Controlled Protein Release from Polyethyleneimine-Coated Poly(L-lactic Acid)/Pluronic Blend Matrices

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Protein release from degradable polymer matrices, composed of poly(L-lactic acid) and its blends with Pluronic surfactant, was investigated with and without the aqueous coating of an adsorptive water-soluble polymer, polyethyleneimine (PEI). PEI is a highly branched cationic polymer containing primary, secondary, and tertiary amino groups in its backbone. The treatment of PEI for PLA/Pluronic blend films exhibited a remarkable decrease in the "burst" release of protein at an initial stage and a significant extension in the protein release period. Protein release profiles could be controlled by varying PEI treatment time and its concentration. Our results suggest that PEI diffuses into the polymer matrices and crosslinks protein molecules by ionic interactions. Such a PEI–protein network near the surface region of matrix may act as a diffusional barrier for further release of protein molecules.

KEY WORDS: protein release; poly(L-lactic acid); polyethyleneimine; coating; burst effect.

INTRODUCTION

Recently, long-term delivery of peptide and protein drugs has attracted much attention because recombinant DNA technology allows mass production of therapeutically important proteins (1,2). Various polymer matrices have been utilized for controlled release of proteins (3,4). However, a large amount of the initially loaded protein is normally released at an early period (i.e., burst effect), which is a serious problem in many cases. The burst phenomenon appears to be, in part, due to the formation of pores and cracks in the polymeric matrices during the device fabrication. This phenomenon is particularly acute when devices are prepared by solvent evaporation. Rapid solvent evaporation causes a highly porous structure in the matrix, so that loaded protein molecules are rapidly released through the aqueous channels (5). In particular, when using a biodegradable polymer as a matrix, it has been often found that protein diffuses out at an early stage of incubation regardless of the degradation of the matrix (6). Slow removal of the solvent is one way to reduce the burst effect (5). In addition, protein loading and particle size play important roles in controlling the initial release of the protein (5).

In a previous report (7), we blended a biodegradable polymer, poly(L-lactic acid) (PLA), with a series of Pluronic surfactants, triblock copolymers of ethyleneoxide/propyleneoxide/ethyleneoxide, to modify the morphology in the matrices. It was found that PLA/Pluronic L-101 blends particularly exhibited a significant reduction in the initial protein burst due to the formation of a less porous surface morphology. Pluronic surfactants have been used safely in humans as coatings for suture materials. In this study, to control the burst effect further, we coated these matrices with a water-soluble, cationic polymer. Polyethyleneimine (PEI) has been chosen as a model polymer. Protein release patterns were examined by varying PEI treatment time and its concentration.

MATERIALS AND METHODS

Materials

PLA (MW 100,000) was obtained from Polysciences. GPC analysis revealed a weight-average and number-average molecular weight of 71,000 and of 60,000, respectively. Pluronic L-101 (MW 3800) which contains 10% (by weight) of ethylene oxide segment was kindly donated by BASF Corp. Polyethyleneimine (PEI) with a molecular weight of 70,000 was from Polysciences. Fluorescein-labeled bovine serum albumin (FITC-BSA) and FITC-dextran (MW 70,000) were purchased from Sigma Chemical Co. All other reagents were analytical grade.

Blend Film Preparation

PLA was dissolved in 3 ml of methylene chloride with varying amounts of Pluronic L-101. The total amount of polymer mixture was 0.5 g. For FITC-BSA-loaded blend films, 20 mg of FITC-BSA dissolved in 0.5 ml of 0.1 *M* phosphate buffer (pH 7.4) was emulsified in the above polymer solution with a vortex mixer and then cast onto presilanized glass petri dishes and the solvent was evaporated at room temperature overnight under vacuum. FITC-BSA-loaded polymer blend films were punched out in the shape of discs (6.35 mm in diameter and about 0.5 mm in thickness).

PLA Coating of Protein-Loaded Blend Film by Compression Molding

Using a Teflon molder (inner diameter, 1 cm), the protein-loaded homo-PLA film was placed in between different amounts of finely grinded PLA powder and then compressed with a vise manually. This compressed molder was incubated at 70°C, above the glass transition temperature of PLA (59°C), for 15 min.

Polyethyleneimine Treatment

Protein-loaded PLA/L-101 blend films were immersed in PEI aqueous solution prepared from distilled water at room temperature using a rotating incubator. After incubation for a fixed time, wet films were blotted with Kimwipe tissue and dried under vacuum overnight. As controls, the same blend films were incubated in distilled water for the same period and then dried.

