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Polydepsipeptides. 11. Conformational Analysis of Polydepsipeptides Containing Methyl, Isopropyl, and Isobutyl Side Chains

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ABSTRACT: The comparative study of the thermal denaturation of polydepsipeptides containing alanine, valine, or leucine residues permits the determination of the relative stability imparted to ordered, helical structures of these different alkyl groups. Thermal melting of four sequence-specific polymers in organic solvents at low temperature was observed by means of circular dichroism. Poly[L-Ala-(*S*)-Lac] and poly[L-Val-(*S*)-Lac] were both observed to undergo order–disorder transitions, while poly[L-Ala-(*S*)-hydroxyisovaleric acid] remained disordered in all solvents studied. The effect of the isopropyl side chain is, therefore, sequence dependent and valine imparts greater or lesser stability than alanine, depending on the specific polymer. The polydepsipeptide poly[L-Leu-L-Leu-(*S*)-Lac] also undergoes an incomplete helix-to-coil transition in trifluoroethanol, while poly[L-Ala-L-Ala-(*S*)-Lac] does not. Thus, leucine imparts greater stability to these polymers than alanine in organic solvents.

Introduction

Previous studies of polydepsipeptides containing alanine and lactic acid have indicated that these polymers can be used to measure the effect of sequence, chirality, and number of hydrogen bonds on polypeptide helical structure and stability.[2-4] Polymers have been prepared which contain L-alanine and (*S*)-lactic acid in ratios of either two amino acids to one hydroxy acid or with equal numbers of amino and hydroxy acids. Polydepsipeptides of this type offer several advantages as compared to randomly polymerized polypeptides in the study of helical structure and stability since they are soluble in a wide range of organic solvents, undergo conformational transitions in single solvents, and are of known sequence.

We have also recently reported the helix-to-coil transitions of polydepsipeptides containing protected polar side chains such as γ-methylglutamic acid in polymers such as poly[$(Glu\text{-}OMe)_2$-(*S*)-Lac].[5] These materials were found to exhibit essentially the same stability to thermal denaturation in tetrahydrofuran as poly[L-alanyl-L-alanyl-(*S*)-lactic acid] {poly[$(Ala)_2$-Lac]}. In trifluoroethanol, however, polydepsipeptides which contain the protected polar side chains are significantly more stable than polymers containing alanine and lactic acid.

Such functional group dependence of helical structure and stability on amino acid type remains an important question in polypeptide chemistry. The characteristics of the bulk of the side chain, whether it is hydrophobic, hydrophilic, ionic, or neutral, have all been invoked in explaining observed conformational tendencies in natural and synthetic polypeptides. Introduction of different side chains into depsipeptides may allow the separation of the effects of hydrogen-bonding and side-chain contributions. Changing the bulk of alkyl side chains in polydepsipeptides probes a different set of factors contributing to helical stability than does introduction of polar side chains. This comparison may be carried out by preparing polymers in which alanine has been replaced by valine (methyl to isopropyl) or leucine (methyl to isobutyl). Poly(valine) itself has proven difficult to study in the past because of its insolubility and tendency to aggregate. Random copolymers of methionine and valine also show aggregation.[6] At low mole fractions of valine the methionine α helix is partially disrupted. Aggregation is a problem in block copolymers of valine and D,L-lysine.[7] The block copolymers poly(Val,Lys) and poly(Lys,Val,Lys) were observed by Scheraga and co-workers to assume self-aggregating β structures in water but to be partially helical in methanol. Qualitative studies in aqueous solutions were carried out for random copolymers of valine and (hydroxypropyl)-glutamine.[8] This work indicated that valine residues in a random copolymer do not support helix formation at room temperature but support partial helicity at elevated temperatures. Valine is therefore regarded as a helix breaker. The conformational tendencies of this amino acid residue are also of interest because of its occurrence in ionophore antibiotics,[9] in which the hydrophobic side chains may participate in the ion transport properties of these compounds.[10]

Polydepsipeptides containing leucine further increase the size of the alkyl side chain by adding an additional methylene to form the isobutyl moiety. There is some question, however, as to how this affects the structure of the alanine α-helix or the relative stabilities of helices formed by alanine and leucine. In aqueous solutions it has been observed that leucine imparts greater helical stability than alanine.[11,12] This arises primarily from the entropic contributions of the hydrophobic side chain since the

