Synthesis and folding characteristics of polydepsipeptides

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Statement of Purpose:

Biomaterials for tissue engineering and regenerative medicine are designed to approximate the correct cellular environment and allow for the regeneration of damaged or diseased tissue. These materials should be biocompatible, simple to synthesize, and have degradation products that are bioresorbable. Polyesters, such as poly(lactic acid (Lac)), satisfy the above conditions, but their biologic activity is uncontrolled, and they lack biomimetic properties. Biological functionality can be incorporated to synthetic polyesters as polydepsipeptides (PDPs) (Figure 1), or modified polypeptides. This work describes the solution phase synthesis, characterization, and theoretical and analytical folding of polydepsi(glycine (Gly)). We are also exploring the synthesis of functional PDP cyclic intermediates via resin chemistry, and the secondary folding properties of the subsequent PDP.

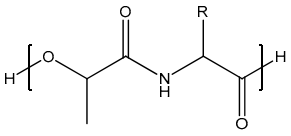

Figure 1: One repeating unit of Lac-based PDP

Methods:

Solution phase synthesis: PDPs are products of the ring-opening polymerization of cyclic intermediates. Synthesis of these monomers has been achieved via both solution and solid phase methods, the later with the support of a resin. In the first method, bromopropionyl glycine dissolved in dimethylformamide (DMF) was added to a slurry of finely ground potassium carbonate in DMF at 65ºC for 24 hours under nitrogen. Upon reaction termination, potassium carbonate was removed and DMF was evaporated.

Resin-based synthesis: Functional cyclic intermediates have been synthesized with resin chemistry. First, a linear monomer is synthesized with Lac loading onto a trityl chloride resin, then mixed overnight with fluorenylmethyl carbamate (Fmoc)-peptide, diisopropylcarboiimide (DIC), and dimethylaminopyridine (DMAP). The Fmoc-amide group and depsipeptide are removed, and cyclization of the linear monomer occurs on a carbodiimide (CDI) functionalized polystyrene resin. The cyclic products are purified with high performance liquid chromatography (HPLC) and characterized with proton NMR and mass spectroscopy.

Polymerization: Dry toluene was added to cyclic intermediate with SnO_2 in a siliconized vessel for polymerization. The toluene was evaporated, and the reaction proceeded at 120ºC for 24 hours.

Degradation is evaluated *in vitro*. The PDP is suspended in 2 ml phosphate buffer solution (PBS) for up to 6 weeks at 37 C, and the pH is maintained at pH 7 with 0.1 N NaOH. Upon data collection, samples are frozen, dried under vacuum, and analyzed for changes in molecular weight.

The ordered, secondary conformations of the PDP were evaluated with circular dichroism (CD). Samples were prepared with a concentration of 0.1 mg/ml in the appropriate buffer at room temperature unless otherwise noted. Additional samples were prepared in 10% 0.1 N NaOH/0.1 M KH2PO4 buffer at pH 6, 7, 8.2, and 10.9.

Results / Discussion:

Our group has synthesized the cyclic monomer of Gly with 93% conversion from the linear monomer in solution and characterized this molecule by proton NMR spectroscopy in deuterated DMSO. The relevant chemical shifts are: δ= 4.97 (q, 1H), 3.92 (d, 1H), 4.18 (d, 1H), 1.37 (d, 3H). NMR analysis of polydepsi(Gly) reveals a homogenous environment upon polymerization.

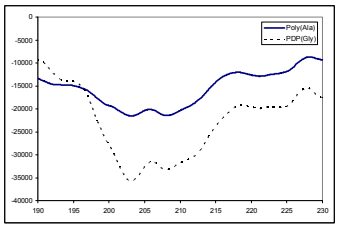

Figure 2: CD spectra of poly(Ala) and polydepsi(Gly) in water at 25 C. X axis: wavelength (nm); y-axis: ellipticity (deg cm^2/dmol)

The folding properties of polydepsi(Gly) were analyzed with CD, under the hypothesis that maintenance of secondary structure in a synthetic material may give rise to biomimetic properties. The folding of polydepsi(Gly) is similar to the polypeptide control, poly(Ala), which folds into a polyproline II (PPII) structure, characteristic of a strong negative band from 200-210 nm[1] (Figure 2). Helix-to-coil transitions dominate as polydepsi(Gly) is heated to 85°C. Half of the peptide bonds of poly(Ala) are replaced by ester bonds in the tested PDP, which may be responsible for the thermal instability of the secondary structure. Changes in pH did not show any significant effects on the secondary folding of polydepsi(Gly).

Experimental results of polydepsi(Gly) secondary folding is confirmed with previous results[2], indicating conformations that resemble the kind of secondary structure found in naturally occurring proteins can be obtained by controlling the chemistry of PDP side groups. We have shown a simple mechanism for a synthetic material that can exhibit biological characteristics similar to its natural counterpart.

References:

1. Shi Z, Olson CA, Rose GD, Baldwin RL, Kallenbach NR. *Proc Nat Aca Sci*, 2002;99(14):9190-9195.
2. Zhang JJ, King M, Suggs L, Ren PY. *Biomacro*, 2007;8(10):3015-3024.