Microparticles of Novel Branched Copolymers of Lactic Acid and Amino Acids: Preparation and Characterization

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Abstract □ The preparation and characterization of microparticles produced from a new class of functionalized, biodegradable, comblike graft copolymers is presented. The copolymers are polyester–polyamino acid hybrids, composed of a poly(L-lactic acid–*co*-L-lysine) (PLAL) backbone, and poly(L-lysine), poly(D,L-alanine) or poly(L-aspartic acid) side chains extending from the lysine residues of PLAL. The microparticles have been characterized with regard to their surface properties, morphology, and size. Thus, electron spectroscopy for chemical analysis data and results of Zeta potential measurements suggest that the polyamino acid side chains tend to concentrate at the surface of the particles. Also, analyses by environmental scanning electron microscopy and confocal scanning laser microscopy indicate that particles carrying poly(lysine) chains have an unusual porous structure, most probably due to the combined effects of the amphiphilic, polyelectrolyte, and chemical nature of the composing copolymer, as well as of the particular preparation technique employed. The capabilities of the microparticles to serve as carriers in controlled drug release and delivery devices were demonstrated by encapsulation and release of rhodamine B, a low molecular weight drug model.

1. Introduction

The use of synthetic degradable polymers in drug delivery systems has dramatically expanded in the past decade. Several "old" drugs have gained renewed interest by improving their pharmacokinetic profile upon incorporation in polymer-based controlled-release systems.[1] Also, such systems, in the form of microcapsules, microparticles, and nanoparticles were found to be useful carriers for many low molecular weight compounds, peptides, and proteins, whose efficacy as drugs is highly dependent on the mode of their delivery to the body.[2−7]

One of the most advanced strategies in biopharmaceutics for the delivery of drugs and vaccines relies on the use of polymeric nano- and microparticles as carriers, mainly those composed of poly(lactic acid) (PLA) or poly(lactic acid−*co*-glycolic acid) (PLGA).[8] These degradable polyesters are nontoxic, well tolerated by living tissues, and degrade hydrolytically at controllable rates to yield naturally occurring metabolites. The procedure for incorporation of bioactive agents in these polymers is generally rather simple and reproducible. However, cases have been reported in which the conformational stability of incorporated proteins was impaired.[9,10]

The successful utilization of polymeric particles as drug carriers is highly dependent on their distribution in the body. Because of the surface characteristics of the particles, this distribution may not always be favorable. Thus, it has been extensively demonstrated that nanoparticles with a hydrophobic surface (e.g., PLA and PLGA) are rapidly taken up by the cells of the reticuloendothelial system (RES).[11−13] On the other hand, particles with a more hydrophilic surface can avoid this uptake to a greater extent, thus achieving a prolonged lifetime in the circulation and a better chance to efficiently deliver the therapeutic agent.[11−13] Toward this goal, such hydrophilic characteristics have been provided by modifying the surface of the otherwise hydrophobic particles with a hydrophilic polymer, either by chemical attachment or physical adsorption.[11−21]

These examples of modification of the preexisting PLGA copolymer represent an interesting approach for several applications. To advance this approach even further, it could be highly beneficial to prepare particles from an amphiphilic polymer that also possesses functional sites where chemical modifications can be carried out, and to utilize them for tailoring specific surface characteristics such as charge, hydrophilicity or targeting capabilities. For this purpose, we have prepared nanoparticles (data not presented) and microparticles from a new family of functional, degradable graft copolymers (PLAL-Lys, PLAL-Asp, and PLAL-Ala)[22,23] and from the linear copolymer PLAL.[24] The incorporation of poly(amino acid) side chains and PLA backbone in a hybrid copolymeric structure provides a unique opportunity to combine the attractive properties of these two classes of important biomedical polymers. PLAL-Lys and PLAL-Asp possess a large number of amino and carboxylic acid functional groups, respectively, and these may be utilized for further chemical modification (e.g., direct attachment of targeting moieties or drugs). At neutral pH, the poly(amino acid) side chains are positively charged (PLAL-Lys), negatively charged (PLAL-Asp), or neutral (PLAL-Ala). Here, we present the preparation of these microparticles and their characterization with regard to their surface properties (functionalization and charge), morphology, and size. In addition, incorporation in these particles of rhodamine B as a drug model and its release are also presented, and the data are considered for assessing the potential use of these particles as drug carriers. The use of PLAL-Lys microparticles as dry powder aerosols for pulmonary drug delivery has been investigated.[25,26]

2. Experimental Section

Materials—Poly(vinyl alcohol) (PVA; 88% hydrolyzed, MW 25 000) was obtained from Polysciences (Warrington, PA), rhodamine B base from Sigma (St. Louis, MO), and rhodamine B

