Pharmaceutical Research, Vol. 4, No. 1, 1987

Report

Effects of Plasma Proteins on Degradation Properties of Poly(L-lactide) Microcapsules

Kimiko Makino,[1] Hiroyuki Ohshima,[1] and Tamotsu Kondo[1,2]

Received May 22, 1986; accepted September 29, 1986

The degradation rate of poly(L-lactide) microcapsules in an aqueous medium was accelerated by the addition of albumin, γ-globulins, and fibrinogen. These proteins form adsorption layers on the surface of poly(L-lactide) microcapsules. At the interface between the microcapsules and the adsorbed protein layers, the value of the electric potential is expected to increase in magnitude, i.e., become highly negative compared with that at the interface between the microcapsules and the bulk buffer solution containing no plasma protein. This potential increase causes an increase in H^+ concentration at that interface, which may result in an acceleration of the hydrolytic degradation rate of poly(L-lactide) microcapsules. Also, the presence of plasma proteins can increase the solubility of poly(L-lactide), causing the poly(L-lactide) molecules to exist in an expanded form. This effect may also accelerate the degradation of poly(L-lactide) microcapsules.

KEY WORDS: poly(L-lactide) microcapsules; plasma proteins; urea; degradation; hydrolysis.

INTRODUCTION

Poly(L-lactide), a synthetic biodegradable polymer, is used in surgical sutures and implantable materials. As previously shown (1,2), poly(L-lactide) microcapsules are hydrolytically degraded into lactic acid in aqueous solutions. Its *in vitro* degradation rate was found to be affected by pH, ionic strength, and buffer concentration in aqueous solutions (1,2).

Besides these physicochemical properties of the dispersion medium, plasma proteins which interact strongly with the microcapsules are expected to affect the degradation rate. In a previous paper (3), we have reported that plasma proteins such as albumin, γ-globulins, and fibrinogen adsorb onto the surface of poly(L-lactide) microcapsules through a hydrophobic interaction in such a way that the outer part of the adsorbed protein layer is rich in positively charged groups and the inner part is rich in negatively charged groups, which causes a variation of the zeta potential of plasma protein-coated poly(L-lactide) microcapsules.

We report here the effects of these plasma proteins on the degradation of poly(L-lactide) microcapsules in relation to the potential distribution across a microcapsule/adsorbed protein layer interface and the increased solubility of poly(L-lactide) caused by the presence of proteins.

[1] Faculty of Pharmaceutical Sciences and Institute of Colloid and Interface Science, Science University of Tokyo, Shinjuku-ku, Tokyo 162, Japan.

[2] To whom correspondence should be addressed at Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12 Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan.

MATERIALS AND METHODS

Materials

Poly(L-lactide) and lactate oxidase were kindly provided by Mitsui Toatsu Kagaku Corp. and Toyo Jozo Co., Ltd., respectively. Bovine serum albumin (Fraction V), bovine serum γ-globulins (Corn Fraction 2), and bovine serum fibrinogen (Type 1-S) (Sigma Chemical Co.) were used without further purification. Chromatographically purified fluorescein-conjugated bovine serum albumin (FITC-BSA) was purchased from Cappel Corp. and rhodamine-conjugated bovine serum γ-globulin (BGG/TRITC) was purchased from Nordic Immunology Co. To prepare FITC-conjugated fibrinogen following the method of Maeda *et al.* (4), fluorescein isothiocyanate (isomer 1) (Sigma Chemical Co.) was used. All other chemicals were of the analytical reagent grade.

Preparation of Poly(L-lactide) Microcapsules

Poly(L-lactide) microcapsules containing water were prepared by the interfacial deposition technique as described previously (1).

Degradation of Poly(L-lactide) Microcapsules

In the degradation experiments, poly(L-lactide) microcapsules were dispersed in various solution media at a final concentration of 0.15% (v/v), and the mixtures were kept at 37°C.

