

Synthesis and Characterization of Biocompatible and Biodegradable Poly (ϵ -Caprolactone-*b*- γ -Benzylglutamate) Diblock Copolymers

Keywords: biodegradability • biocompatibility • biomaterial poly(ϵ -caprolactone) • polypeptide • poly- γ -benzylglutamate • block copolymer

INTRODUCTION

The successful block polymerization of a lactone or lactide and an amino acid might be major progress towards the preparation of biocompatible and biodegradable hydrogels and surfactants. Since the 70's, some aliphatic polyesters, e.g., polylactides (PLA) and poly(ϵ -caprolactone) (PCL), are well-known for their low toxicity and their hydrolytic and enzymatic biodegradability.¹⁻³ Accordingly, the association of these polyesters to a polypeptide block would provide versatile original materials. Indeed, the rate of biodegradation and the amphiphilicity of such di- and tri-block copolymers might be controlled by the nature and composition of the two components.

The ring-opening polymerization of unsubstituted lactones⁴ as well as of amino acid-*N*-carboxyanhydrides (NCA)⁵ has proved very efficient to control the molecular characteristics and the end-functionalization of the related

polyesters and polypeptides. Furthermore, NCA's are known to be polymerized by aliphatic primary amines in such a way that the initiator is attached to the growing chain and the propagation is living. ω -Amino prepolymers (PA-NH₂) are thus potential macroinitiators for the NCA's polymerization, which opens the way to P(A-b-peptide) copolymers, where PA can be polymethylmethacrylate,⁶ polybutadiene,⁷ polyethylene oxide,⁸ polystyrene,⁹ or polypropylene oxide.¹⁰ This communication reports the original preparation of completely biodegradable polypeptide-based block copolymers initiated by ω -amino PCL.

RESULTS AND DISCUSSION

Functional aluminum alkoxides such as Et₂Al—O—R—X (where X is a functional group) are effective initiators for the living polymerization of ϵ -caprolactone (ϵ -CL) and lactides leading to α -hydroxy, ω -X chains.⁴ Although halogen atoms, tertiary amines, and double bonds (e.g., of the methacrylic type) have been successfully

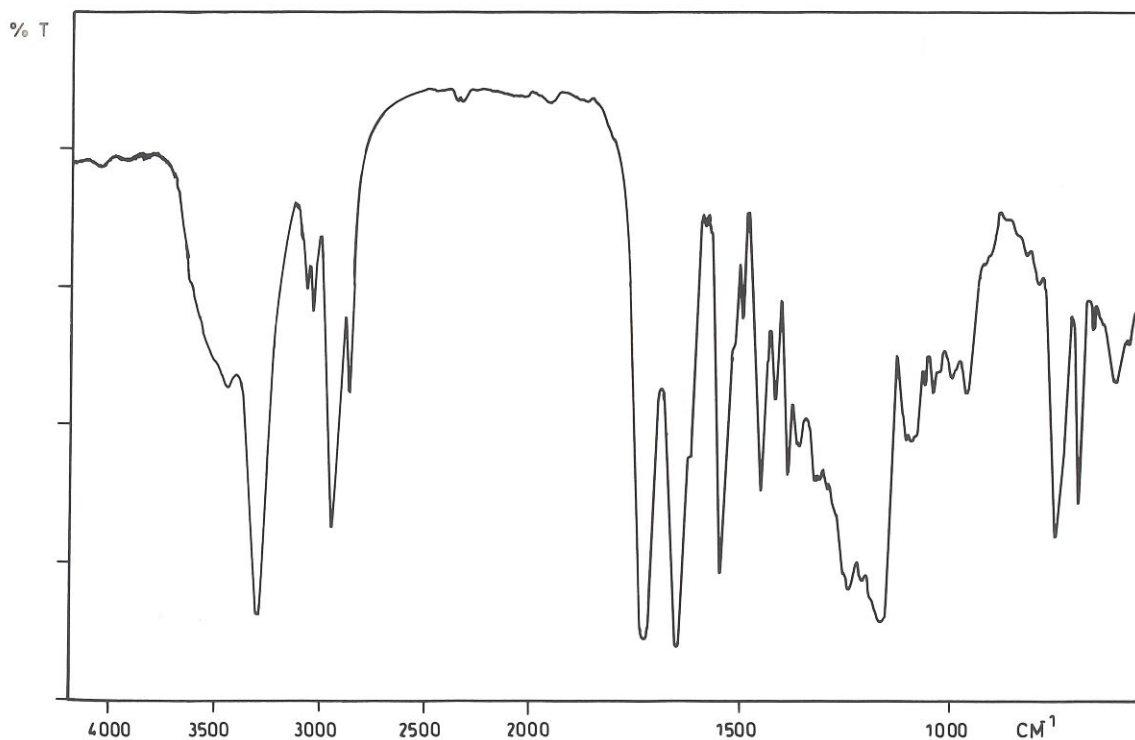
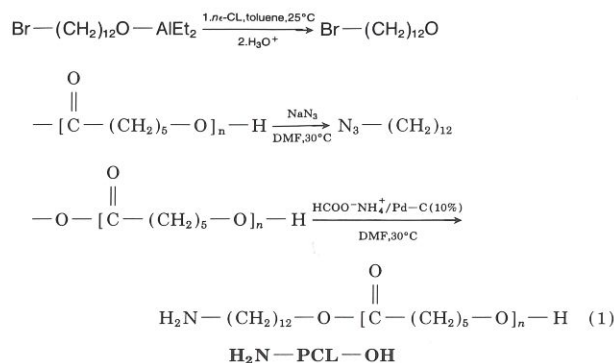
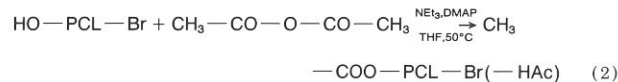


