Modifying Oligoalanine Conformation by Replacement of Amide to Ester Linkage

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Abstract: Oligo(lactic acid) is an ester-analogue of short oligoalanine sequence and adopts a rigid left-handed helical structure (Hongen *et al. Macromolecules* **2014**, *47*, 5313). In this study, oligo(lactic acid) was incorporated into oligoalanine sequences and their conformations were studied by vibrational circular dichroism and electronic circular dichroism spectroscopy. The results suggested that oligo(lactic acid) moiety in these sequences maintain a left-handed helix and increase the conformational propensity of the oligoalanine moiety to form a left-handed PPII-like helix. The importance of the chirality of oligo(lactic acid) moiety for the oligoalanine conformation was also studied. The results obtained in this study should be useful in developing ester-containing oligopeptides that function better than normal peptides.

Keywords: polyproline type II helix, β-strand, lactic acid, vibrational circular dichroism, electronic circular dichroism

Introduction

Polyproline type II (PPII) helix is a left-handed 3_1-helix found in various proteins such as collagen, elastin, and casein.[1-3] PPII helix does not form intramolecular hydrogen bonds, unlike right-handed α-helix, which forms hydrogen bonds between the C=O group of the *i*th residue and the NH group of the (*i*+4)th residue. As a result, PPII adopts a rather extended structure and its carbonyl and amino groups are readily accessible by other molecules for intermolecular interactions. These structural properties have rendered PPII numerous possibilities as functional motifs for protein-protein interactions, molecular scaffold, peptide-based drugs, antifreeze agent, and so on.[4-6] However, designing a peptide sequence forming a PPII helix without proline residues is sometimes difficult because of the lack of stabilizing hydrogen bonds. For example, oligoalanines may adopt multiconformational states including not only PPII[8-10] but also α-helix,[12,13] β-strand and the resultant self-assembled fibrils.[13,14] As another example, we have witnessed that antifreeze glycopeptide (AFGP) H-[Ala-Thr-Ala]$_n$-OH exists as PPII when its Thr residues are glycosylated with *N*-acetylgalactosamine, while it shows an electronic circular dichroism (ECD) spectrum typical for disordered structures when it is unglycosylated.[15,16] A method to facilitate the formation of PPII helix should benefit medicinal chemistry and biochemistry using peptides and their analogues.

Modification of amide linkages in the peptide main chain to different ones (e.g. urea, carbamate, and aminoxy) has been effective in regulating their secondary structures and hence their functions.[17,18] Considering future applications to biological systems, ester linkage is promising because of (1) low toxicity, (2) easy availability (e.g. solid-phase synthesis using α-hydroxy acids, genetic code expansion, etc.),[19-21] and (3) ability to alter polypeptide conformation due to the impaired hydrogen bonding property.[19,22] Among many α-hydroxy acids, lactic acid (Lac) is often incorporated into oligopeptides: for example, a recent study found that substitution of an Ala residue to Lac changed the conformations of pentapeptides and heptapeptides from the 11-helix to the 14/15-helix.[22] Meanwhile, to the best of our knowledge, there has been no study on the conformations of oligopeptides containing di- and longer oligo(lactic acid)s. Recently, the solution conformation of poly(L-lactic acid), a biodegradable thermoplastic polyester, was studied by Ho's and our groups by means of vibrational circular dichroism (VCD) spectroscopy.[23,24] A negative-positive VCD couplet at around 1760 cm^{-1} observed under several conditions suggested that poly(L-lactic acid) adopt a fairly stable left-handed 10_3-like helix in solution,[24-26] unlike multiconformational oligoalanines. Moreover, conformational studies on several oligo(L-lactic acid)s indicated that four ester linkages is sufficient to fix their helicity.[24] These results inspired us to use oligo(L-lactic acid) sequences as a rigid structural unit for ester-containing peptides.