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strength of the hydrogen bonds formed by leucine were determined to be less than that of alanine. In dichloroacetic acid–dichloroethane solvent mixtures, however, leucine-containing polymers were observed to melt thermally at low temperatures than alanine-containing polymers.[13] It is also known that poly(leucine) melts at a lower mole fraction of trifluoroacetic acid in chloroform than poly(alanine).[14] Moreover, in random copolymers of glutamic acid and leucine it has been shown that the polymers melt at lower mole fractions of acid than poly(glutamic acid) with <4% leucine but that, with greater amounts of leucine, the copolymers become increasingly more stable than poly(glutamic acid).[15] The effect of leucine residues in a helix appears to depend upon not only the solvent but also the composition of the polymer.

Polydepsipeptides which contain equal numbers of amino and hydroxy acids have also been prepared.[16] These materials are not observed to form α helices at room temperature but may assume R_{10} helices at lower temperatures. This structure has been predicted to consist of concatenated β-I-like turns in which the amides are intramolecularly hydrogen bonded. Semiempirical energy calculations suggest ϕ,ψ angles for the amide residues of +51° and −94°, while the ϕ,ψ angles for the esters are approximately −144° and +30°.[17] In a sense, the amino acid residues may be thought to be in positions two and four of a β turn while the hydroxy acid residues occupy positions one and three. This unusual structure would imply that the amides are in a left-handed conformation and that the amide and ester transition moments point in different directions along the helix. Theoretical calculations of the CD spectrum of this conformation have also been carried out.[17] These suggest a positive dichroism centered near 218 nm with an intensity of 10 000 (deg cm^2)/dmol and a negative dichroism of −10 000 (deg cm^2)/dmol at 197 nm. It has also been suggested that the helical segments were short and, based upon the observed thermodynamics of poly[$(Ala)_2$-Lac], that poly(Ala-Lac) should melt at −35 °C in chloroform and −40 °C in tetrahydrofuran.[3,4]

In this paper, we report the conformational transitions of poly[L-alanyl-(*S*)-lactic acid] {poly(Ala-Lac)}, poly[L-valyl-(*S*)-lactic acid] {poly(Val-Lac)}, and poly[L-leucyl-L-leucyl-(*S*)-lactic acid] {poly[$(Leu)_2$-Lac]} in chloroform, tetrahydrofuran, and trifluoroethanol. The observed spectra of poly-[L-alanyl-(*S*)-hydroxyisovaleric acid] {poly(Ala-Hiv)} are also reported. These transitions are analyzed in terms of the relative stability imparted to ordered, helical structures by these different alkyl side chains.

Experimental Section

The matrix-mediated polymerizations of poly(Ala-Lac) and poly(Val-Lac) have been previously described.[16] Measurements of intrinsic viscosities were carried out in dichloroacetic acid. The constants for the Mark–Houwink equation were taken from analogous polypeptides. The polymers used in this study were found to have intrinsic viscosities of 0.19 dL/g for poly(Ala-Lac) and 0.42 dL/g for poly(Val-Lac). This would imply molecular masses of approximately 24 000 and 64 000 daltons, respectively. The sample of poly[$(Leu)_2$-Lac] was made available by BioResearch Inc. and was also polymerized in the solid state on Celite. This polymer exhibited an intrinsic viscosity of 0.83 dL/g, implying a molecular mass of approximately 138 000 daltons. Poly(Ala-Hiv) was prepared by a matrix-mediated polymerization of the monomer TFA [L-alanyl-(*S*)-α-hydroxyisovaleric acid]$_2$-PCP. The sample was deposited on the Celite and polymerized at 80 °C for 24 h. It was then postpolymerized at 110 °C for 2 days. The resulting polydepsipeptide was washed from the solid support with trifluoroacetic acid and precipitated with ether. The precipitate was washed with ether and fractionally precipitated from trifluoroethanol with water. The fraction employed in this study was an off-white powder with an intrinsic viscosity of 0.63 dL/g. This implies a molecular mass of more than 50 000 daltons.

