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International Journal of Pharmaceutics 178 (1999) 183–192

international journal of pharmaceutics

Modified polypeptides containing γ-benzyl glutamic acid as drug delivery platforms

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Received 19 May 1998; received in revised form 2 November 1998; accepted 2 November 1998

Abstract

We previously reported the development of diffusion-controlled biodegradable polypeptides for drug delivery purposes. In this paper, we describe the synthesis of three modified polypeptides that contain γ-benzyl glutamic acid as the common structural backbone. The properties of these polymers were characterized with regard to their potential application as drug delivery platforms. Procainamide hydrochloride, a hydrophilic drug, and protamine sulfate, a low molecular weight protein, were used as model drugs for examining release rate profiles from these polymers. The homopolymer of poly(γ-benzyl-L-glutamic acid), PBLG, showed a highly helical configuration and a moderate release rate of procainamide. Modification of structural attributes by random copolymerization of the D- and L- isomers of γ-benzyl glutamic acid produced poly(γ-benzyl-D,L-glutamic acid), PBDLG, which displayed a significantly slower release of procainamide when compared to PBLG. The modification of polymer bulk hydrophobicity by copolymerization of PBLG (A) with poly(ethylene glycol) (B) yielded an ABA triblock copolymer exhibiting much faster release rates for both procainamide and protamine than those demonstrated by the other two polymers. Using this triblock copolymer, protamine release rates ranging from 2 weeks to approximately 2 months were obtained by simply varying the polymer processing conditions and protein particle size. A nearly complete release of protein was obtained from the triblock copolymer blends and this occurred without reliance upon degradation of the polymer backbone. Fickian diffusion-controlled release mechanisms were implied for release of procainamide and protamine from these polypeptide formulations based on the linear relationship displayed between cumulative drug release and the square root of time.

Keywords: Delayed-action preparations; Polymers; Polypeptides; Polyglutamic acid; Polyethylene glycols; Protamine sulfate

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1. Introduction

Synthetic polypeptides continue to be of interest as drug delivery platforms because of their capacity to be both biocompatible as well as biodegradable to naturally-occurring biological products (Anderson et al., 1985). The large number of amino acids along with their range of physical and chemical properties render this class of polymers useful for the design of novel drug delivery systems. By appropriate selection of amino acid residues and sequences, a variety of polypeptides can be designed to possess varying degrees of hydrophobicity, structural attributes, and electrostatic properties. This feature offers the potential that specific polypeptide polymers possessing requisite physicochemical properties could ultimately be tailor-made to yield optimum delivery for particular drugs.

Two obstacles are commonly cited as limiting the suitability of polypeptides for drug delivery applications: their biological degradation and potential toxicity (Anderson et al., 1985; Sanders, 1991). A number of approaches have been reported for addressing these potential pitfalls. Because of the stability of the amide backbone towards hydrolysis, the catalytic activity of native enzymes is usually relied upon for polypeptide degradation (Silman and Sela, 1967; Pytela et al., 1990). An alternative approach is to incorporate labile chemical bonds into the polypeptide backbone, thereby introducing hydrolytic instability to the polymer chain. To this end, the synthesis of copolymers of amino acids with either α-hydroxy acids (Katakai and Goodman, 1982) or anhydrides (Domb, 1990) has been described. The concerns over polypeptide toxicity are generally minimized by careful selection of amino acid residues and by limiting the number of different amino acids in the polypeptide backbone (Marck et al., 1977; Anderson et al., 1985).

