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Polydepsipeptides. III. Theoretical Conformational Analysis of Randomly Coiling and Ordered Depsipeptide Chains

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ABSTRACT: A theoretical conformational analysis of polydepsipeptides comprised either of alternating glycine and glycolic acid units or of alternating alanine and lactic acid units is described. Conformational energy maps of depsipeptide structural units are presented and compared to similar maps for peptide and lactic acid units. Asymptotic characteristic ratios $C_x = \langle r^2\rangle_0/xl^2$ of 2.27 and 3.80 were calculated for randomly coiling poly(glycine-glycolic acid) and poly(L-alanine-L-lactic acid), respectively. Conformational analysis of ordered poly(L-alanine-L-lactic acid) revealed a low energy helical form very similar to the standard right-handed polypeptide α helix. The polydepsipeptide helix is of low energy in spite of the fact that it is stabilized by one-half the number of hydrogen bonds of the polypeptide α helix. Statistical mechanical aspects of the helix-to-random coil transitions of polypeptides and polydepsipeptides are compared.

High molecular weight polydepsipeptides comprised of alternating L-valine and L-lactic acid residues have recently been synthesized in our laboratory.[1] A preliminary experimental and theoretical conformational analysis of these polymers has been described.[1]

The conformational properties of polydepsipeptides are of particular interest because of the close analogy between such molecules and polypeptides. Since both the amide and ester groups strongly favor the planar trans conformation, the skeletal geometry of the two types of chains is very similar. The steric and dipolar factors that determine the conformational energy of peptides and depsipeptides are also closely related because of the close similarity of their structural units. There are differences between important features of these structural units, however. Comparison of the conformational properties of polypeptides and polydepsipeptides will allow assessment of the importance of these features in determining polymer chain conformation. In particular, hydrogen bonding characteristics of polypeptides and polydepsipeptides will differ since every second amide NH group of the former chains is replaced by an ester O atom of the latter. This replacement will primarily affect the nature of ordered polydepsipeptide structures stabilized by hydrogen bonds. Random chain dimensions will also be affected by elimination of certain steric interactions that involved the replaced amide hydrogen atom.

In this paper we describe our calculations of the conformational energies of the structural units of poly(glycine-glycolic acid) and of poly(L-alanine-L-lactic acid). Theoretical values for the mean square end-to-end distance of unperturbed randomly coiling depsipeptide chains are reported. A theoretical analysis of ordered polydepsipeptide structures is also described.

Theoretical Methods

Structural Parameters. A polydepsipeptide chain segment is shown in Figure 1 with the repeating unit i, composed of an α-amino and an α-hydroxy acid residue, indicated. The amide and ester groups are assumed fixed in their planar trans conformation[2,3] so that depsipeptide chain conformations are determined by the torsional angles ϕ_{ai}, ψ_{ai} and ϕ_{hi}, ψ_{hi} describing rotations about the $N\text{–}C_i^\alpha$ and $C_i^\alpha\text{–}C'$ skeletal bonds of the α-amino acid and about the $O\text{–}C_i^\alpha$ and $C_i^\alpha\text{–}C'$ skeletal bonds of the α-hydroxy acid, respectively, for each repeating unit i of the chain. Virtual bonds l_a and l_h of Figure 1 join consecutive C^α atoms.

For convenience of conformational energy calculations, the chain is divided into the structural units AA and HA that are also shown in Figure 1. Coordinate systems are fixed to amide and ester groups with their origins at C′ and their x axes aligned along the C′–N or C′–O bonds. The y axes are directed in the plane of the amide or ester groups so that O=C′ oxygen atoms have positive y coordinates. The z axes are chosen to complete right-handed orthogonal coordinate systems.

The bond angles and bond lengths used in the calculation are presented in Table I. They are essentially those derived by Flory and coworkers from analysis of the structure of model amides and esters.[2,3]

The structure of the two units is quite similar, except for the skeletal bond angle at the ester oxygen atom of the α-

Table I
Depsipeptide Structural Parameters

Bond length, Å				Bond angle, deg			
Amino acid		Hydroxy acid		Amino acid		Hydroxy acid	
NC^{α}	1.47	OC^{α}	1.44	$C'NC^{\alpha}$	123	$C'OC^{\alpha}$	113
$C^{\alpha}C'$	1.53	$C^{\alpha}C'$	1.53	C'NH	123		
C'=O	1.22	C'=O	1.22	$NC^{\alpha}C'$	109.5	$OC^{\alpha}C'$	109.5
C'O	1.34	C'N	1.32	$NC^{\alpha}H$	109.5	$OC^{\alpha}H$	109.5
$C^{\alpha}H$	1.00	$C^{\alpha}H$	1.00	$NC^{\alpha}C^{\beta}$	109.5	$OC^{\alpha}C^{\beta}$	109.5
$C^{\alpha}C^{\beta}$	1.54	$C^{\alpha}C^{\beta}$	1.54	$C^{\alpha}C'=O$	121	$C^{\alpha}C'=O$	121
				$C^{\alpha}C'O$	114	$C^{\alpha}C'N$	114

hydroxy acid unit which is 10° smaller than the corresponding angle of the α-amino acid unit.

The random coil state represents the array of chain conformations that is generated by all possible combinations of values for the ϕ,ψ torsional angles. Thus properties of the random state are averages over all allowed conformations.[4] Ordered structures are formed when all repeat units have identical conformations. Such chain structures must be helical.[5]

Conformational Energy Calculations. The conformational energy of a random depsipeptide chain was calculated, following procedures developed for treating random polypeptide[2] and poly(α-hydroxy acid)[3] chains, as a sum of independent contributions $V_a(\phi_{ai},\psi_{ai})$ and $V_h(\phi_{hi},\psi_{hi})$, from each structural unit i, that depend exclusively on the torsional angles ϕ_{ai},ψ_{ai} and ϕ_{hi},ψ_{hi}. The energy terms V_a and V_h were each calculated according to the semiempirical potential functions of eq 1. The terms $V_{\phi}(\phi_i)$ and

$$V(\phi_i,\psi_i) = V_{\phi}(\phi_i) + V_{\psi}(\psi_i) + \sum_{jk}[V_{r,jk}(\phi_i,\psi_i) + V_{l,jk}(\phi_i,\psi_i) + V_{c,jk}(\phi_i,\psi_i)] \quad (1)$$

$V_{\psi}(\psi_i)$ represent intrinsic torsional potentials for rotation about the angles ϕ_{ai} and ψ_{ai} or ϕ_{hi} and ψ_{hi}; the terms $V_{r,jk}$, $V_{l,jk}$, and $V_{c,jk}$ represent repulsive, London, and Coulombic nonbonded interactions, respectively. These interactions are calculated between atoms j and k whose internuclear separation depends only on the torsional angles ϕ_{ai}, ψ_{ai} or ϕ_{hi}, ψ_{hi} of Figure 1. Thus V_a and V_h represent the conformational energy of structural units AA and HA, respectively.

