ELSEVIER

International Journal of Pharmaceutics 159 (1997) 197–206

international journal of pharmaceutics

Polypeptides for controlled release applications: Synthesis and preliminary characterization and release studies

Thomas J. Cook, Gordon L. Amidon, Victor C. Yang *

College of Pharmacy, The University of Michigan, 428 Church St., Ann Arbor, Michigan 48109-1065, USA

Received 30 December 1996; received in revised form 15 July 1997; accepted 28 August 1997

Abstract

The objective of this study is to evaluate the feasibility of synthesizing biodegradable polypeptide polymers of sufficient hydrophobicity so that release of solute is diffusion controlled. The benefit of such a diffusion-controlled, biodegradable polypeptide system is that the main advantages of biodegradable (avoiding the retrieval of the implanted device) and non-degradable polymers (greater structural integrity) are both exploited. In this study, one sequential polypeptide and two random co-polypeptides with different hydrophobicities were synthesized using active ester intermediates. The polymers were characterized with regard to their chemical composition, molecular weight and hydrophobicity. The in vitro release of a model steroid, 17α-ethynylestradiol, from compressed disks of these polymers followed a square root of time dependence indicating a diffusion controlled release mechanism. The drug release rates could be varied over several orders of magnitude and covered the therapeutic range for an implantable device delivering the steroid drug on the order of 10 mg per day. The results of these studies indicate that polypeptide co-polymers can be made with sufficient hydrophobicity to provide release rates in the therapeutic range for parenteral drug delivery. © 1997 Elsevier Science B.V.

Keywords: Poly(amino acids); Sequential polypeptides; Random co-polymers; Controlled release

1. Introduction

Controlled release implants that use a biodegradable polymer matrix have enjoyed popularity in pharmaceutical research (Leong et al., 1985). While a number of these materials are still being developed in preliminary research as well as clinical trials, only one particular member of this class of polymers, polyesters of glycolic and lactic acid, is approved for human use. The hurdle of biocompatibility is particularly daunting for biodegradable polymers since the polymer as well as its major degradation products should be deemed safe. Since the polymer and its degradation products are subject to evaluation for toxicity

* Corresponding author. Tel: + 1 313 7644273; fax: + 1 313 7632022; e-mail: vcyang@umich.edu

PII S0378-5173(97)00308-6

lycine), poly(phenylalanine), sodium azide and 17α-ethynylestradiol (EE2) were purchased from Sigma (St. Louis, MO). Pentachlorophenol (PCPOH), 1,3-dicyclohexylcarbodiimide (DCC), and trifluoroacetic acid (TFA) were purchased from Aldrich (Milwaukee, WI). *N-t*-Butyloxycarbonyl-L-serine-*O*-benzyl ether (Boc-SerOBzl) was purchased from Bachem (Philadelphia, PA). Solvents of reagent grade were purchased from Mallinckrodt (Paris, KY) and stored over molecular sieves (4A beads, 4-8 mesh, Aldrich). Triethylamine (TEA) was purchased from J.T. Baker (Phillipsburg, NJ). Tritiated 17α-ethynylestradiol was purchased from Dupont/NEN (Bsoton, MA). Distilled, deionized water was used throughout the studies.

2.2. Synthesis of polypeptides

For the synthesis of the polypeptides, standard solution phase peptide chemistry techniques were used. A general synthetic scheme (Fig. 1) was designed to facilitate the synthesis of multiple products and to minimize racemization.

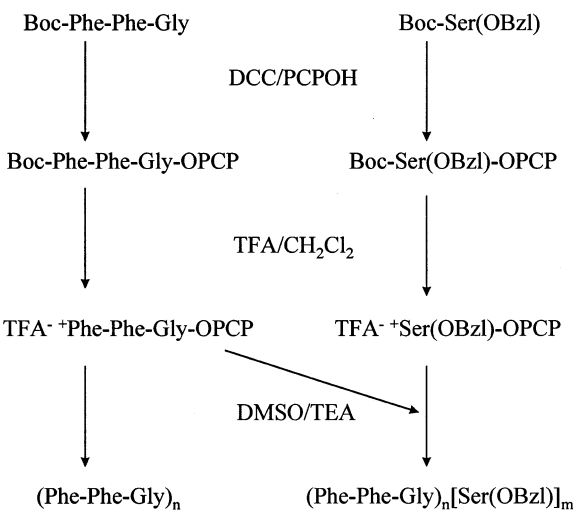

Fig. 1. Reaction scheme for the synthesis of sequential and random polypeptides used in this study. See text for abbreviations.