In this current work, we prepared several hexamers composed of oligo(lactic acid) and oligoalanine, and studied their conformations by using VCD and ECD spectroscopy. The results indicated that the left-handed helical conformations of oligo(lactic acid)s in these hexamers are indeed rigid and also induce PPII helix formation of adjacent oligoalanine moieties. The chiroptical spectra were analyzed in an empirical manner, because our preliminary DFT (density functional theory) geometry optimization calculations of the oligoalanine moiety of these hexamers resulted in β-strand, not PPII, conformations.

Materials and Methods

All the samples were prepared by means of solution-phase synthesis using HATU and HOBt (see Supporting Information). The purity of each molecule was confirmed by ^{1}H and ^{13}C NMR spectroscopy. VCD and IR spectra were measured for 16 and 6000 scans, respectively, using a BioTools Chiral*ir*-2X. All

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spectra were recorded using a 100-μm CaF_2 cell at a resolution of ca. 8 cm^{-1} at ambient temperature, and were presented after subtracting solvent spectra obtained under the identical experimental conditions. Prior to the VCD measurements using CD_3OD, each sample was once dissolved in an excess amount of CD_3OD, heated for 10 mins, and then dried by vacuum pump to perform amide H-D exchange. ECD and UV spectra were measured on a Jasco J-820 spectrometer using a 0.1 cm quartz cell.

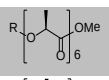
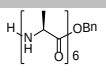
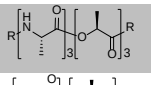
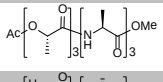
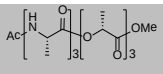
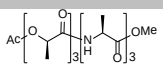
Results and Discussion

Synthesis and General Properties of Hexamers

Several hexamers were synthesized by combining a Lac trimer and Ala trimer as listed in Table 1. ^{L}Ala trimer was placed before or after ^{L}Lac trimer (**3** and **4**) to study the difference in the influences in the peptide conformation. Using ^{D}Lac and ^{L}Ala, diastereomeric Ac-^{L}Ala$_3$-^{D}Lac$_3$-OMe (**5**) and Ac-^{D}Lac$_3$-^{L}Ala$_3$-OMe (**6**) were also synthesized to examine the influences of the helicity of Lac trimer on the conformation of ^{L}Ala trimer. H-^{L}Ala$_6$-OBn (**2a**) was used as a standard hexaalanine because the other ^{L}Ala hexamers synthesized in this study were not soluble enough for spectral measurements in water (pH 1 to 13), CH_3OH, trifluoroethanol, DMF, $CHCl_3$, and so on. Some oligoalanines have been known difficult to dissolve because of aggregate formations.[14]

Aiming at future applications of ester-substituted oligopeptides in various biological fields, we first carried out a cell viability assay of H-^{L}Ala$_3$-^{L}Lac$_3$-OH (**3a**) against mouse embryonic fibroblast cells and confirmed that it was indeed low in toxicity (Figure S1)**.** Initially, we also planned to test the antifreeze activity (an activity to inhibit the growth of ice crystal at low temperature) of the hexamers synthesized in this work because of their expected conformational similarity with an AFGP hexapeptide that forms a PPII helix.[15] However, this plan was abandoned due to the insolubility of **3a** in pure water. Despite this observation, **3a** showed much higher solubility than **2a** and H-^{L}Ala$_6$-OH in various solvents such as CH_3OH, 50% CH_3OH-H_2O, and acetonitrile, which exemplified the usefulness of ester substitution for modifications of oligopeptide properties. In order to obtain more insights into the properties of ester-containing peptides, the conformations of the hexamers were further studied.

