Internal dynamics of poly(glutamic acid) arborescent polymers probed by pyrene excimer formation

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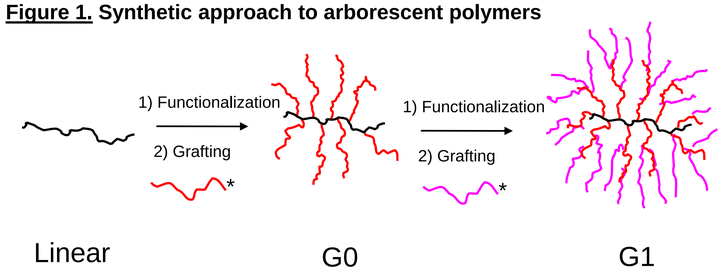
Introduction

Arborescent polymers are obtained by a "grafting onto" method which produces essentially monodispersed samples (Scheme 1). The synthesis of arborescent poly(L-glutamic acid) (PGA) has been previously reported.[1] The globular topology of these macromolecules resembles the tertiary structure of a protein, which makes them useful as model compounds to probe the effect of crowding on chain dynamics.

Figure 1. Synthetic approach to arborescent polymers

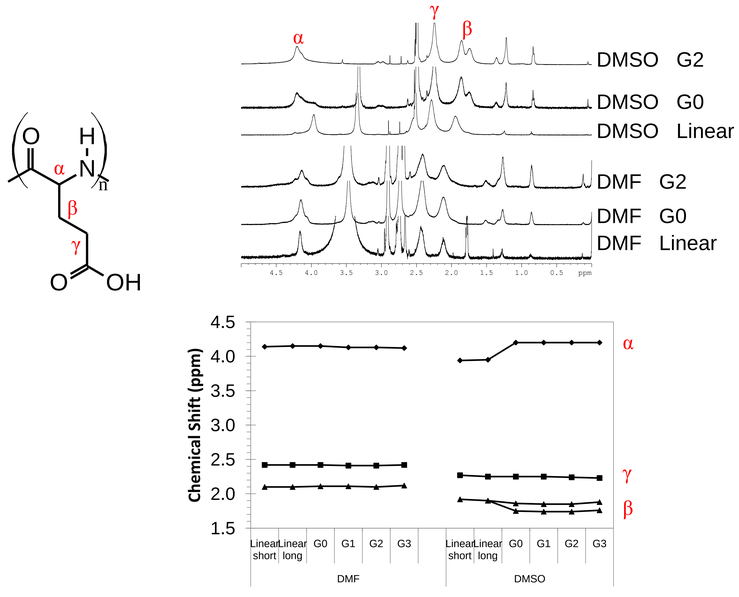


Background

Fluorescence has been used to probe the chain dynamics of linear PGA randomly labeled with pyrene in N,N-dimethylformamide (DMF).[2,3] Application of this analysis technique to macromolecules with a branched architecture, that exhibit randomly coiled and α-helical secondary structures, is expected to provide valuable information on the effect that crowding inside proteins has on the side-chain dynamics of short stretches of amino acids.

NMR experiments were first conducted to find solvents that induce conformation changes in arborescent PGAs. The NMR peaks for the protons attached to the alpha and beta carbons of the glutamic acid units were found to shift to different positions in DMF and dimethylsulfoxide (DMSO), indicating distinct microenvironments. These results confirm that DMF induces an α–helical conformation in the branched PGA molecules, in analogy to linear PGA, while a predominantly randomly coiled conformation is observed in DMSO. This analysis takes into account the chemical shift differences observed in the two different solvents and are analogous to results reported for the helix-to-coil transition of poly(γ-benzyl glutamate) in a mixture of chloroform and trifluoroacetic acid.[4]

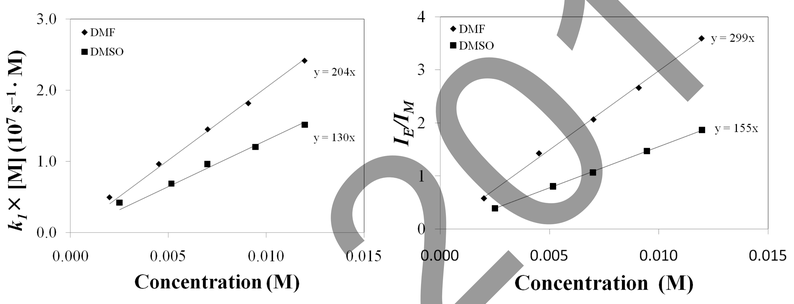
Figure 2. NMR results showing the helix to coil transition of PGA with the addition of branched PGA chains. PGA in DMF has an α-helix conformation. Top: NMR spectra. Bottom: Chemical shifts of peaks. Inset: Glutamic acid repeat unit with labeled carbon atoms.



Introduction of a Fluorophore

Since the method to probe the internal dynamics of arborescent PGA relies on pyrene excimer formation, it is important to assess whether DMF and DMSO are suitable solvents for these fluorescence studies. Fluorescence data were obtained for 1-pyrenemethanol at concentrations ranging from 2 to 12 mM. Results obtained through Birks' Scheme analysis of time-resolved fluorescent decays of these solutions indicate that both DMSO and DMF are efficient at forming excimer (Figure 3). The product of the rate constant for excimer formation, k_1, and the concentration of 1-pyrenemethanol in solution, [M], indicates that excimer formation in DMF is more efficient. Furthermore, the fluorescence intensity ratio of the excimer to monomer (I_E/I_M) also indicates that excimer formation is enhanced in DMF, and that DMSO is likewise a suitable solvent for this project. Any differences between I_E/I_M for the polymers randomly labeled with pyrene and these homogenous solutions can serve to probe the internal dynamics of the arborescent polypeptides.