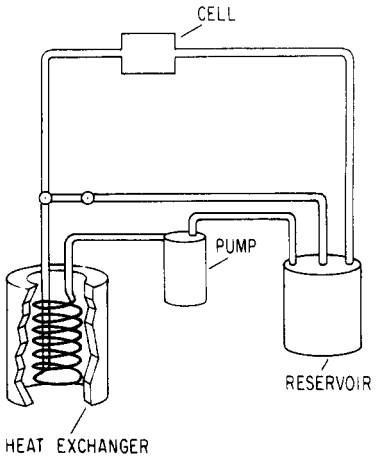

Figure 1. Cryostat for operation from −70 to 0 °C. The working fluid is 2-propanol and the heat exchanger rests in a dry ice/2-propanol bath.

The solvents used were trifluoroethanol (Aldrich, Gold Label), tetrahydrofuran (Aldrich, reagent grade), and chloroform (Malinckrodt, spectroscopic grade). The tetrahydrofuran was refluxed over sodium in an inert atmosphere for 2 h and then fractionally distilled under nitrogen onto Linde 4A molecular sieves. It was stored in a dark glass bottle sealed by a rubber septum. The solvent was withdrawn by syringe through the septum. The chloroform was stored over molecular sieves and used without further purification.

Circular dichroism spectra were obtained with a Cary 61 spectropolarimeter which has been extensively modified. Signal averaging was achieved by means of a TI 980A minicomputer. Temperatures from −10 to +100 °C were maintained by means of a Lauda 2k/R bath which was held constant to ±0.1 °C. Temperatures of −70 to −10 °C were maintained with the cryostat shown schematically in Figure 1. This consisted of a 10-L Nalgene Dewar for the thermal reservoir. Four meters of $^1/_3$-cm copper tubing was empolyed as the heat exchanger and was connected to a Micropump Model 7004-90. The working fluid for the cryostat was 2-propanol. The thermal bath was dry ice/2-propanol. The temperatures within the cell were continuously monitored by two Yellow Springs Instrument calibrated thermistors (no. YSI44011 to −30 °C and no. YSI44001A to −70 °C) which were implanted in a cooling block beside the sample cell. The accuracy of the thermistors was determined to be ±0.2 °C to −30 °C and ±0.4 °C to −70 °C. These temperatures and the temperature of the Cary 61 modulator were monitored and controlled by means of a Biocomputronics digital controller.

All samples were prepared with concentrations expressed as weight:volume and ranged from 0.40 to 0.02 mg/mL, depending upon the path length of the cell. The molecular weights employed in calculating the molar ellipticities were the mean average molecular weight/residue of the depsipeptide "monomers". For example, the molecular weight of the "monomer" L-Ala-(*S*)-Lac is 143 and the mean average molecular weight/residue is, therefore, 71.5. No effect upon the CD spectra was observed with changes in concentration, with the exception of poly(Val-Lac) in trifluoroethanol. The CD cells used were Helma 0.1 mm and 1.0 cm. Since many of the observed transitions take place 50 or 60 °C below room temperature, the actual concentration of the samples would vary according to the thermal coefficients of expansion of the solvents. It was assumed that these values were the same as for pure solvent and the literature values for each solvent were used to correct the observed molar ellipticities. The corrections were never greater than 1%.

Results and Discussion

Temperature-Dependent Spectra of Polydepsipeptides Containing One Amino Acid and One Hydroxy Acid per Repeat. The circular dichroism spectra

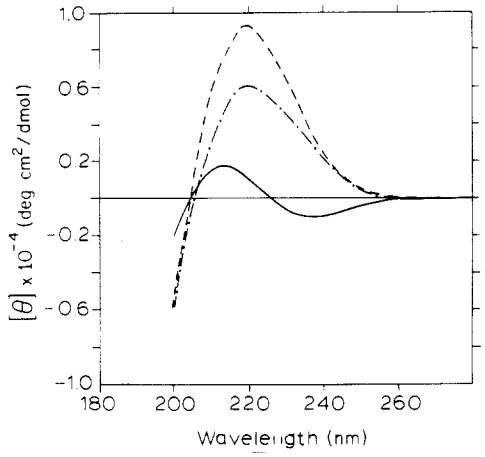

Figure 2. Comparison of the CD spectra of poly(Ala-Lac) (-·-), poly(Val-Lac) (---), and poly(Ala-Hiv) (—) at −50 °C in tetrahydrofuran.