2.2.1. Boc-Phe-Phe-Gly-OPCP

To a solution of *N-t*-Boc-L-Phe-L-Phe-Gly (0.5 g) and pentachlorophenol (PCPOH; 0.852 g) in ethyl acetate (2 ml) and dimethylformamide (0.3 ml) at 0°C, 1,3-dicyclohexylcarbodiimide (DCC; 0.2194 g) was added. After 1 h at 0°C, the ice water bath was removed and the mixture stirred at room temperature for 2 h. The suspension was cooled to −10°C, filtered, and the solid was washed three times with 10-ml portions of cold *p*-dioxane and ethyl ether. The white solid was transferred into 45 ml of isopropanol with stirring for 4 h, filtered, and washed with 30 ml of isopropanol to give 0.61 g of pure white Boc-Phe-Phe-Gly-OPCP. Melting point (MP) = 207–212°C (m/e = 718, C:H:N: calculated 52.02, 4.23, 5.87; experimental 51.75, 4.43, 5.78).

2.2.2. TFA^- $^+$Phe-Phe-Gly-OPCP

A solution of equal volumes of trifluoroacetic acid and dichloromethane was added to the Boc-protected tripeptide ester until the solid dissolves. This provides a large excess of TFA. The reaction solution was stirred for 1–2 h at room temperature. The solvents were removed by rotavapor at 37°C. The residual oil was triturated to powder with ethyl ether (10 ml), filtered and washed with ethyl ether (10 ml × 4), then dried in vacuo for a few hours. This intermediate was synthesized immediately prior to polymerization (yield > 90%).

2.2.3. Poly(Phe-Phe-Gly)

The polymerization was carried out in dimethylsulfoxide (DMSO) at room temperature, in concentrated solutions of the tripeptide pentachlorophenyl ester salts with TEA as catalyst. To 1 mmol of TFA^- $^+$Phe-Phe-Gly-OPCP dissolved completely in 1 ml of DMSO, 2 mmol of TEA was added dropwise with stirring. The polymerizing mixtures converted into a highly viscous gel in a matter of minutes. A second addition of TEA (2 mmol) was made one day later and then the polymerization was allowed to proceed at room temperature for 5 days. The reaction mixture was dried in vacuo for a few hours, and the gel was triturated to powder with ethyl ether (10 ml × 6), and dried in vacuo. To the powder, 8 ml of water was added with stirring for 40 min, then

Table 1
Amino acid and elemental analysis of synthesized polymers

Polymer	Amino acid analysis	Elemental analysis					
	Phe:Gly:Ser (pmol ratio)	C		H		N	
		Calc.	Actual	Calc.	Actual	Calc.	Actual
Poly(Phe-Phe-Gly)	2:1.26:0.00	68.36	62.31	6.02	6.24	11.96	10.88
$Poly(Phe\text{-}Phe\text{-}Gly)_1(SerOBzl)_{0.7}$	2:1.13:0.66	68.19	68.09	6.08	6.27	10.94	11.13
$Poly(Phe\text{-}Phe\text{-}Gly)_1(SerOBzl)_{1.2}$	2:0.96:1.21	68.00	65.65	6.10	6.33	10.41	9.59

washed with water (10 ml), and dried in vacuo overnight. Overall yield 46%. Amino acid analysis and C/H/N element analysis are tabulated in Table 1.

2.2.4. Boc-SerOBzl-OPCP

A solution of Boc-SerOBzl (2.0 g, 6.77 mmol) and pentachlorophenol (5.4 g, 20.3 mmol) in 11.3 ml of ethyl acetate and 1.1 ml of dimethylformamide was cooled to 0°C on an ice water bath. To this solution, 1.4 g (6.77 mmol) of DCC was added. After 1 h of stirring at 0°C, the ice water bath was removed and stirring continued for 2 h. During this time a precipitate forms which was dicyclohexylurea. After filtering and washing with dioxane (5 ml each time), the product of the evaporation step was not a solid, but a gel. A solid was produced by recrystallizing from ethyl ether–petroleum ether. However, this solid demonstrated two spots on TLC, solvent system A. The desired product, Boc-SerOBzl-OPCP, was effectively separated from the pentachlorophenol contaminant by repeated liquid chromatography using Silica Gel 230–400 mesh and a gradient elution of chloroform with 1% v/v acetic acid as component A and methanol with 1% v/v acetic acid as component B. Evaporation of the solvent on a rotary evaporator and recrystallization from ethyl ether/petroleum ether produced the chromatographically pure compound (A: $R_f = 0.94$, MP = 90–92°C). The product was confirmed by mass spectroscopy.