Although sometimes regarded as a liability, the stability of the polypeptide backbone towards hydrolysis could serve certain useful purposes. The enhanced stability of polypeptides can influence the mechanisms and kinetics of drug release by affecting the timescales over which both drug diffusion and polymer degradation occur (Hopfenberg, 1982). Drug release from hydrolytically labile polymers such as poly(D,L-lactide-co-glycolide) (PLGA) can proceed by both a diffusion-controlled mechanism (Paul and McSpadden, 1976) and a degradation-controlled mechanism (Lee, 1980; Hutchinson and Furr, 1988; Shah et al., 1992). Thus, variations in polymer degradation rates could lead to irreproducible drug release patterns thereby affecting both the efficacy and toxicity profile of the drug therapy. In addition, the onset of polymer degradation could cause structural deterioration of the implanted device. As a result, the risk of dose-dumping is increased should either the partially degraded system collapse or require retrieval when unexpected reactions occur during treatment. For these reasons, uncoupling the mechanism of drug release from polymer degradation would be a desirable alternative. Polypeptide polymers, which are less prone to hydrolysis and can thus provide structural integrity over a long period of time during which drug release may proceed through a diffusion-controlled mechanism, appears to be an outstanding choice.

Previous work conducted in our laboratory demonstrated that simple changes in the ratio of hydrophilic to hydrophobic amino acid residues within the polypeptide backbone could modify the diffusion-controlled drug release properties of such a material (Cook et al., 1997). In the current study, we further explored the possibility of altering the drug release profile of a polypeptide through modifications of the bulk polymer hydrophobicity and structural attributes. Poly(γ-benzyl-L-glutamic acid) (PBLG) was chosen as the model polypeptide because it contains a highly helical structure in the solid-state (McKinnon and Tobolsky, 1966; Baier and Zisman, 1970). The structural attributes of this polymer were modified by random copolymerization of the D- and L- isomers of γ-benzyl glutamic acid to produce poly(γ-benzyl-D,L-glutamic acid) (PBDLG). In addition, the bulk polymer hydrophobicity of PBLG was modified by polymerization of PBLG blocks (A) with a hydrophilic pre-polymer block of poly(ethylene glycol) (PEG) (B) to form an ABA triblock copolymer (PBLG–PEG–PBLG). The physicochemical properties of these three syn-

thetic polypeptides were characterized, and drug release studies were performed using two model hydrophilic drugs, namely, procainamide hydrochloride and protamine sulfate.

2. Materials and methods

2.1. Materials

Tetrahydrofuran (THF), dioxane, hexane, triethylamine (TEA), and procainamide hydrochloride were obtained from Aldrich (Milwaukee, WI). *Bis*(trichloromethyl) carbonate (triphosgene) was purchased from Fluka Chemical Corporation (Ronkonkoma, NY). Diethyl ether and chloroform were obtained from Fisher Scientific (Pittsburgh, PA). Methylene chloride, dichloroacetic acid (DCA), deuterated chloroform, poly(D,L-lactic-co-glycolic acid) 75:25 (PLGA), γ-benzyl-L-glutamic acid, and protamine sulfate (Grade III) were purchased from Sigma (St. Louis, MO). γ-Benzyl-D-glutamic acid was obtained from Bachem Bioscience (King of Prussia, PA) and diamino poly(ethylene glycol) (diamino-PEG; molecular weight 3.4 kDa) was obtained from Shearwater Polymers (Huntsville, AL). Disposable Acrodisc® 1 μm CR PTFE syringe filters were supplied by Gelman Sciences (Ann Arbor, MI).

The solvents THF and dioxane were rigorously dried by distillation over sodium metal, and TEA was dried by distillation and stored over molecular sieve. Methylene chloride was dried by distillation immediately prior to use. Amino acid and diamino-PEG were dried under vacuum over phosphorous pentoxide. Other solvents were anhydrous grade and were used directly without any additional treatment.