Figure 1. IR Spectrum of the copolymerization product (film).

used as the X functional group,^{11,12} a primary amine failed to provide the expected amino-terminated polyester. Indeed, diethylaluminum 3-amino, 1-propoxide led to the formation of α,ω -hydroxy PCL.¹³ Recently, an alternative pathway using an alkylbromide as the functional group led to the expected ω -NH₂ PCL.¹⁴ It was based on the conversion of the bromo end-group into an azido group which was subsequently reduced into the primary amine [eq. (1)]:

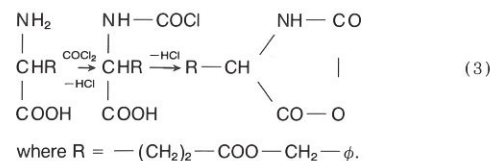


Before it can initiate the NCA polymerization, the hydroxyl end-group of α -hydroxy, ω -amino PCL has to be protected in order to avoid any side nucleophilic attack of the monomer. The protective reaction has been achieved onto the α -bromo, ω -hydroxy PCL by a quantitative reaction with acetic anhydride under basic conditions [eq. (2)]:



Obviously this protective acetate end-group does not perturb the course of the forthcoming reactions leading to ω -NH₂ PCL.

In a first series of experiments, γ -benzylglutamate NCA (BG-NCA) has been selected as the amino acid comonomer. BG-NCA has the advantage of being synthesized at a very high yield ($\geq 95\%$) by direct phosgenation of the benzyl ester of the parent amino acid¹⁵ [eq. (3)]:



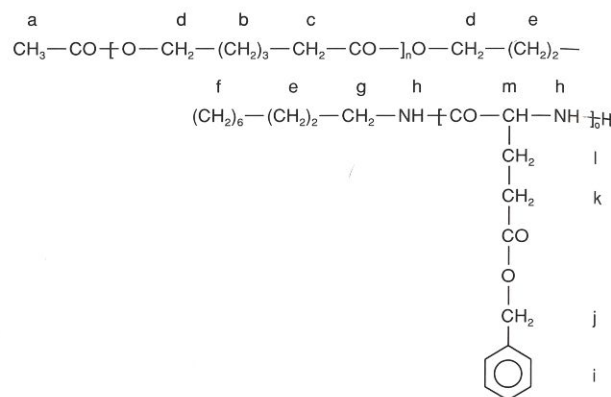
Furthermore, the polybenzylglutamate can be easily hydrogenated into polyglutamic acid (PGA), i.e., a hydrophilic polypeptide. P(ϵ -CL-b-GA) is thus a potential surfactant, whereas P(ϵ -CL-b-GA-b- ϵ -CL) triblock copolymers might be swollen by water and thus give rise to hydrogels.

The copolymerization of BG-NCA has been initiated by a low molecular weight α -acetyl, ω -primary amino PCL ($\bar{M}_n = 5700$, $\bar{M}_w/\bar{M}_n = 1.15$) in dried chloroform at room temperature for 1 h. The copolymerization product has

been precipitated in diethylether and recovered with a conversion of ca. 90%.

The ring-opening polymerization of BG-NCA ($\nu_{\text{CO}} = 1770$ and 1865 cm^{-1}) has been assessed by the IR spectrum which clearly shows the formation of the polypeptide block ($\nu_{\text{CO}} = 1650 \text{ cm}^{-1}$, $\nu_{\text{CO-NH}} = 1549 \text{ cm}^{-1}$) (Fig. 1). The absorption at 1729 cm^{-1} is characteristic of both PCL ($\nu_{\text{CO}} = 1725 \text{ cm}^{-1}$)¹¹ and polybenzylglutamate ($\nu_{\text{CO}} = 1730 \text{ cm}^{-1}$)¹⁵.