2.2.5. $TFA^-\ {}^+SerOBzl\text{-}OPCP$

Synthesis was performed according to the same procedures described above in Section 2.2.2. The TFA salt was used directly for subsequent polymerization.

2.2.6. $Poly(Phe\text{-}Phe\text{-}Gly)_1(SerOBzl)_{1.2}$ and $Poly(Phe\text{-}Phe\text{-}Gly)_1(SerOBzl)_{0.7}$

These two random copolymers were synthesized following the procedures described above in Section 2.2.3. In two separate polymerizations, the ratios of the starting monomers, $TFA^-\ {}^+$Phe-Phe-Gly-OPCP and $TFA^-\ {}^+$SerOBzl-OPCP, were controlled to be 1:2 and 1:1, respectively. The polymerizations were carried out in concentrated solutions of DMSO (a total of 1 mmol monomers in 1 ml DMSO) with TEA (2 mmol) as the initiator. The yield of $Poly(Phe\text{-}Phe\text{-}Gly)_1(SerOBzl)_{1.2}$ was 16% and the yield of $Poly(Phe\text{-}Phe\text{-}Gly)_1(SerOBzl)_{0.7}$ was 43%. Amino acid composition and C/H/N element analyses are listed in Table 1.

2.3. Amino acid analysis

Amino acid analyses of the synthesized polymers were conducted by the Protein and Carbohydrate Structure Core Facility at the University of Michigan. In brief, vapor phase hydrochloric acid hydrolysis was performed in an ABI Model 420H instrument. After drying under argon, the sample was derivatized with phenylisothiocyanate and

transferred to an ABI Model 130A for chromatographic separation. The results are expressed in a picomole ratio and are listed in Table 1.

2.4. Molecular weight measurements

The molecular weights of Poly(Gly) and Poly(Phe-Phe-Gly) were determined by measuring the intrinsic viscosity of polymer solutions. Published reports on the viscosity of poly(amino acids) (Doty et al., 1956; Brack and Trudelle, 1985) were used for the molecular weight determination. The equation based on solutions of poly(γ-benzyl-L-glutamate) in dichloroacetic acid was used to calculate the molecular weight: $[\eta]M_o = 66n^{0.87}$ where $[\eta]$ is the intrinsic viscosity, M_o is the mean residue weight (MW of an average repeating unit), and n is the average number of residues in a polymer chain (Brack and Trudelle, 1985). The intrinsic viscosities of the polymer solutions were measured following the single determination method of Solomon and Ciutâ (1962). Solutions of Poly(Phe-Phe-Gly) and Poly(Gly) were made in dichloroacetic acid at a concentration of approximately 0.2% (w/v). Before measuring, the solutions were filtered through a coarse fritted disk filter. Viscosity measurements of the polymer solutions and pure dichloroacetic acid were carried out using an Ubbelohde viscometer (Cannon CUSMU size 75) at a temperature of 25°C held stable in a water bath. For comparison purposes, the molecular weight of the three synthesized polypeptides (i.e. Poly(Phe-Phe-Gly), Poly(Phe-Phe-Gly)$_1$ (SerOBzl)$_{1.2}$ and Poly(Phe-Phe-Gly)$_1$(SerOBzl)$_{0.7}$) were also measured by laser desorption mass spectroscopy (performed at the Protein and Carbohydrate Structure Core Facility at the University of Michigan).

2.5. Hydrophobicity estimation

The π-value scale of Fauchère et al. (1988) was used to estimate the relative hydrophobicity of the synthesized and the commercial polypeptides. This π-value is an amino acid hydrophobicity scale based on the octanol/water partition coefficient of amino acid analogues. By blocking the amine terminus with an acetyl group and making the carboxy terminus an amide, charge contributions from zwitterionic species were avoided. The scale is normalized to the glycine analogue, which has been assigned a π-value of zero. To calculate the π-value, Fauchère et al. (1988) subtracted the octanol/water partition coefficient ($P_{o/w}$) of *N*-acetylglycine-amide from each corresponding amino acid analogue (i.e. π-value = log $P_{o/w}$ (*N*-acetyl-amino acid-amide) − log $P_{o/w}$ (*N*-acetyl-glycine-amide)) (Fauchère et al., 1988). In this study, the estimated hydrophobicity of the side chains for an average repeating unit in the polymers is the sum of the π-values for the amino acids in an average repeat unit divided by the average number of amino acids in a repeat unit.