2.2. Preparation of monomers

The monomers, amino acid *N*-carboxyanhydrides (NCA), were prepared as previously described (Daly and Poche, 1988). In brief, THF was added to dry γ-benzyl glutamic acid and warmed to 55°C with stirring. Triphosgene was added to amino acid at a 1:3 molar ratio, respectively, and stirred at 55°C for 3 h. The solution was occasionally purged with dry nitrogen to remove HCl produced in the reaction. The product was precipitated using hexane and stored at −20°C overnight. Typically, the product was purified three times by recrystallization or until a constant melting point was achieved. The measured melting points of γ-benzyl-l-glutamic acid NCA and γ-benzyl-D-glutamic acid NCA monomers were 90–93°C and 90–92°C, respectively, which were in good agreement with literature values (i.e. 96–97°C; Daly and Poche, 1988).

2.3. Polymer synthesis

PBLG was prepared by polymerization of NCA monomers (3% concentration by weight) in dioxane at room temperature according to the method reported previously (Blout and Karlson, 1956). TEA was added as initiator at a monomer:initiator ratio of 25:1. Polymerization was continued for either 3 days or until a viscous product was obtained, whichever came first.

PBDLG was prepared by random copolymerization of a 2:1 molar ratio of the D- to L-isomers of γ-benzyl glutamic acid NCA. Polymerization was carried out in a methylene chloride/dioxane solution (1:1 by volume) containing approximately 3% monomer concentration. TEA was used as initiator at a monomer:initiator ratio of 50:1. Polymerization was allowed to proceed for 14 days.

The ABA triblock copolymer was prepared by polymerization at room temperature of the γ-benzyl-L-glutamic acid NCA (A blocks) with diamino-PEG (B block) in methylene chloride according to procedures described previously (Cho et al., 1990).

Unless otherwise stated, all polymers were isolated in diethyl ether, dried, and dissolved in methylene chloride. After filtering through a 1-μm PTFE filter, the final polymer products were precipitated in ether and then dried under vacuum.

2.4. Polymer characterization

A Cannon–Ubbelohde capillary viscometer was used to measure kinematic viscosities of poly-

mer solutions in dichloroacetic acid at 25°C. Relative viscosities were taken as the ratio of drop times between solution and solvent. Intrinsic viscosities were determined and then used to estimate molecular weights according to the method described by Doty et al. (1956).

The molecular weight of the ABA block copolymer was estimated using NMR. In brief, the average number of γ-benzyl glutamic acid residues per polymer chain was first determined by integration using the 3.4-kDa diamino-PEG as an internal standard. The shift at 5.3 ppm from the ethene protons of PEG was used to calibrate the integral of the shift at 7.2 ppm from the benzyl protons. Once this value was obtained, the average number of γ-benzyl glutamic acid residues per chain was determined. The molecular weight of the ABA triblock was then estimated from the molecular weights of the poly(γ-benzyl glutamic acid) blocks and the PEG pre-polymer (molecular weight 3.4 kDa).

Thermal transitions of polypeptide samples (3–10 mg) were analyzed by differential scanning calorimetry using a Perkin–Elmer DSC-7 calorimeter at a scanning rate of 10°C/min and a temperature range between −20°C and 220°C. Scans were also performed up to the decomposition temperatures (approximately 315°C for PBDLG and 325°C for PBLG) to examine evidence of large-scale crystallinity in the samples.

Contact angle measurements were performed using a Rame–Hart goniometer (Mountain Lakes, NJ). Droplets of 40 μl double-distilled water were placed on solvent-cast polymer films which had been thoroughly dried under vacuum (Bascom, 1988). Measurements on at least six droplets were taken within 5 min after placing the liquid on the film. To confirm the accuracy of this method, contact angles were also measured on Parafilm® (American National Can, Neenah, WI). The measured value of 106 ± 2° on Parafilm® was in excellent agreement with that (109°C) reported in the literature (Zografi and Tam, 1976).

Circular dichroism (CD) spectra were measured with a Jasco J-710 spectropolarimeter (Tokyo, Japan) on samples with concentrations at or below 0.5 mg/ml, using a scan rate of 20 nm/min and a cell path length of 1.0 mm. Data were collected down to 210 nm using four accumulations. Solvent subtraction and noise suppression were used to treat the spectra.