Intrinsic torsional potentials were considered, after Flory and coworkers,[2,3] to be threefold with minima for each bond at 60, 180, and 300° when measured from the cis conformation according to recent IUPAC recommendations.[6] Thus, $V_{\phi_a} = (V_{\phi_a}^0/2)(1 + \cos 3\phi_a)$ with V_{ψ_a}, V_{ϕ_h}, and V_{ψ_h} given by identical equations. The barrier heights $V_{\phi_a}^0$, $V_{\psi_a}^0$, $V_{\phi_h}^0$ and $V_{\psi_h}^0$ of 1.5, 1.0, 1.0 and 1.0 kcal, respectively, were those suggested by Flory and coworkers.[2,3]

The repulsive and London van der Waals interaction terms were calculated according to the commonly accepted "6–12" potential function; $V_{r,jk} = A_{jk}/R_{jk}^{12}$, $V_{l,jk} = -C_{jk}/R_{jk}^{6}$, where R_{jk} represents the distance separating atoms j and k. Parameters C_{jk} were determined in the usual manner from atomic polarizabilities and effective numbers of electrons using the Slater–Kirkwood equation.[2,7] The repulsive parameters A_{jk} were fixed, following the procedure of Brant, Miller, and Flory,[2] by requiring that the total van der Waals potential $V_{r,jk} + V_{l,jk}$ be minimized at an internuclear distance 0.2 Å greater than the sum of the van der Waals radii of atoms j and k. The β-methyl group of both the L-alanine and L-lactic acid units was considered spherical with a van der Waals radius $R_{CH_3}^0 = 1.85$ Å.

Electrostatic interaction between polar amide and ester groups was calculated from a sum of terms $V_{c,jk} = 332\epsilon_j\epsilon_k/DR_{jk}$ with partial changes ϵ_j taken as those assigned by Brant, Miller, and Flory[2] for amide and Brant, Tonelli, and Flory[3] for ester groups. Their recommended value for the local dielectric constant $D = 3.5$ was also employed here.

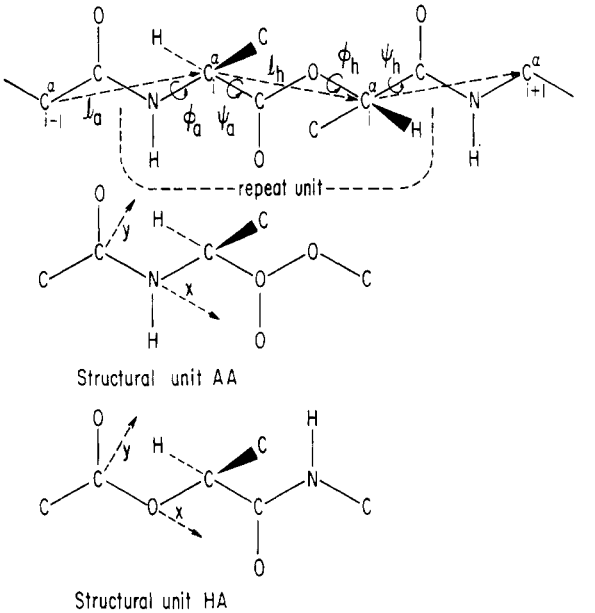

Figure 1. A segment of a polydepsipeptide chain showing repeat unit i, torsional angles ϕ_a,ψ_a and ϕ_h,ψ_h, and virtual bonds l_a and l_h. The structural units AA and HA are also illustrated.

In contrast to random coiling depsipeptides whose conformational properties are determined by short-range interactions, the conformational energy of a repeat unit in an ordered depsipeptide chain contains contributions from interactions that extend to many neighboring repeat units. In order to include contributions from such long-range interaction the conformational energy of a depsipeptide repeat unit in the interior of a long ordered chain was obtained according to the accounting procedure of Brant[8] as a sum of the self energy of the repeat unit and its interaction energy with each of the units that succeed it in the chain. The repeat unit self energy is comprised of the torsional potentials of eq 1 and nonbonded interactions between atoms of the repeat unit. Interunit energy is comprised exclusively of nonbonded interactions. In order to include hydrogen bonding interactions in ordered depsipeptide chains, the nonbonded potential functions of eq 1 were employed with the modification suggested by Brant[8] of the van der Waals interaction between amide NH and carbonyl CO groups.

Conformational Analysis of Random Coiling Polydepsipeptides. Mean square unperturbed end-to-end distances $\langle r^2\rangle_0$ of polydepsipeptide chains were calculated according to the matrix multiplication technique of Flory[4]

$$\langle r^2\rangle_0 = 2\mathbf{J}^* \prod_{i=1}^{x} \mathbf{g}_i\mathbf{J} \quad (2)$$

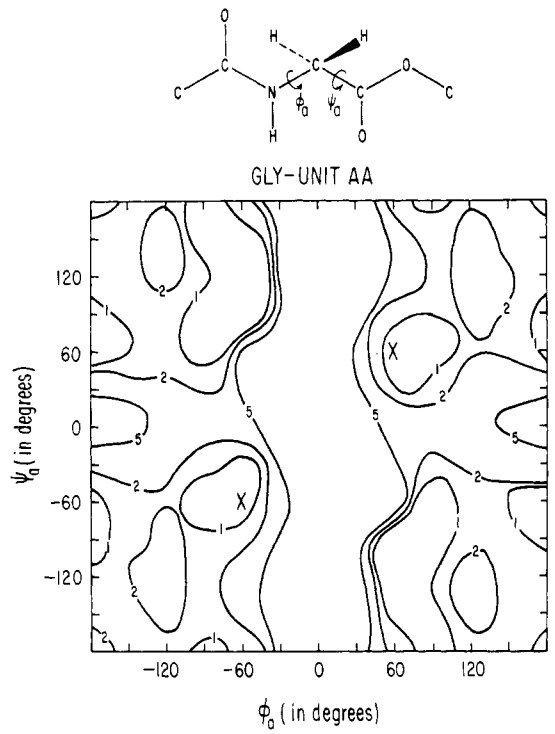

Figure 2. Conformational energy map for the glycine unit AA calculated according to eq 1.

