

Inhibition of Bovine Serum Albumin Adsorption by Poly(ethylene glycol) Soft Segment in Biodegradable Poly(ethylene glycol)/Poly(L-lactide) Copolymers

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SYNOPSIS

The objective of this study was to investigate the effects of the incorporation of ether linkages into polylactide (PLLA) chains and the time of biodegradation on the behavior of protein adsorption. The content of poly(ethylene glycol) (PEG) in PLLA/PEG copolymers is from 4.4 to 18.3 wt %, and the length of the PEG soft segment is 1000, 2000, and 6000 daltons. The bovine serum albumin (BSA) adsorption onto the biodegradable PLLA/PEG copolymers was carried out using ultraviolet spectroscopy. The surface tension of PLLA and PLLA/PEG was measured using a contact angle. The data show that the incorporation of PEG segments makes the copolymer more polar and, therefore, leads to a reduction of protein adsorption. As the hydrolysis of polymers proceeds, both PLLA and PLLA/PEG turn out to be more polar. However, the initial compositions of degraded PLLA/PEG have a weak influence on the protein adsorption onto its hydrolyzed surface with a substantially long duration of hydrolysis. This phenomenon is attributed to the hydrophobic interaction between polar PLLA/PEG and BSA. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Hydrophilic poly(ethylene oxide) (PEO) is hardly able to adsorb protein.¹⁻³ PEO has been commonly grafted onto hydrophobic substrates. Studies on the protein adsorption on PEO-grafted polymers reveal that the intermolecular interactions causing the adsorption are mainly steric repulsion and hydrophobic interaction, both of which are more significant than are van der Waals forces, leading to low amounts of protein adsorbed. The hydrophobic interaction, in addition, makes the entropy of adsorption increase,^{4,5} favoring the change of free energy. The protein adsorption can also be reduced through steric repulsion, due to the compression of PEO chains. Moreover, the length of the PEO graft is a factor influencing the intermolecular forces during adsorption and the amount of protein adsorption.⁶ Jeon et al.⁷ theoretically calculated the optimum

PEO density on the polymer surface and the optimum segmental length of PEO, resulting in the minimum protein adsorption. However, this theory has not yet been tested experimentally.

Young⁸ and Chan and Brash⁹ reported that the low-surface free energy of polymers, namely, poly(vinyl chloride), polyethylene, silicone rubber, and polyetherurethane, results in low bovine serum albumin (BSA) adsorption. Straaten and Peppas^{10,11} correlated the protein-polymer interaction energy with the BSA adsorption for poly(vinylidene fluoride), poly(methyl methacrylate), poly(vinyl chloride), and poly(ethylene glycol) (PEG)-grafted polymer and showed that the PEG graft surfaces exhibited lower BSA adsorptions.

Lok et al.¹² investigated BSA adsorption onto siloxane rubbers, poly(methyl methacrylate), poly(styrene sulfonate) (PSS), and PEO and found an extremely low adsorption for PEO. They also showed that the negatively charged hydrophilic polymer, i.e., PSS, leads to a lower rate of BSA adsorption than does the neutral hydrophilic substance having the same critical surface tension.

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Table I Number-average Molecular Weight (\bar{M}_n), PEG Contents, and Chain Length of PEG for PLLA/PEG Copolymers

Sample No.	PEG Wt % in Feed	\bar{M}_n of PEG	PEG Wt % in Copolymer	\bar{M}_n of PLLA/PEG
S-1	2	2000	4.40	24,100
S-2	3	2000	5.41	19,812
S-3	5	2000	9.60	15,783
S-4	10	2000	18.30	9,801
S-5	10	1000	13.40	6,070
S-6	10	6000	12.70	30,780

To the best of our knowledge, there are few reports on the protein adsorption onto PEO block copolymers. The biodegradable polylactide (PLLA) and its copolymers have been widely utilized for surgical suture and drug-release devices, and their physicochemical behavior has been studied intensively. The objective of this work was to study mainly the effect of segment length of PEG blocks and PEG content on the BSA adsorption in PLLA/PEG block copolymers and to relate the BSA adsorption to the surface polarity of hydrolyzed copolymers.