Protein Release Studies

FITC-BSA-loaded blend samples were incubated in 5 ml

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of PBS, pH 7.4, at 37°C. The amount of released FITC-BSA at various time intervals was assayed by monitoring the absorbance at 495 nm (4).

RESULTS AND DISCUSSION

In a preliminary attempt to reduce the burst effect of proteins from PLA matrices, the film was coated with PLA to form an additional polymer layer. The film was dipped into PLA-dioxane solution and then transferred to distilled water to precipitate the PLA onto the surface of the film while dioxane is dissolved in water (8). The major problem using this technique was nonuniform coating of the PLA on the surface with porous cracks, and the protein burst was still found. Repeated coating may help reduce the burst effect. However, the blend films will become thick.

An alternative approach is to coat PLA powder on both sides of the films via compression molding above the softening temperature (glass transition temperature) of the PLA. This allows the PLA powder to fuse together, forming a pellet. Figure 1 shows the effect of PLA coating on BSA release. With an increase in the amount of PLA powder used for coating, delayed BSA release profiles can be seen. This is probably due to a long diffusion pathlength through the fused PLA powder, which still has porous channels due to physical fusion. However, this compressed pellet has a bulky volume compared to the thin layer of the PLA film. As the coating amount of PLA powder increased to 25, 50, 75, and 100 mg, the changes in the thickness of the compressed pellets were 1.1, 1.6, 2.3, and 3.7 mm, respectively.

A third approach is to modify the polymer morphology by blending PLA with other polymers. As described previously (7), Pluronic L-101 blending with PLA significantly modifies the release profiles of BSA. This might be attributed to the formation of a less porous morphology in the blend film as evidenced via scanning electron microscopy (7). In the blend, water–nonionic surfactant interactions may allow the formation of a liquid crystalline phase embedded in the PLA amorphous region, which keeps protein molecules from rapid release through aqueous pores. Thus, the burst effect can be reduced significantly and was dependent on the amount of L-101 incorporated (7).

When protein-loaded PLA and its Pluronic blend matrices are treated with the aqueous solution of PEI, it can be expected that PEI polymer chains will adsorb onto and/or diffuse into the polymer matrices, thereby ionically cross-linking protein molecules present near the surface region. This cross-linked protein layer may play a role as a diffusion barrier in retarding further release of protein molecules existing inside the matrices. Indeed, when these PLA/L-101 blend films were treated with a 1% aqueous solution of PEI, a more pronounced decrease in the protein burst as well as a significant extension of the release was seen (Fig. 2), compared to PEI untreated blend samples (7).

In order to examine the extent of PEI network formation with BSA and its effect on BSA release, we changed two parameters of PEI treatment: treatment time and PEI concentration. First, PLA/L-101 (90/10) blend films were treated with 1% aqueous solutions of PEI for different time periods (Fig. 3). It can be seen that the protein burst effect is more significantly reduced the longer the PEI treatment time is. It is obvious that with longer treatment times, water-soluble PEI can penetrate deeper into the blend matrices and form a thicker layer of PEI-BSA network. However, it is not clear why the treatment time of 120 min shows faster albumin release than that of either 60 or 90 min. This is probably due to nonuniform distribution of albumin in the blend films. Second, the PLA/L-101 (90/10) blend films were treated with different concentrations of aqueous PEI for a fixed time period (60 min) (Fig. 4). The treatment with higher PEI concentration allows the slow release of BSA with a minimal burst effect, again suggesting that the cross-linked network of BSA-PEI is more extensive and dense. Although we have changed the BSA release profiles by controlling the PEI treatment conditions, appreciable amounts of the loaded protein may not be released due to the formation of the PEI-BSA cross-linked layer. Thus, it will be important to optimize the PEI treatment as well as the protein loading amount to minimize the loss of the loading BSA amount. A thin and dense PEI–protein layer is desirable to reduce the burst effectively and to lengthen the release period. To establish further the existence of BSA–PEI interactions, FITC-labeled dextran, which is neutral in nature and has a similar MW to BSA, was loaded in PLA microspheres and then dextran release profiles were examined with and without the

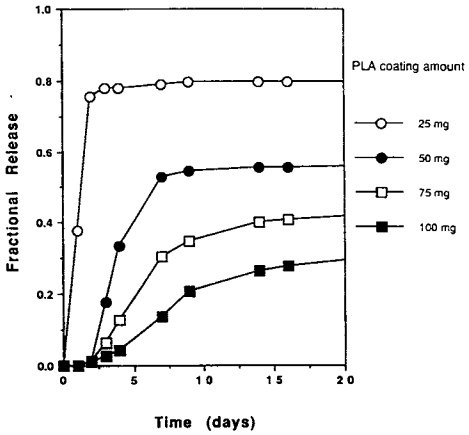

Fig. 1. BSA release profiles from PLA-coated pellets. Different amounts of PLA powder to be coated were placed on both sides of BSA-loaded PLA film and then compressed at 70°C.