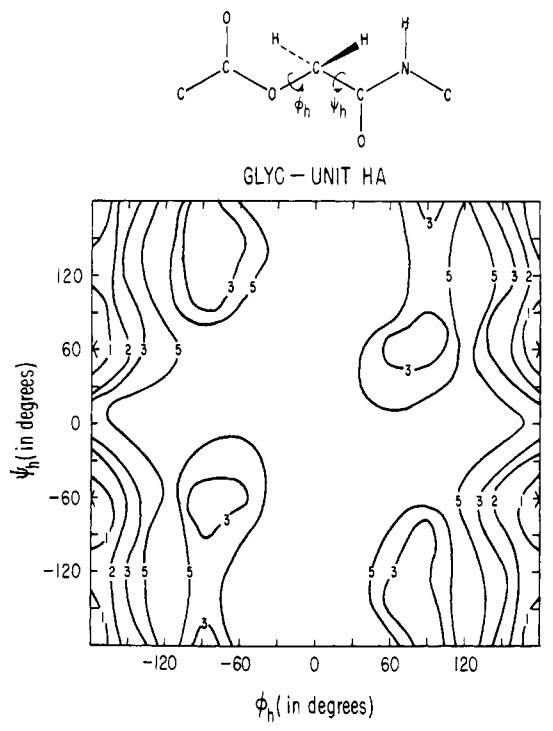

Figure 3. Conformational energy map for the glycolic acid unit HA calculated according to eq 1.

from eq 2, 3, 4. The matrices $\mathbf{g}_i$, one for each repeat unit i in a chain of x repeat units, are given by the product

$$\mathbf{g}_i = \mathbf{G}_{ai}\mathbf{G}_{hi} \tag{3}$$

where the matrices $\mathbf{G}_{ai}$ and $\mathbf{G}_{hi}$ are associated with the α-amino and α-hydroxy acid residues of the repeat unit i. Since the energy contributions $V_{ai}(\phi_{ai}, \psi_{ai})$ and $V_{hi}(\phi_{hi}, \psi_{hi})$ are independent of the conformation of neighboring units[2,3] the G matrices can be calculated as indicated by eq 4,[4] where $\langle \mathbf{T}_a \rangle$ is the conformational average of the trans-

$$\mathbf{G}_{ai} = \begin{bmatrix} 1 & \mathbf{l}_a^T & \langle \mathbf{T}_a \rangle & \mathbf{l}_a^2/2 \\ 0 & & \langle \mathbf{T}_a \rangle & \mathbf{l}_a \\ 0 & & 0 & 1 \end{bmatrix}_{ai} \tag{4}$$

formation matrix $\mathbf{T}_{ai}(\phi_{ai}, \psi_{ai})$ which relates coordinate systems fixed to amide group i and the succeeding ester group; the vector $\mathbf{l}_{ai}$ connects the origins of the amide and ester bond coordinate systems. The matrix $\mathbf{G}_{hi}$ is given by an analogous expression using the transformation $\mathbf{T}_{hi}(\phi_{hi}, \psi_{hi})$ that relates coordinate systems fixed to ester group i and the succeeding amide group and the vector $\mathbf{l}_h$ that connects the origins of the two systems. **J** and **J*** are, respectively, a column of four zeros followed by unity and a row formed by an element of unity followed by four elements of zero.

Conformational averages of the transformation matrices $\mathbf{T}_{ai}$ and $\mathbf{T}_{hi}$ were approximated by the sum of eq 5 taken at

$$\langle \mathbf{T}_{ai} \rangle = \frac{\sum_{\phi=0}^{2\pi}\sum_{\psi=0}^{2\pi} T_{ai}(\phi_{ai}, \psi_{ai}) \exp(-V_{ai}(\phi_{ai}, \psi_{ai})/RT)}{\sum_{\phi=0}^{2\pi}\sum_{\psi=0}^{2\pi} \exp(-V_{ai}(\phi_{ai}, \psi_{ai})/RT)} \tag{5}$$

30° intervals of ϕ and ψ. Unless otherwise stated the averaging was performed for t = 25°.

Conformational Analysis of Ordered Polydepsipeptides. Conformational analysis of ordered polydepsipeptide chains was restricted to helical structures in which each amide H atom participates in an intramolecular hydrogen bond to either an amide or ester carbonyl oxygen. Following Bragg, Kendrew, and Perutz[9] polydepsipeptide helices are distinguished by the number of atoms R that form their characteristic hydrogen bonded rings. Hydrogen bonding to ester carbonyl oxygen atoms occurs for ring sizes R = 5, 7, 11, 13, 17, 19, . . . while rings of size R = 8, 10, 14, 16, 20, 22, . . . are produced by hydrogen bonding to amide carbonyl oxygens. Chain structures with specific values of R up to 14 were identified by examination of molecular models. Those structures having sterically allowed values for ϕ_a,ψ_a and ϕ_h,ψ_h, as determined by reference to the conformational energy maps for the individual hydroxy and amino acid structural units, were selected for further analysis. The four-dimensional energy surfaces in the vicinity of these allowed helices were explored by conformational energy calculations at 15° intervals of ϕ_a,ψ_a and ϕ_h,ψ_h. Low-energy conformations found by this procedure were chosen as starting structures for energy minimization using the Fletcher and Powell[10] modification of Davidson's minimization technique.