The diffusion coefficient of benzoic acid in PBLG and PBDLG was estimated using polymer films cast from chloroform and thoroughly dried under vacuum. A side-by-side diffusion apparatus was used which contained 0.01 M citrate buffer, pH 3.0, in both chambers and a drug-saturated donor half-cell. The cumulative mass of benzoic acid transported across the films at 25°C was determined by UV absorbance at 228 nm. Diffusion coefficients were calculated according to the method of Martin (1993).

2.5. Drug release rate experiments

Drug was incorporated into polymer at a loading of 10% by weight. The polymer was first dissolved in a suitable solvent (e.g. chloroform) and then the appropriate amount of drug was added. The suspension was blended to a thick paste while the volatile solvent was evaporated. The blended mixture was thoroughly dried under vacuum and pressed at an elevated temperature of either 120 or 140°C and a pressure of 15 000 lbs using a hydraulic Carver press (Mountain Lakes, NJ). A stainless steel shim was used during this procedure to form a heat-pressed sheet of constant thickness, typically 0.7–0.9 mm. From this heat-pressed sheet, individual samples were cut to a size of 1 cm^2 and weighed. The drug loading in the blend was estimated by measuring the total amount of drug extracted from triplicate samples.

Release rate experiments were performed by suspending a drug-loaded polymer system in 20 ml of 0.1 M phosphate buffer, pH 7.4, at 37°C in a shaking (60 cycles/s) water bath (Precision Scientific, Chicago, IL). At each time interval, the sample was transferred to a new vial containing a fresh buffer solution. Drug concentrations were measured by UV absorbance at 278 and 204 nm for procainamide hydrochloride and protamine sulfate, respectively. Data was presented as the mean ± S.D. of the cumulative percent drug released from triplicate systems.

The particle size of procainamide and protamine was either the same as supplied by the

manufacturer or was manipulated by lyophilization or manual grinding with a pestle and mortar. Particle size was analyzed using an AeroSizer® LD (Amherst Process Instruments Incorporated, Amherst, MA) and the geometric mean particle diameter was calculated assuming a density value of 1.05 mg/ml.

3. Results and discussion

Previously we have demonstrated that modification of polypeptide hydrophobicity through selection of amino acids and their composition could influence the diffusion-controlled release of a model steroid drug (Cook et al., 1997). In the current work, we further examine whether modification of polypeptide bulk hydrophobicity as well as structural attributes can be used to regulate drug release profiles. To this end, modified polypeptides were synthesized using γ-benzyl-L-glutamic acid as the common structural backbone. PBLG was selected as the parent polymer because of the presence of a well-characterized helical structure in the solid-state (McKinnon and Tobolsky, 1966; Hiltner et al., 1972). The effects of polypeptide structural modifications on drug release were studied by synthesizing PBDLG through random copolymerization of the D- and L-isomers of γ-benzyl glutamic acid (Wada, 1961; Koleske and Lundberg, 1969). To examine the effects of modification of bulk polymer hydrophobicity on drug release, an ABA triblock copolymer containing (A) blocks of PBLG and (B) blocks of PEG of molecular weight 3.4 kD was synthesized (PBLG–PEG–PBLG).

The physicochemical characteristics of the polypeptides prepared in this study are summarized in Table 1. The synthetic polypeptides had relatively high molecular weights: approximately 110 kDa for PBLG, 150 kDa for PBDLG, and 41 kDa for PBLG–PEG–PBLG. Although the molecular weight of the triblock copolymer was the lowest among the three polypeptides, it nevertheless represented a composition of the targeted monomer ratio of 1:1:1 (PBLG–PEG–PBLG). As shown, incorporation of the PEG block into the polymer considerably decreased bulk hydrophobicity as reflected by the presence of the lowest contact angle value for the triblock copolymer. On the other hand, modification of structural attributes did not introduce any significant change in the polymer hydrophobicity between PBLG and PBDLG.