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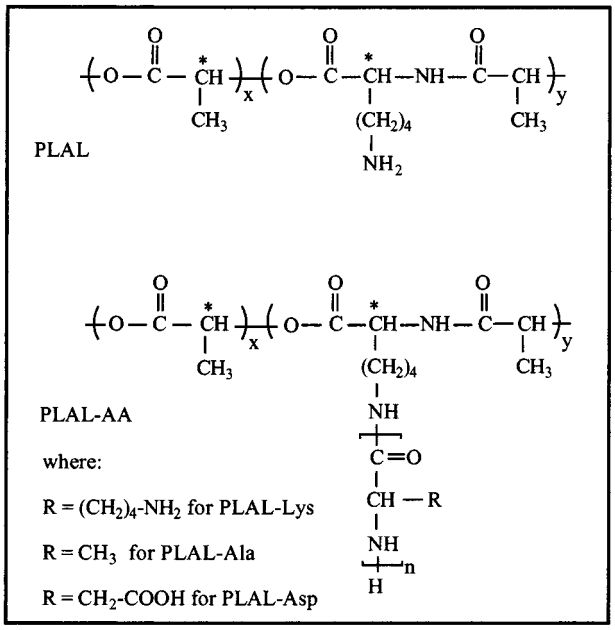

Scheme 1

isothiocyanate from Research Organics (Cleveland, OH). Phosphate buffered saline solution (PBS, 1 mM in KH_2PO_4, 10 mM in Na_2HPO_4, 137 mM in NaCl, 3 mM in KCl) was prepared with a pH of 7.4 unless otherwise noted.

Methods—*Synthesis of Copolymers*—PLAL was synthesized as described previously by Barrera et al.,[24] and PLAL-Lys, PLAL-Asp, and PLAL-Ala were synthesized as described by Hrkach et al.[22,23] The chemical structures of these copolymers are presented in Scheme 1. PLAL is a linear copolymer with approximately 2 mol % lysine randomly situated in a PLA backbone. The ϵ-amino groups of these lysine units are utilized as initiating sites for building poly(amino acid) chains from the PLAL backbone, thus leading to a comblike graft copolymer structure for PLAL-Lys, PLAL-Asp, and PLAL-Ala with total amino acid content of 15−20 mol %.

Preparation of Microparticles—Microparticles were prepared by the single emulsion/solvent evaporation method. One hundred milligrams of polymer were dissolved in 1 mL of dimethyl sulfoxide (DMSO) and subsequently mixed with 3 mL of methylene chloride. This solution was emulsified in 100 mL of PVA solution (5% w/v) for 60 s, using a homogenizer at 7500 rpm. The resulting emulsion was stirred continuously for 3 h in an open beaker to remove the organic solvents. The precipitated microparticles were centrifuged at 1000x *g* for 10 min, washed three times with distilled water, lyophilized, and stored under desiccation at room temperature.

For the preparation of particles incorporating rhodamine B, the same procedure was followed but 10 mg of rhodamine B were dissolved in CH_2Cl_2 before mixing with the polymer solution in DMSO.

Surface Modification—To investigate the structure of the PLAL-Lys and PLAL microparticles and their potential for attachment of targeting moieties or drugs, rhodamine B moieties were chemically attached to the lysine ϵ-amino groups on the surface of the microparticles. Two milligrams of microparticles were suspended in 1.5 mL of PBS and 0.5 mg of rhodamine B isothiocyanate (RITC) was added to the suspension. After stirring for 3 h, the suspension was centrifuged and washed three times with methanol to remove unreacted RITC. Microparticles were then suspended in water and analyzed by confocal microscopy (Biorad, MRC 600).

Characterization of Microparticles—*A. Size Distribution*—After lyophilization, a small amount of particles was resuspended in aqueous solution (Isoton solution) and the size distribution was measured using a Coulter Counter (Coulter Multisizer II, Coulter Electronics Ltd.). The average diameter was calculated as a mean value of the volume distribution.

B. Surface Charge—To evaluate the presence of electrostatic charges on the surface of the particles, the Zeta potential was determined by laser doppler anemometry (Zetasizer 3, Malvern Instruments, Malvern, UK) after suspending the particles in PBS at pH 7. The measurements were taken at 20 °C for 20 s, with an applied voltage of 150 V.

C. Surface Chemical Composition—For the chemical characterization of the particle surface, electron spectroscopy for chemical analysis (ESCA, also known as X-ray photoelectron spectroscopy, XPS, Model 5100, Perkin Elmer, with a magnesium anode as X-ray source at 15 kV and 300 W) was used. Aqueous suspensions of particles were mounted on metal stubs, and then the water was evaporated under vacuum over phosphorus pentoxide for at least 12 h. The angle between the sample surface and the analyzer was set to 45°. An elemental survey spectrum (0−1000 eV) was acquired for each sample.

