Synthesis and purification of a poly(ethylene oxide)–poly(γ-benzyl-L-glutamate) diblock copolymer bearing tyrosine units at the block junction

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An AB-type diblock copolymer, composed of poly(ethylene oxide) as the A block and poly(γ-benzyl-L-glutamate) as the B block, was prepared by the polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) using α-(2-aminoethyl)-ω-methoxypolyoxyethylene as a macroinitiator. *o*-Benzyl-L-tyrosine *N*-carboxyanhydride (BT-NCA) was used to introduce a tyrosine function at the block junction.

(Keywords: block copolymers; poly(amino acid); polypeptide; drug carriers)

Introduction

Block copolymers comprising poly(ethylene oxide) (PEO) and poly(amino acid) or polypeptide sequences have recently attracted considerable interest[1–8]. In solution, and under physiological conditions, the block copolymers behave as aggregates or micelles. It is believed that these have great potential as drug carriers. The polypeptide acts as a carrier for covalently bonded hydrophobic drug molecules whilst the hydrophilic PEO block is able to confer water 'solubility' and stability to the whole system[3]. PEO has been used many times as a biocompatible material, and the polypeptide sequence is potentially biodegradable; both are important considerations for drug delivery use.

Here we report on the synthesis of AB-type diblock copolymers composed of PEO as the A block and poly(γ-benzyl-L-glutamate) as the B block. *o*-Benzyl-L-tyrosine has been incorporated at the block junction to facilitate radiolabelling of the block copolymer with ^{125}I for subsequent *in vitro* or *in vivo* investigation. Recently, syntheses of PEO–poly(aspartic acid)[1–4], PEO–poly(L-lysine)[5] and PEO–poly(γ-benzyl-L-glutamate)[6–8] have been described in the literature. All were prepared using similar methodology producing materials that contained a broad distribution of polymer molecular weights and inevitably some homopolymer. Consequently the aim of our work was to prepare a copolymer without homopolymer impurities.

Results and Discussion

Polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) was achieved using α-(2-aminoethyl)-ω-methoxypolyoxyethylene (POE-NH_2) as a macroinitiator. *o*-Benzyl-L-tyrosine *N*-carboxyanhydride (BT-NCA) was used to introduce a tyrosine function at the block junction. POE-NH_2 was purchased from Fluka with a number-average molecular weight of 5000 g mol^{-1} and amine content >0.17 mmol g^{-1}, according to the manufacturer's data. G.p.c. analysis of the polymer ($\bar{M}_n = 4400$ g mol^{-1}, polydispersity 1.4) in tetrahydrofuran (THF) revealed the presence of high molecular weight impurities. All g.p.c. analyses of polymer samples were performed on a Millipore g.p.c. 150 chromatograph comprised of three Merck Lichrogel columns arranged in series, PS400 (10 μm), 40 (10 μm) and 4 (10 μm), and equipped with both refractive index and u.v. ($\lambda_{max} = 254$ nm) detectors. The columns were calibrated with PEO standards of known molecular weights and of low polydispersity.

Commercially available POE-NH_2 generally contains considerable amounts of impurities, details of which have been thoroughly discussed by Leonard and Dellacherie[9]. High molecular weight impurities obviously result from the presence of water in the polymerization of ethylene oxide monomer. As a result, some polymer chains contain two hydroxyl groups which are subsequently converted into amino groups. The problem is particularly important in the case of higher molecular weight samples[10]. For the polymer to be used as a macroinitiator it is desirable to minimize the presence of difunctional impurities in order to avoid the formation of triblock copolymers. Prior to use, it was therefore necessary to carefully fractionate[9] the POE-NH_2 by repeated precipitation of the polymer with diethyl ether from solution in CH_2Cl_2/EtOH (1:1). The first fraction, which contained mostly high molecular weight difunctional polymer chains, was removed by filtration. Upon subsequent precipitation (the low molecular weight impurities remained in the filtrate), a polymer was collected and dried *in vacuo* overnight at 50°C. The resulting polymer had a molecular weight of ~5700 g mol^{-1} with a polydispersity of 1.25, a somewhat higher molecular weight and lower polydispersity than the original sample.