Figure 2 shows the ¹H-NMR spectrum of the recovered block copolymer. The relative intensities of the aromatic



Proton	Assignment (ppm)	Intensity
h	8, 3	13, 34
i	7, 25	170, 54
j	5, 04	61, 41
d, m	4, 05	112, 75
a, c, k	2-3	213, 2
b, e, l	1, 9-1, 3	285, 91
f	1, 26	19, 86
g	3, 46	1, 32

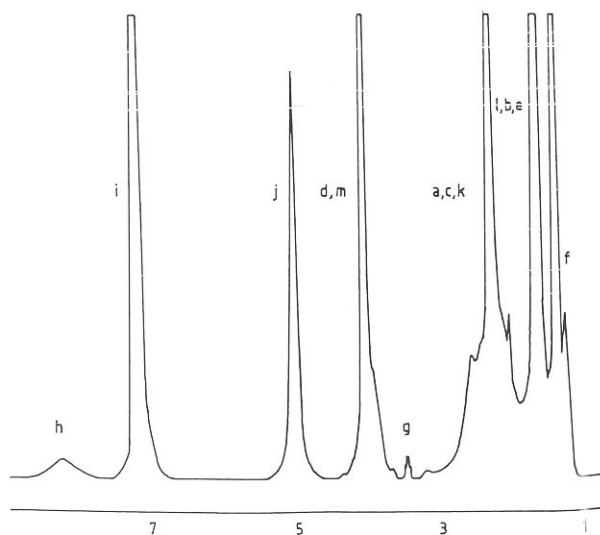


Figure 2. ¹H-NMR spectrum of the copolymer resulting from the polymerization of BG-NCA initiated with a α -acetyl, ω -amino PCL.

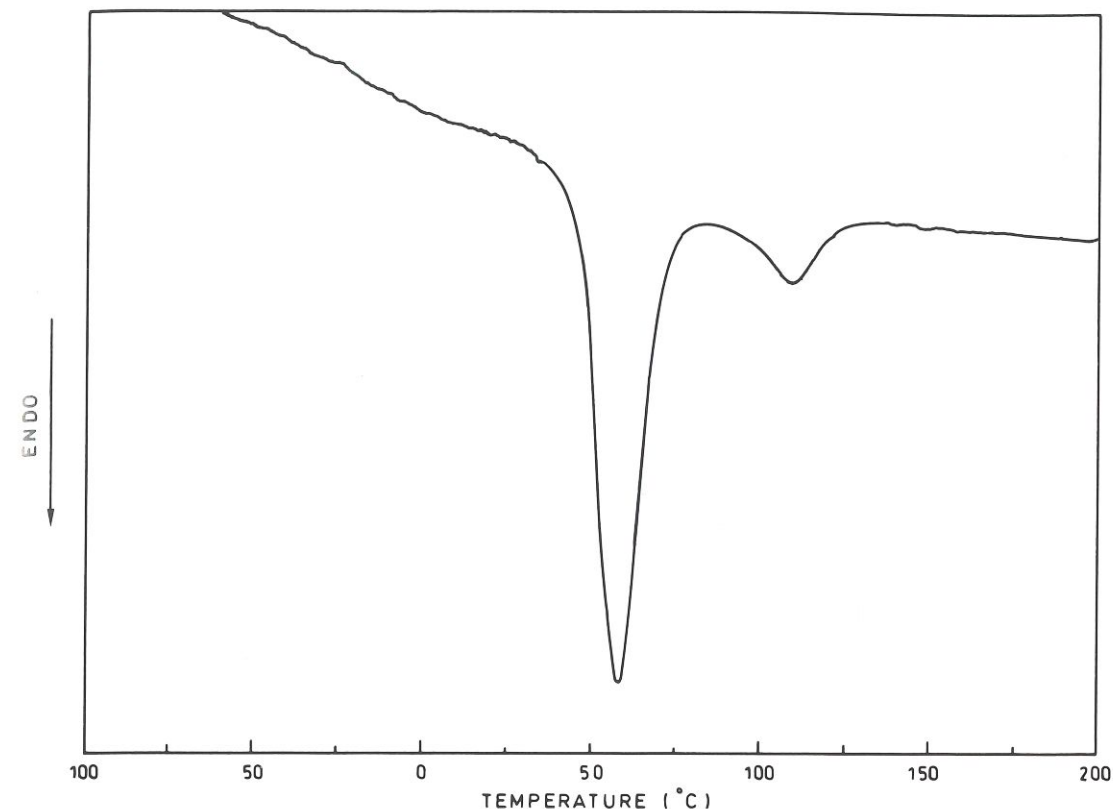


Figure 3. Typical DSC curve (first scan) of the P[CL-b-BG] (20°C/min).

protons (i) and protons (d + m) at 4.05 ppm allow the molecular weight of the polypeptide block to be estimated at $9200 \pm 10\%$ which is in close agreement with the theoretical \bar{M}_n (10^4) taking into account the degree of conversion.