2.6. Disk formation and release study

Polymer disks containing EE2, a commonly used steroid for hormonal contraception, and a trace amount of radiolabeled EE2 were prepared following a published procedure (Cohen et al., 1984). A solution of radiolabeled and unlabeled EE2 was made so that the drug concentration was 25 mg/ml and the ratio of cold to hot drug was 80.96 mg/μCi. An aliquot of this solution was added to each polymer powder in a scintillation vial (Research Products International) so that the percent loading was approximately 20%. The ethanol was evaporated via a vacuum pump. Each polymer/drug mixture was ground in a glass mortar and pestle, passed through a 250-μm sieve, and vortexed to evenly disperse the components. Portions were weighed (40–50 mg) and placed into the stainless steel dies (made by the University of Michigan Physics Shop). The dies and powder were heated in a dry oven (Blue M, Model C-3991-Q) at 50°C for at least 15 min. Each die was taken separately and compressed on a laboratory press (Carver Model B) at 1000 lb (1×10^9 dyn/cm^2) for 15 min. The remaining polymer/drug mixture was weighed into scintillation vials for future radioactivity counting to determine the precise percent loading. Each disk was placed in a scintillation vial and 20 ml of PBS solution at pH 7.4 containing 0.05% sodium azide was added. The vials were placed in a shaking

water bath and shaken continuously at 80 rev./min at 37°C. At each time point, the total volume of PBS was removed and replaced with a fresh solution. An aliquot of the sample (3 ml) was used for scintillation counting. The release was followed over a period of 90 days. No cloudiness from microbial growth was noted during this time period.

3. Results and discussion

The primary method of chemically synthesizing polypeptides is through the active ester intermediate of a peptide. While the strength of this approach lies in the creation of sequential polypeptides, the active ester synthetic pathway can also be used to produce random copolymers. Reviews by Goren (1974) and by Jones (1977) summarize the effects of the variables involved with sequential polypeptide synthesis through active ester polycondensation. The choice of monomer and active ester is very crucial. An appropriate combination of these factors will maximize polymerization while minimizing or eliminating the risk of racemization at the carboxy terminus. Where feasible, a glycine or proline is most desirable at the carboxy terminus. The achiral glycine and sterically hindered proline preempt the risk of racemization. The pentachlorophenol ester moiety is one of the most reactive leaving groups for this type of synthesis. In addition, when the carboxy terminus cannot be glycine or proline, the pentachlorophenol ester is particularly attractive because of its excellent polymerizing to racemizing activity (Kovacs et al., 1972). In an attempt to maximize the polymer yield and eliminate the risk of racemization, the pentachlorophenol ester was used in this study, and the main tripeptide monomer was chosen so that the carboxy terminus is glycine.

One sequential polypeptide, Poly(Phe-Phe-Gly), was synthesized in this initial study. Sequential polypeptides are the most attractive type of the amino acid polymers. Their specific amino acid sequence is known and can be manipulated to confer different bulk properties on the final polymer. To avoid cyclization in the polymerization reaction, the monomer for a sequential polypeptide should have at least three amino acid residues in the sequence (Jones, 1977). Our approach with a tripeptide monomer gives us potential control over the properties of the polymer. Appropriate choice of amino acid sequence will provide polymers with varied degrees of hydrophobicity and, possibly, varied degrees of secondary structure.

Two random copolymers containing the Phe-Phe-Gly tripeptide sequences were also synthesized in this study. The serine residues, with the –OH groups protected by benzyl ether linkages, were used to produce the copolymers with the Phe-Phe-Gly residues for several reasons. One is that the rather hydrophobic benzyl protecting group can enhance the overall hydrophobicity of the synthesized copolymers. Another reason is that the benzyl protecting groups on the copolymers can be either completely or partially hydrolyzed to produce the desired hydrophobicity to the copolymers. The third reason is that the –OH groups of the 'de-blocked' serine residues on the copolymers could potentially be used as linkers for attachment of various drugs.

The yields of these synthesized polymers ranged from approximately 16% up to 46%. The amino acid analysis of the products confirms the composition of these polymers (Table 1). The analysis also suggests different reactivities of the monomers used. The serine benzyl ether pentachlorophenol ester appears less reactive than the tripeptide active ester. The evidence lies in comparing the starting reaction ratio to the final amino acid ratio in the polymers. When a 1:1 or a 1:2 molar ratio of the tripeptide to the serine moiety was polymerized, the serine monomer incorporates to a lesser extent than the tripeptide monomer (Table 1). While the overall effect of this polymerization would be a random copolymer, the different activities of monomers may lead to long stretches of one repeat unit (Odian, 1981). Solutes diffusing through block copolymers can exhibit different rates of diffusion based on the length of the block segments (Kumaki et al., 1985; Sato et al., 1985). Thus, the effect of unequal reaction activities may alter the release of excipients from the polymer matrices.