TABLE 1 The structures of the hexamers

	Structure [a]	
1 (R^1 = Ac) **1a** (R^1 = Me)	R^1-^{L}Lac$_6$-OMe	
2a	H-^{L}Ala$_6$-OBn	
3 (R^1 = Ac, R^2 = OMe) **3a** (R^1 = H, R^2 = OH)	R^1-^{L}Ala$_3$-^{L}Lac$_3$-R^2	
4	Ac-^{L}Lac$_3$-^{L}Ala$_3$-OMe	
5	Ac-^{L}Ala$_3$-^{D}Lac$_3$-OMe	
6	Ac-^{D}Lac$_3$-^{L}Ala$_3$-OMe	

[a] Ala = -NHCH(CH_3)CO-; Lac = -OCH(CH_3)CO-; Bn = -CH_2Ph

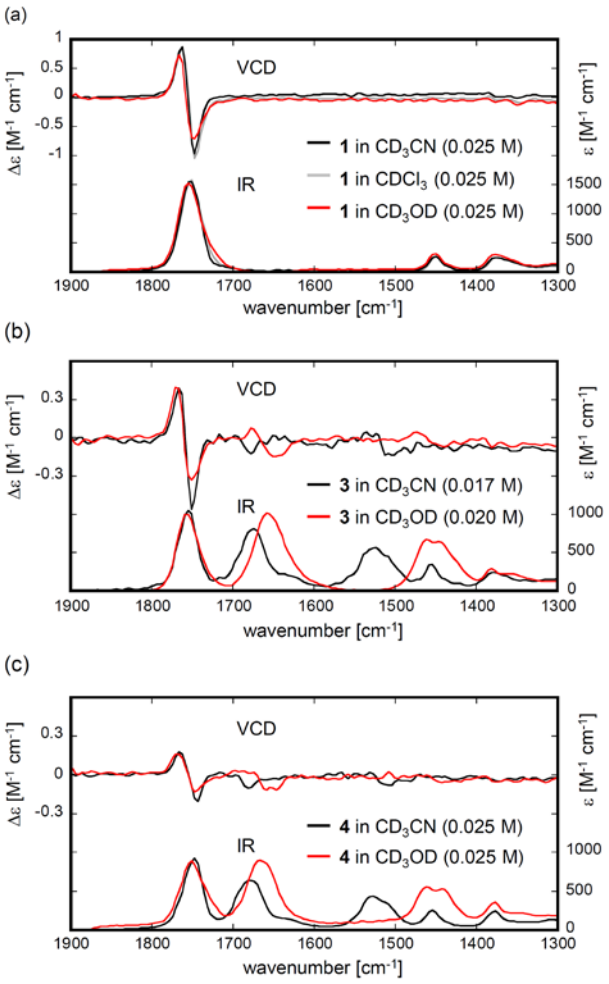


FIGURE 1 VCD (top) and IR (bottom) spectra of (a) **1**, (b) **3**, and (c) **4** in CD_3CN (black), $CDCl_3$ (gray), and CD_3OD (red).

Conformations of the oligoester moiety of hexamers

The VCD spectra of **1**, **3**, and **4** were measured first in $CDCl_3$ and CD_3CN to analyze the conformation of the oligoester moiety through comparison with previously studied **1a** in $CDCl_3$.[24] Especially, we were interested to see, if any, differences in the helicity of **3** and **4**, as an ester C=O is capable of acting as a weak hydrogen bond acceptor with an NH group.

As shown in Figure 1a, ^{L}Lac hexamer **1** measured in $CDCl_3$ (gray line) and CD_3CN (black line) exhibited a strong IR and VCD signals in the ester C=O stretching region at around 1750 cm^{-1}. In accordance with our previous observation on **1a**, the negative-positive VCD couplet (from lower to higher frequencies) suggested a left-handed helical structure.[23-26] Meanwhile, heterogeneous hexamers **3** and **4** in CD_3CN presented three characteristic IR absorption bands originating from ester C=O stretching (~1750 cm^{-1}), amide I (~1675 cm^{-1}), and amide II vibrational modes (~1525 cm^{-1}) (black lines in Figure 1b and 1c, Table 2). The $\Delta\varepsilon$ values of the ester carbonyl VCD couplet of **3** and **4** were smaller than those of **1**, as expected from the smaller numbers of ester linkages (seven, four, and three consecutive ester linkages for **1**, **3**, and **4**, respectively). Nonetheless, both **3** and **4** exhibited a negative-positive couplet, which is indicative of the left-handed helical properties of their ^{L}Lac$_3$ moiety. No shift was recognized for the ester C=O IR peak positions between **1** and **3** (1755 cm^{-1}), which should reflect the absence of hydrogen bonds involving ester C=O groups (Table 2). A minor red-shift observed for **4** (1747 cm^{-1}) seemed within a range of red-shifts