D. Morphology—The morphology and structure of the microparticles were characterized using environmental scanning electron microscopy (ESEM, ElectroScan), and confocal scanning laser microscopy (MRC 600, BioRad Company, with rhodamine filter). For ESEM, samples were mounted with double-sided adhesive tape on metal stubs coated with gold−palladium to a thickness of 200−400 Å, and analyzed at 3−10 kV. For confocal microscopy, samples were first labeled with rhodamine isothiocyanate and suspended in water.

E. Incorporation and Release of Rhodamine B—To assess the incorporation efficiency and release characteristics of low molecular weight agents, rhodamine B base was used as a drug model. The incorporation efficiency was evaluated by measuring the fluorescence intensity (excitation: 554 nm; emission: 574 nm) of the solution obtained after complete degradation of 10 mg of particles in 0.1 M NaOH at 37 °C and by relating the results obtained to the fluorescence of a standard solution of rhodamine B in the same solvent. For these measurements a spectrofluorimeter (Photon Technology International) was used.

For the release studies, a known amount of dried microparticles were suspended in 5 mL of PBS in a plastic tube and incubated at 37 °C under continuous shaking. One milliliter of the supernatant from each tube was collected periodically after centrifugation and replaced each time with fresh PBS to mimic infinite sink conditions. The fluorescence intensity of the samples was measured as indicated for determination of loading and compared with a standard solution of rhodamine B base in the same solvent.

Table 1—Mean Diameters of Plain and Rhodamine-Loaded Particles

	plain particles	rhodamine-loaded particles	
constitutive polymer	average diameter (μm)	average diameter (μm)	dye loading (%w/w)
PLAL	4.9	6.1	2.2
PLAL-Lys	7.4	6.7	2.2
PLAL-Ala	6.2	8.5	18.7
PLAL-Asp	6.0	5.5	14.2

3. Results and Discussion

3.1. Particle Size Distribution—The size distribution of particles made of PLAL-Lys, PLAL-Asp, PLAL-Ala, or PLAL are presented in Figure 1. The PLAL microparticles show the narrowest size distribution and lowest mean diameter, whereas microparticles prepared from the graft copolymers exhibit a broader size distribution and a slightly larger average diameter. Nevertheless, it can be concluded that the commonly used single emulsion/solvent evaporation technique can be utilized effectively for the preparation of microparticles with relatively low average size ($<10\ \mu$m) from the new class of PLAL-amino acid graft copolymers. Table 1 summarizes the mean diameters of these particles, as well as of those in which rhodamine B base was incorporated. We note that, although different loading levels were achieved with various particles, incorporation of dye molecules had only a minimal effect on the average size of the particles.

3.2. Surface Chemical Composition—The presence of amino acid residues at the surface of the particles was confirmed by ESCA (Figure 2). The small nitrogen peak

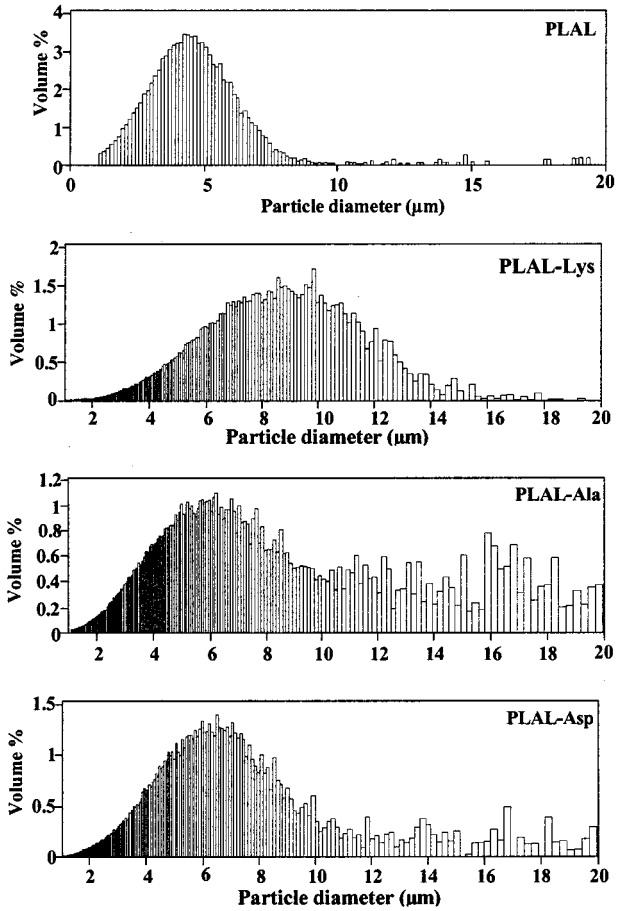

Figure 1—Size disributions of microparticles made of PLAL, PLAL-Lys, PLAL-Ala, and PLAL-Asp.