Viscosity measurements provide an indication of the molecular weight of the relatively hydrophilic polymers. Results show that the intrinsic viscosities of Poly(Gly) and Poly(Phe-Phe-Gly) in dichloroacetic acid were 7.402 ± 0.041 and 7.141 ± 0.012 cP, respectively. These values correspond to a molecular weight of 4019 and 9258 Da for Poly(Gly) and Poly(Phe-Phe-Gly), respectively, using a standard curve equation for poly(γ-benzyl glutamate) (Brack and Trudelle, 1985). Laser desorption mass spectrometry performed on Poly(Phe-Phe-Gly) provided results consistent with the viscosity data. Despite the difference in chemical compositions, the two random co-polymers (i.e. Poly(Phe-Phe-Gly)$_1$(SerOBzl)$_{1.2}$ and Poly(Phe-Phe-Gly)$_1$(SerOBzl)$_{0.7}$) nevertheless yielded a similar range of average molecular weight ($\sim 10\,000$ Da), as revealed by the laser desorption mass spectroscopic method. These molecular weights obtained are consistent with other polymerization of amino acids and peptides using the active ester approach (Brack and Trudelle, 1985). The molecular weight of Poly(Phe) had already been provided by the manufacturer ($\sim 15\,000$ Da), and was thus not further measured.

Of the many theoretical scales for estimating the hydrophobicity of proteins, the π-value scale of Fauchère et al. (1988) was used to estimate the relative hydrophobicity of the synthesized and the commercial polypeptides. This scale was chosen since it uses a physical parameter, the partition coefficient of amino acid analogues, to estimate the hydrophobicity of amino acid side chains. Table 2 shows the π-values of glycine, phenylalanine, and *O*-benzylated serine residues, the building blocks of the polymers under investigation. From the values for these amino acids, an estimated π-value for the average repeating unit in each polymer is calculated. As shown in Table 2, the hydrophobicity of the polymers follows the sequence of Poly(Phe) > Poly(Phe-Phe-Gly)$_1$(SerOBzl)$_{1.2}$ > Poly(Phe-Phe-Gly)$_1$(SerOBzl)$_{0.7}$ > Poly(Phe-Phe-Gly) > Poly(Gly), primarily due to the hydrophobic nature of the phenylalanine and the *O*-benzylated serine residues.

Fig. 2 shows the release profiles of EE2 from the five different polypeptide disks under the in vitro setting. In all cases, EE2 was slowly but continuously released over a period of 90 days. The linear release pattern observed when the fractional release was plotted against the square root of time (see the inset of Fig. 2) implicitly indicates a diffusion-controlled release process that has been observed by many other investigators (Ritger and Peppas, 1987). Selected samples of the release media were tested for the presence of polymer degradation products (amino acids or peptide fragments) using a fluorescent detection method (CBI-Amine Assay Kit, Oread Laboratories). The release media exhibited a fluorescent reading equivalent to blank solution (data not shown) demonstrating that polymer degradation was not a factor in the release process.

Fig. 3 clearly demonstrates that the rate of drug release from the polymer is dependent on the hydrophobicity of the polymer; increasing when reducing the hydrophobicity of the polymer. A linear relationship with a correlation coefficient of 0.75 was observed for all the polymers except for Poly(Gly), when the drug release rate was plotted against the π-values. It is not clear why the release of EE2 from Poly(Gly) is actually slower than that from Poly(Phe-Phe-Gly), despite the fact that Poly(Phe-Phe-Gly) is presumably more hydrophobic based on the calculated π-values ($\pi = 1.19$ for Poly(Phe-Phe-Gly) as compared to zero for Poly(Gly)). Since Poly(Gly) is a homopolymer, drug release from it may be related to the secondary structure of the polypeptide, rather than the tertiary structure expected for all the other

Table 2
π-Values for amino acids and estimated π-values for polymers

Amino acid or polymer	π-Value or estimated π-value for average repeating unit
Gly	0.00
Phe	1.79
SerOBzl	2.34
Poly(Gly)	0.00
Poly(Phe-Phe-Gly)	1.19
Poly(Phe-Phe-Gly)$_1$ (SerOBzl)$_{0.7}$	1.42
Poly(Phe-Phe-Gly)$_1$ (SerOBzl)$_{1.2}$	1.52
Poly(Phe)	1.79

