phi < 19°$, and poly(γ-benzyl L-glutamate) $5° < \alpha < 15°$, $12° < \gamma < 15°$.[9b] We are now examining the relationships between draw ratios and orientation which we will report elsewhere.

Both circular dichroism and infrared spectroscopy reveal a conformation for poly[(L-Ala)$_2$-L-Lac] in which the amide groups adopt an α-helix-like conformation. The standard hydrogen-bonding pattern for the α helix results in hydrogen bonds only between amide groups; the ester groups are not restrained by intramolecular hydrogen bonds. The transverse orientation of the ester group may relieve strain contacts of the tightly packed helical conformation; it certainly increases opportunities for favorable ester carbonyl–solvent interactions. Apparently, stereochemical compatibility is insufficient to confine a residue to an α-helical conformation even in the interior of a helical segment. A helical unit requires the stabilizing influence of hydrogen bonds.

Molecular models of poly[(L-Ala)$_2$-L-Lac] which are consistent with these considerations can be readily constructed. The ester groups of Kendrew models of α-helical poly[(L-Ala)$_2$-L-Lac] can be twisted transverse to the helical axis without significant disruption of the helix. Figure 3 illustrates two such hypothetical models. The figure on the left represents poly[(L-Ala)$_2$-L-Lac] in the undistorted α-helical conformation and that on the right an α-helical segment with partially distorted transverse ester residues. We are currently using semiempirical energy calculations to explore the consequences of such helix distortion on intramolecular energy.

Statistical Mechanics of the Polydepsipeptide Helix-to-Coil Transition

The conformational characteristics of poly(L-Ala-L-Lac), poly[(L-Ala)$_2$-L-Lac], and poly(L-Ala) have been established. Poly(L-Ala-L-Lac) is not helical at room temperature either in dilute organic solution or in the solid state. Poly[(L-Ala)$_2$-L-Lac] adopts an α-helix-like conformation in chloroform which can be denatured thermally or by solvents such as trifluoroethanol or trifluoroacetic acid. Poly(L-Ala) forms a heat stable α helix in chloroform which requires substantial quantities of trifluoroacetic acid to destroy its secondary structure.

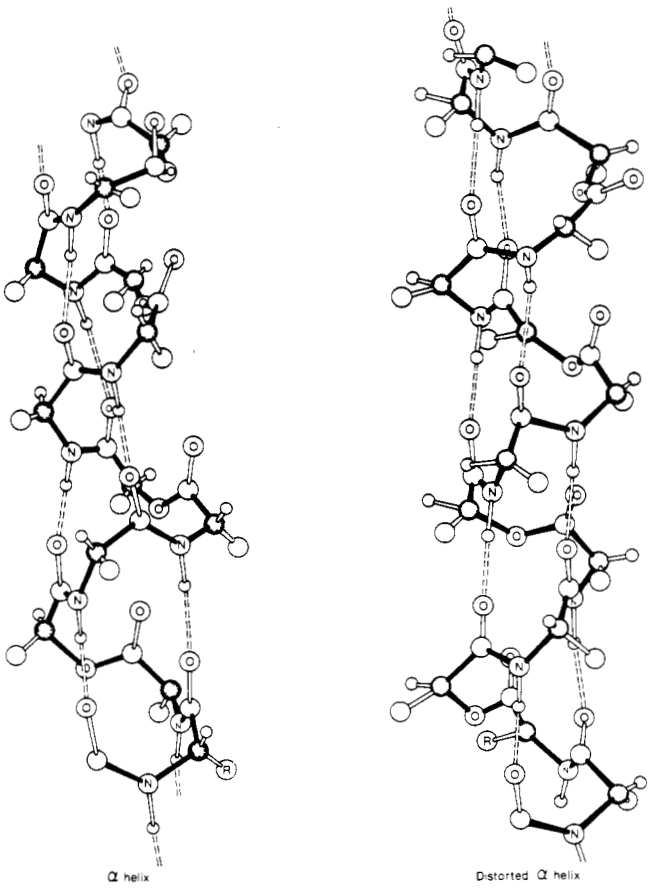

Figure 3. The α-helix and distorted α-helix conformations of poly(L-alanyl-L-alanyl-L-lactic acid).