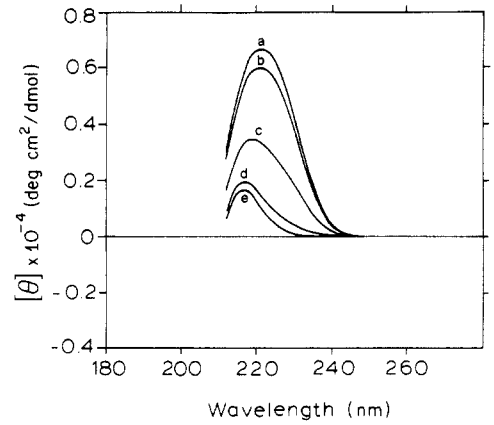

Figure 3. Temperature-dependent CD spectra of poly(Ala-Lac) in chloroform at (a) −53, (b) −42.50, (c) −33, (d) −20, and (e) +13 °C.

of poly(Ala-Lac), poly(Val-Lac), and poly(Ala-Hiv) at −50 °C in tetrahydrofuran are shown in Figure 2. Poly(Ala-Lac) and poly(Val-Lac) each exhibit positive dichroism centered at 217 and 218 nm, respectively, and negative bands below 200 nm. Spectra of this type are predicted for the R_{10} helical structure of polydepsipeptides. Poly(Ala-Hiv) does not exhibit intense positive dichroism at 220 nm and appears disordered. If the solutions containing these three polymers are allowed to come to room temperature it is observed that poly(Ala-Hiv) retains the same CD spectrum and that the other two polydepsipeptides become disordered.

Temperature-dependent CD spectra for poly(Ala-Lac) in chloroform are shown in Figure 3. Similar spectra for poly(Val-Lac) in trifluoroethanol are shown in Figure 4. For poly(Ala-Lac) the positive band near 217 nm increases in intensity as the temperature is lowered. The maximum of this transition shifts from 217 nm to 218 nm in tetrahydrofuran to 220 nm in chloroform. Similar shifts are noted for poly(Val-Lac) and arise from the solvent and conformational sensitivity of the $n\pi^*$ transition. The limiting dichroism for poly(Ala-Lac) is 6140 ± 100 (deg cm^2)/dmol in chloroform and 8300 ± 100 (deg cm^2)/dmol in tetrahydrofuran. For poly(Val-Lac) the limiting dichroism is 9300 ± 100 (deg cm^2)/dmol in tetrahydrofuran and 9000 ± 100 (deg cm^2)/dmol in trifluoroethanol. In each case the observed intensity for the high-temperature disordered state was 1600 ± 10 (deg cm^2)/dmol.

From Figures 3 and 4 it is apparent that poly(Val-Lac) remains ordered at a higher temperature than poly(Ala-Lac). The approximate midpoint of the melting of poly(Val-Lac) in tetrahydrofuran is −10 °C while that for poly(Ala-Lac) in this solvent is approximately −40 °C. Furthermore, while poly(Val-Lac) undergoes a helix-to-coil transition in trifluoroethanol with a midpoint near −17 °C, poly(Ala-Lac) remains disordered to the freezing point of the solvent. The bulk of the isopropyl side chain is not the sole determining factor in the stability of poly(Val-Lac) since poly(Ala-Hiv) remains disordered in *all* solvents. The effect of the isopropyl side chain must be sequence dependent.

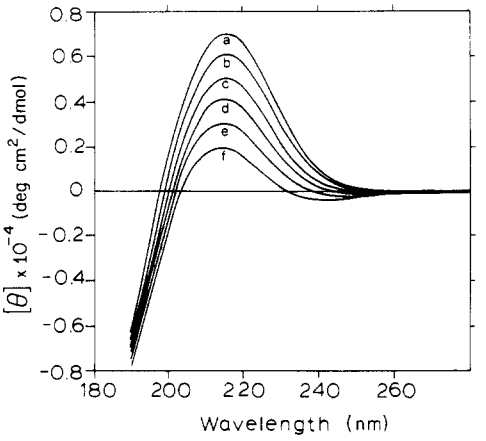

Figure 4. Temperature-dependent CD spectra of poly(Val-Lac) in trifluoroethanol at (a) −49, (b) −32, (c) −15, (d) −5, (e) +5, and (f) +8 °C.