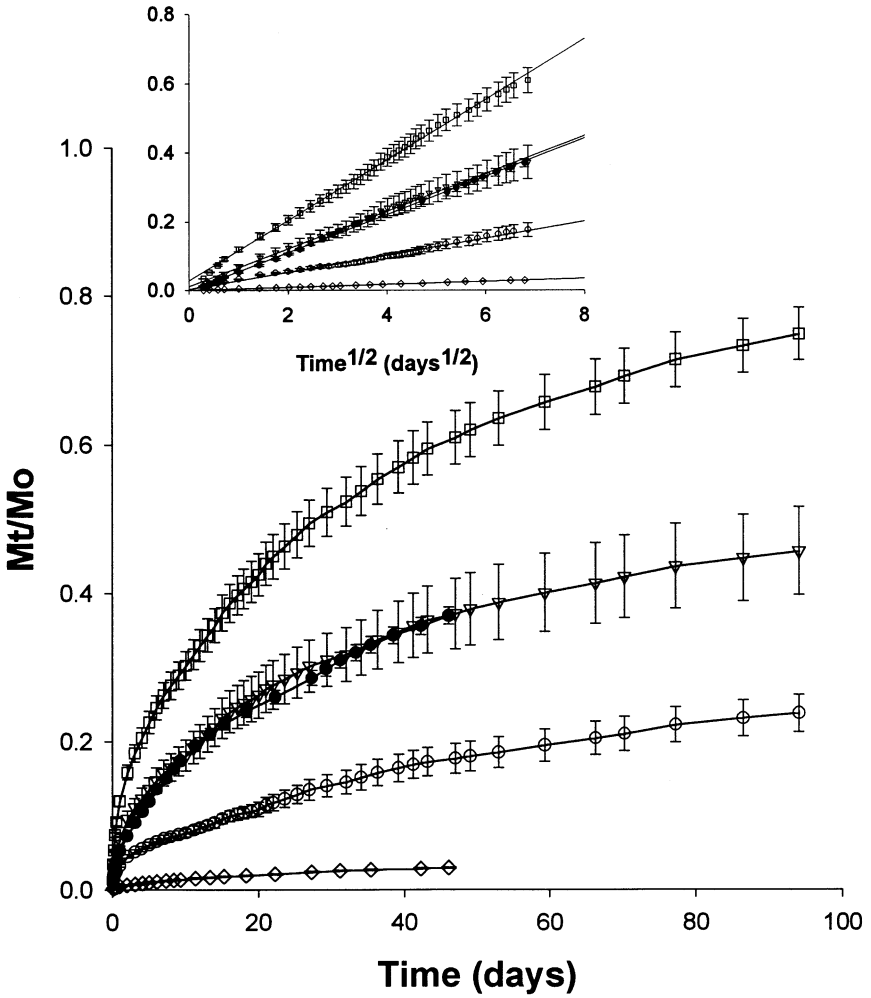

Fig. 2. Release of EE2 from polypeptide matrices. Poly(Gly) [●] and Poly(Phe) [◇], Poly(Phe-Phe-Gly)$_1$(SerOBzl)$_{1.2}$ [▽], Poly(Phe-Phe-Gly) [□] and Poly(Phe-Phe-Gly)$_1$(SerOBzl)$_{0.7}$ [○]. The inset represents the plot of EE2 release versus the square root of time.

three synthetic heteropolymers. In addition, the release of the hydrophobic EE2 drug from the polymer matrix may be governed by the surface morphology of the polymers. Due to the highly hydrophilic nature of glycine, the surface of Poly(Gly) is expected to be different from the other three synthetic polymers. Indeed, scanning electron microscopy on specially formulated microparticles of Poly(Gly) revealed abnormal morphology (rough surfaces resemble to roses) which was dramatically different from that (i.e. rather smooth surfaces) observed with the other synthetic polymers (data are not shown). Furthermore, the relatively small molecular weight ($\sim$4000 Da) for Poly(Gly) when compared to other polymers ($\sim$10 000 Da) may also play a role in the rapid release of the entrapped drug. It should be pointed out that the therapeutic dose of 17α-ethynylestradiol for contraceptive purposes ranges from 20 to 35 μg/day. In this preliminary release study, the release rates from the polymer matrices spanned this range for much of the 90 days.

A potential concern with the use of poly(amino acids) is the risk of an immunogenic reaction. The question of immunogenicity cannot be answered a

priori. Some researchers have suggested that peptide polymers with three or greater different amino acids should not be used for biomedical applications (Anderson et al., 1985). Others, however, have demonstrated the biocompatibility of such polymers (Wood et al., 1986; Urry et al., 1991). Therefore, each particular peptide polymer must be tested for an immune reaction. In this preliminary study, biocompatibility experiments were unwarranted.