The ester group, because of its close structural similarity to the amide group, is accommodated in the polypeptide α-helix without significant alteration of van der Waals and electrostatic interactions.[2] The primary effect of substitution of an ester for an amide in a helical segment, therefore, is the loss of stabilization energy contributed by the hydrogen bond of the replaced amide NH group. Accordingly, a thermodynamic analysis of the conformational properties of the alanine–lactic acid polydepsipeptides provides insight into the importance of hydrogen bonding to α-helix stability. We have applied Lifson's statistical thermodynamic analysis of the polypeptide helix-to-coil transition, based on sequence generating functions, to polydepsipeptides.[6] Modifications to account for the depsipeptide ester group are described below with specific reference to poly(L-Ala-L-Lac).

The hydrogen-bonding pattern expected for α-helical poly(L-Ala-L-Lac) is illustrated in Figure 4. Atoms hydrogen-bonded in the α-helical conformation are joined by dashed lines. Identification of the atoms of the amino acid (AA) and hydroxy acid (HA) structural units as indicated in Figure 4 was made for convenience of enumeration of polymer conformational states. The structural units should not, of course, be identified with monomer units used for the polydepsipeptide synthesis. The α-helical conformation is characterized by hydrogen bonds between the carbonyl oxygen atom of the HA structural unit and the NH atom of the second succeeding AA unit.

The two types of structural units AA and HA do not necessarily have identical conformations in the helical state. Instead, the repeat units, each comprising an AA and an HA structural unit, of an ordered polydepsipeptide chain segment adopt identical conformations. Following Zimm and Bragg,[13] Lifson and Roig,[14] and others,[15] we divide the conformational space of each type of structural unit AA and HA into two

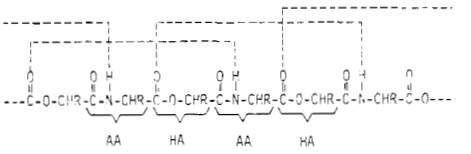

Figure 4. A schematic diagram of α-helical poly(L-alanyl-L-lactic acid).

states, a helical and a random coil state. The conformation of the helical state is defined by the helical repeat unit conformation. The random coil state represents the remaining portion of conformational space.

The shortest helical chain segment which contains a hydrogen bond is the three-unit sequence HA:AA:HA. Here a single hydrogen bond links the carbonyl oxygen of the left terminal HA unit with the NH atom of the second succeeding AA unit (Figure 4). One additional hydrogen bond is formed for every helical repeat unit HA:AA or AA:HA added to the left or right end, respectively, of the existing helical segment. Isolated segments of one or two helical structural units are too short to contain a stabilizing hydrogen bond. We expect, therefore, that such segments will occur very infrequently. Accordingly, we ignored these segments during enumeration of polydepsipeptide conformational states for our statistical thermodynamic analysis. The analogous assumption has been employed for analysis of polypeptide helix-to-coil transitions without significant error.[15]

An AA unit on either end of a helical segment is not fixed in its helical conformation by a hydrogen bond. It is very unlikely, therefore, that helical segments will have terminal AA structural units. Rather, HA units will occur almost exclusively at the ends of helical segments. Neglect of helical segments with terminal AA units is equivalent to neglecting isolated helical structural units.

Statistical weights are assigned to depsipeptide conformational states in a manner similar to that used by Lifson and Roig[14] for the analysis of polypeptide helix-to-coil transitions. The shortest allowed helical sequence HA:AA:AA is assigned to statistical weight $\gamma(w\upsilon)$ representing respectively contributions from the three helical structural units HA, AA, and HA. We have arbitrarily included the contribution from the single stabilizing hydrogen bond in the statistical weight w for the helical AA unit. Accordingly, it is very similar to the parameter used by Lifson and Roig[14] to represent α-helical peptide units. The factors γ and υ account for HA units which are immobilized in a helical conformation but are not associated with a hydrogen bond. They are thus analogous to Lifson and Roig's parameter υ. Although we distinguish the N-terminal helical HA units, statistical weight γ, from the remaining helical HA units, statistical weight υ, it is recognized that these two classes of HA units have similar conformations and statistical weights.

