

## Crosslinking of poly( $\gamma$ -dec-1-enyl-L-glutamate) and poly( $\gamma$ -dec-1-enyl-co- $\gamma$ -alkyl-L-glutamate) through interchain olefin metathesis

Drew S. Poché, Laried M. Malter, Jeannine M. Perrault

Department of Chemistry and Physics, Southeastern Louisiana University,  
Hammond, LA 70402, USA

Received: 25 May 1999/Accepted: 17 June 1999

### Summary

Poly( $\gamma$ -dec-1-enyl-L-glutamate) and poly( $\gamma$ -dec-1-enyl-co- $\gamma$ -alkyl-L-glutamate), helical polypeptides with pendent, terminal C-C double bonds in the side chains, were crosslinked through an intermolecular olefin metathesis reaction using Grubbs' ruthenium catalyst. Polymer samples were crosslinked at a variety of concentrations and with varying content of pendent C-C double bonds. Base promoted hydrolysis of the resulting gels was carried out to determine the nature of the chemical crosslink in the gel.

### Introduction

In recent years Ru complexes developed by Grubbs and co-workers (1-5) have been used to catalyze ring closing olefin metathesis reactions to form 5-8 membered carbocycles or heterocycles. Furstner and Langemann (6) also found that large macrocycles could be formed using the same catalyst. In contrast to these intramolecular reactions, Clark and Ghadiri (7) carried out an intermolecular olefin metathesis using Grubbs Ru catalyst to covalently link cyclic peptides into a supramolecular, cylindrical structure. The latter two studies demonstrated the potentially broad application of the Grubbs catalyst in olefin metathesis reactions and suggested its usefulness in crosslinking certain polymers containing pendent C-C double bonds. However, it is apparently necessary to have a polar functional group (e.g., a carbonyl group) in close proximity to the C-C double bond which coordinates with the Ru in the catalyst to aid in properly positioning the C-C double bond for the metathesis reaction (6).

Herein we describe the use of Grubbs' catalyst to crosslink poly( $\gamma$ -dec-1-enyl-L-glutamate) and poly( $\gamma$ -alkyl-L-glutamate)s (PALGs) containing varying amounts  $\gamma$ -dec-1-enyl-L-glutamate repeating units (8) through an intermolecular olefin metathesis reaction. Reactions involving the modification of the pendent double bond of these polymers have

been previously described (8). The structures of these polymers, represented schematically in Figure 1, are known (9) to exist as stiff, rod-like polymers in organic solvents due to their  $\alpha$ -helical secondary conformation. This fact is useful in computing chain dimensions as described later in this paper. Although the crosslinking of PALGs can be accomplished by heating the polymer in the presence of a diamine (10-11), the intermolecular olefin metathesis reaction described here offers a gentler, faster (typically a few minutes) method for crosslinking without potentially deleterious reactions occurring on the backbone of the polypeptide. The method is broadly applicable for the crosslinking of a variety of PALGs by simply copolymerizing the N-carboxyanhydride (NCA) monomer of choice with the appropriate quantity of  $\gamma$ -dec-1-enyl-L-glutamate NCA monomer.