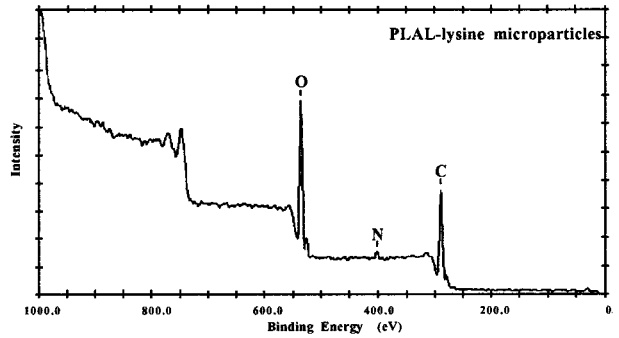

Figure 2—ESCA survey spectrum of PLAL-Lys microparticles.

at approximately 400 eV proves the presence of lysine residues at the outer layer of the particles, because these residues are the only nitrogen-containing ones in the copolymer. This result is in agreement with the hypothesis that the preparation procedure employed promotes orientation of the amino acid chains of the copolymer toward the outer layer of the particles. The relatively small size of the nitrogen peak in the ESCA spectrum is most likely due to the presence of PVA molecules in the outermost layer of the particles. As already stated, PVA was used as a surfactant to stabilize the microparticle structure during preparation. Thus, quantitative analysis of the nitrogen content, based on the ESCA spectrum, was not attempted.

ESCA spectra, similar to that of PLAL-Lys, were also obtained for PLAL-Asp and PLAL-Ala microparticles. However, for PLAL particles, the nitrogen peak was negligible due to the very low nitrogen content of their constitutive polymer (spectra not shown).

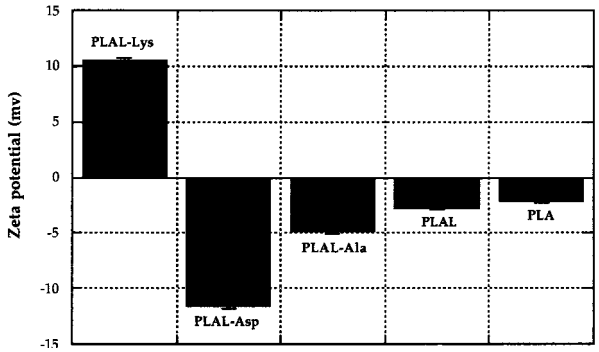

Figure 3—Zeta potential of microparticles made of PLAL, PLAL-Lys, PLAL-Ala, PLAL-Asp, and PLA.

3.3. Zeta Potential—Figure 3 shows the Zeta potentials of the microparticles in PBS at pH 7. The graph emphasizes the difference in surface charge among the microparticles studied. Thus, PLAL-Lys microparticles carry a positive charge, as expected from the presence of protonated ϵ-amino groups of the lysine residues at the particle surface. PLAL-Asp, PLAL-Ala and PLAL, on the other hand show negative charges. The large negative value for PLAL-Asp is attributed to the β- carboxylate groups of the aspartic acid residues. The apparently unexpected small negative charges exhibited by PLAL and PLAL-Ala particles are probably due to the terminal carboxylate groups of the backbone of their constitutive polymers. This view is supported by the fact that a similar, small negative charge is also exhibited by PLA particles. These results provide additional evidence for the presence of amino acid side chains on the surface of the particles, resulting from the extension of the hydrophilic portion of the comblike graft copolymer into the outer aqueous phase during preparation. More important, they demonstrate the versatility of this new class of materials. The ability to change the charge characteristics (type and strength) of the microparticles can be utilized to tailor their use for various drug delivery systems.

3.4. Particle Morphology and Surface Modification—ESEM and confocal microscopy were utilized to study the structure and morphology of the particles. As seen in Figure 4, ESEM reveals that PLAL particles show a smooth surface and a spherical structure, similar to what is observed with PLA or PLGA particles (data not shown). Apparently, the low amount of lysine (about 2 mol %) present in the PLAL chain does not significantly affect the particle morphology. On the other hand, PLAL-Ala and PLAL-Asp particles have a slightly corrugated surface. Most likely, the extension of the hydrophilic amino acids chains into the aqueous phase during particle preparation promotes the formation of the rougher surface exhibited by these particles, compared with that of PLAL, PLA, or PLGA particles prepared by the same technique. In strong contrast to the microparticles just mentioned, PLAL-Lys particles have an unusual shape and also a porous, spongelike structure. It is likely that the large amount of lysine in the copolymer structure, in the form of grafted poly(lysine) side chains, imparts specific characteristics to the polymer and to the resulting particles. The fact that only microparticles made of PLAL-Lys exhibit this structure is highly relevant. From these results it is possible to conclude that the porous structure does not stem solely from the presence of an amphiphilic or amino acid-based polymer in general, but specifically from the presence of lysine chains (and perhaps other poly-cationic chains).