EXPERIMENTAL

Preparation of PLLA/PEG Copolymers and Their Films

L-Lactic acid (Merck) was dimerized to form L-lactide, with a melting temperature of 98°C. Then, lactide was copolymerized with PEG oligomers at 180°C, with stannous octoate as the catalyst. The reaction time was 10 h. The molecular weight of the PEG segment was 1000, 2000, and 6000. The weight percentage of PEG in the feed varied from 2 to 10. The procedure was similar to that used by Zhu et al.,¹³ Deng et al.,¹⁴ and Cohn et al.^{15,16} PLLA homopolymer (Medisorb®) was supplied by DuPont. The molecular weight was measured with a high-performance liquid chromatograph (Shimadzu LC-6A/RID-6A). The PEG content in copolymers was determined by ¹H-NMR (Bruker AC200) based on the chemical shifts at 1.57 for —CH₃ in PLLA, 3.64 for —CH₂— in PEG, and 5.16 for —CH in PLLA.

PLLA/PEG copolymers were cast into films having 100 μm in thickness to undergo the hydrolysis reaction at pH 7 and 37°C. The surface energy of the polymer samples at various time intervals of hydrolysis was measured with a Face contact angle goniometer (CA-D, Kyowa), using water, β-thiodigly-

col, ethylene glycol, glycerol, and formamide. The contact angle readings were taken after the liquid drops of 2 mm in diameter were in contact with the polymer surface for 10 min. The surface polarity of polymers, represented by the polar component of surface tension, determined by the Girifalco–Good–Fowkes method, was modified by Kaebler, and Moacanin¹⁷:

$$\gamma_{lv}(1 + \cos \theta) = 2(\gamma_{lv}^p \times \gamma_s^{p1/2} + 2(\gamma_{lv}^d \times \gamma_s^d)^{1/2} \quad (1)$$

where γ_{lv} is the surface tension of liquid; γ_{lv}^p , the polar energy contribution to γ_{lv} ; γ_s^p , the polar energy contribution to γ_s of polymer; γ_s^d , the dispersion energy contribution to γ_s of polymer; and θ , the con-

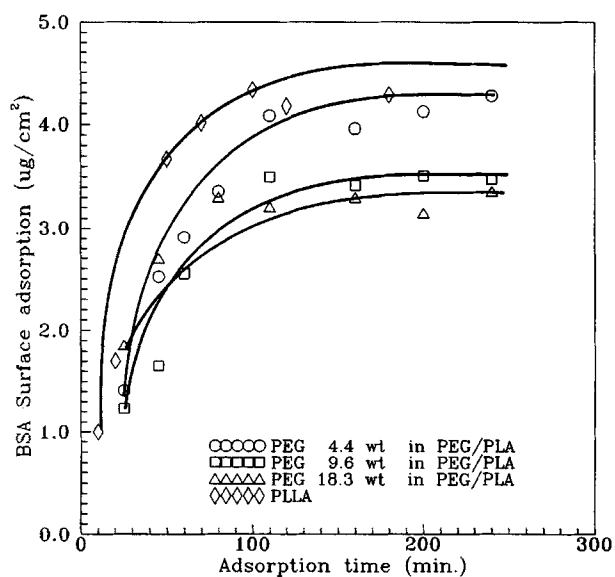


Figure 1 BSA surface adsorption vs. adsorption time for PLLA/PEG copolymers, chain length of PEG = 2000, and BSA concn = 20 μg/mL, at pH 7 and 25°C.

tact angle of liquid with polymer. γ_{lv}^p was computed from the plot of $(1 + \cos \theta)$ against $(\gamma_{lv}^p)^{1/2}$.

For water, β -thiodiglycol, ethylene glycol, glycerol, and formamide, the values γ_{lv} are 72.8, 54.0, 48.2, 63.4, and 58.4 dyn/cm, respectively. The values of γ_{lv}^p are 51.0, 15.6, 30.7, 26.4, and 40.3 dyn/cm, respectively.

Measurement of BSA Adsorption

The buffer solution, with pH 7, consists of 0.01 M tris-(hydroxymethyl) aminoethane (Sigma Co.) dissolved in 3.6 M ammonium sulfate. The UV-VIS spectrometer (UV-240, Shimadzu) was used to determine the concentration of BSA in solution with the aid of a calibration curve made from known solutions. The original and hydrolyzed PLLA/PEG films were cut to fit into a Petri dish of a diameter of approximately 9 cm, and then 25 mL of BSA solution was drawn into the dish to contact the polymer on one side. The concentration of BSA in solution was determined at various times and the amount adsorbed on the polymer was calculated from the initial and final amounts of protein in the solution.

RESULTS AND DISCUSSION

Protein Adsorption on Surface

Adsorption for PLLA/PEG Copolymers before Hydrolysis

The PEG content in the feed and in the copolymers and their number-average molecular weight are listed in Table I.