Results and Discussion

Conformational Characteristics of Depsipeptide Structural Units. Conformational energies V_a and V_h are presented in Figures 2–5 as a series of contour lines joining conformations of equal energy. The contour lines are drawn at 1-kcal intervals relative to the low-energy conformation indicated by a cross in each of the figures. Energy contours greater than 5 kcal are not included. The energy maps of Figures 2–5 can be compared to similar maps of Brant, Miller, and Flory[2] for glycine and L-alanine and of Brant, Tonelli, and Flory[3] for L-lactic acid structural units. Differences between depsipeptide and peptide energy maps arise primarily because of reduced steric interactions involving C′–O compared to C′–NH and because the C′–O–C skeletal

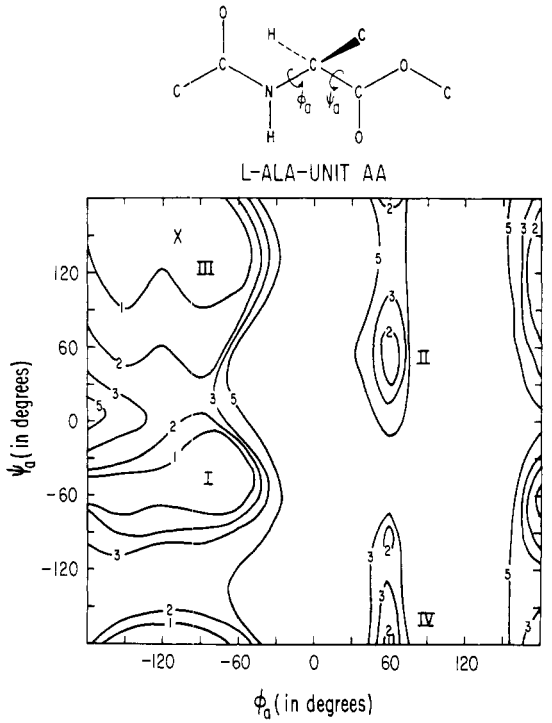

Figure 4. Conformational energy map for the L-alanine unit AA calculated according to eq 1.

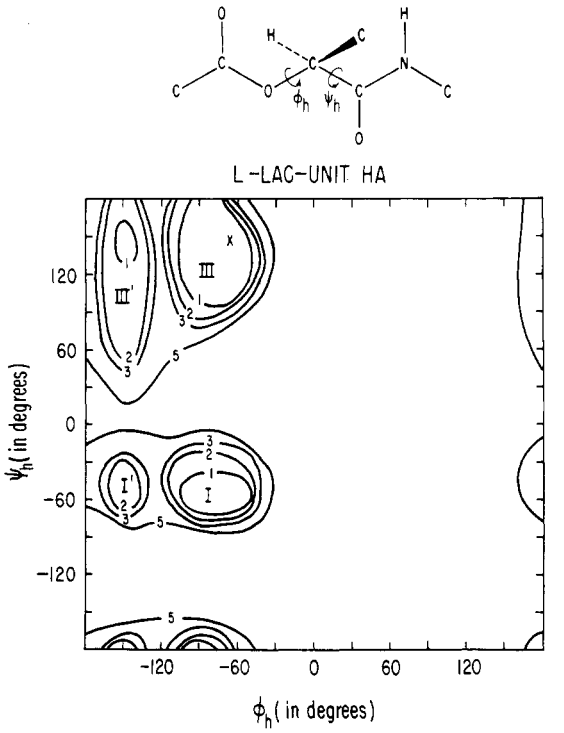

Figure 5. Conformational energy map for the L-lactic acid unit HA calculated according to eq 1.

bond angle is 10° smaller than the corresponding amide bond angle.

Conformational energy maps for glycine (Gly) unit AA and for glycolic acid (Glyc) unit HA are depicted in Figures 2 and 3. Strong steric repulsions involving C'O–NH and C'O–O exclude conformations with ϕ_a or $\phi_h \sim 0°$. The energy surfaces of Figures 2 and 3 are distinguished mainly by the energy barrier at ψ_a, $\psi_h \sim 0°$ separating the upper and lower regions, the barrier at $\psi_a \sim 0°$ being generally lower than the one at $\psi_h \sim 0°$. The low-energy conformations of Figure 2 are found in the lower left and upper right quadrants in contrast to their locations in the upper left and lower right quadrants in the glycine peptide energy map. This difference probably results from the reduced importance of electrostatic interactions involving ester groups compared to amide groups.

Averaged transformation matrices $\langle \mathbf{T}_a \rangle_{Gly}$ and $\langle \mathbf{T}_h \rangle_{Glyc}$ calculated according to eq 5 for the glycine unit AA and the glycolic acid unit HA are presented in eq 6 and 7, respectively.

$$\langle \mathbf{T}_a \rangle_{Gly} = \begin{bmatrix} -0.0103 & 0.2491 & -0.0001 \\ 0.1546 & 0.2081 & 0.0002 \\ -0.0002 & -0.0008 & -0.0377 \end{bmatrix} \quad (6)$$

$$\langle \mathbf{T}_h \rangle_{Glyc} = \begin{bmatrix} 0.3845 & 0.8541 & 0.0001 \\ -0.0165 & -0.0309 & 0.0001 \\ 0.0002 & 0.0002 & 0.0127 \end{bmatrix} \quad (7)$$

Conformational energy maps for the L-alanine derivative of unit AA and the L-lactic acid derivative of unit HA are presented in Figures 4 and 5, respectively. Allowed regions of the maps are marked by Roman numerals following conventions used for peptide and lactic acid energy surfaces. The low-energy conformation for both units was found in region III in agreement with L-alanine and L-lactic acid energy calculations.

The energy barrier at $\psi_a \sim 0°$ separating regions I and III of Figure 4 is lower than the corresponding barrier of the L-Ala unit of a polypeptide chain. Region II for L-Ala unit AA is more extensive than for the L-Ala peptide unit. In addition, the allowed region IV of Figure 4 is not found in the L-Ala peptide energy map. These differences arise primarily from decreased steric interactions of the oxygen atom of the ester as compared to those of the N–H group. The energy map of Figure 5 for the L-Lac unit HA is similar to that for the L-Lac unit AA except for the higher energy barrier at $\psi \sim 0°$ of the former unit compared to the latter.