The results of confocal microscopy analysis of PLAL-Lys and PLAL particles are shown in Figure 5. Covalent attachment of the fluorescent marker to the particles was

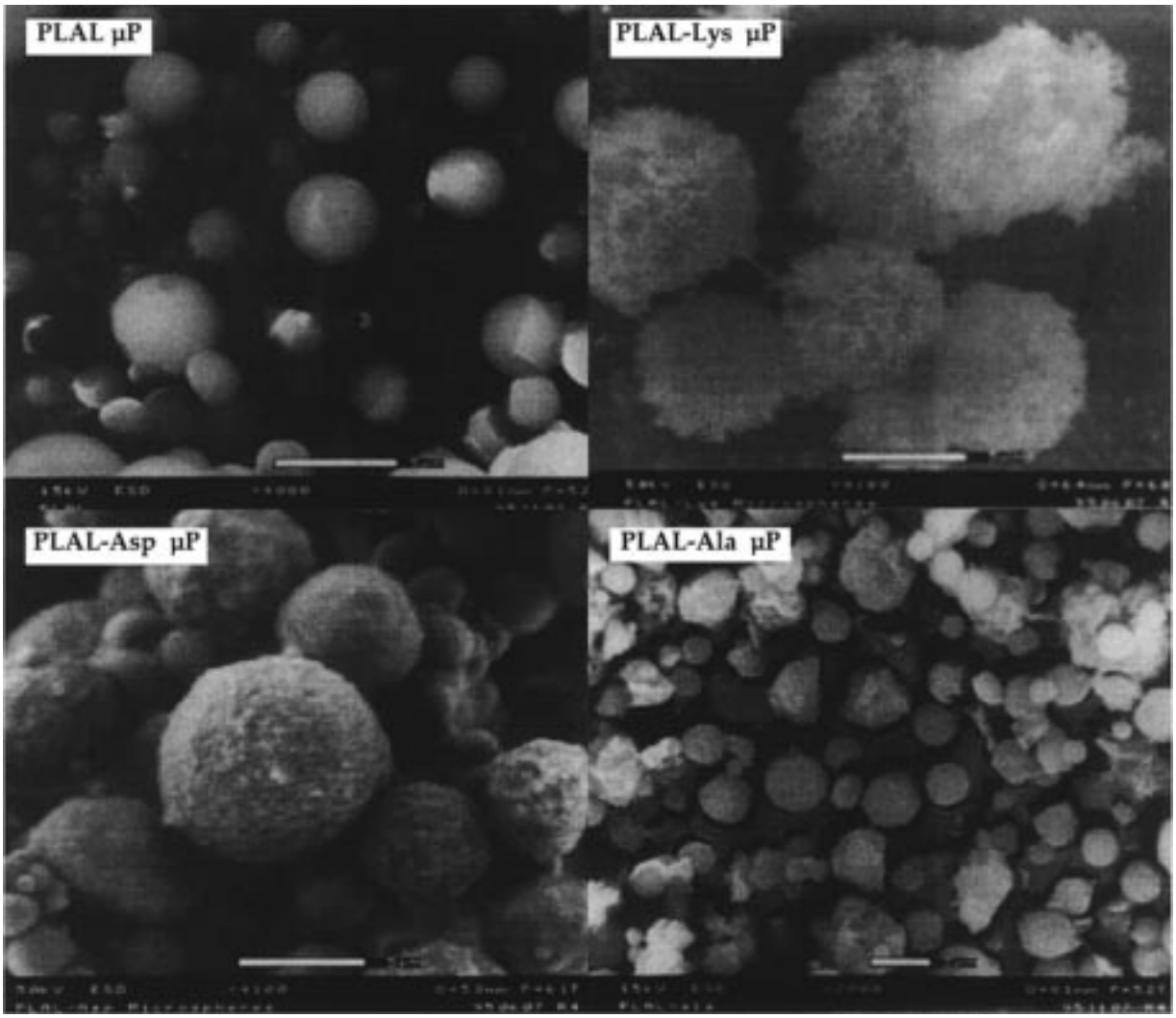

Figure 4—ESEM pictures of microparticles made of PLAL (magnification, ×4000), PLAL-Lys (x4100), PLAL-Ala (x2000), and PLAL-Asp (x4100). All scale bars = 5 μm.

accomplished through the reaction of rhodamine isothiocyanate with the lysine ϵ-amino groups on the surface of the particles. Under the same experimental conditions, PLA particles yield no fluorescent response (a completely dark field) confirming covalent attachment of rhodamine moieties at the surface amine groups of PLAL-Lys and PLAL and ruling out nonspecific adsorption of the dye to the particle surface. The successful coupling of rhodamine proves the availability of the surface amino groups for further chemical modification. This characteristic imparts great versatility to this new class of functional particles, and may be utilized to improve cellular targeting and uptake of peptides/proteins, anticancer drugs, and other therapeutic agents that require or could benefit from site-specific delivery.