Consistent with findings by other investigators (Kail et al., 1962; Koleske and Lundberg, 1969), our results from thermal analysis show two transitions for PBLG resembling glass transition baseline shifts (Table 2). Hiltner et al. (1972) indicated that while these transitions were similar in appearance to glass transition relaxations commonly observed for random coil polymers, the helical structure of PBLG would, nevertheless, prevent the occurrence of such large-scale relaxations in the polymer backbone. As a consequence, the transitions observed in PBLG were not expected to reflect the true glass transitions. Interestingly, the high temperature transition seen in the PBLG homopolymer was also observed in the triblock copolymer PBLG–PEG–PBLG. It should be pointed out that the existence of helical structure for polymers containing PBLG was confirmed by the presence of the characteristic double minima

Table 1
Physicochemical properties of poly(γ-benzyl-L-glutamic acid)-based polypeptides

Polymer sample	[η] (dl/g)[a]	Molecular weight (daltons)	Contact angle (°)
Poly(γ-benzyl-L-glutamate) (PBLG)	0.67	110 000[b]	77 ± 3
Poly(γ-benzyl-D,L-glutamate) (PBDLG)	0.89	150 000[b]	77 ± 4
ABA Triblock Copolymer (PBLG–PEG–PBLG)	0.75	41 000[c]	64 ± 3

[a] Kinematic viscosities for polymer samples dissolved in dichloroacetic acid.
[b] Molecular weight was determined according to Doty et al. (1956).
[c] Molecular weight was determined by NMR analysis.

Table 2
Thermal, structural, and diffusion characteristics of poly(γ-benzyl-L-glutamic acid)-based polypeptides

Polymer sample	Transition temps. (°C)	CD maxima/ minima[a]	Position of CD peaks (nm)	Benzoic acid diffusion coeffieients (cm^2/s)
PBLG	−7, 135	Double minima	212, 222	1.48×10^{-10}
PBDLG	nd[b]	Single maximum	224	1.18×10^{-10}
PBLG–PEG–PBLG	130	Double minima	212, 222	n/a[b]
Poly(benzyl-D-Glutamate)	n/a[b]	Double maxima	212, 222	n/a[b]

[a] Samples were prepared in dioxane for spectral analysis between 210 and 260 nm.
[b] nd refers to not detected whereas n/a refers to not analyzed.

(W. Curtis Johnson, 1990) in circular dichroism spectra (Table 2). The PBLG polymer and others containing the PBLG blocks have been documented to exhibit highly helical configurations in both solution and the solid state (McKinnon and Tobolsky, 1966; Cho et al., 1990).

In contrast, the random copolymer PBDLG exhibited none of the thermal transitions observed with PBLG. In addition, the CD spectra yielded only a single maximum, implicating the presence of a random coil configuration in solution (W. Curtis Johnson, 1990). Obtaining a truly random coil configuration would require specific polymerization conditions to produce a perfectly alternating arrangement of the D- and L-isomers. This would be an improbable task for the synthesis of the PBDLG copolymer considering the distinct reactivity ratios of these two monomers. Earlier studies by Wada (1961) on the synthesis of PBDLG indicated that copolymerization, in fact, resulted in a disruption of the helical configuration rather than formation of a purely random coil structure. Based on these findings and results from circular dichroism analysis, it appears that we have likely produced a polymer containing an appreciable but probably not a complete degree of random coil configuration. A strong indication of such a structural modification would be a change in the diffusivity of small molecules through these polymers. Measurements of the diffusion coefficient (Table 2) indeed disclosed a relatively slower diffusion of benzoic acid through the PBDLG film relative to PBLG. No evidence of polymer crystallinity was observed in either of these two polypeptides when they were examined by differential scanning calorimetry. Despite the lack of large-scale crystallinity, the low magnitude in diffusion coefficients nevertheless implicated the existence of certain regions of organized structure in the solid-state of these polymers, a finding that was in agreement with that of McKinnon and Tobolsky (1966).