The next largest helical sequence HA:AA:HA:AA:HA is assigned a statistical weight $\gamma(w\upsilon)^2$. Each additional repeat unit HA:AA or AA:HA added to the helical segment is accounted for by inclusion of a factor $w\upsilon$ in the statistical weight for the segment. Thus we assign a helical segment comprising j AA units, j hydrogen bonds, and $(j+1)$ HA units a statistical weight $\gamma\,(w\upsilon)^j$. Lifson's sequence generating function $V(x)$[6] becomes therefore:

$$V(x) = \sum_{j=1}^{\infty} \frac{\gamma(w\upsilon)^j}{x^j} = \frac{\gamma(w\upsilon)}{(x - w\upsilon)} \qquad (1)$$

We have assigned statistical weights U_a and U_h to structural units AA and HA, respectively, in the random coil conformational state. Because helical segments end at HA units, sequences of randomly coiling residues must begin and end

at AA units. Accordingly, the statistical weight of a random coil sequence comprising j HA and $(j + 1)$ AA structural units is $U_a (U_a U_h)^j$. We have chosen the randomly coiling repeat unit AA:HA as a reference state by setting $U_a U_h = 1$. With these considerations, Lifson's sequence generating function $U(x)$[6] is readily calculated as follows:

$$U(x) = \sum_{j=1}^{\infty} \frac{U_a}{x^j} = \frac{U_a}{x - 1} = \frac{1}{U_h(x - 1)} \tag{2}$$

Lifson has shown that the partition function Z for sufficiently long chains is given as

$$Z = x^N \tag{3}$$

where x is the following largest root of the equation

$$U(x)V(x) - 1 = 0 \tag{4}$$

and N is the number of repeat units per chain. Combining eq 1, 2, and 4 yields

$$x = \tfrac{1}{2}\{1 + s + [(1 - s)^2 + 4s\sigma]^{1/2}\} \tag{5}$$

where we have substituted $s = wv$ and $\sigma = \gamma/U_h$. A variety of helix-to-coil characteristics can be calculated from the partition function. Of considerable interest is the fraction θ_h of amide units in the helical state.

$$\theta_h = \frac{1}{N}\frac{\partial \ln Z}{\partial \ln w} = \frac{w}{x}\frac{\partial x}{\partial w} \tag{6}$$

Substitution of eq 5 into eq 6 yields the following expressions:

$$\theta_h = \frac{s}{c}\frac{(s + c + 2\sigma - 1)}{(s + c + 1)}$$
$$c = [(1 - s)^2 + 4s\sigma]^{1/2} \tag{7}$$

The effects of temperature and solvent on the helix-to-coil transition of poly[(L-Ala)$_2$-L-Lac] are determined by their influence on s and σ. It is clear, from the definition of the statistical weights given above, that $-RT \ln (s) = -RT \ln (wv)$ represents the free-energy change accompanying the addition of a previously random AA:HA repeat unit to a helical segment. This free-energy change comprises contributions from the AA and HA units. Since we have associated the stabilizing hydrogen bond of the repeat units with the AA units, its contribution resembles that for a helical unit in the corresponding polypeptide chain, poly(L-Ala). Accordingly:

$$-RT \ln (w) = \Delta H^\circ_t - T\Delta S^\circ_t \tag{8}$$

$$w = \exp\left[\frac{\Delta H^\circ_t}{RT^\circ_p}\left(1 - \frac{T^\circ_p}{T}\right)\right] \tag{9}$$

where ΔH°_t and ΔS°_t are the enthalpy and entropy change, respectively, for the formation of a helical unit in the poly(L-Ala) chain and T°_p is the helix-to-coil transition temperature for this polymer. To aid comparison of their helix-to-coil transitions, we have equated the enthalpy and entropy of helix formation of AA units in poly(L-Ala) and poly(L-Ala-L-Lac) chains. The validity of this assumption depends upon the similarity of the environment of helical AA units in the two chains. Its final analysis, therefore, must await detailed structural determination by x-ray diffraction. Meanwhile we rely on our theoretical and experimental conformational analyses for indicating general similarity of helical peptide and depsipeptide chains.