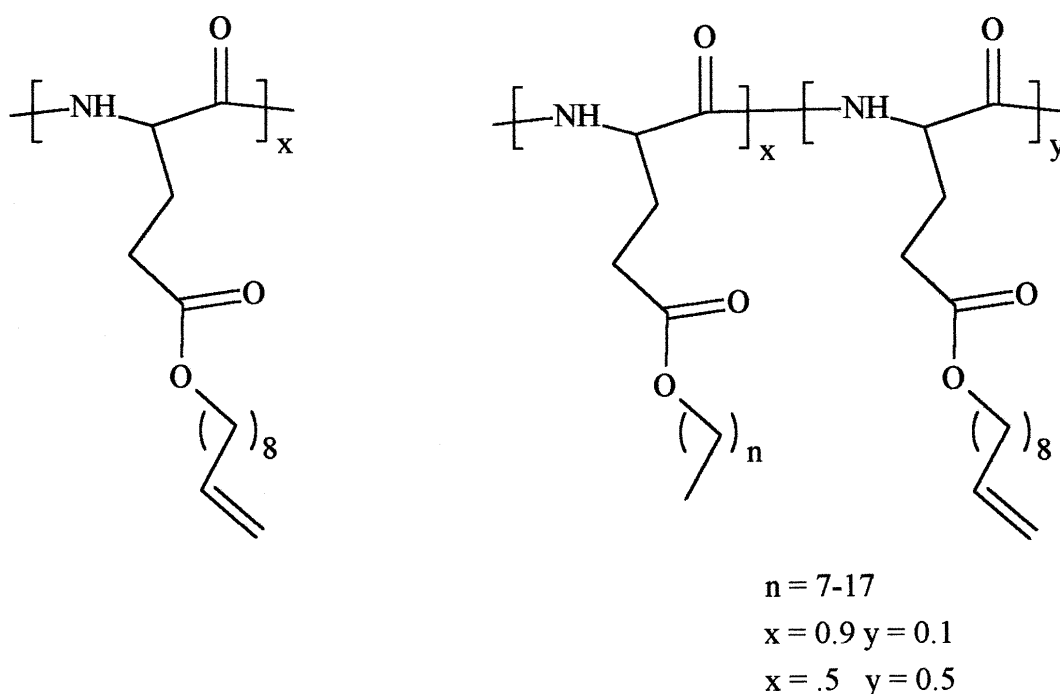


Figure 1. Structure of the PALGs used in the crosslinking reactions.

## Experimental

The chain dimensions reported in Figure 3 were measured using a ThermoSeparations model KMX-6 low angle laser light scattering detector in the "batch" mode (HeNe laser, 633 nm). Scattering was measured from tetrahydrofuran (THF) solutions in the concentration range of 0.2-20 mg/mL. The THF was distilled from potassium metal immediately prior to use. The refractive index increment,  $dn/dc$ , of poly( $\gamma$ -dec-1-enyl-L-glutamate) was measured at 633 nm on a Brice-Phoenix differential refractometer and found to be  $0.082 \pm 0.004$  mL/g. Light scattering data were then used to calculate chain dimensions in the usual manner, with the weight average molar mass,  $M_w$ , extracted

from y-intercept of a plot of reduced scattering vs. concentration, and the osmotic second virial coefficient,  $A_2$ , extracted from the slope of the linear fit.

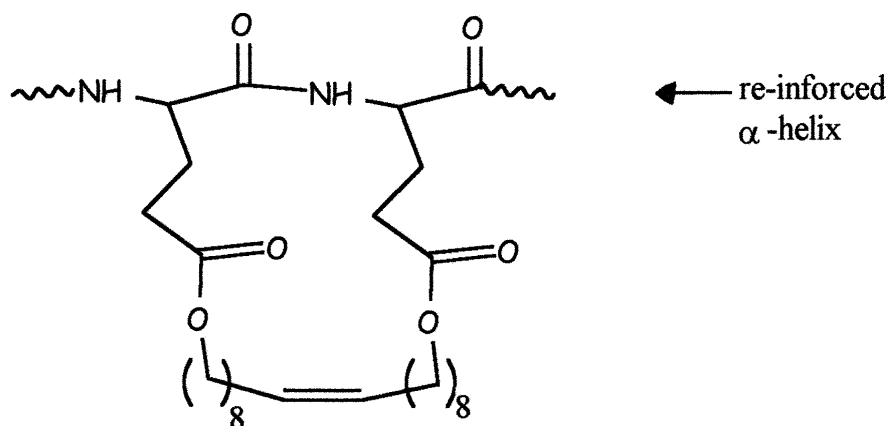
The synthesis of the polymers shown in Figure 1 is reported elsewhere (12). Grubbs' catalyst, benzylidene-bis(tricyclohexylphosphine)dichlororuthenium, was purchased from Fluka Chemical Co. and used as received. A typical example of the crosslinking reaction is as follows: A solution of 0.2 g of polymer in 4 mL of dichloromethane (DCM) was prepared in a glass vial with a teflon lined cap. It was then mixed with 10 mg of Grubbs catalyst dissolved in 1 mL of dichloromethane. The capped vial was placed in a water bath at 40°C until a stiff gel was noted. Typically, reaction times well under 30 minutes produced the gel. The DCM catalyst solution was initially a bright purple color. After the reaction, the gel was yellow or brown presumably due to entrapped, oxidized catalyst residue. To date, we have not found a solvent suitable for removing the spent catalyst residue from the gel.