The capacity of these particles for binding functional molecules onto their surface is very high indeed. For example, for PLAL-Asp particles with an aspartic acid content of 20 mol % and assuming that all the β-COOH groups of Asp residues are present at the surface, a capacity of 2.7 mmol-COOH/g is calculated. And if each carboxyl group would bind one molecule of dye of opposite charge and of molecular weight 440 (similar to that of rhodamine B base) a loading capacity of about 55% (w/w) dye could be accommodated. A similar calculation, when performed for PLAL-Lys particles contaning 20 mol % Lys residues, yields 2.4 mmol-NH_2/g and 50% (w/w) labeling capacity.

The structural characteristics of the PLAL-Lys particles have highly valuable implications in the area of pulmonary drug delivery as well. The flight characteristics of dry powders are crucial for the efficient delivery of therapeutic agents to the lungs, for either local or systemic therapy. In vitro aerosolization experiments carried out in our laboratory show that 57 ± 1.9% of the PLAL-Lys particles are in the respirable fraction, whereas only 9.3 ± 0.6% of the nonporous PLA or PLGA particles of the same size are respirable. Furthermore, in vivo studies in rats show that the pulmonary delivery of testosterone with PLAL-Lys particles results in prolonged release compared with nonporous particles.[25]

3.5. Incorporation Efficiency and Release Characteristics—In Table 1, data relating the average size of microparticles and their efficiency in incorporating rhodamine B are presented. We note that all the microparticles have essentially similar size distribution and mean diameter. However, their efficiency for loading this low molecular weight drug model seems to be significantly dependent on the chemical nature of the constitutive polymer and its interaction with the drug model molecule. Thus, the basic dye rhodamine B is efficiently incorporated (14.2%) in microparticles made of the acidic, negatively charged PLAL-Asp, but only very little dye (2.2%) is accommodated by particles made of the basic, positively charged PLAL-Lys. The highest incorporation efficiency (18.7%) was

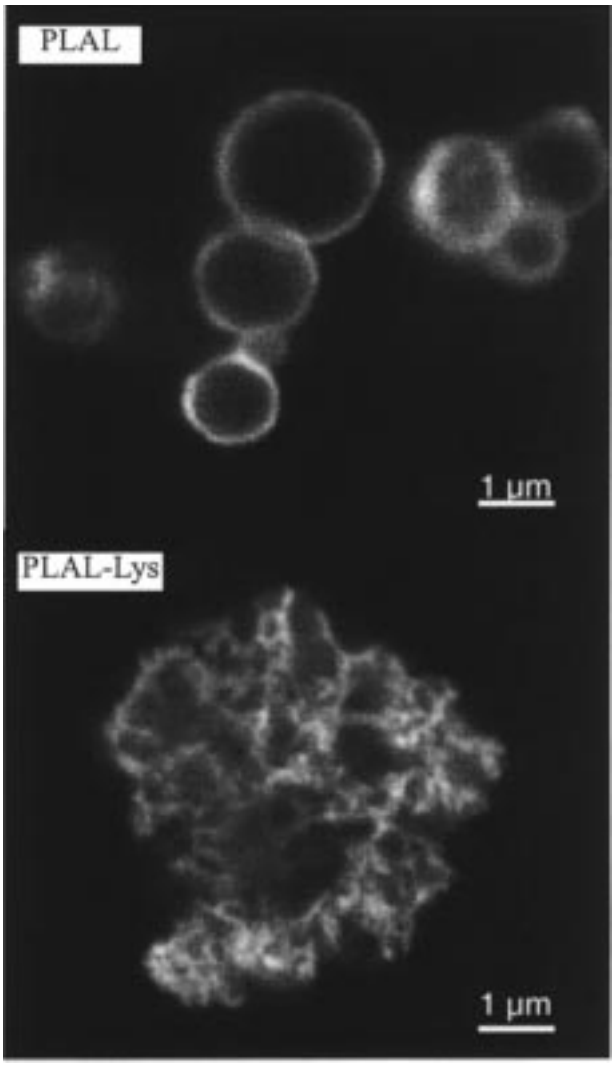

Figure 5—Confocal microscopy pictures of PLAL-Lys and PLAL microparticles, after labeling with rhodamine B isothiocyanate.

reached with the essentially neutral PLAL-Ala, whereas the similarly neutral PLAL particles exhibit very low efficiency (2.2%), similar to PLAL-Lys particles. These results indicate that, in addition to electrostatic interactions, dye incorporation efficiency is also to be related to other factors, such as hydrophobicity/hydrophilicity balance and dye partition characteristics.