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Effect of Plasma Proteins

As dispersion media of poly(L-lactide) microcapsules, protein solutions were prepared in the following way. Various amounts of plasma proteins were dissolved in a phosphate buffer solution (pH 7.6), the ionic strength of which was adjusted to 0.154. Sodium azide was added to the solutions to make its final concentration 1% (w/v).

For solutions containing urea, 0.1 M urea was added to the above-mentioned phosphate buffer solution, and the resultant solution was used as the medium for preparing protein solutions.

Estimation of Degradation

The degree of degradation of poly(L-lactide) microcapsules was estimated (i) from the rate of decrease in the weight-averaged molecular weight ($\overline{M}_w$) and the ratio of the weight-averaged molecular weight to the number-averaged molecular weight ($\overline{M}_w/\overline{M}_n$); (ii) from the weight loss of poly(L-lactide) evaluated by gel permeation chromatography (GPC); and (iii) from the amount of lactic acid generated as the final product in bulk solution, determined at different degradation periods by the use of a lactate sensor (2). In the GPC procedure, chloroform was chosen as an eluent and the columns used were Shodex AC803 and AC804 (Shokotsusho, Japan). The flow rate was 1 ml/min, and an RI detector was employed.

Determination of Protein Adsorption

Various amounts of plasma proteins, together with corresponding fluorescent proteins, were dissolved in the phosphate buffer solution, the ionic strength of which was adjusted to 0.154 by the addition of NaCl.

To each solution, a specified amount of poly(L-lactide) microcapsules were dispersed, and the mixture was shaken for 2 hr at 37°C. These samples were then centrifuged at 3000 rpm for 10 min. The clear supernatants were removed, and the concentrations of proteins remaining in the solutions were measured with a recording spectrofluorophotometer RF502 (Shimadzu Corp.) at suitable wavelengths: for FITC-BSA and FITC-fibrinogen, ex. 490 nm and em. 520 nm; and for BGG/TRITC, ex. 554 nm and em. 573 nm. The specific surface area of the microcapsules was estimated from the average diameter (1.5 μm) and the sedimentation volume of the microcapsules.

In the determination of the effect of urea on protein adsorption, the same procedures as above were employed except that the medium used was the phosphate buffer solution to which 0.1 M urea was added.

RESULTS AND DISCUSSION

The presence of albumin, γ-globulins, or fibrinogen in the phosphate buffer solution caused little effect on the effective cleavage of poly(L-lactide), leading to a decrease in $\overline{M}_w$. The increase in $\overline{M}_w/\overline{M}_n$ with time in the presence of the proteins also differed little from that for the control solution containing no protein. As the increase in $\overline{M}_w/\overline{M}_n$ suggests that degraded poly(L-lactide) molecules of intermediate molecular weights that are soluble in chloroform but not in aqueous solution are produced with the lapse of time, the production rate of the degraded poly(L-lactide) molecules was little affected by the addition of proteins.

As previously reported (2), poly(L-lactide) molecules of intermediate molecular weights are considered to be degraded into lactic acid. Figures 1a and b show, respectively, the weight loss of poly(L-lactide) microcapsules with time in albumin and γ-globulin solutions of various concentrations. The weight loss in the concentrated albumin solution (30 mg/ml) was observed in the period between 25 and 80 days. In the concentrated γ-globulin solution (30 mg/ml), the weight loss was seen in the period between 40 and 70 days. However, at low concentrations with both albumin and γ-globulins, no appreciable weight loss was detected. In fibrinogen solutions, the weight loss was not detected at all. This suggests that poly(L-lactide) molecules of intermediate molecular weights observed in concentrated albumin solutions can be degraded further and liberated from the matrix. However, those observed in concentrated fibrinogen solutions seem not to be degraded further or to be liberated from the matrix so rapidly. In concentrated γ-globulins solutions, the rate of degradation of poly(L-lactide) molecules of intermediate molecular weights to that of molecules of lower degrees of polymerization that are soluble in the aqueous phase seems to be fast. This explains the observed result that no appreciable increase in $\overline{M}_w/\overline{M}_n$ was detected, in spite of the fact that the weight of poly(L-lactide) was lost between 40 and 70 days (Fig. 1b).