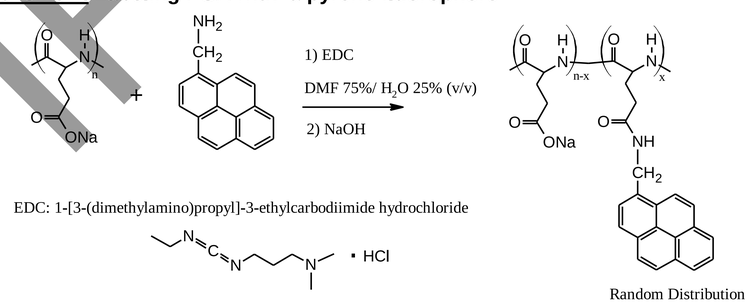
Figure 3. Florescence analysis of 1-pyrenemethanol in DMF and DMSO. Left: Birks' Scheme results. Right: Fluorescence intensity ratio.



Poly(L-glutamic acid) labeled with pyrene

1-Pyrenemethylamine was randomly and covalently attached to a low percentage of PGA structural units according to Scheme 2. Excess pyrene was removed by liquid-liquid extraction in hexane and dialysis in methanol and water. Size exclusion chromatography with a fluorescence detector was used to confirm that the samples were free of unattached fluorescent labels.

Scheme 2. Labeling PGA with a pyrene fluorophore



Determination of the pyrene content of the sample was achieved using UV-Vis spectroscopy by applying the Beer-Lambert Law

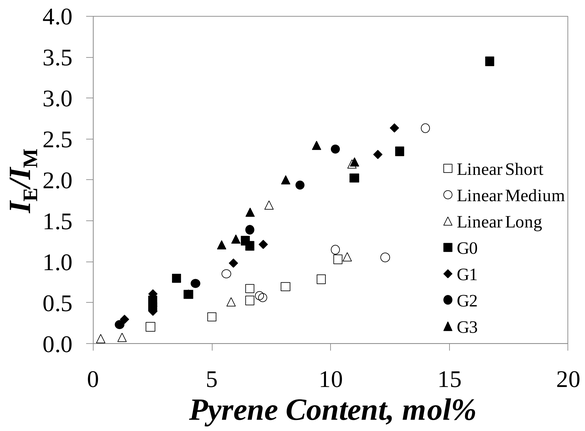
$$Abs = \varepsilon [Py] l$$

where ε= 40 000 L mol^{-1} cm^{-1} for N-(1-pyrenylmethyl)acrylamide in DMF.[2]

Steady-State Fluorescence

As increasing amounts of pyrene are attached onto the PGA, more excimer is formed. The polymer samples in DMSO (Figure 4) indicate that linear PGA forms less excimer as a result of its rigid helical conformation. The arborescent polymers, occurring in a randomly coiled conformation, have higher chain mobility favouring excimer formation, and yielding a steeper slope. The general trend deviates somewhat from linearity due to local clustering of the pyrene moieties enhancing excimer formation.

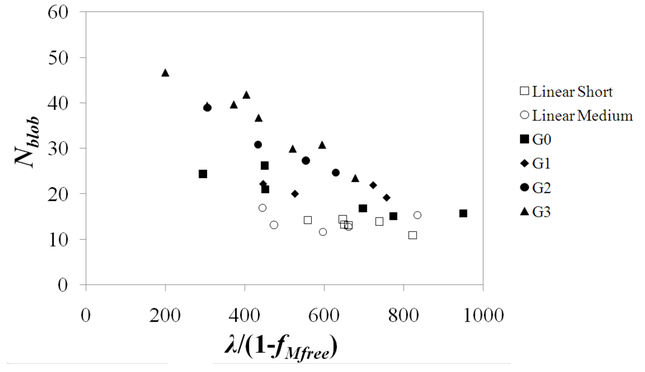
Figure 4. Excimer formation expressed as I_E/I_M as a function of pyrene content in DMSO. λ_{ex} = 344 nm, [Pyrene] ≈ 2.5x 10^{-6} M



Data Analysis

Quantitative information describing the internal dynamics of the poly(L-glutamic acid) samples can be obtained from the analysis of the time-resolved fluorescence decays. The amount of excimer formed is a measure of the internal dynamics of the macromolecules probed by the excited pyrene monomer. The Fluorescence Blob Model (FBM) can be used to analyze the fluorescence decays. Unlike Birks' Scheme which is used to study pyrene end-labeled polymers, the FBM accounts for the distribution of rate constants for excimer formation associated with random labeling of the chain with pyrene. Analysis of the fluorescence decays for the arborescent PGA is an on-going process. The preliminary results shown in Figure 5 demonstrate a difference in the overall size of the polymer coils. N_{blob} is a parameter derived from the FBM which describes the number of repeat units confined to a finite region called a *blob*, defined as the volume of the macromolecule probed by an excited pyrene before relaxing back to the ground state. In Figure 5, it is clear that as the generation number of the molecules increases, the blob size increases, reflecting a more crowded interior for the arborescent polypeptides.

Figure 5. N_{blob} as a function of pyrene content for the labeled PGA samples in DMSO.



Acknowledgements and References

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3. Duhamel, J.; Kanagalingam, S.; O'Brien, T.; Ingratta, M. *J. Am. Chem. Soc.* 125, **2003,**12810-12822.
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