Figure 1 shows the relationship between the amount adsorbed and time of adsorption. An equilibrium of the Langmuir type is reached after about 150 min. The amount adsorbed decreases with the PEG content for all copolymers.

Figure 2 indicates the relationship between adsorption and time. It is obvious that, with PEG in copolymers equal to 12.7–18.3 wt %, the equilibrium adsorption is decreased with the length of the PEG segment. This point regarding the effect of chain length on the BSA adsorption agrees with the theoretical prediction by Jeon et al.⁷ Furthermore, referring to Table I leads us to note that copolymers S-5 and S-6 are of comparable PEG content, i.e., 13.4 vs. 12.7 wt %, but have a substantial difference in the equilibrium adsorption ($3.59 \mu\text{g}/\text{cm}^2$ against $2.93 \mu\text{g}/\text{cm}^2$) due to the influence of the length of the soft segment.

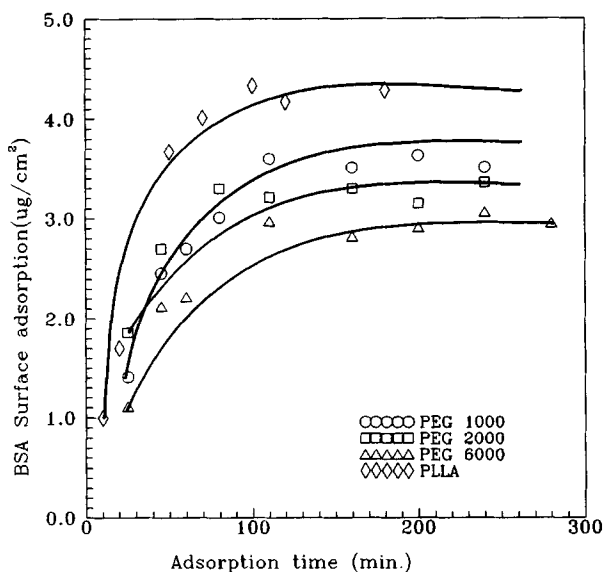


Figure 2 BSA surface adsorption vs. adsorption time for PLLA/PEG copolymers, with content of PEG = 10 wt %, BSA concn = $20 \mu\text{g}/\text{mL}$, at pH 7 and 25°C .

Figure 3 indicates that, while the BSA concentration in bulk is increased from 10 to $40 \mu\text{g}/\text{mL}$, the equilibrium adsorption goes up accordingly. Notably, for the initial stage of adsorption, the highest protein concentration leads to less affinity for the polymer surface relative to the protein concentration of $20 \mu\text{g}/\text{mL}$. The equilibrium data of Figures 1–3 are summarized in Table II, indicating the effects of PEG content in feed, length of PEG segment, and protein concentration in the bulk.

Influence of Hydrolysis on BSA Adsorption

Figure 4 shows the plot of equilibrium adsorption with contact time equal to 2 h against the time of hydrolysis at pH 7, 37°C , and BSA concentration = $40 \mu\text{g}/\text{mL}$. The PLLA homopolymer demonstrates a slight increase of adsorption along with the hydrolysis time. On the other hand, S-1 and S-4 copolymers exhibit a significant reduction of protein adsorption with hydrolysis time. Both copolymers seem to approach the same level of adsorption.

Surface Energy of Copolymers

Effect of Copolymer Composition

Figure 5 shows the plot of contact angle of copolymers as a function of PEG content. The increase of wettability is understandable in terms of the hydrophilic component of PEG in copolymers. It should

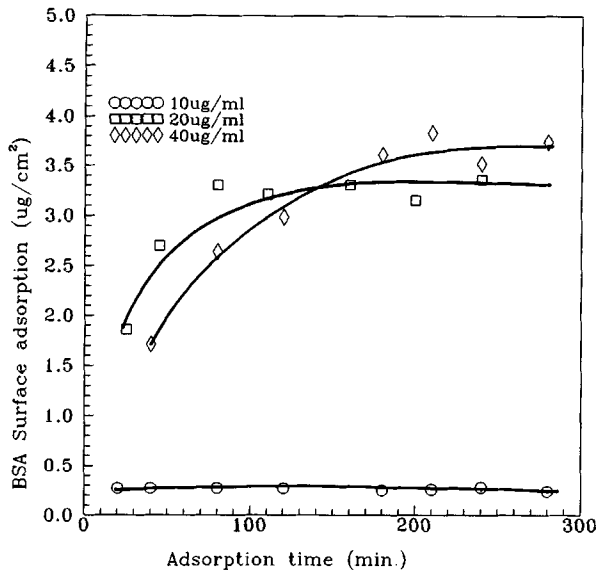


Figure 3 BSA surface adsorption vs. adsorption time for PLLA/PEG copolymers' content of PEG = 18.3 wt %, chain length of PEG = 2000, at pH 7 and 25°C, for BSA concn = 10, 20, and 40 µg/mL.