The release profiles of rhodamine B from PLAL− and PLAL−poly (amino acid) microparticles over a period of 8 weeks are shown in Figure 6. All of the PLAL−poly(amino acid) microparticles exhibited a basically similar release behavior; namely, an initial burst in the first 2−3 days when some 35% of the incorporated dye is liberated, followed by a continuous much slower process, which was monitored only until day 56. Up to this point, only 45−50% of the dye is released and, obviously, the process continues further on. The release profile of rhodamine B from PLAL microparticles follows, in principle, the same time course as already described for the PLAL−poly(amino acid) microparticles. Yet it differs in the quantitative aspects of it: thus, only 6% dye is released during the initial burst of 1 day and only 25% of the dye is released by day 56, during the subsequent low rate process.

From these results it can be concluded that the very presence of poly(amino acid) side chains into a branched copolymer with PLA backbone may affect the drug loading capacity of these particles (see Table 1) yet may have a

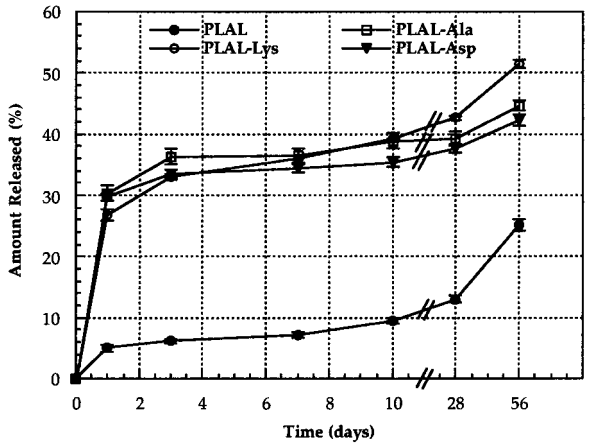

Figure 6—Release profile of rhodamine B from PLAL, PLAL-Lys, PLAL-Ala, and PLAL-Asp microparticles.

significant influence on the release of drugs from microparticles composed of these polymers. However, the specific properties of the various poly(amino acids) considered did not significantly affect the release process because the pertinent profiles for PLAL-Lys, PLAL-Ala, and PLAL-Asp microparticles are all similar. Obviously, depending on specific drug−polymer interactions, the release characteristics may be different when drug molecules of various structure, hydrophobicity, and charge are used.

Further studies focusing on the release of various drugs from these microparticles will be carried out to gain a better understanding of the role of the amino acid chains on the structure and properties of the microparticles and on the mechanism of drug release from them.

4. Conclusions

Microparticles made of PLAL, PLAL-Lys, PLAL-Ala, or PLAL-Asp were prepared and investigated. Due to their specific structure, this new class of copolymers imparts interesting surface (charge, external functional groups) and morphological (porosity) characteristics to the microparticles. The chemical attachment of molecular markers on the surface of PLAL and PLAL-Lys microparticles has also been accomplished. This attachment capability demonstrates the potential use of these particles for attaching various functional moieties, including targeting elements required for site specific controlled drug delivery.

References and Notes

1. *Biodegradable Polymers as Drug Delivery Systems*; Chasin, M., Langer, R., Eds.; Marcel Dekker: New York, Basel, Hong Kong, 1990; Vol. 45.
2. Heller, J. Polymers for controlled parenteral delivery of peptides and proteins. *Adv. Drug Del. Rev.* **1993**, *10*, 163−204.
3. Couvreur, P.; Puisieux, F. Nano- and microparticles for the delivery of polypeptides and proteins. *Adv. Drug Del. Rev.* **1993**, *10*, 141−162.
4. Couvreur, P.; Dubernet, C.; Puisieux, F. Controlled drug delivery with nanoparticles: current possibilities and future trends. *Eur. J. Pharm. Biopharm.* **1995**, *41*(*1*), 2−13.
5. Brannon Peppas, L. Recent advances on the use of biodegradable microparticles and nanoparticles in controlled drug delivery. *Int. J. Pharm.* **1995**, *116*, 1−9.
6. Davis, S. S.; Illum, L.; Moghimi, S. M.; Davies, M. C.; Porter, C. J.; Muir, I. S.; Brindley, A.; Christy, N. M.; Norman, M. E.; Williams, P.; Dunn, S. E. Microspheres for targeting drugs to specific body sites. *J. Controlled Release* **1993**, *24*, 157−163.