We expect that the free-energy change $-RT \ln (v)$ will have a negligible enthalpy contribution since a stabilizing hydrogen bond is not associated with a helical HA unit. Therefore we write

$$v = \exp(\Delta S^\circ/R) \tag{10}$$

where ΔS° is the entropy change that occurs when an HA unit is added to the helical segment. The statistical weight σ is similarly related to the entropy of formation of the N-terminal helical HA unit.

Values for the statistical weights w, v, and σ are readily calculated from the indicated thermodynamic parameters according to eq 9 and 10. In the simplest case of poly(L-Ala-L-Lac) dissolved in an inert solvent, such as chloroform, the thermodynamic parameters will be effectively independent of temperature. The presence of a denaturing solvent, such as trifluoroacetic acid, as a second component in the depsipeptide solvent system will require consideration of the effect of both temperature and solvent composition on the thermodynamic parameters. Substitution of the required statistical weights thus obtained in eq 7 yields the helix-to-coil transition for poly(L-Ala-L-Lac).

The preceding considerations are easily extended to the helix-to-coil transition of poly[(L-Ala)$_2$-L-Lac]. The equations developed for poly(L-Ala-L-Lac) can be directly applied to poly[(L-Ala)$_2$-L-Lac] with the simple redefinition of s as w^2v to account for the two AA structural units and one HA structural unit in a repeat unit. The helix content, θ_h, is calculated from eq 7 with the appropriate modification of s. The terms w and v retain their values from eq 9 and 10. Lifson's unmodified theory for polypeptide helix-to-coil transition can be used with the parameters w and v determined above to calculate the transition for poly(L-Ala).

The effect of the ester groups on the polydepsipeptide helix melting temperature is readily calculated. For poly(L-Ala-L-Lac)

$$-RT \ln (wv) = \Delta H^\circ - T\Delta S^\circ = \Delta H^\circ_t(1 - T/T^\circ_d) \tag{11}$$

where ΔH° and ΔS° refer to the formation of a helical L-Ala-L-Lac repeat unit and T°_d is the melting temperature of this polydepsipeptide. We have assumed that the enthalpy change results exclusively from the formation of the helical hydrogen bond. Since $w = 1$ when $T = T^\circ_p$, we obtain

$$T^\circ_d = T^\circ_p/(1 + RT^\circ_p \ln (v)/\Delta H^\circ_t) \tag{12}$$

Similar considerations allow us to write eq 13 for the melting temperature of poly[(L-Ala)$_2$-L-Lac].

$$T^\circ_d = T^\circ_p/(1 + RT^\circ_p \ln (v)/2\Delta H^\circ_t) \tag{13}$$

We can also calculate the sharpness of the depsipeptide helix-to-coil transition. Here we require $(\partial \theta_h/\partial T)_{s=1}$, the rate of change of the helical content at the transition point $s = 1$.

$$\left(\frac{\partial \theta_h}{\partial T}\right)_{s=1} = \left(\frac{\partial \theta}{\partial s}\right)_{s=1}\left(\frac{\partial s}{\partial T}\right)_{s=1} \tag{14}$$

We find $(\partial \theta_h/\partial s)$ by differentiation of eq 7.

$$\left(\frac{\partial \theta_h}{\partial s}\right)_{s=1} = \tfrac{1}{4}\sigma^{1/2}$$

The term $(\partial s/\partial T)_{s=1}$ is determined from the appropriate definition of s. For poly(L-Ala-L-Lac)

$$\left(\frac{\partial \theta_h}{\partial T}\right)_{s=1} = \frac{\Delta H^\circ_t}{4RT^\circ_d{}^2\sigma^{1/2}} \tag{15}$$

and for poly[(L-Ala)$_2$-L-Lac]

$$\left(\frac{\partial \theta_h}{\partial T}\right)_{s=1} = \frac{\Delta H^\circ_t}{2RT^\circ_d{}^2\sigma^{1/2}}$$

It should be remembered when using eq 12, 13, 15, and 16 that the melting temperature T°_d must refer to the specific polydepsipeptide under consideration; all other parameters have the same meaning in each of these equations.