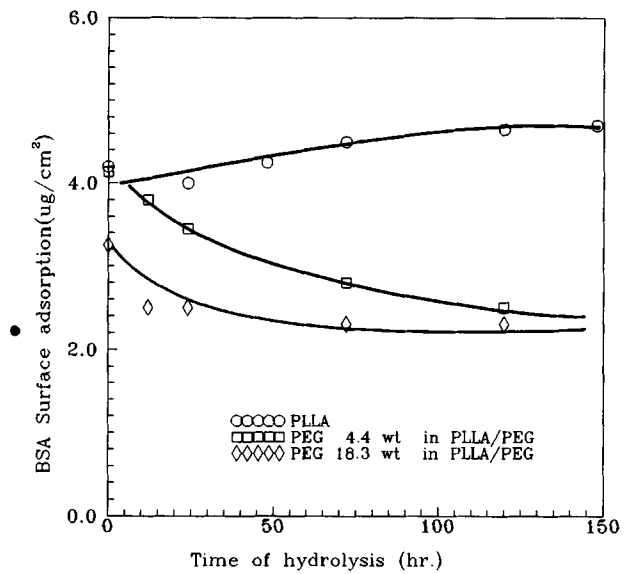


Figure 4 BSA surface adsorption vs. time of hydrolysis for PLLA homopolymer and PLLA/PEG copolymers, chain length of PEG = 2000, at adsorption time = 2 h, pH 7, and 25°C.

be mentioned that the water contact angles are not equilibrium values and are not constant values with time for contact times between the liquid drop and the polymer greater than 10 min. Data for four other liquids are time-independent up to 10 min. It should also be pointed out that samples S-1 and S-4 in Figure 5 have the same length of the PEG segment, but different PEG contents (see also Table I). In conjunction with Table II, it seems that the higher hydrophilicity causes a lower protein adsorption over the range of copolymer composition used in this study. The effect of PEG content on the polar component of surface tension for S-1 and S-4 copolymers and PLLA is shown in Figure 6.

Surface Energy of Hydrolyzed Copolymers

Figure 7 gives the relationship between the polar component of surface tension (γ_s^p) of polymers and

hydrolysis time, indicating an increase with hydrolysis time from 0 to 720 min. Moreover, the more hydrophilic copolymer S-4 possesses a constant level of γ_s^p after 400 min.

The results clearly show that the more PEG-rich copolymers result in more polar surfaces and, hence, in less protein adsorption. The greater length of the PEG segment and higher surface density of PEG result in a lower van der Waals interaction with the protein, as indicated by Jeon et al.⁷ It should be pointed out that the PEG segments in copolymers are terminally attached at both ends,¹³ making the effect of steric repulsion minimal.

CONCLUSIONS

This study proves the inhibition of protein adsorption by PEG in PLLA/PEG copolymers. It also

Table II BSA Surface Adsorption ($\mu\text{g}/\text{cm}^2$) at Equilibrium vs. BSA Concentration in Bulk for PLLA Homopolymer and PLLA/PEG Copolymers at 25°C and pH 7

BSA Concn ($\mu\text{g}/\text{mL}$)	PLLA Homopolymer	S-1	S-2	S-4	S-5	S-6
10	—	1.02	0.85	0.27	0.59	0.22
20	4.42	4.13	3.47	3.29	3.59	2.93
40	—	4.63	3.89	3.67	3.93	3.38

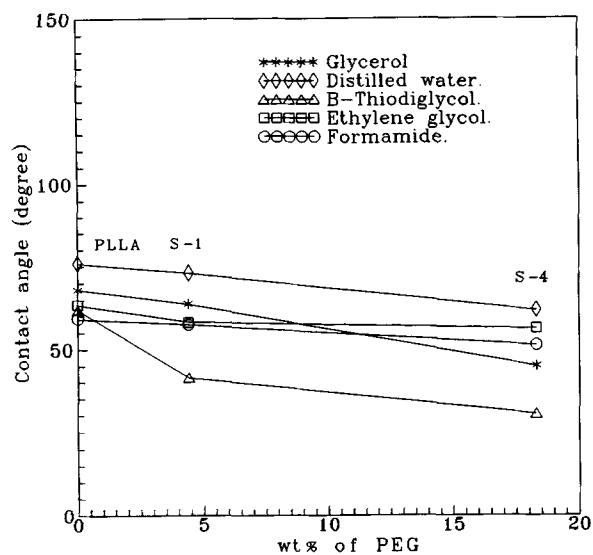


Figure 5 Variation of contact angle of PLLA/PEG copolymers, taken with five liquids for PLLA/PEG copolymers vs. weight percentage of PEG, at pH 7 and 25°C.