intrinsic to shorter oligo(lactic acid)s.[24] Possibilities of significant hydrogen bonds involving the ester groups of **4** were also excluded by the similar VCD patterns between **3** and **4** in the amide I and II regions. These observations led us to speculate that the $^{L}Lac_3$ moieties of **3** and **4** were hardly involved in hydrogen bonding with their $^{L}Ala_3$ moieties and maintained left-handed helical properties.

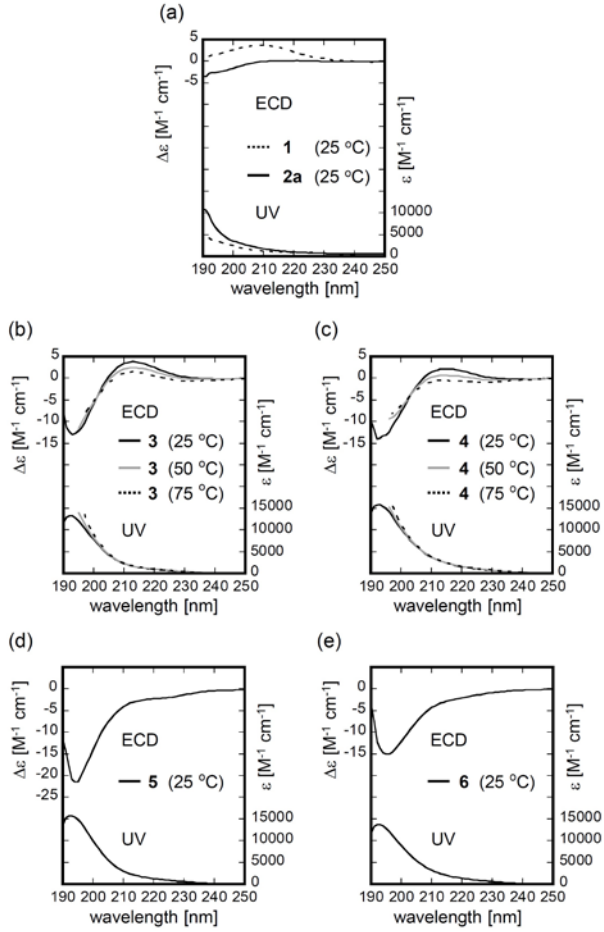


FIGURE 2 ECD (top) and UV (bottom) spectra of (a) **1** and **2a**, (b) **3**, (c) **4**, (d) **5**, and (e) **6** in 50% CH_3OH-H_2O measured at various temperatures.

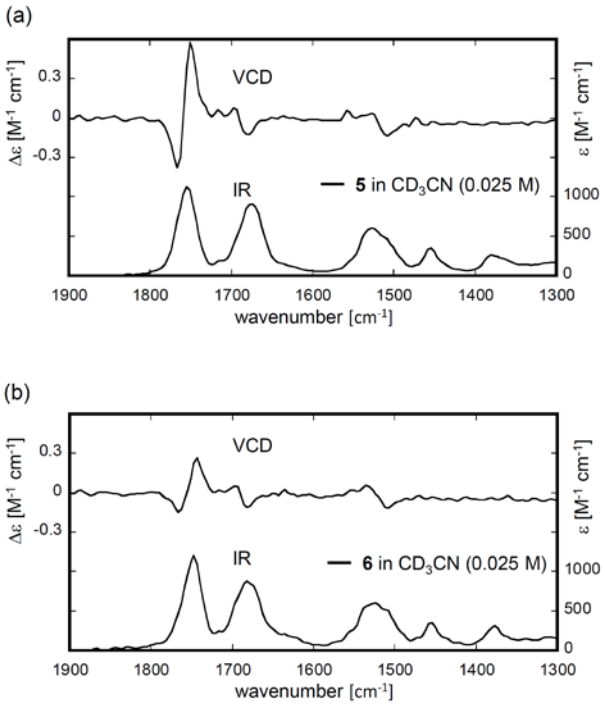


FIGURE 3 Comparison of VCD spectra of (a) Ac-$^{L}Ala_3$-$^{D}Lac_3$-OMe (0.025 M), (b) Ac-$^{D}Lac_3$-$^{L}Ala_3$-OMe (0.025 M). All spectra were measured in CD_3CN.