This study presents the first step in exploring the possibility of synthesizing biocompatible and biodegradable polypeptide polymers with sufficient hydrophobicity so that drug release from these carriers can be diffusion controlled. Preliminary results proves the concept is both logical and feasible. Model polypeptide polymers with different hydrophobicity were successfully synthesized, and the release of a model drug, EE2, from these polymers follows a diffusion controlled pattern which is in accordance with the hydrophobicity of the polymer carrier. The fact that EE2 can be released at a therapeutic dose over a span of 90 days suggests that, with an appropriately tailored hydrophobicity for the polymer carrier, a variety of drugs could be released at the rates that meet with specific clinical or therapeutic need. Because of the presence of a great variety of amino acids with markedly different hydrophobicity, it is envisioned that polypeptide polymers with any desired hydrophobicity and drug release rate could be tailor-made. Further studies towards this goal are currently in progress in our laboratory, with the goal to design certain specific polypeptide polymers for the delivery of a number of clinically important low molecular weight drugs and small peptides.

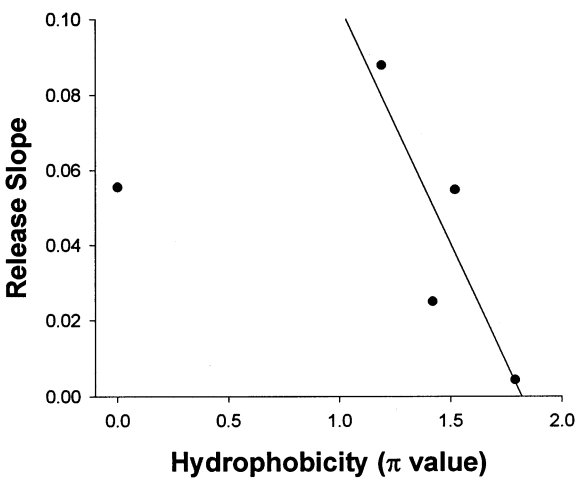

Fig. 3. Relationship between EE2 release rates (slopes of the release versus square root of time plot, Fig. 2 inset) and the hydrophobicity of the polypeptide polymers. Release rates are plotted against the estimated π-values determined using the Fauchère et al. (1988) scale (for details, see text and Table 2). Except for Poly(Gly), all data points were fitted by linear regression (Sigma Plot).

Acknowledgements

Financial support from the following sources is gratefully acknowledged: American Foundation for Pharmaceutical Education Fellowship, NIH Training Grant (5T32-GM07767-15), the University of Michigan College of Pharmacy (Lilly Endowment Fellowship and Upjohn Fellowship), and NIH Grants HL 38353, HL 55461, and GM 37188.

References

Anderson, J.M., Spilizewski, K.L., Hiltner, A., 1985. Poly-α-amino acids as biomedical polymers. In: Williams, D.F. (Ed.), Biocompatibility of Tissue Analogs. CRC Press, Boca Raton, FL, pp. 67–88.

Brack, A., Trudelle, Y., 1985. Molecular weight determination of polypeptides from viscosity measurements. Polym. Commun. 26, 369–370.

Cohen, J., Siegel, R.A., Langer, R., 1984. Sintering technique for the preparation of polymer matrices for the controlled release of macromolecules. J. Pharm. Sci. 73, 1034–1037.

Dhall, G.I., Krishna, U., Sivaraman, R., Datey, S., Gaur, L.N., Mehta, S., Roy, M., Saxena, N.C., Yadav, S.C., Viswanath, P., Saxena, B.N., 1991. Phase II clinical trial with biodegradable subdermal contraceptive implant Capronor (4.0 cm single implant). Contraception 44, 409–417.

Dickinson, H.R., Hiltner, A., Gibbons, D.F., Anderson, J.M., 1981. Biodegradation of a poly(α-amino acid) hydrogel I. In vivo. J. Biomed. Mater. Res. 15, 577–589.

Doty, P., Bradhury, J.H., Holtzer, A.M., 1956. Polypeptides. IV. The molecular weight, configuration and association of poly-γ-benzyl-L-glutamate in various solvents. J. Am. Chem. Soc. 78, 947–954.

Fauchère, J.L., Charton, M., Kier, L.B., Verloop, A., Pliska, V., 1988. Amino acid side chain parameter for correlation studies in biology and pharmacology. Int. J. Pept. Protein Res. 32, 269–278.