A typical example of film formation is as follows: A ca. 10% w/v solution of the polymer in DCM was poured into a shallow glass dish. The dish was warmed until the solution ceased to flow. It was then uncovered to allow the DCM to evaporate from the thin gel. An optically clear film or membrane resulted.

The crosslink moiety formed from the metathesis reaction (shown in Figure 4, structure **A**) was isolated from the gel using base catalyzed hydrolysis of the gel. The gel was refluxed for 3 days in ca. 20% aqueous KOH. The cooled reaction was extracted with hexane to isolate the crosslink. After removing the hexane by evaporation, the residue was vacuum dried overnight and analyzed by FT-IR and  $^1\text{H}$  and  $^{13}\text{C}$  NMR to verify the structure of the crosslink.

## Results and Discussion

The gel produced by the intermolecular olefin metathesis qualitatively appeared quite stiff and tough. We also noted no flow of the material when the reaction vial containing it was inverted for an extended period of time (e.g., several weeks). We applied Grubbs catalyst to both poly( $\gamma$ -dec-1-enyl-L-glutamate) and poly( $\gamma$ -decyl-L-glutamate) containing varying quantities of the pendent C-C double bond. We also varied the concentration of the polymer to determine the lower concentration limit necessary to form a gel. Poly( $\gamma$ -dec-1-enyl-L-glutamate) crosslinked readily at concentration exceeding 5% w/w. A 1% w/w solution did not appear to gel but the usual color change from purple to brown indicating spent catalyst was noted. The possibility of intrachain crosslinking (making big "loops" on the chain backbone) was considered but there is presently no data available to verify this structure. However, it is worth speculating about this possibility since such a structure would provide a chain architecture with a "reinforced"  $\alpha$ -helix. Figure 2 shows an over-simplified structure that schematically represents this loop formation. At very low concentrations of polymer, it is conceivable such a structure could form although it seems unlikely that pendent C-C double bonds on *adjacent* repeat units would metathesize due to their positions on an  $\alpha$ -helical backbone.



**Figure 2. Suggested structure formed by intrachain olefin metathesis reaction. Metathesis of double bonds on adjacent repeat units is probably not realistic.**

PALG copolymers containing  $\geq 10$  mol% decenyl side chains were observed to form gels in the metathesis reaction. When the PALG backbone was "loaded" with as little as 1 mol% decenyl side chains, no gelation was observed. At present, we are investigating methods of removing the spent catalyst residue from the gel without damaging its structure. Dialysis of any of these gels against a variety of solvents has failed to remove the residue.

For this study, we were interested in working with moderate to high molecular weight materials. It is known (13) that rods with an axial ratio (length/diameter) exceeding about 5 are capable of forming lyotropic liquid crystals. One of our goals in making these PALG gels is to "lock" an ordered macrostructure, due to the formation of a liquid crystalline phase, into the crosslinked network. It was useful, therefore, to have some knowledge of the chain dimensions prior to crosslinking. The chain dimensions can be computed from static light scattering data as demonstrated in Figure 3 for a typical poly( $\gamma$ -dec-1-enyl-L-glutamate) sample. These data were represented in a reduced scattering vs. concentration plot from which the weight average molar mass ( $M_w$ ), the osmotic second virial coefficient ( $A_2$ ), and the diameter of mutual exclusion ( $d_m$ ) were computed. The rod diameter was calculated using the Zimm-Schulz-Onsager (14-16) relationship below:

$$A_2 = \pi N_A d_m L^2 / 4M_w^2$$

where,  $N_A$  is Avogadro's number

$L$  is the length of the rod

This relationship relates the dimensions of a rod-like polymer to  $A_2$ . Since the value of  $A_2$  for a polymer-solvent-temperature combination is directly accessible through static

light scattering measurements, the dimensions of the rod can be readily computed. The value of  $L$  was computed using  $L = 0.15\text{nm}(M_w)/M_o$ , where  $0.15\text{ nm}$  is the known pitch/residue for an  $\alpha$ -helix and  $M_o$  is the repeat unit molar mass. The diameter computed was  $20\text{ \AA}$ , indicating that the C10 side chains are somewhat "folded" or "pushed down" along the polypeptide backbone in solutions of THF (17).

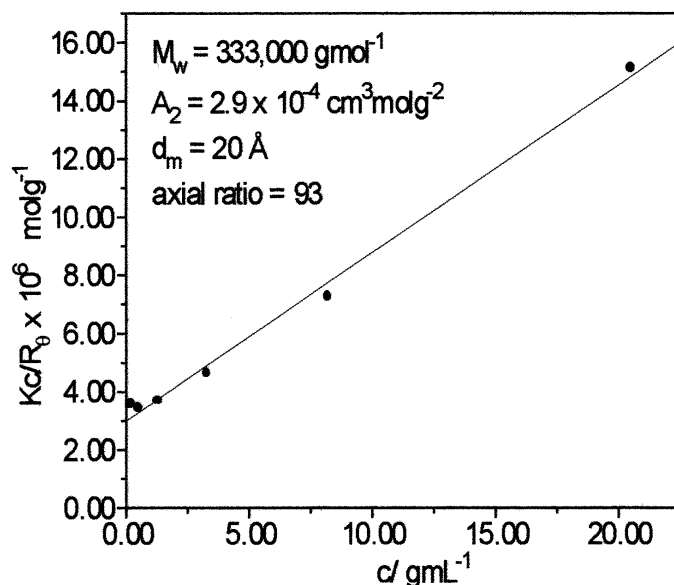


Figure 3. Chain dimensions for poly( $\gamma$ -dec-1-enyl-L-glutamate)

As shown in Figure 4, the crosslink tethers the macromolecular chains through ester linkages. Therefore, a sample of the gel was hydrolyzed in order to isolate the crosslink moiety in the polymer network. FT-IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data indicated a structure consistent with a usual olefin metathesis using Grubbs' catalyst. That is, the spectroscopic data indicated the pendent C-C double bonds in the side chains of the polymer reacted to form a new C-C bond with expulsion of ethylene. The structure, **A**, of the crosslink is shown in Figure 4.