The release of a low molecular weight model hydrophilic drug, procainamide hydrochloride, from the three polypeptide matrices is illustrated in Fig. 1. The polymer-drug blends were heat-pressed at 120°C, and the drug possessed a unimodal particle size distribution with geometric mean diameter of 4.0 ± 2.8 microns. As shown in Fig. 1(A), 90% of the drug was released from the PBLG matrix in about 1 month, whereas only 1 week was required to completely release drug from the PBLG–PEG–PBLG matrix. While polymer molecular weight may contribute to the different release rates exhibited by these two matrices, the considerably higher hydrophilicity in PBLG–PEG–PBLG is believed to be the predominate factor for the much faster release of this hydrophilic drug. These results, along with our earlier findings (Cook et al., 1997), demonstrate that increasing polypeptide hydrophobicity at either the monomeric level or at a bulk scale can enhance the release rate of low molecular weight drugs. Further studies are in progress to better understand how molecular weight would influence the release profiles of drugs.

Alternatively, modifying polypeptide structure can also influence the release rate of a drug. As shown in Fig. 1(A), release of procainamide hydrochloride from PBDLG was significantly slower

than from PBLG matrices; less than 50% of the drug was released from PBDLG after a period of 1 month. This notable difference in drug release rate in conjunction with previous diffusivity data and circular dichroism spectra suggest that disruption of the helical structure of PBLG has imparted significant change on the release and transport properties of PBDLG. Procainamide release from all three polypeptide platforms appeared to be governed by a diffusion-controlled mechanism as reflected by the presence of a linear

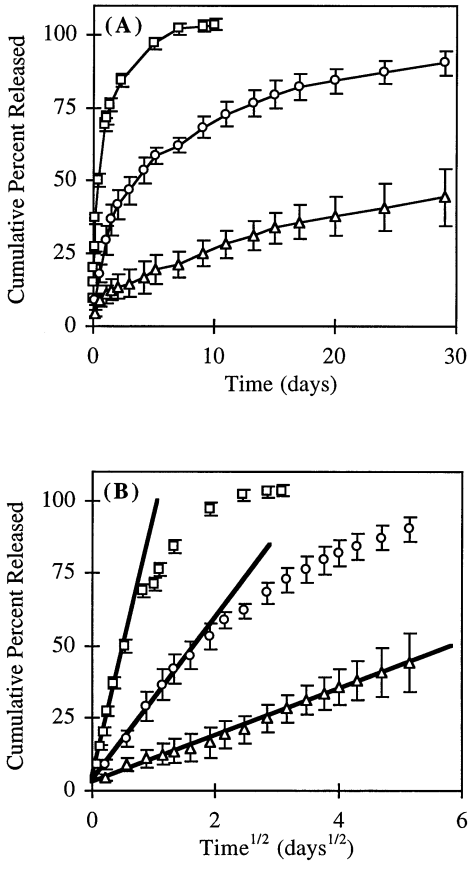

Fig. 1. Effect of time on the release of procainamide hydrochloride from poly(γ-benzyl-L-glutamic acid)-based polypeptides. Cumulative percent drug released was plotted against (A) time; and (B) square root of time. The data are shown for (.) PBLG–PEG–PBLG, (/) PBLG, and (0) PBDLG matrices. Polymer blends contained 10% (w/w) drug and were processed at 120°C. Solid lines in the square root of time plot (B) represent the linear regressions of the first 60% of the cumulative drug release.