7. Cohen, S.; Yoshioka, T.; Lucarelli, M.; Hwang, L. H.; Langer, R. Controlled delivery systems for proteins based on poly-(lactic acid/glycolic acid) microspheres. *Pharm. Res.* **1991**, *8* (*6*), 713−720.
8. Alonso, M. J.; Cohen, S.; Park, T. J.; Gupta, R. K.; Siber, G. R.; Langer, R. Determinants of release rate of tetanus vaccine from polyester microspheres. *Pharm. Res.* **1993**, *10* (*7*), 945−953.
9. Schwendeman, S. P.; Cardamone M.; Klibanov A.; Langer R.; Brandon M. R. In *Microparticulate Systems for the Delivery of Proteins and Vaccines*; Cohen, S., Bernstein, H., Eds.; Marcel Dekker: New York, 1996; pp 1−49.
10. Iwata, M.; McGinity, J. W. Dissolution, stability and morphological properties of conventional and multiphase poly(D,L-lactic acid-*co*-glycolic acid) microspheres containing water soluble compounds. *Pharm. Res.* **1993**, *10*, 1219−1227.
11. Gref, R.; Minamitake, Y.; Peracchia, M. T.; Trubetskoy, V.; Torchilin, V.; Langer, R. Biodegradable long-circulating polymeric nanospheres. *Science* **1994**, *263*, 1600−1603.
12. Illum, L.; Davis, S. S. The organ uptake of intravenously administered colloidal particles can be altered using a nonionic surfactant (Poloxamer 338). *FEBS Lett.* **1984**, *167*, 79−82.
13. Illum, L.; Davis, S. S.; Muller, R. H.; Mak, E.; West, P. The organ distribution and circulation time of intravenously injected colloidal carriers sterically stabilised with block-copolymer poloxamine 908. *Life Sci.* **1987**, *40*, 367−374.
14. Stolnik, S.; Dunn, S. E.; Garnett, M. C.; Davies, M. C.; Coombres, A. G. A.; Taylor, D. C.; Irving, M. P.; Purkiss, S. C.; Tadros, T. F.; Davis, S. S.; Illum, L. Surface modification of poly(lactide-*co*-glycolide) nanospheres by biodegradable poly(lactide)-poly(ethylene glycol) copolymers. *Pharm. Res.* **1994**, *2*(*11*), 1800−1808.
15. Gref, R.; Domb, A.; Quellec, P.; Blunk, T.; Muller, R. H.; Verbavatz, J. M.; Langer, R. The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. *Adv. Drug Del. Rev.* **1995**, *16*, 215−233.
16. Stolnik, S.; Illum, L.; Davis, S. S. Long circulating microparticulate drug carriers. *Adv. Drug Del. Rev.* **1995**, *16*, 195−214.
17. Kwon, G. S.; Kataoka, K. Block copolymer micelles as long-circulating carriers for delivery of therapeutic and diagnostic agents. *Adv. Drug Del. Rev.* **1995**, *16*, 295−309.
18. Moghimi, S. M. Mechanisms regulating body distribution of nanospheres conditioned with pluronic and tetronic block-copolymers. *Adv. Drug Del. Rev.* **1995**, *16*, 183−193.
19. Torchilin, V. P.; Trubetskoy, V. S. Which polymers can make nanoparticulate drug carriers long-circulating? *Adv. Drug Del. Rev.* **1995**, *16*, 141−155.
20. Rooser, M.; Kissel, T. Surface-modified biodegradable albumin nano- and microspheres. I. Preparation and characterization. *Eur. J. Pharm. Biopharm.* **1993**, *39*(*1*), 8−12.
21. Hrkach, J. S.; Peracchia, M. T.; Domb, A.; Lotan, N.; Langer, R. Nanotechnology for biomaterials engineering: structural characterization of amphiphilic polymeric nanoparticles by ^{1}H NMR spectroscopy. *Biomaterials* **1997**, *18*, 27−30.
22. Hrkach, J. S.; Ou, J.; Lotan, N.; Langer, R. Synthesis of poly(L-lactic acid-*co*-L-lysine) graft copolymers. *Macromolecules* **1995**, *28*, 4736−4739.
23. Hrkach, J. S.; Ou, J.; Lotan, N.; Langer, R. Poly(L-lactic acid-*co*-amino acid) graft copolymers: A class of functional biodegradable biomaterials. In *Hydrogels and Biodegradable Polymers for Bioapplications*; Ottenbrite, R. M.; Huang, S. J., Park, K., Eds.; ACS Symposium Series No. 627; American Chemical Society: Washington, DC, 1996; Ch. 8, pp 93−102.
24. Barrera, D. A.; Zylstra, E.; Lansbury, P. T.; Langer, R. Synthesis and RGD peptide modification of a new biodegradable copolymer: Poly(lactic acid-*co*-lysine). *J. Am. Chem. Soc.* **1993**, *115*, 11010−11011.
25. Edwards, D. A.; Hanes, J.; Caponetti, G.; Hrkach, J. S.; Ben-Jebria, A.; Eskew, M. L.; Mintzes, J.; Deaver, D.; Lotan, N.; Langer, R. Large porous particles for pulmonary drug delivery. *Science* **1997**, *276*, 1868−1871.
26. Caponetti, G.; Hrkach, J. S.; Edwards, D. A.; De la Cruz, C. P.; Heidel, J. D.; Lotan, N.; Colombo, P.; Langer, R, manuscript in preparation.

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