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$n \rightarrow \pi^*$ interactions in poly(lactic acid) suggest a role in protein folding

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Poly(lactic acid) (PLA) is a versatile synthetic polyester. We noted that this depsipeptide analog of polyalanine has a helical structure that resembles a polyproline II helix. Using natural bond orbital analysis, we find that $n \rightarrow \pi^*$ interactions between sequential ester carbonyl groups contribute 0.44 kcal mol^{-1} per monomer to the conformational stability of PLA helices. We conclude that analogous $n \rightarrow \pi^*$ interactions could direct the folding of a polypeptide chain into a polyproline II helix prior to the formation of hydrogen bonds between backbone amides.

Polyesters have found widespread utility due to their inexpensive preparation and structural integrity.[1] Poly(lactic acid) (PLA) is a recyclable polyester that can be prepared by condensing lactic acid, a renewable resource (Fig. 1).[2–4] The thermal and structural properties of PLA can be adjusted by varying the ratio of L- and D-lactic acid monomers, or by altering the polymer processing conditions. These ensuing materials have received significant attention amongst macromolecular scientists, especially for use in biocompatible and biodegradable devices.[2–5]

Interest in tuning properties of PLA has motivated the determination of its structure in atomic detail. These analyses have revealed the existence of conformational isomers, which arise from different preparation conditions.[6,7] The α form of PLA has received the most attention, due to its high stability.

Fibre diffraction of α-PLA has proven challenging, and the ensuing structure has been revised several times since its first report.[8–12] Recently, data from neutron diffraction and NMR spectroscopy have been used to complement X-ray diffraction data.[13,14] Though refinement of the structural model continues, the overall topology appears to be consistent between studies (Fig. 2A).

We became interested in PLA due to its similarity to polyalanine. Indeed, PLA is the depsipeptide counterpart to polyalanine wherein each amide linkage is replaced by an ester. The amide-to-ester modification has proven useful for revealing the contribution of hydrogen bonds to the structure and stability of peptides and proteins because incorporation of the ester linkage deletes a backbone hydrogen-bond donor and reduces the strength of a hydrogen bond with the carbonyl oxygen.[15–20]

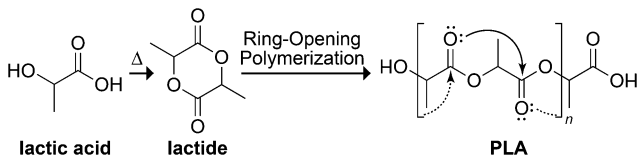

Fig. 1 Synthetic route to PLA. Curved arrows indicate putative $n \rightarrow \pi^*$ interactions.

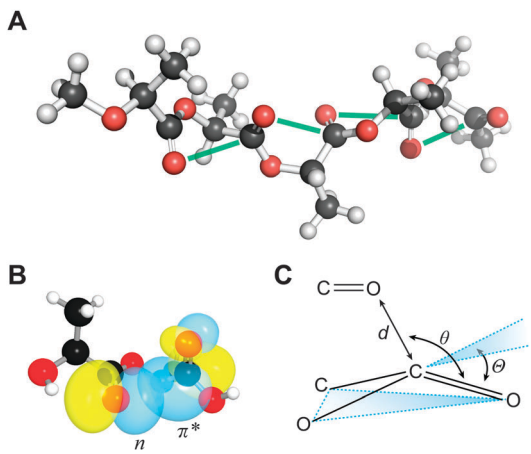

Fig. 2 $n \rightarrow \pi^*$ Interactions in PLA. (A) Five-residue segment of α-PLA from L-lactic acid (*i.e.*, isotactic PLLA).[12] Green lines show putative $n \rightarrow \pi^*$ interactions. (B) Overlap of the *n* and π^* orbitals in di(L-lactic acid) (CCDC refcode: DUZMER). (C) Structural parameters describing the $n \rightarrow \pi^*$ interaction and the resulting pyramidalization of the acceptor carbonyl.

By examining the structure of a peptide-like polymer that is incapable of forming intramolecular hydrogen bonds, we sought to isolate other interactions that bias the conformation of peptide chains. In particular, we wished to determine the role of the $n \rightarrow \pi^*$ interaction in dictating the conformational geometry of PLA. In an $n \rightarrow \pi^*$ interaction, the filled *p*-type lone pair (*n*) of a carbonyl oxygen overlaps with the empty π^* antibonding orbital of a nearby

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carbonyl group (Fig. 1 and 2B). This overlap allows for orbital mixing and the subsequent release of energy. Such an interaction occurs when the donor oxygen contacts the acceptor carbonyl carbon within the sum of their van der Waals radii ($r_O + r_C$ = 3.22 Å), and along the Bürgi–Dunitz trajectory for nucleophilic addition ($\angle O\cdots C{=}O = \sim 109^\circ$).[21] We have estimated that $n\rightarrow\pi^*$ interactions between amides likely contribute 0.27 kcal mol^{-1} of stabilization energy per interaction,[22] and we have shown that these interactions are present in protein structures, especially helices.[23] The question remains, however, does the $n\rightarrow\pi^*$ interaction bias a peptide toward a particular conformation, or is the interaction an artefact of a particular structural motif? By examining the structure of a polymer devoid of hydrogen bonds, we hoped to ascertain the relevance of the $n\rightarrow\pi^*$ interaction to macromolecular conformation.