Side-chain hydrophobic effects have been previously noted for valine-containing compounds in water. These effects significantly increase the entropy of the system. In some manner the effect of the side chain in polar organic media must also be different for compounds containing valine as compared to compounds which contain α-hydroxyisovaleric acid. This could arise from the slightly different ester geometry. It is known that alanine is much more prevalent than valine in α-helical regions of proteins but that valine is much more likely than alanine to be found in β sheets.[19] The two are very similar in their likelihood to be found in β turns. Thus, the R_{10} structure is more like an extended than an α-helical conformation and inclusion of the isopropyl side chain of valine in the amino acid sequence provides significant stabilization of an ordered structure.

Temperature-Dependent Spectra of Poly[(Leu)$_2$-Lac]. The spectrum of poly[(Leu)$_2$-Lac] in trifluoroethanol at −50 °C is shown in Figure 5. A spectrum of poly(Ala) in this solvent at this temperature is also shown in Figure 5 for comparison. The CD spectrum of poly[(Leu)$_2$-Lac] exhibits a negative trough at 205 nm and shoulder near 220 nm, which are expected for α helices. The dichroism of both, however, is about one-half to one-third that normally observed. In addition to the loss of intensity in these two bands, the positive dichroism below 200 nm, which results from the parallel component of the $\pi\pi^*$ couplet, is not observed. The spectra appear nonconservative above 185 nm. A similar loss of intensity is observed for poly[Glu(OMe)$_2$-Lac] in this solvent.[5] Polypeptides in this solvent or in other polar media such as water or hexafluoro-2-propanol which are α helical do not show this reduction in the $\pi\pi^*$ exciton.[18] This type of polydepsipeptide CD spectrum was not observed for poly[(Ala)$_2$-Lac] in chloroform or tetrahydrofuran[3] but may be observed in polar solvents for the following reasons. It is well-known for amides and esters that $n\pi^*$ transitions undergo blue shifts and $\pi\pi^*$ transitions red shifts in their

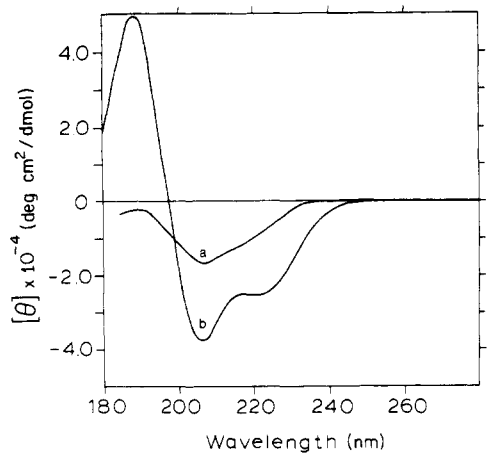

Figure 5. CD spectra of (a) poly[$(Leu)_2$-Lac] and (b) poly(Ala) in trifluoroethanol at −50 °C.

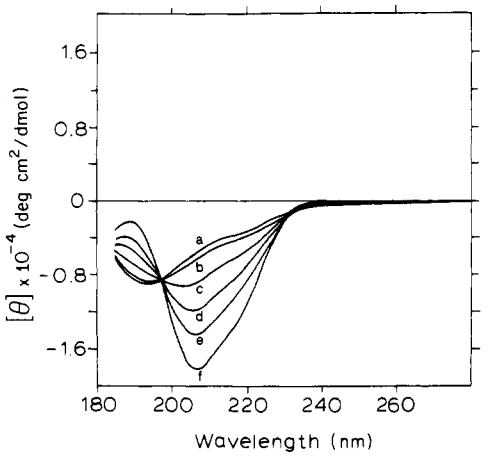

Figure 6. Helix-to-coil transition of poly[$(Leu)_2$-Lac] in trifluoroethanol at (a) −12, (b) −20, (c) −32, (d) −46, (e) −48, and (f) −53 °C.