Goren, H.J., 1974. Sequential polypeptide synthesis. CRC Crit. Rev. Biochem. 2, 197–225.

Heller, J., 1990. Development of poly(ortho esters) — A historical overview. Biomaterials 11, 659–665.

Jones, J.H., 1977. Sequential polypeptide synthesis. In: Weinstein, B. (Ed.), Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, Vol. 4. Marcel Dekker, New York, pp. 29–63.

Kovacs, J., Mayers, G.L., Johnson, R.H., et al., 1972. On the problem of racemization during the synthesis of sequential polypeptides. In: Lande, S. (Ed.), Progress in Peptide Research, Vol. 2. Gordon and Breach, New York, pp. 185–193.

Kumaki, T., Sisido, M., Imanishi, Y., 1985. Antithrombogenicity and oxygen permeability of block and graft copolymers of polydimethylsiloxane and poly(α-amino acid). J. Biomed. Mater. Res. 19, 785–811.

Leong, K.W., Brott, B.C., Langer, R., 1985. Bioerodible polyanhydrides as drug-carrier matrices. I. Characterization, degradation and release characteristics. J. Biomed. Mater. Res. 19, 941–955.

Marck, K.W., Wildevuur, C.R.H., Sederel, W.L., Bantjes, A., Feijen, J., 1977. Biodegradability and tissue reaction of random copolymers of L-leucine, L-aspartic acid, and L-aspartic acid esters. J. Biomed. Mater. Res. 11, 405–422.

McGrath, K.P., Fournier, M.J., Mason, T.L., Tirrell, D.A., 1992. Genetically directed syntheses of new polymeric materials. Expression of artificial genes encoding proteins with repeating -(AlaGly)$_3$ProGluGlu-elements. J. Am. Chem. Soc. 114, 727–733.

Minoura, N., Aiba, S., Fujiwara, Y., 1984. Copolymer matrices composed of L-leucine and *N*5-(3-hydroxypropyl)-L-glutamine as carrier of drugs. Makromol. Chem. Rapid Commun. 5, 643–648.

Odian, G., 1981. Principles of Polymerization, 2nd edn. Wiley, New York.

Ritger, P.L., Peppas, N.A., 1987. A simple equation for description of solute release I. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. J. Control. Release 5, 23–36.

Sato, H., Nakajima, A., Hayashi, T., Chen, G.-W., Noishiki, Y., 1985. Microheterophase structure, permeability, and biocompatibility of A-B-A triblock copolymer membranes composed of poly(γ-ethyl L-glutamate) as the A component and polybutadiene as the B component. J. Biomed. Mater. Res. 19, 1135–1155.

Sidman, K.R., Schwope, A.D., Steber, W.D., Rudolph, S.E., 1980a. Use of synthetic polypeptides in the preparation of biodegradable delivery systems for narcotic antagonists. In: Willette, R.E., Barnett, G. (Eds.), Naltrexone: Research Monograph, 28th edn., pp. 214–231.

Sidman, K.R., Schwope, A.D., Steber, W.D., Rudolph, S.E., Poulin, S.B., 1980b. Biodegradable, implantable sustained release systems based on glutamic acid copolymers. J. Membr. Sci. 7, 277–291.

Sidman, K.R., Steber, W.D., Schwope, A.D., Schnaper, G.R., 1983. Controlled release of macromolecules and pharmaceuticals from synthetic polypeptides based on glutamic acid. Biopolymers 22, 547–556.

Solomon, G.F., Ciutâ, I.Z., 1962. Détermination de la viscosité intrinsèque de solutions de polymères par une simple détermination de la viscosité. J. Appl. Polym. Sci. 6, 683–686.

Urry, D.W., Prasad, K.U., 1985. Syntheses, characterizations, and medical uses of the polypentapeptide of elastin and its analogs. In: Williams, D.F. (Ed.), Biocompatibility of Tissue Analogs. CRC Press, Boca Raton, FL, pp. 89–116.

Urry, D.W., Parker, T.M., Reid, M.C., Gowda, D.C., 1991. Biocompatibility of the bioelastic materials, poly(GVGVP) and its γ-irradiation cross-linked matrix: Summary of generic biological test results. J. Bioact. Compat. Polym. 6, 263–282.

Wood, S.A., Lemons, J.E., Prasad, K.U., Urry, D.W., 1986. In vitro calcification and in vivo biocompatibility of the cross-linked polypentapeptide of elastin. J. Biomed. Mater. Res. 20, 315–335.