The purified macroinitiator was used to initiate

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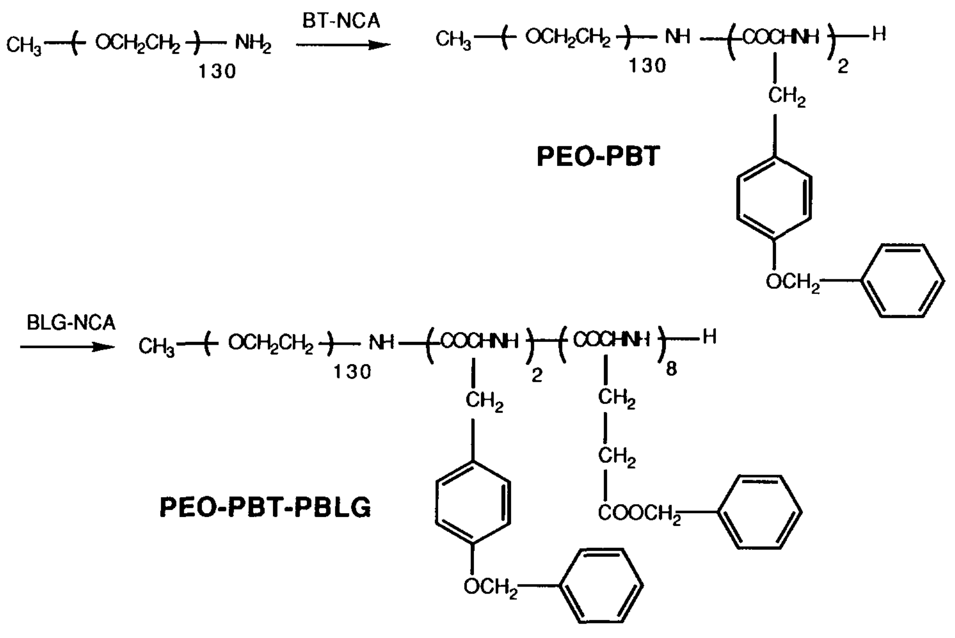

Scheme 1

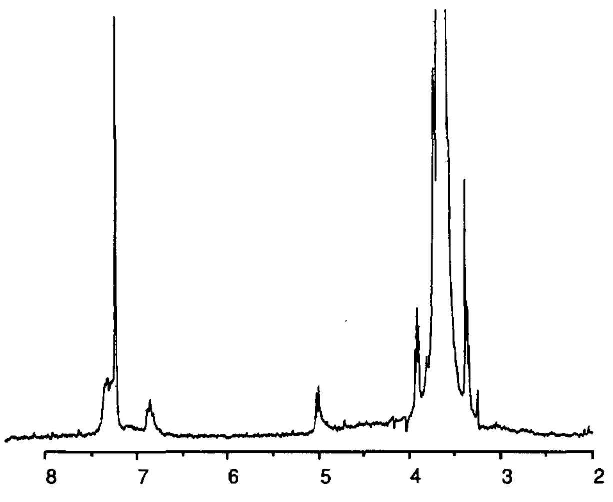

Figure 1 ^1H n.m.r. spectrum of polymer

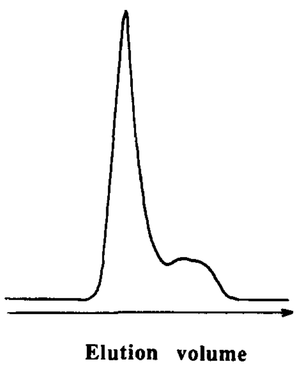

Figure 2 G.p.c. analysis of PEO–PBT–PBLG

the polymerization of BLG-NCA and BT-NCA (*Scheme 1*). The monomers were prepared from the corresponding α-amino acids and phosgene. Phosgene was generated *in situ* from triphosgene (Fluka) according to the procedure of Daly and Poche[11]. The NCAs were recrystallized twice from THF/hexane mixtures. The observed ^1H n.m.r. and melting point data agreed with those reported[11]. BLG-NCA and BT-NCA were prepared in yields of 81 and 51%, respectively.