shows that the BSA adsorption on block copolymers during the hydrolysis process becomes virtually the same, which is attributable to the controlling role played by the PEG segment. For 150 h of hydrolysis, it is inferred that the PEG content increases, to give ultimately the same protein adsorption. This phenomenon is corroborated by the surface tension data, in which the surface polarity increases with time and tends to reach the saturation level. In light of the theory by Andrade and de Gennes, the reduction of protein adsorption for copolymers is understood in terms of hydrophobic interactions between the protein and the polymer, which causes a decrease of Gibbs free energy. The stronger interaction caused by increasing amounts of PEG, the smaller the pro-

Table III Data of Contact Angle (Degree) Using Five Liquids for PLLA Homopolymer and PLLA/PEG Copolymers at 25°C

Liquids	PLLA		
	Homopolymer	S-1	S-4
Water	68.0	63.8	45.0
β -Thiodiglycol	62.0	41.5	30.5
Ethylene glycol	63.5	58.5	56.0
Glycerol	76.0	73.0	61.5
Formamide	59.0	58.0	51.0

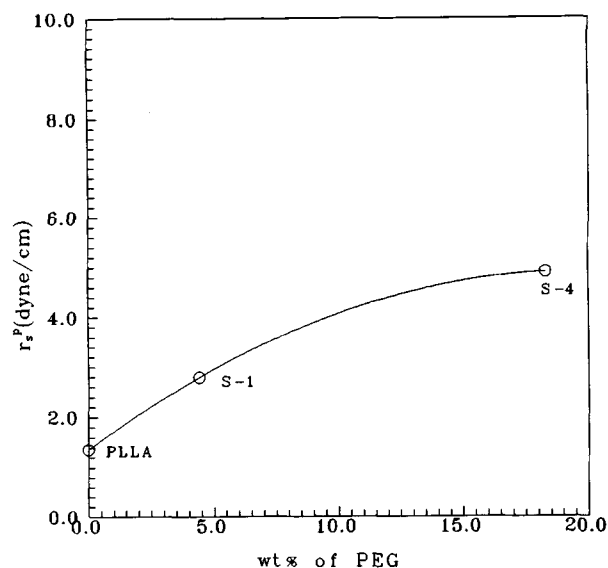


Figure 6 Polar component of surface tension for PLLA/PEG copolymers vs. weight percentage of PEG.

tein adsorption. However, in the case of weakly polar PLLA homopolymer, the surface polarity increases slightly during hydrolysis. This may increase the van der Waals forces between PLLA and BSA, making the adsorption increase to a slight extent.

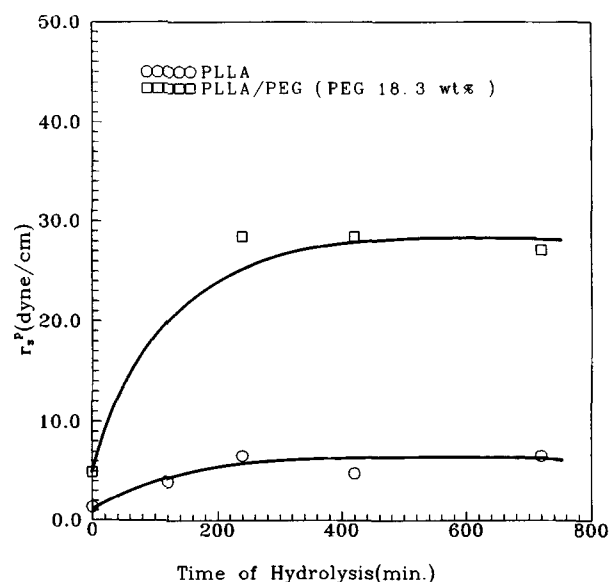


Figure 7 Effect of time of hydrolysis on polar component of surface tension for PLLA and PLLA/PEG copolymer with PEG content = 18.3 wt % and chain length of PEG = 2000, at adsorption time = 2 h, pH 7, and 37°C.

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