In order to make sure that no homopolymers contaminate the P(ϵ -CL-b-peptide) copolymer, SEC and selective fractionation experiments have been conducted. Unfortunately, polybenzylglutamate is strongly adsorbed on the SEC columns which excludes this method. Furthermore, the solubility of PCL and PBG in common solvents are so close that selective fractionation fails at this stage. Nevertheless, a decisive observation in favor of the block copolymerization is certainly the ¹H-NMR signal at 3.46 ppm (protons g, see Fig. 2) which is characteristic of an amide linkage between PCL and polypeptide component. Moreover, the comparison of the intensity of those *N*-amide methylene protons ($\delta H_g = 3.46$ ppm) with the intensities of the benzyl protons ($\delta H_j = 5.04$ ppm) and the PCL protons ($\delta H_d = 4.05$ ppm), respectively, attests for the blocky structure of the copolymer in absence of any homopolymers.

The DSC curve of P(ϵ -CL-b-BG) copolymer is shown on Figure 3. Two melting endotherms are clearly observed attesting the crystallization of both PCL ($T_m \sim 60^\circ\text{C}$) and PBG ($T_m \sim 110^\circ\text{C}$).

The preliminary results reported in this communication convincingly show that ω -aliphatic primary amino PCL

is an efficient macroinitiator for the ring-opening polymerization of amino acid *N*-carboxyanhydrides. ¹H-NMR analysis supports the selective formation of P(ϵ -CL-b-peptide) block copolymer. Since that diblock bears a primary amine at the end of the polypeptide component, a coupling reaction could lead to P(ϵ -CL-b-peptide-b- ϵ -CL) triblock molecules. Characterization and potentialities of these biocompatible and biodegradable multicomponent materials will be reported in the near future, as well as the extension of the method to polylactides.

EXPERIMENTAL PART

Materials

ϵ -CL (Janssen Chimica) was dried over calcium hydride for 48 h at room temperature and distilled under reduced pressure just before use. Phosgene solution in toluene (1.93M) was purchased from Fluka. γ -Benzylglutamate (Aldrich) and 4-dimethylamino pyridine (Janssen) were dried by three azeotropic distillations of toluene before use. Triethylamine (Janssen) and acetic anhydride (Aldrich) were dried over BaO and CaCl₂, respectively, for a week and distilled under reduced pressure. Tetrahydrofuran (THF) were dried by refluxing over benzophenone Na complex. Chloroform was successively dried over

CaCl_2 , P_2O_5 , and distilled under reduced pressure. γ -Benzylglutamate *N*-carboxyanhydrides were prepared using the classical method.¹⁴

Synthesis of ω -Primary Amino PCL

ϵ -CL polymerization and conversion of ω -bromo PCL into ω - NH_2 PCL has been reported elsewhere.¹⁵

Esterification of PCL-OH

PCL-OH was esterified by acetic anhydride (5 equiv) in dry THF, in the presence of triethylamine (5 equiv) and a catalytic amount of 4-dimethylaminopyridine (0.2 equiv), for 48 h at 50°C. The polyester was purified by three successive precipitation-dissolution cycles (THF-methanol).

Copolymerization of γ -Benzylglutamate *N*-Carboxyanhydride

ω -Amino PCL was used as an initiator for the polymerization of BG-NCA. This macroinitiator was dried by two azeotropic distillations of toluene and dissolved in CHCl_3 (5%). Copolymerization was carried out under nitrogen in a previously flamed glass reactor connected to an oil valve. After a 1 h reaction time at 25°C, the copolymer was recovered by precipitation in diethylether.

Polymer Characterization

^1H -NMR spectrum was recovered in CDCl_3 on a Bruker AM400 apparatus at 25°C. Size exclusion chromatography was achieved in THF by using a Hewlett-Packard 1090 liquid chromatograph equipped with a Hewlett-Packard 1037 A refractometer under detector. IR spectrum was recorded on a Perkin-Elmer 1600 series FTIR. Differential scanning calorimetry (DSC) measurement was carried out with a DSC Dupont 9000 apparatus calibrated with ultrapure indium and gallium (heating rate: 20°/min).

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