As a further illustration of the effect of hydrogen bonding

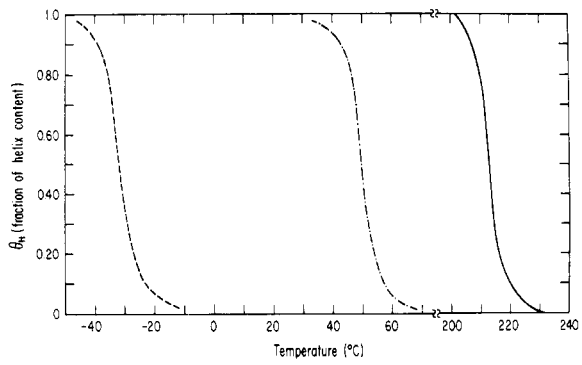

Figure 5. Calculated helix-to-coil melting in chloroform for: (- - -) poly(L-alanyl-L-lactic acid); (– • –) poly(L-alanyl-L-lactic acid); (—) poly(L-alanine).

on polypeptide helix stability, we compare in Figure 5 helix-to-coil melting curves calculated for poly(L-Ala-L-Lac), poly[(L-Ala)$_2$-L-Lac], and poly(L-Ala) in an inert solvent. Klotz and Franzen[16] have measured the enthalpy of formation of the peptide hydrogen bond in chloroform. We use their value of −4.2 kcal/mol for $\Delta H°_t$ in the calculations for Figure 5. For v we use the value of 0.012 determined by Ingwall et al.[17] for poly(L-Ala). Because of their similarity, we set $\sigma = v$ for the calculations of Figure 5. The melting temperature of poly(L-Ala), $T°_p$, was calculated from eq 12 and the melting temperature $T°_d$ = 323 K was observed here for poly[(L-Ala)$_2$-L-Lac] in chloroform. Although these parameters are approximate, they serve our purpose in illustrating hydrogen-bonding effects shown by the curves in Figure 5. Calculations based entirely on experimentally observed parameters will be reported in the future when more extensive measurements have been completed.

The curves of Figure 5 clearly reveal the profound effect of hydrogen bonding on helix stability. Melting temperatures of −32, 50, and 214 °C are indicated for poly(L-Ala-L-Lac), poly[(L-Ala)$_2$-L-Lac], and poly(L-Ala), respectively. The calculations of Figure 5 are consistent with the observed conformational properties of these polymers. The large differences in melting temperatures are dramatic indicators of the importance of hydrogen bonding in the determination of helix stability. The melting of poly(L-Ala-L-Lac) is predicted to occur at an experimentally accessible temperature. We are currently performing experiments with a specially designed low-temperature cell to test the calculations of Figure 5. Because of its extremely high melting temperature (>200 °C), it is now clear why the thermally induced helix-to-coil transition of poly(L-Ala) in an inert organic solvent has not been observed.

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Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. 17. Threonine Parameters from Random Poly(hydroxybutylglutamine-*co*-L-threonine)[1]

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ABSTRACT: The synthesis and characterization of water-soluble random copolymers containing L-threonine with N^5-(4-hydroxybutyl)-L-glutamine and the thermally induced helix–coil transitions of these copolymers in water are described. The incorporation of L-threonine was found to decrease the helix content of the copolymers in water at all temperatures. The Zimm–Bragg parameters σ and s for the helix–coil transition of poly(L-threonine) in water were deduced from an analysis of the melting curves of the copolymers in the manner described in earlier papers. The computed values of s indicate that L-threonine destabilizes helical sequences at all temperatures in the range of 0–70 °C.

The use of the "host–guest" technique for the evaluation of the helix–coil stability constants of various amino acids in water has been illustrated in earlier papers of this series, the latest of which was paper 16.[3] In the present paper, this approach is extended to L-threonine. In the "host–guest" technique, a water-soluble α-helical host homopolymer with nonionizable side chains is selected, and various amounts of a guest residue are incorporated into it to form random

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