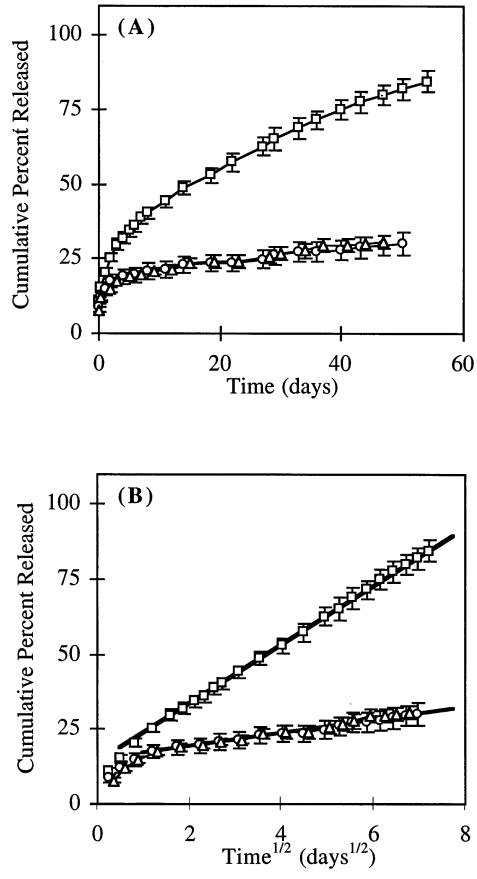

Fig. 2. Effect of time on the release of protamine sulfate from poly(γ-benzyl-L-glutamic acid)-based polypeptides. Cumulative percent drug released was plotted against (A) time; and (B) square root of time. The data are given for (.) PBLG–PEG–PBLG, (/) PBLG, and (0) PBDLG matrices. Polymer blends contained 10% (w/w) drug and were processed at 140°C. Drug was treated to particle size reduction by manual grinding. Solid lines in the square root of time plot (B) represent the linear regressions of the first 60% of the cumulative drug release.

relationship between drug release and the square root of time (Fig. 1(B)). The broad range of procainamide release rates, varying from 1 week with the PBLG–PEG–PBLG polymer to well over 1 month with PBDLG, has demonstrated both the utility and feasibility of our concept of utilizing modified polypeptides to provide regulated and diffusion-controlled drug release rates.

Release profiles of the model hydrophilic protein drug protamine sulfate from the three

polypeptide matrices are illustrated in Fig. 2. In these studies, the drug-polymer blends were processed at 140°C (i.e. above the high temperature thermal transition of the PBLG-containing polymers) and the drug was ground drug to contain an average particle size of 3.7 ± 2.3 microns. As indicated, the release profiles from PBLG and PBDLG matrices were nearly identical, with only 25% of the loaded drug being released after 50 days. In contrast, protamine release from the more hydrophilic PBLG–PEG–PBLG matrix was significantly faster, with over 80% of the loaded drug being released during the same time period. Based on these results, copolymerization of the polypeptide with a hydrophilic PEG block was apparently a feasible approach to achieve more desirable release rates for large protein drugs such as protamine. The linearity shown in the plots using the square root of time (Fig. 2(B)) again implies the presence of a diffusion-controlled release mechanism from these matrices.

The effect of particle size on drug release was also examined. Protamine was prepared by lyophilization to produce a mean particle size of 1.9 ± 1.5 microns, and the drug-polymer blends were processed at 140°C. The release profile of ground protamine from Fig. 2 was included for comparison. As shown in Fig. 3, release of lyophilized protamine was significantly faster than that of ground protamine suggesting that particle size of protein drugs can be used to regulate their release rates.

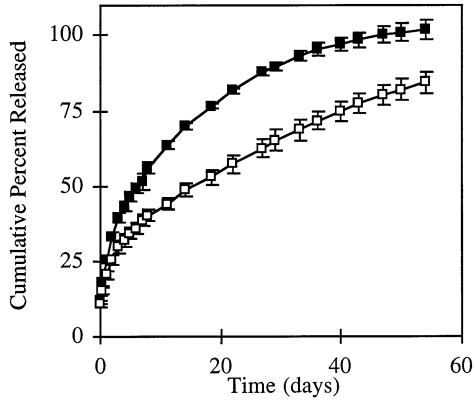

Fig. 3. Effect of particle size on the release of protamine sulfate from PBLG–PEG–PBLG matrices. Polymer blends contained 10% (w/w) drug and were processed at 140°C. Protamine was either (+) lyophilized powder or (.) a manually-ground preparation.