absorption energies when molecules containing these chromophores are transferred from nonpolar to polar media. In a polar solvent such as trifluoroethanol, the $\pi\pi^*$ and $n^*\pi$ transitions of the amide chromophores would be expected to occur at 189 and 209–211 nm, respectively. The ester chromophores exhibit transitions at different wavelengths. The ester $n\pi^*$ is observed near 205 nm in polar media while the $\pi\pi^*$ would be expected to occur near 170–175 nm. In an α-helical conformation, the ester $n\pi^*$ and the parallel lobe of the amide $\pi\pi^*$ would, therefore, be nearly degenerate in energy. In addition, exciton–exciton interaction would be expected between the amide and ester $\pi\pi^*$. It has recently been postulated that such interactions may account for the loss of the positive 190-nm band in poly(proline II) spectra.[2]

As was observed for poly(Val-Lac), it is possible thermally to denature poly[$(Leu)_2$-Lac] in trifluoroethanol. A set of melting curves for this polymer in trifluoroethanol are shown in Figure 6. As the temperature is lowered the 203-nm trough and 217-nm shoulder become increasingly negative while the 187-nm peak increases in intensity. The latter band actually exhibits positive dichroism only at the lowest recorded temperature.

From the melting spectra in Figure 6 we may see that the isobutyl side chains of poly[$(Leu)_2$-Lac] stabilize what we believe to be an α helix just as the isopropyl side chains of poly(Val-Lac) stabilize an ordered structure in that polymer. This polydepsipeptide undergoes a partial conformational transition while poly[$(Ala)_2$-Lac] remains disordered at this temperature. We have reported in preliminary results that poly[$(Ala)_3$-Lac] also undergoes a conformational transition in trifluoroethanol,[21] implying that an alanine-containing polydepsipeptide with three hydrogen bonds per residue is as stable as a leucine-containing polymer with only two hydrogen bonds per three residues. If these polymers assume similar, ordered structures, leucine would appear to form a more stable helix than alanine. This conclusion is in accord with observed helix-to-coil transitions of leucine-containing polymers in water but does not agree with similar studies in mixed organic solvents.[22] The differences may arise, however, from the manner in which the two experiments were carried out. In the previous study leucine residues were randomly copolymerized with γ-benzyl glutamate and the conformational transitions observed in DCA–DCE solutions. This is significantly different from melting of a sequential polymer of known composition in a single solvent. To date, the two techniques have not yielded concordant results. Another possibility is that inclusion of leucine into the polydepsipeptide chain forms a sequential polymer with properties different from those of either poly(Ala) or poly(Leu). Earlier studies indicated that leucine destabilized the poly(Glu) helix at some mole fractions and increased it at others.[15] The helix formed by a combination of Ala and Leu residues is not the same as that of either homopolymer. This is far from a tautology. It is an underlying premise of our studies on polydepsipeptides that introduction of small changes in homopolymers (such as replacing some of the amides with esters in our work) or introduction of varying amounts of a guest amino acid into a host chain (as in Scheraga's approaches[23]) will allow the determination of the thermodynamics of melting of individual amino acids. If the structure formed by the helical copolymer or copolydepsipeptide is unlike that of either parent macromolecule, this premise may be false. Direct comprison of the two different experimental techniques is therefore difficult at the present time.

Conclusions

The hydrophobic amino acids valine and leucine exhibit a more complex set of conformational tendencies in polydepsipeptides than can be explained only in terms of their bulk. Both preferentially stabilize polydepsipeptide ordered structures to a greater extent than does alanine, but they do not stabilize the same secondary structures. As is found for proteins, valine exhibits a tendency to be in extended structures while leucine prefers helical structures. The fact that valine and hydroxyisovaleric acid containing polymers exhibit such different behavior and that poly(Val-Lac) is only metastable in trifluoroethanol suggests that the different reports in the literature of the conformational tendencies of this amino acid result from a sensitivity to sample composition and experimental conditions. In a more general sense, the observation that polydepsipeptides with fewer hydrogen bonds melt at lower temperatures and do not appear to adopt a helical conformation is consistent with our earlier predictions of the influence of hydrogen bonding on secondary structure and stability.