Averaged transformation matrices $\langle \mathbf{T}_a \rangle_{Ala}$ and $\langle \mathbf{T}_h \rangle_{Lac}$ calculated according to eq 5 for L-Ala unit AA and L-Lac unit HA are presented in eq 8 and 9, respectively.

$$\langle \mathbf{T}_a \rangle_{Ala} = \begin{bmatrix} 0.2064 & 0.3250 & -0.1538 \\ 0.2447 & 0.0561 & 0.0496 \\ -0.2350 & -0.6186 & -0.0868 \end{bmatrix} \quad (8)$$

$$\langle \mathbf{T}_h \rangle_{Lac} = \begin{bmatrix} -0.0152 & 0.0978 & -0.5120 \\ 0.5994 & 0.0915 & -0.0877 \\ -0.1714 & -0.8229 & -0.0339 \end{bmatrix} \quad (9)$$

Regional partition functions for L-Ala unit AA and L-Lac unit HA were calculated as sums of the terms $e^{-V(\phi,\psi)/RT}$ taken at 30° intervals of ϕ and ψ over areas of Figures 4 and 5 enclosed by the 3 kcal mol^{-1} energy contours. The statistical weight contributed by each region was calculated as the ratio of the appropriate regional partition function to the sum of all regional partition functions. Products of these statistical weights for conformations of L-Ala unit AA and of L-Lac unit HA, representing statistical weight contributions from the associated conformations of the depsipeptide repeat unit, are presented in Table II. A qualitative indication of the end-to-end dimension of each repeat unit conformation is also included.

The two largest repeat unit statistical weights, which together comprise 68% of the repeat unit partition function, occur for the conformations AA-III, HA-III and AA-I, HA-III. The difference between the statistical weights of these

Table II
Statistical Weights for Conformations of the L-Ala-L-Lac Depsipeptide Repeat Unit

AA	HA I	HA I′	HA III	HA III′	Σ_{HA}
I	0.057 Compact	0.014 Compact	0.251 Compact	0.040 Compact	0.362
II	0.002 Extended	0 Compact	0.007 Extended	0.001 Extended	0.010
III	0.097 Extended	0.024 Extended	0.429 Extended	0.068 Extended	0.618
IV	0.002 Moderate	0 Extended	0.008 Moderate	0.001 Extended	0.010
Σ_{AA}	*0.158*	*0.038*	*0.695*	*0.110*	

Table III
Theoretical Asymptotic Characteristic Ratios C_∞ of Several Polydepsipeptides

Depsipeptide	C_∞
Poly(glycine-glycolic acid)	2.27
Poly(L-alanine-L-lactic acid)	3.80
Poly(L-alanine-D-lactic acid)	1.55
Poly(D-alanine-L-lactic acid)	1.55

two conformations results from different contributions from region III compared to region I of Figure 4 for the L-Ala unit AA. Since the repeat unit conformation AA-III, HA-III is extended and AA-I, HA-III is compact, chain dimensions are expected to be sensitive to features which affect the relative statistical weight values of these two dominant conformations.

Unpreturbed Dimensions of Random Coiling Polydepsipeptides. In order to facilitate comparison with polypeptides and poly(L-lactic acid), characteristic ratios $C_x = \langle r^2\rangle_0/xl^2$ of polydepsipeptide chains were calculated with values of the degree of polymerization x equal to the total number of both α-amino and α-hydroxy acid structural units. Likewise, the length of the virtual bond l = 3.75 Å was taken as the average of the length of the two bonds l_a and l_h.

Asymptotic characteristic ratios C_∞ calculated for selected polydepsipeptides are presented in Table III. The characteristic ratio C_∞ = 2.27 for poly(glycine-glycolic acid) is very similar to the value 2.16 calculated for poly(glycine).[2] This value is also close to the free rotation values C_∞ = 1.93 and 1.92 of polypeptide[2] and poly(L-lactic acid)[3] chains, respectively. Poly(L-alanine-L-lactic acid) has a characteristic ratio C_∞ = 3.80 intermediate between C_∞ = 9.27 calculated for poly(L-alanine)[2] and C_∞ = 2.13 calculated for poly(L-lactic acid).[3] The racemic depsipeptides poly(L-alanine-D-lactic acid) and poly(D-alanine-L-lactic acid) have identical characteristic ratios C_∞ = 1.55 that are less than the free rotation value. The results presented in Table II suggest that depsipeptide chain dimensions are sensitive to the relative energies of regions I and III of the L-Ala unit AA. This is illustrated by the results of characteristic ratio calculations in which the electrostatic energy contribution, which generally favors region III over region I, was set equal to zero. If electrostatic energy is ignored, the theoretical characteristic ratio of poly(L-alanine-L-lactic acid) is reduced to C_∞ = 1.93.

A negative value of the temperature coefficient d ln $\langle r^2\rangle_0/dT = -1.47 \times 10^{-3}$ was calculated for poly(L-alanine-L-lactic acid). Evidently the population of repeat units in the compact conformation AA-I, HA-III is increased with increasing temperature at the expense of the more extended conformation AA-III, HA-III. Accordingly, the value of the temperature coefficient is particularly sensitive to energy differences between conformations of the L-Ala unit AA in regions I and III.

The conformational characteristics of random coiling polydepsipeptides reported here have important features in common with those reported for polypeptides and for poly(lactic acid). In particular, the random chain dimensions of poly(glycine) and poly(glycine-glycolic acid) are nearly identical. Because of the absence of a suitable solvent it has not been possible to compare the theoretical characteristic ratio of poly(glycine) to the appropriate experimental quantity. It is our belief that the solubility characteristics of poly(glycine-glycolic acid) will be less restrictive. Favored conformations of the depsipeptide structural units L-Ala-AA and L-Lac-HA correspond to similar low-energy conformations observed for peptide and lactic acid structural units. The two depsipeptide repeat unit conformations AA-I, HA-III and AA-III, HA-III which dominate the structure of random poly(L-alanine-L-lactic acid) are closely related to conformations that are important for polypeptide and poly(lactic acid) chains. The chain end-to-end distance of random poly(L-alanine-L-lactic acid) and its temperature coefficient are sensitive to the difference in statistical weights of regions AA-I and AA-III. This is illustrated by the strong dependence of the characteristic ratio C_∞ on electrostatic interactions. An experimental analysis of the dimensions of randomly coiling polydepsipeptides is currently in progress in our laboratory. A comparison of the theoretical and experimental results will be presented in a subsequent publication.