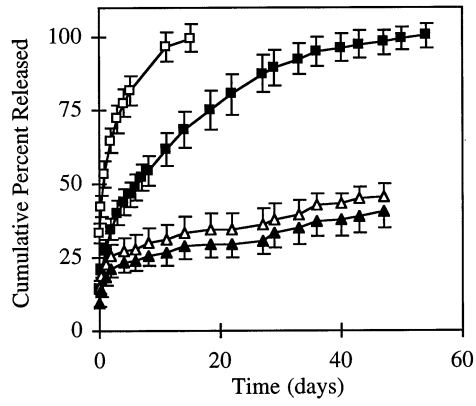

Fig. 4. Effect of temperature on the release of protamine sulfate from various polymer matrices. The data are given for (.) PBLG–PEG–PBLG processed at 120°C; (+) PBLG–PEG–PBLG processed at 140°C; (0) PLGA processed at 120°C; and (&) PLGA processed at 140°C. Protamine was used as supplied by manufacturer without any manipulation of particle size.

As previously discussed, the PBLG–PEG–PBLG copolymer contained a high thermal transition temperature at about 130°C. Drug-polymer blends were therefore processed at temperatures bracketing this transition to examine if processing can also influence drug release. Fig. 4 presents results of protamine release from polymer blends processed at either 120 or 140°C. Protamine was used as received without further manipulation of the particle size, with an average diameter of 6.3 ± 4.3 microns. Similar to the results seen with procainamide, a rapid release of protamine was achieved over a 2-week period from blends processed at 120°C. In contrast, polymer blends processed at 140°C, a temperature above the thermal transition temperature of the polymer, yielded a much slower release of protamine over a period of approximately 50 days.

It should be pointed out that although the 130°C thermal transition is not generally considered identical to a glass transition temperature

(Hiltner et al., 1972), drug release from the ABA triblock copolymer nevertheless appeared to be influenced by changes in the processing temperature around this transition. Interestingly, protamine release from similarly prepared PLGA matrices was markedly slower and was not significantly influenced by processing temperature (Fig. 4). This finding, however, is somewhat anticipated since PLGA does not possess thermal transitions around this temperature range. Instead, the glass transition temperatures of PLGA are typically below 100°C (Kopecek and Ulbrich, 1983).

In summary, we have demonstrated that polypeptides modified by altering either their structural attributes or bulk hydrophobicity can provide a broad range of release characteristics for hydrophilic small molecule and protein drugs. Structural modification via copolymerization of D- and L- amino acid isomers was effective in producing polypeptides possessing different release characteristics for low molecular weight drugs such as procainamide. In contrast, modification of polymer bulk hydrophilicity by incorporation of hydrophilic polymer blocks was useful in changing the release profiles of both small molecular weight drugs as well as protein drugs such as protamine. Protein release rates ranging from 1 week to nearly 2 months were successfully obtained from the ABA triblock copolymer (PBLG–PEG–PBLG) without reliance upon mechanisms of polymer degradation to facilitate release. In addition, protein particle size and polypeptide processing temperatures can also play a role in regulating drug release rates.

Acknowledgements

We would like to acknowledge NIH Grants GM-07767 and HL-55461 and the American Foundation for Pharmaceutical Education (AFPE) for financial assistance. We also thank Dr Yuehua Zhang for helpful discussions, Amy Miles for technical assistance in release rate experiments, and Dr Vijendra Singh for editorial advice. We are grateful to Dr John Crison at TSRL, Inc. (Ann Arbor, MI) for providing assistance in particle size analysis.

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