The conclusions and discussions of these polymers have been largely qualitative in nature. The principal barrier to a quantitative discussion is a lack of corroborative evidence that the low-temperature forms of the polymers are indeed R_{10} helices for poly(Ala-Lac) and poly(Val-Lac) and α helix for poly[$(Leu)_2$-Lac]. Although the spectra for the former two polymers agree quite well with our earlier prediction, this may be fortuitous. Still, the melting temperatures of poly(Ala-Lac) in chloroform and tetrahydro-

furan are near those predicted earlier from the melting of poly[(Ala)$_2$-Lac]. We are currently reexamining our theoretical predictions of the circular dichorism thought to be exhibited by polymers in the R_{10} helical conformation.

Acknowledgment. We gratefully acknowledge the financial support of the National Science Foundation (Grant CHE-77 08920) and the National Institutes of Health (Grant RR-00757). We also thank Mr. Joseph Taulane for his aid in constructing the low-temperature cryostat and in maintaining the Cary 61.

Registry No. poly(Ala-Lac) (homopolymer), 53777-54-1; poly(Ala-Lac) (SRU), 53745-64-5; poly(Val-Lac) (homopolymer), 54664-24-3; poly(Val-Lac) (SRU), 54711-82-9; poly[(Leu)$_2$-Lac] (homopolymer), 78426-69-4; poly[(Leu)$_2$-Lac] (SRU), 78426-76-3; poly(Ala-Hiv) (homopolymer), 78426-67-2; poly(Ala-Hiv) (SRU), 78437-72-6.

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Conformational Transition of a Water-Soluble Poly(diacetylene): Potassium Salt of Poly[4,6-decadiyne-1,10-diol bis([carboxymethyl]urethane)]

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ABSTRACT: The conformational transition of poly(diacetylene), (=RC—C≡C—CR=)$_x$, was studied on a poly(electrolytic diacetylene), poly(3KAU) (R = -(CH$_2$)$_3$OCONHCH$_2$COOK), in aqueous solution by measurements of visible absorption spectra, potentiometric titration, and shear rate dependence of intrinsic viscosity. Visible absorption spectra for the 10^{-2} M solutions of poly(3KAU) showed that the effective conjugation length of the main chain becomes longer as the degree of neutralization α' decreases. Correspondingly, the color of the solutions changed from yellow to violet with decreasing α'. Potentiometric titrations resulted in distinct conformational transition curves similar to those observed for poly(methacrylic acid) and poly(α-glutamic acid). The enthalpy change ΔH of the conformational transition was estimated from the titration experiments to be -8 kJ per carboxylic acid group. These results are interpreted by a planar-nonplanar conformational transition proposed by Patel. The intrinsic viscosity [η] for the violet solutions of poly(3KAU) in the low-α' region strongly depended on the shear rate, while [η] for the yellow solutions in the high-α' region did not. This may be explained by the fact that the whole chain conformation is rodlike in the low-α' region and coillike in the high-α' region.

Introduction

Poly(diacetylenes) have conjugated backbones of a resonance admixture of acetylenic (=RC—C≡C—CR=)$_x$ and butatriene (—RC=C=C=CR—)$_x$ structures, exhibiting a characteristic color.[1] Although poly(diacetylenes) had been thought to be insoluble in common organic solvents because of their conjugated backbone, Patel succeeded in synthesizing a series of soluble poly(diacetylenes), in which R = -(CH$_2$)$_n$OCONHCH$_2$COO-(CH$_2$)$_m$CH$_3$ with n = 1-4 and m = 1 or 3.[2] They are referred to as poly(nACMU), standing for the number of methylene groups, n, and [(alkoxycarbonyl)methyl]-urethane side chains. Patel et al. also found that the yellow to orange color of the solution of poly(3BCMU) (n = 3, m = 3) and poly(4BCMU) (n = 4, m = 3) changes to blue and red on addition of a poor solvent, respectively. They attributed this color change to a conformational transition of poly(nACMU). The conformation in a poor solvent such

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