Figures 2a–c show the amount of lactic acid released in the bulk solution as a function of the degradation period in various protein solutions. For all proteins, as their concentration increased, the released amount of lactic acid increased at any time period. In other words, poly(L-lactide) molecules of intermediate molecular weights, either soluble or insoluble in the aqueous solution, were produced more rapidly in concentrated protein solutions than in the diluted ones.

As mentioned above, the presence of plasma proteins in the phosphate buffer solution accelerated the cleavage of ester bonds in poly(L-lactide) molecules. This action of plasma proteins is considered to originate from their adsorption onto the microcapsule surface. Hence, we have measured the adsorption amount of the proteins. The results are shown in Figs. 3a–c, which confirm that the proteins used in the present work are indeed adsorbed on the microcapsule surface. We see that the protein concentrations at adsorption plateau were about 2 mg/ml for albumin, 10 mg/ml for γ-globulins, and 2 mg/ml for fibrinogen. In our previous

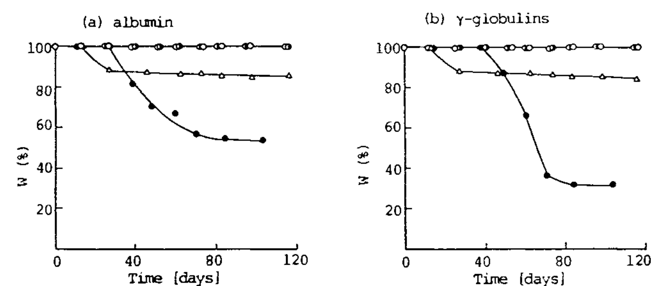

Fig. 1. Effects of albumin and γ-globulins on the weight loss of poly(L-lactide) microcapsules. Protein concentration: (●) 30 mg/ml; (◑) 5 mg/ml; (○) 0.1 mg/ml; (△) 0 mg/ml.

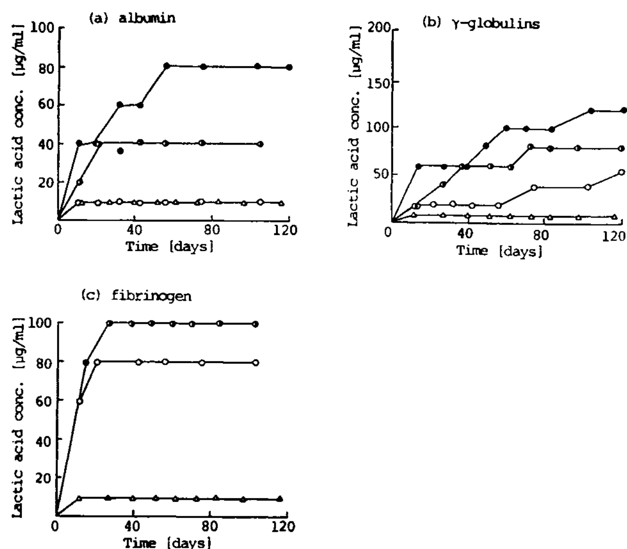

Fig. 2. Amounts of lactic acid generated from poly(L-lactide) microcapsules at different plasma protein concentrations. Symbols are the same as in Fig. 1.

