Synthesis and folding characteristics of polydepsipeptides

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Statement of purpose: In building synthetic, biologically-based materials, researchers have investigated strategies to exploit secondary structures to encourage functionality similar to naturally occurring proteins[1]. Polydepsipeptides (PDPs) have alternating ester and peptide bonds and have previously been shown to maintain regular secondary folding[2]. This study aims to synthesize and investigate the secondary folding characteristics of polydepsi(Gly), polydepsi(Lys), and polydepsi(Asp) showing that regular secondary structure can be modeled and maintained within PDPs.

Methods:

Reagents. Bromopropionyl-glycine (BPG), and poly-L-alanine (poly(Ala)) were purchased from Sigma Aldrich (St. Louis, MO). Fmoc-L-Lys(Z)-OH, Fmoc-L-Asp(OBzl)-OH, and dimethylaminopyridine (DMAP) were from Novabiochem/EMD Biosciences (San Diego, CA). Trityl chloride resin and diisopropylcarbodiimide (DIC) were from Anaspec (San Jose, CA).

Polydepsi(Gly). The cyclic intermediate was prepared with BPG dissolved in excess DMF to a slurry of potassium carbonate and DMF under nitrogen at 65ºC for 24 hours. Potassium carbonate was removed by centrifugation. The cyclic intermediate was concentrated and polymerized with $SnOct_2$ in a siliconized vessel at 120ºC for 24 hours under vacuum.

Polydepsi(Lys) and polydepsi(Asp). The polymers were synthesized using solid phase chemistry on a trityl chloride resin. The resin was functionalized with L-lactic acid using DIPEA in DCM and coupled to the desired Fmoc-peptide with DMAP and DIC. The N- and C-terminal groups were exposed with 20% piperidine in DFM or 1% TFA in DCM, respectively. The deprotected groups were then coupled with DIC to achieve a depsipeptide repeat (Figure 1). The deprotection and coupling method are repeated until 12 depsipeptide units are achieved.

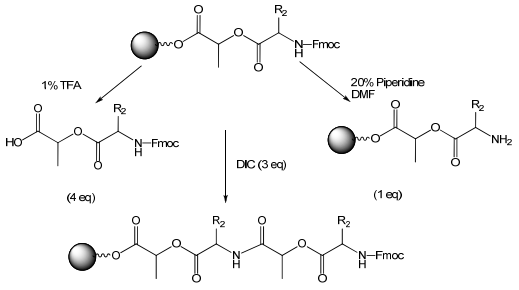

Figure 1. Stepwise growth of polydepsipeptides. R_2 = Lys(Z) or Asp(OBzl).

Circular Dichroism. Polydepsi(Gly) and poly(Ala) were dissolved in water and dichloroacetic acid respectively 0.1, 0.05, or 0.005 mg/ml and concentrated in 90% trifluoroethanol. CD data was collected at room temperature unless noted, within 180-250 nm. Collected data that exceeded 800 V was dismissed as noise. FTIR spectra of the PDPs and controls were collected on polyethylene cells. All samples (0.1 mg/ml) were referenced with varying buffers within the wavenumber range of 400-4000 cm^{-1}.

Results/Discussion: The synthesis of cyclodepsi(Gly) and its corresponding PDP are confirmed with H-NMR and are consistent with the literature[3]. Intermediates of polydepsi(Lys) and polydepsi(Asp), specifically of 2 and 4 repeats have been confirmed with mass spectroscopy. The larger masses of the longer repeats are analyzed with MALDI. CD spectra of polydepsi(Gly) and poly(Ala) revealed α-helix structure with maximum ellipticity at 190 nm and minima at 208 and 222 nm for the control in 90% TFE and a β-sheet structure with a maximum ellipticity at 198 nm and minimum at 218 nm in 60% TFE. Polydepsi(Gly) in 90% TFE reveals a β-sheet structure with a slight transition to a β-turn conformation, evident at 209 nm (Figure 2). The FTIR spectra share similar peak patterns for polydepsi(Gly) and poly(Ala), specifically at 1658 cm^{-1} and 1567 cm^{-1}.

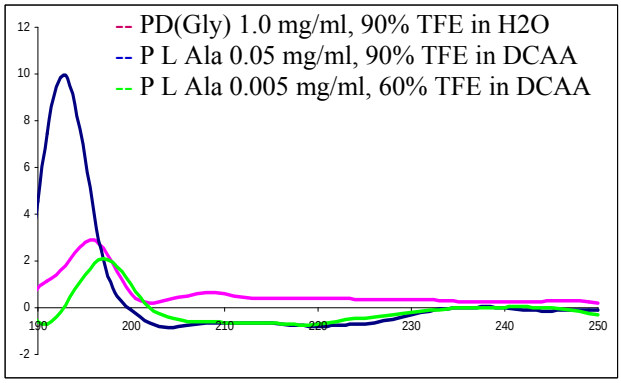

Figure 2. CD of polydepsi(Gly) and poly(Ala)

Computational evaluation of depsipeptide oligomers are consistent with our results[4]. Alternating oligodepsipeptide sequences (12mers), upon simulated annealing and replica exchange molecular dynamics simulations, are both right- and left-handed helices for the Gly-based oligomer and PPII for the Lys-based oligomer. The folding analysis of the 12-mer depsi(Lys) will be compared to these results.

Conclusion: A major motivation towards this current synthesis of PDPs lies in the relative ease in tailoring functionality within the chemical structure. Despite substitutions of the amide bond at alternating positions by ester functionality, polydepsi(Gly) folds into naturally occurring secondary structures that are sensitive to specific environmental conditions. Having the ability to control the folding of a molecule allows for tailored materials with specific functionalities.

References:

1. Li, J. H. et al, *Chinese Science Bulletin* 2008
2. Becktel et al, *Macromolecules* 1981
3. Barrera, D. A. et al, *Macromolecules* 1995
4. Zhang, J. J. et al, *Biomacromolecules* 2007