Ordered Polydepsipeptide Structures. Low-energy helical structures were found with hydrogen-bonded rings of size R = 10 or R = 13. Five- and seven-membered rings restrict only the conformation of α-amino or α-hydroxy structural units. Irregular or random chains would result from the conformational flexibility of the unrestricted structural units. Hence stabilization by long-range interactions or cooperative effects is precluded. It is unlikely that stabilization energy from the weak hydrogen bonds of the five- and seven-membered rings can adequately compensate for their unfavorable steric interactions in the absence of long-range stabilizing forces. We were unable to find helical structures with 8- or 11-membered hydrogen-bonded rings that were free of prohibitive steric contacts. Conformational energy calculations revealed that helical structures with R = 14 also suffer severe steric interactions. We have assumed that helices with $R > 14$ are precluded by similar steric interactions that result from their small helical pitch.

The conformational characteristics and total energy of an interior repeat unit of the two minimum energy helical structures with R = 10 and R = 13 are presented in Table IV. Energies are measured from that of the low-energy extended form $\{\phi_a,\psi_a,\phi_h,\psi_h\}$ = (−85°,143°,−76°,170°) found by minimization from the conformation of lowest energy for the individual structural units. Conformational energy

Table IV
Conformational Characteristics and Energy of Two Minimum Energy Polydepsipeptide Helices

	Dihedral angles, deg							
R	ϕ_a	ψ_a	ϕ_h	ψ_h	n[a]	d,[b] Å	Hydrogen bonding	V, kcal/mol
10	51	−94	−144	30	1.78	4.50	(CONH) $\xrightarrow{2^c}$ (CONH)	1.84
13	−65	−35	−63	−47	1.81	2.89	(COO) $\xrightarrow{3^c}$ (CONH)	−7.91

[a] Number of repeat units required for a complete turn about the helix axis. [b] Displacement along the helix axis per repeat unit. [c] Number of intervening α-carbon atoms between the indicated hydrogen-bonded groups.

Table V
Conformational Energy Characteristics of Two Low Energy Polydepsipeptide Helices

	Helix	m										
	R	0	1	2	3	4	5	6	7	8	9	10
$(V_m - V_{m^*})$	10	−3.52	0.32	−0.02	−0.01	−0.01	0					
	13	0.91	2.19	0.33	0.15	0.09	0.06	0.04	0.03	0.02	0.02	0.01
$(V_{e,m} - V_{e,m^*})$	10	0.30	−0.04	−0.02	−0.01	−0.01	0					
	13	1.88	0.60	0.28	0.15	0.09	0.06	0.04	0.03	0.02	0.02	0.01

characteristics of the two low-energy helices are listed in Table V. The quantity $V_m - V_{m^*}$ given in the first two rows for values of m between 0 and 10 is defined as[8]

$$V_m - V_m^* = \left(\sum_{n=0}^{m^*} V_n\right) - V_m^* \qquad (10)$$

where the term V_n is the interaction energy between repeat units i and $i + n$ and V_{m^*} is the asymptote of V_m. $V_{n=0}$ represents the self energy of a depsipeptide repeat unit. Thus defined V_m is the increase in total helical energy that occurs upon adding a depsipeptide repeat unit to a helical sequence of length m. The values of $V_{e,m} - V_{e,m^*}$ presented in the third and fourth rows of Table V were calculated according to eq 10 by considering only electrostatic contributions to the conformational energy.

The skeletal dihedral angles $\{\phi_a,\psi_a\}$ = (51°,−94°) and $\{\phi_h,\psi_h\}$ = (−144°,30°) of the R = 10 helix occur in regions IV of Figure 4 and III′ of Figure 5, respectively, of the amino acid and hydroxy acid conformational energy maps. Conformations of peptides corresponding to region IV are of high energy because of steric interactions between neighboring C=O and NH groups. Polypeptides are therefore prevented from forming the R = 10 type helix of Table IV by these interactions. The R = 10 helix is left handed with 1.78 depsipeptide repeat units (Figure 1) per helix turn and a 4.50-Å translation along the helix axis for each repeat unit. For comparison with the corresponding parameters of polypeptide helices, the former number should be multiplied by 2 and the latter divided by 2. It can be seen from Figure 6a that the amide C=O and N—H bonds are directed roughly parallel to the helix and the ester C=O bonds are approximately perpendicular to the helix axis. Hydrogen bonding occurs between neighboring amide groups.

As indicated in Table IV, the depsipeptide repeat unit energy of the R = 10 helix is 1.8 kcal/mol higher than the energy of the extended form. It is possible that the relative stabilities of the R = 10 helix and the extended form could be reversed by slight adjustments of the geometrical or energy parameters. For example, relaxing the requirement for planar amide and ester groups would eliminate some steric repulsions of the R = 10 helix. It should also be pointed out that the conformational energy calculations presented here are only approximate for depsipeptides in solution since energy contributions from solute–solvent interactions have not been included.

A moderate strength hydrogen bond of −3.27 kcal/mol links adjacent amide groups of the R = 10 helix. This hydrogen bond is essentially linear with an O···N contact distance of 2.75 Å. Results presented in the first and third rows of Table V indicate that interactions between depsipeptide repeat units of the R = 10 helix are generally unfavorable. The interaction between unit i and $i + 1$ is most severe. It accounts for 3.84 kcal/mol in spite of the favorable hydrogen-bonded interaction between these units. This unfavorable interaction results primarily from steric repulsions between the carbonyl oxygen atom of the amide group of unit i and the succeeding ester oxygen atom. The R = 10 helix energy is decreased slightly by the favorable interaction between units i and $i + 2$ that results from van der Waals attractions between the H and C^β side-chain atoms of the amino acid residue of repeat unit $i + 2$ and the atoms of the hydroxy acid residue of unit i. Interunit interactions in the R = 10 helix are of relatively short range because of its extended form and the perpendicular orientation of adjacent amide and ester dipole moments. More than 99% of the total interunit energy is accounted for by interactions between repeat units i and $i + 1$ and i and $i + 2$. Results presented in the third row of Table V illustrate the relatively minor role played by electrostatic interactions in determining the R = 10 helix energy.