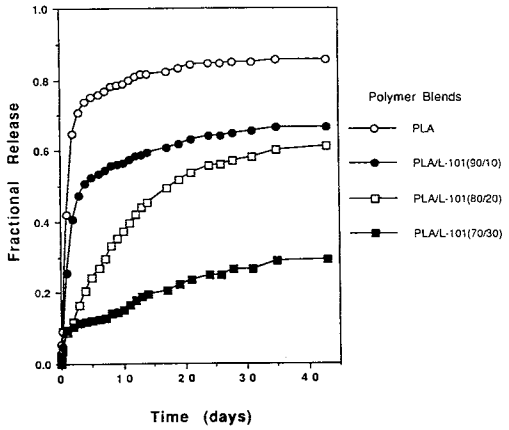

Fig. 2. BSA release profiles from PLA/Pluronic L-101 blends with the treatment of 1% PEI for 5 min.

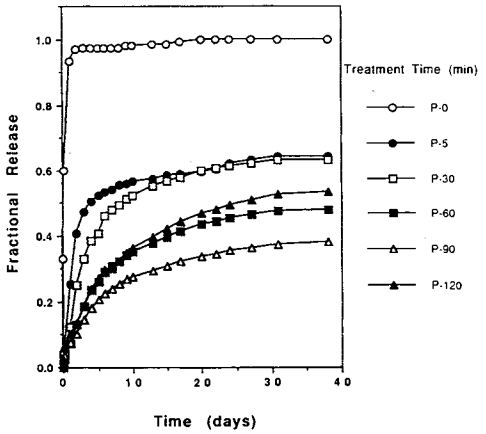

Fig. 3. BSA release profiles from PLA/Pluronic L-101 (90/10, w/w) blend film as a function of PEI treatment time. PEI concentration is 1%.

PEI treatment (1% concentration with 5 min). As a control, FITC-BSA-loaded PLA microspheres were used. It was observed that after 30 days, BSA release was reduced to 94.6% with the PEI treatment relative to the PEI untreated sample, while dextran release was reduced to 11.3% with the PEI treatment, supporting that, indeed, PEI cross-links BSA molecules in the matrix.

In order to utilize the PEI-coated polymer matrices as implantable devices, *in vivo* PEI safety should be considered. To our knowledge, there have been no human studies about the biocompatibility of PEI. However, several potential clinical applications of PEI have been reported (11–13). PEI has been used to inhibit the growth of tumor cells in mice at nontoxic levels to the host (11). It has also been known that PEI forms complexes with serum lipids and accelerates the hydrolysis of long-chain fatty acid esters at physiological pH (12). More recently, Starburst dendrimer microparticles, which contain many PEI segments in their backbone, were utilized as monoclonal antibody carriers for

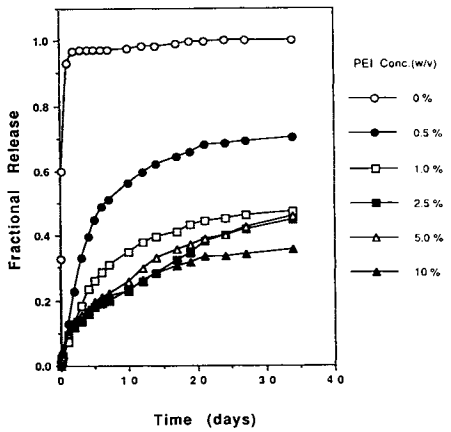

Fig. 4. BSA release profiles from PLA/Pluronic L-101 (90/10, w/w) blend film as a function of PEI concentration. PEI treatment time is 60 min.

diagnostic and therapeutic purposes in an *in vivo* animal model (13), as well as synthetic peptide vaccines in mice (14). In no case were any cytotoxic effects of these polycations observed. In this respect, clinical usage of PEI may be possible at its minimum toxic level. Alternatively, other polycations that behave similarly to PEI could be used for these coatings.

In conclusion, aqueous coating of PEI is an effective way to reduce the protein burst effect and to extend the release period by ionically cross-linking the negatively charged protein molecules near the surface region of the matrix. This method may be useful in controlling protein release profiles from polymeric matrices.

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