In order to confirm the presence of benzyltyrosine units in the copolymer, the usual polymerization procedure was slightly modified. First, BT-NCA was allowed to react with POE-NH_2 in $CHCl_3$ (which previously had been washed with H_2O to remove EtOH, dried with $CaCl_2$ and distilled). Because of the low solubility of BT-NCA in $CHCl_3$ the polycondensation was performed in a dimethylformamide (DMF)-rich solvent mixture (the DMF had been dried with Silicagel and distilled three times under reduced pressure, 55°C/3.07 kPa). After 24 h, a sample of the reaction mixture was removed for analysis. BLG-NCA was subsequently added to the reaction mixture to complete the reaction (see *Scheme 1*). Typically, 0.22 g (0.74 mmol) of BT-NCA was dissolved in 5 ml of freshly distilled DMF. POE-NH_2 (2 g, 0.25 mmol) was dissolved in 15 ml of DMF and 5 ml of $CHCl_3$. The BT-NCA solution was added to the initiator solution. After 24 h at 40°C a sample of the reaction mixture was removed and a polymer product isolated by precipitation with diethyl ether. G.p.c. analysis using u.v. detection ($\lambda_{max} = 254$ nm) indicated that the polymer contained aromatic groups (BT). In the ^1H n.m.r. spectrum, which is shown in *Figure 1*, the ratio of the intensities of the $-CH_2-$ signal (benzylic at 5 ppm) and of the $-CH_2CH_2O-$ signal (at 3.6 ppm) confirmed the presence of approximately two tyrosine units per polymer chain.

BLG-NCA (1.4 g, 5.32 mmol) was added to the remaining reaction mixture and the polymerization left at 40°C for a further 2 days. To recover the product, the reaction mixture was added dropwise into 100 ml of dried diethyl ether. A precipitate was recovered and dissolved in a minimal volume of CH_2Cl_2/EtOH mixture (1:1) and the solution was again precipitated with diethyl ether. The solid obtained was dried *in vacuo* to give 2.5 g of PEO–PBT–PBLG copolymer. By g.p.c. analysis this contained a significant amount of low molecular weight impurities, most likely PBLG homopolymer (see *Figure 2*). Consequently, the copolymer was treated with cold

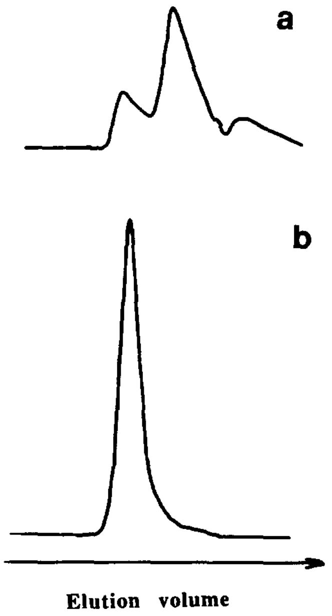

Figure 3 G.p.c. analysis of (a) soluble and (b) insoluble fractions

DMF — in which it is insoluble — then filtered, re-dissolved in $CHCl_3$, and finally precipitated with diethyl ether. The g.p.c. chromatograms of the soluble and insoluble fractions are shown in *Figure 3*.

Conclusions

It is clear that the purification to remove non-DMF soluble material was successful. The final block copolymer reflects the low polydispersity of the starting macroinitiator, POE-NH_2. Using both g.p.c. and ^1H n.m.r. techniques we found that PEO–PBT–PBLG has a polydispersity of 1.26 and contains approximately 72% of PEO ($M_{PEO} = 5700$, $M_{PBT} = 500$, $M_{PBLG} = 1750\,g\,mol^{-1}$). Finally, PEO–PBT–PBLG is soluble in water, although not completely dissolved; according to our preliminary results, the copolymer forms micelles with a diameter of 8–10 nm in water.

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