The sum $\Sigma_{m=0}^{m=m^*}(V_m - V_{m^*})$ represents the total excess conformational energy contribution of terminal units compared to interior units. Its value of −3.24 kcal/mol for the R = 10 helix indicates that terminal units are more stable than interior ones for this depsipeptide structure. The electrostatic contributions to the excess energy of chain termini, +0.23 kcal/mol, is only 7% of the total and acts to stabilize interior compared to terminal units.

The skeletal dihedral angles $\{\phi_a,\psi_a\}$ = (−65°,−35°) and $\{\phi_h,\psi_h\}$ = (−63°,−47°) of the R = 13 depsipeptide helix are similar to the angles $\{\phi,\psi\}$ = (−58°,−47°) of the standard right-handed polypeptide α helix.[11] The corresponding left-handed polydepsipeptide helix has an unfavorable energy due to repulsions that involve the ester C=O group. These repulsions are relieved in polypeptides because of the larger skeletal bond angle at the amide N atom compared to the angle at the ester O atom. The results of Table IV allow comparison of the number of hydroxy and amino acid structural units per turn of the R = 13 helix, $2 \times n$ = 3.62, and the average displacement along the helix axis per structural unit, $d/2$ = 1.45 Å with the corresponding parameters n = 3.60 and d = 1.50 Å of the standard right-handed polypeptide α helix.[11] A segment of the R = 13

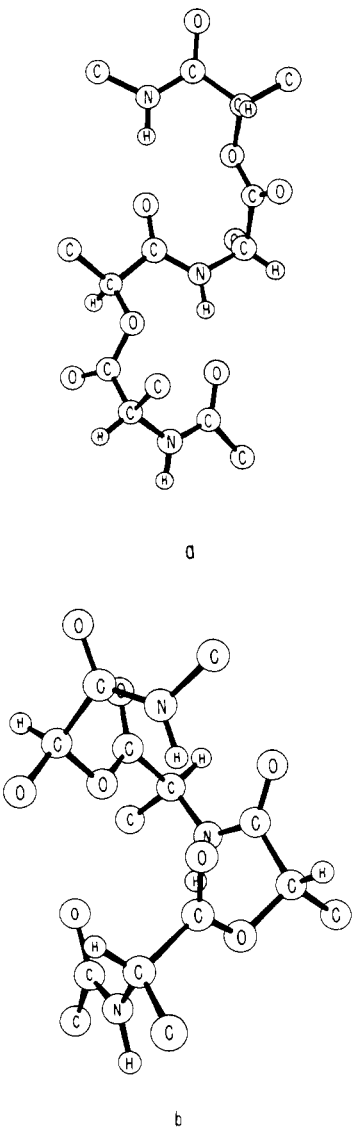

Figure 6. (a) Schematic diagram of the $R = 10$ polydepsipeptide helix. (b) Schematic diagram of the $R = 13$ polydepsipeptide helix.

depsipeptide helix is shown in Figure 6b. The amide and ester C=O bonds are approximately parallel to the helix axis. Hydrogen bonding occurs between ester carbonyl oxygen atoms and amide NH atoms that are separated by three α carbons.

The energy of an interior repeat unit of the $R = 13$ helix is 7.91 kcal/mol below that of the low-energy extended form. This stabilization energy is comparable to that found by Brant[8] for the polypeptide α helix in spite of the fact that the depsipeptide helix has only one-half the number of hydrogen bonds of the polypeptide helix. The importance of van der Waals and electrostatic interactions to the formation of the α helix is illustrated by this comparison.

The hydrogen bond of the $R = 13$ depsipeptide helix contributes an energy of -2.71 kcal/mol. Its O···N distance is 2.88 Å. Energy contributions from interunit interactions in the $R = 13$ helix are listed in the second row of Table V. In contrast to the $R = 10$ helix the terminal units of the $R = 13$ helix are less stable than interior units. The stabilization of interior units results primarily from long-range electrostatic interactions between the parallel dipole moments of the amide and ester groups. Effective interunit interactions occur between atoms separated by five or less intervening units. However, weaker interactions extend further. The excess conformational energy at a chain terminus $\Sigma_{m=0}^{m=m^*}(V_m - V_{m^*}) = 3.87$ kcal/mol, of which 3.20 kcal/mol is contributed by electrostatic interactions, illustrates the instability of terminal compared to interior repeat units. Terms $V_m - V_{m^*}$ for the $R = 10$ and 13 helices are compared in Figure 7 for values of m between 0 and 10.

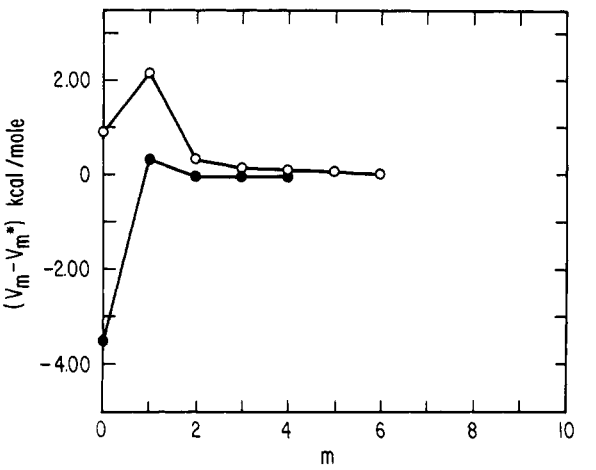

Figure 7. The excess energy $(V_m - V_{m^*})$ due to end effects of a helical depsipeptide repeat unit plotted against the number of units m that separate it from the helix end.

The results of Table V and Figure 7 suggest that the $R = 10$ helix is more stable for short depsipeptide chains of two or three repeat units than it is for longer chains. This is in contrast to the expected increase in stability of the $R = 13$ helix with increasing chain length. The effect of solvents on conformational stability is also likely to differ for the two helices. Examination of Figures 6a and 6b reveals that the polar amide and ester groups of the $R = 10$ helix are more accessible to solvation than are those of the $R = 13$ helix. This is especially true of the perpendicularly oriented ester groups of the $R = 10$ helix. Thus, in the absence of energy contributions involving side chains, the $R = 13$ helix will be most stable in nonpolar solvents that interact weakly with the amide and ester groups. On the other hand, the stability of the $R = 10$ helix will be greatest in more polar solvents that can solvate the exposed ester groups.