Upon inspection of the structure of α-PLA,[12] we observed that its backbone torsion angles (Table 1) bear striking similarity to those of the polyproline II helix, which has backbone torsion angles of $\phi(C_{i-1}'\text{–}N_i\text{–}C_i^\alpha\text{–}C_i') = -75^\circ$ and $\psi(N_i\text{–}C_i^\alpha\text{–}C_i'\text{–}N_{i+1}) = 150^\circ$, and the strands of a collagen triple helix.[24] We showed previously that these torsion angles allow for effective $n\rightarrow\pi^*$ interactions.[23] Indeed, the average O···C distance in the α-PLA structure is 2.98 Å, which is 0.24 Å less than the sum of the van der Waals radii; moreover, the average $\angle O\cdots C{=}O$ is 94°, which is consistent with an $n\rightarrow\pi^*$ interaction.[23]

To evaluate whether or not an $n\rightarrow\pi^*$ interaction is operative in the structure of α-PLA, we conducted natural bond orbital (NBO) analysis of its crystalline structure at the B3LYP/6-311+G(2d,p) level of theory.[25–27] We observed an average $n\rightarrow\pi^*$ energy of 0.44 kcal mol^{-1} per interaction. This value is consistent with a strong $n\rightarrow\pi^*$ interaction between the carbonyl groups of adjacent backbone esters in α-PLA.

To establish further the presence of an $n\rightarrow\pi^*$ interaction, we searched for a structural signature. As the $n\rightarrow\pi^*$ interaction populates the π^* orbital of the acceptor carbonyl, it induces a pyramidalization of the carbonyl group from planar sp^2 geometry (Fig. 2C), which can be detected in high-resolution crystal structures.[22,28–31] Unfortunately, the initial α-PLA structure-determination assumed planarity of the ester bond,[12] thereby obscuring the most definitive signature of an $n\rightarrow\pi^*$ interaction. Later structures do not provide enough resolution to determine pyramidalization accurately.[13] Accordingly, we sought high-resolution structures of lactic-acid oligomers.

We analysed structures of di(L-lactic acid) and tri(L-lactic acid), which were obtained from the Cambridge Structural Database.[32]

Table 1 Structural parameters and $n\rightarrow\pi^*$ energies of lactic acid polymers

Polymer	ϕ (°)	ψ (°)	d^d (Å)	θ^d (°)	$E_{n\rightarrow\pi^*}{}^e$ (kcal mol^{-1})	Θ^d (°)
α-PLA[a]	−63.7	154.4	2.98	93.6	0.44	ND
Di(L-lactic acid)[b]	−69.2	148.0	2.90	102.0	0.67	2.62
Tri(L-lactic acid)[c]	−69.2	163.5	2.98	92.7	0.41	3.40

[a] Values are the mean over five consecutive L-lactic acid residues.[12] For an image, see: Fig. 2A. ND, not determined. [b] Values are the mean from two molecules in the unit cell of CCDC refcode DUZMER. For an image, see: Fig. 2B. [c] Values are the mean from three residues in CCDC refcode DUZMIV. [d] For definitions, see: Fig. 2C. [e] Values are from second-order perturbation theory.

To ensure that these short oligomers are appropriate models for the structure of α-PLA, we compared their backbone torsion angles to those observed in α-PLA and found gratifying agreement (Table 1). We also employed DFT calculations and NBO analysis to estimate the energy of the $n\rightarrow\pi^*$ interaction in these molecules and found that the $n\rightarrow\pi^*$ energies are consistent with those observed in the polymer. Confident that these structures are an accurate reflection of the structure of α-PLA, we then determined the distortion of the backbone esters from planarity, as measured by the angle Θ. In both structures, we observed substantial pyramidalization of the putative $n\rightarrow\pi^*$ acceptor toward the putative donor. In the absence of an attractive interaction, one would expect distortion to occur in the opposite direction, so as to reduce unfavorable Pauli repulsion.[33] Accordingly, the observed pyramidalization is strong evidence of an attractive $n\rightarrow\pi^*$ interaction between the monomeric units in α-PLA.

These observations have broad implications. First, they imply a new means to modulate the structure of organic polymers. We found previously that the nucleophilicity of sulfur in thioamides can be exploited to increase the strength of an $n\rightarrow\pi^*$ interaction[22,28] and that surrogate alkenes and fluoroalkenes can be used to attenuate an $n\rightarrow\pi^*$ interaction.[33] These isosteres[20] could be used to produce polymeric materials with tailored structural and thermal properties. Secondly, as the $n\rightarrow\pi^*$ interaction is likely to reduce the electrophilicity of the acceptor carbonyl by contributing additional electron density,[34,35] it could contribute to the observed hydrolytic stability of PLA. Thirdly, because $n\rightarrow\pi^*$ interactions are extant in PLA even without the potential for intramolecular hydrogen bonding, we conclude that the $n\rightarrow\pi^*$ interaction can operate independently of the geometric constraints imposed by hydrogen-bonding patterns. Finally, the observation of polyproline-like structure in PLA is itself significant, given that this structural motif is prevalent in the unfolded state of proteins.[36–40] During their folding, polypeptide chains are likely to sample highly local interactions sooner than less local ones. Operating between adjacent residues (that is, $i\rightarrow i{+}1$), the $n\rightarrow\pi^*$ interaction is considerably more local than common hydrogen-bonding patterns such as that in the α-helix ($i\rightarrow i{+}4$). Thus, the presence of $n\rightarrow\pi^*$ interactions in the structure of PLA suggests that—before hydrogen bonds can form—the conformation of polypeptide chains can be guided by $n\rightarrow\pi^*$ interactions.[41]

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