Influence of the helicity of oligoester moiety to oligopeptide moiety

Left-handed helical $^{L}Lac_3$ stabilized a left-handed PPII-like conformation of an adjacent $^{L}Ala_3$. This finding interested us to study which $^{L}Ala_3$ conformation (e.g. left-handed PPII, right-handed α-helix, and β-strand) is induced by inversion of the helicity of the oligoester moiety. To this end, we studied the conformations of hexamers **5** and **6**, of which the right-handed helicity of the $^{D}Lac_3$ moiety was confirmed by VCD measurement (Table 2 and Figure 3). The ECD measurements of **5** and **6** were carried out in 50% CH_3OH-H_2O, resulting in a spectral pattern indicative of a β-strand structure (Figure 2d and 2e).[28] Although right-handed helical $^{D}Lac_3$ could not induce a right-handed helical $^{L}Ala_3$ conformation, this study showed the importance of the combination of the chirality of oligoester and oligopeptide moieties.

TABLE 2 Comparison of the peak positions of the exciton couplets of hexamers

	1		**3**		**4**		**5**	**6**
solvent	CD_3CN	CD_3OD	CD_3CN	CD_3OD	CD_3CN	CD_3OD	CD_3CN	CD_3CN
ester C=O stretching								
ν [cm^{-1}] at IR_{max}	1755	1759	1755	1759	1747	1751	1755	1747
ν [cm^{-1}] at $\Delta\varepsilon_1$ [a]	1751	1751	1744	1751	1744	1747	1767	1767
ν [cm^{-1}] at $\Delta\varepsilon_2$ [a]	1767	1767	1763	1771	1767	1767	1751	1744
amide I or I'								
ν [cm^{-1}] at IR_{max}	-	-	1674	1659	1678	1667	1674	1682
ν [cm^{-1}] at $\Delta\varepsilon_1$ [a]	-	-	1678	1643	1862	1659 [b]	(1678) [c]	(1682) [c]
ν [cm^{-1}] at $\Delta\varepsilon_2$ [a]	-	-	-	1682	-	1678 [b]		

[a]Wavenumber at the extrema of the first or second Cotton effect ($\Delta\varepsilon_1$ or $\Delta\varepsilon_2$, respectively). [b]Determined by the measurement at 0.017 M. [c]The first Cotton effect appeared at the same or higher frequencies compared to the IR peak maximum.

Conclusion

In this work, we incorporated oligo(lactic acid)s, a rigid structural motif we previously found, into ^{L}Ala hexamer sequences, and studied their conformations. These model compounds themselves may be difficult to use for biological systems due to the poor solubility to pure water, albeit low toxicity; however, this study provided useful insights into future applications of ester-substituted oligoesters. First, oligo(L-lactic acid) sequences maintained a left-handed helical structure even when incorporated into oligopeptides. Second, left-handed helical $^{L}Lac_3$ induced the formation of a left-handed PPII-like helix of adjacent $^{L}Ala_3$ on both the N- and C-terminal sides. Third, the chirality of oligoester sequences is important for the conformational outcome of neighboring oligopeptides. Last, introduction of ester linkage to oligoalanine increased its solubility to various solvents possibly due to suppression of aggregation. These insights should be useful in developing peptide analogues containing ester linkage with various amino acids and α-hydroxy acids that function better than normal oligopeptides.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher’s website.

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29. The positive ECD band of **3** and **4** observed at around 217 nm was not due to the ECD signals originating from $^{L}Lac_3$ moiety. See Figure S2.

Graphical Abstract