The thermodynamic stabilities of α-helical polypeptides and polydepsipeptides cannot be directly compared on the basis of conformational calculations of the type reported here since entropy effects and solvent interactions are not considered. However, an estimation of the stability of the polydepsipeptide α helix can be obtained from a statistical mechanical analysis of its helix-to-coil transition. For this purpose the depsipeptide chain was considered as a strictly alternating copolymer of α-amino and α-hydroxy acid structural units whose conformational states are determined by torsional rotations ϕ and ψ about the two skeletal single bonds attached to the α-carbon atom of each unit. Following Lifson and Roig[12] we proceed by enumerating the conformational states of the depsipeptide structural units and then assigning statistical weights to each conformational state.

Structural units whose torsional angles ϕ and ψ are appropriate for the $R = 13$ helix are considered in the helical state. A unit in any other conformation is in the random coil state. Useful approximate rules governing conformational properties of partially helical depsipeptide chains were derived from an examination of the hydrogen-bonding characteristics of the $R = 13$ helix. It is unlikely that a helical segment will begin or end with an α-amino acid unit since the α-amino carbonyl oxygens are not involved in hydrogen bonding and these units will not be restricted to the α-helix conformation if they appear at the ends of helical

segments. Therefore, a helical segment containing x hydrogen bonds will be comprised of x α-amino and $x + 1$ α-hydroxy acid structural units. Thus we associate a hydrogen bond with each α-amino acid structural unit in the helical state and assign a statistical weight of w' to such units. Helical α-hydroxy acid structural units are restricted to a small portion of conformation space but do not contribute hydrogen bonds. We have assigned a statistical weight of v' to these units. Amino and hydroxy acid units in the coil state are assigned statistical weights u_a and u_h, respectively. The reference state is chosen so that $u_a u_h = 1$.

A helical segment containing x hydrogen bonds will have a statistical weight $w'^x v'^{x+1} = (w'v')^x v'$. Therefore, $w'v'$ is the equilibrium constant for adding an additional hydrogen bond to a depsipeptide helical segment increasing its length by an amino and hydroxy acid structural unit. The free energy change for this process is thus

$$\Delta G_{c \to h} = -RT \ln (w'v') \quad (11)$$

At the melting temperature T^d of the depsipeptide helix $\Delta G_{c \to h} = 0$ and

$$\Delta G_{c \to h} = 0$$

and

$$-RT^d \ln (w') - RT^d \ln (v') = 0 \quad (12)$$

An approximate comparison of the melting temperature T^d of a depsipeptide helix with the melting temperature T^p of the corresponding polypeptide helix can be made by assuming that the statistical weights w' and v' of the depsipeptide helix have the same values as the statistical weight parameters w and v assigned by Lifson and Roig[12] to analogous conformational states of polypeptides. This appears as a reasonable approximation in light of the close resemblance between the depsipeptide and peptide conformational states that are represented by w' and w, and by v' and v. We consider after Lifson and Roig[12] that w' depends upon temperature accoring to eq 13

$$\Delta G^p_{c \to h} = -RT \ln w' = \Delta H^p - T\Delta S^p \quad (13)$$

where the superscript p refers to the indicated process in polypeptide chains. At the melting temperature of the polypeptide helix

$$\Delta H^p = T^p \Delta S^p \quad (14)$$

Equations 12, 13, and 14 can be combined to yield T^d in terms of thermodynamic parameters for the corresponding polypeptide helix-to-coil transition.

$$T^d = T^p/(1 + RT \ln v/\Delta H^p) \quad (15)$$

Thus polydepsipeptides will have a helix-to-coil transition within ~100° of the peptide transition temperature if

$$0.5 \leq 1 + RT^p \ln v/\Delta H^p \leq 1.5 \quad (16)$$

or

$$\left|\frac{\Delta H^p}{RT^p}\right| > \frac{|\ln v|}{0.5}$$

Employing in eq 16 a value of 0.025 for v that is representative of polypeptide melting[13] and a temperature of 398°K we find that a stable depsipeptide $R = 13$ helix can be expected if ΔH^p, the enthalpy change for the coil-to-helix transition of the corresponding polypeptide, has an absolute value greater than 4.4 kcal/mol. This is a considerably larger enthalpy change than is commonly observed for helix-to-coil transitions in aqueous[14-17] and mixed organic solvents.[18-20] Thus it is likely that $R = 13$ helical polydepsipeptides will be observed only in helix supporting organic solvents such as chloroform.

Examination of the statistical weights assigned to depsipeptide conformational states reveals that v' represents the equilibrium constant for the formation of an interruption in a helical sequence by a process that does not change the number of helical hydrogen bonds. Applequist[21] has shown that the sharpness of the helix-to-coil transition depends inversely on this equilibrium constant. The equilibrium constant for the analogous process in polypeptide α helices is v^2. This difference arises as a consequence of the different number of unformed hydrogen bonds per helical segment for the two types of chain molecules. There are two unsatisfied H bonds for a peptide and one for a depsipeptide helical segment. Since $v' \ll 1$ the cooperativity or sharpness of the depsipeptide helix-to-coil transition will be considerably less than observed for polypeptides.

These considerations suggest that both the random and ordered forms of polydepsipeptides will be experimentally accessible for conformational characterization. Because of the predicted low stability of the α-helical form we expect that polydepsipeptides will exist in the random state in a wider variety of solvents than is the case for polypeptides. Thus the theoretical analysis of the conformational characteristics of randomly coiling polydepsipeptides chain presented in the preceding section can be readily subjected to experimental verification. Furthermore, it is likely that thermally induced helix-to-coil transitions of polydepsipeptides can be observed in pure organic solvents instead of the mixed solvent systems required for polypeptide transitions. Thermodynamic parameters for helix-to-coil transitions in pure nonpolar solvents are more amenable to theoretical analysis than are those for transitions in mixed solvents containing a very polar component. Experimental conformational analyses of both randomly coiling and ordered polydepsipeptides are now in progress in this laboratory. Results from these studies will be reported soon.

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