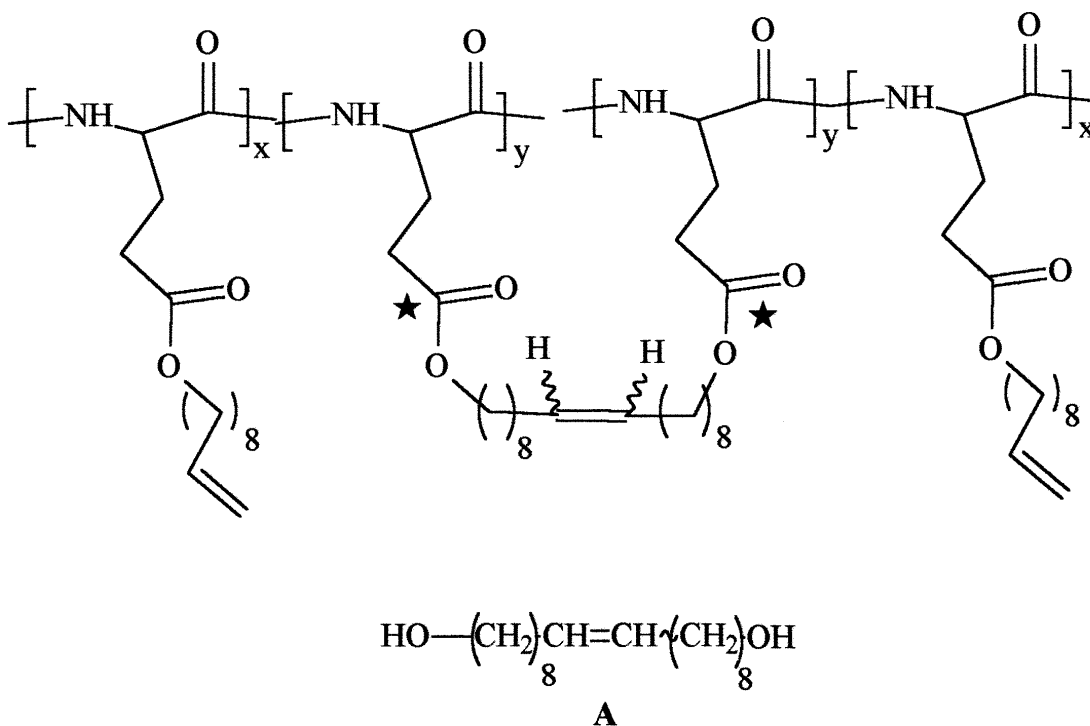


Figure 4. Hydrolysis of the gel at the ester linkages (marked with stars) yields the crosslink moiety. Subscript y represents the number of crosslinks; x, the number of unreacted terminal C-C double bonds.

### Acknowledgement.

The authors wish to thank the Louisiana Educational Quality Support Fund (LEQSF Research Competitiveness Program, LEQSF(1198-00)-RD-A-25) and Research Corporation (Cottrell College Science Award) for financial support of this project. DSP is also grateful for the financial support provided by Faculty Development Grants awarded through the College of Arts and Sciences at Southeastern Louisiana University. JMP acknowledges the financial support of the Federal Work/Study Program.

### References

1. Miller SJ, Blackwell HE, Grubbs RH (1996) *J Am Chem Soc* 118: 9606
2. Fu GC, Grubbs RH (1992) *J Am Chem Soc* 114:5426
3. Fu GC, Grubbs RH (1992) *J Am Chem Soc* 114:7324
4. Fu GC, Grubbs RH (1993) *J Am Chem Soc*. 115:3800
5. Fu GC, Nguyen ST, Grubbs RH (1993) *J Am Chem Soc* 115:9856
6. Furstner A, Langemann K (1997) *J Am Chem Soc* 119:9130
7. Clark TD, Ghadiri MR (1995) *J Am Chem Soc* 117:12364

8. Poché DS, Thibodeaux SJ, Rucker VC, Warner IM, Daly WH (1997) *Macromolecules* 30:8081
9. Block H (1983) *Poly( $\gamma$ -benzyl-L-glutamate) and Other Glutamic Acid Containing Polymers*. Gordon and Breach, New York
10. Aviram AJ (1976) *J Polym Sci Polym Lett Ed* 14:757
11. Kishi R, Sisido M, Tazuke S (1990) *Macromolecules* 23:3779
12. Poché DS, Moore MJ, Bowles J (1999) *Syn Comm* 29(5):843
13. Flory PJ (1982) In: Ciferri A, Krigbaum WR, Meyer RB (eds) *Polymer Liquid Crystals*. Academic Press, New York (p. 40)
14. Zimm BH (1946) *J Chem Phys* 14:164
15. Schulz GV (1947) *Naturforsch* 2a: 348
16. Onsager L (1949) *Ann NY Acad Sci* 51: 627
17. Poché DS, Daly WH, Russo PS (1995) *Macromolecules* 28: 6745