paper (3), it was suggested that these proteins are adsorbed on the surface of poly(L-lactide) microcapsules in such a way that the outer area of the adsorbed proteins (closer to the surrounding solution) is rich in positively charged groups, while the inner area is rich in negatively charged groups. At the same time, the hydrophobic portion of adsorbed proteins must be preferentially in contact with the microcapsule surface. In view of this, we propose a possible explanation for the enhancement of the polymer degradation by plasma proteins as described below. The partition coefficient of electrolyte ions between the hydrophobic region of the adsorbed proteins and the aqueous phase should be very small, resulting in low electrolyte concentrations in that region. Hence, the electric potential there, which is practically equal to the Donnan potential, becomes very large in magnitude, i.e., becomes highly negative (5). Accordingly, the electric potential at the portion of the microcapsule membrane that is in contact with the hydrophobic region of adsorbed proteins can become highly negative. This should be followed by a large increase in the hydrogen ion concentration in that portion, causing an enhanced attack of H^+ on the microcapsule surface. Note that, although the adsorbed amounts of albumin and γ-globulins were almost constant, independent of their concentrations between 5 and 30 mg/ml, the effect of these proteins on the degradation was very dependent on the protein concentration. Also, in the case of fibrinogen, a similar dependence on concentration was observed. This concentration dependence can be understood as follows. The presence of hydrophobic groups in polymer molecules is known to increase the degree of hydrogen bonding so that local regularity of the water structure around the hydrophobic groups is enhanced. Since the proteins used in this work have some hydrophobic groups, when such iceberg formation-enhancing polymers are dissolved in the aqueous phase, a cage of water molecules is formed around the polymer solute, and the cosolute, poly(L-lactide), can then be incorporated within the same cage as the proteins. Such an effect of iceberg formation-enhancing

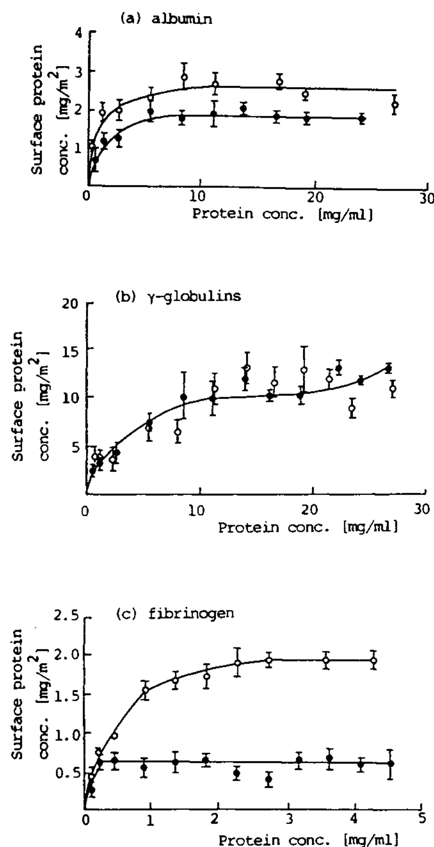

Fig. 3. Adsorption isotherms for plasma proteins on the surface of poly(L-lactide) microcapsules in the presence (●) and absence (○) of urea.

polymers on the solubility of cosolutes has been reported for the solubility of naphtalene in aqueous solution (6). Therefore, as the protein concentration increases, the solubility of poly(L-lactide) can increase, leading to the accelerated degradation.

According to these hypotheses fibrinogen should be more effective on the degradation than γ-globulins and albumin, because fibrinogen is the most hydrophobic protein. The experimental results, however, showed little difference among the three proteins. It is possible that fibrinogen adsorption increases the number of structured water molecules in the matrices of poly(L-lactide), causing limited attacks of OH^- or H^+ on the ester bonds in the polymer chain and, thereby, reducing poly(L-lactide) degradation.

In order to eliminate the effect of structured water, we added urea to the microcapsule/protein systems. Figure 4 shows the decrease in $\overline{M}_w$ of poly(L-lactide) with time measured in four solutions. Only the phosphate buffer solution containing 0.1 *M* urea and 5 mg/ml fibrinogen accelerated the decrease in $\overline{M}_w$ more than the control.

As shown in Fig. 5, in all these protein solutions containing 0.1 *M* urea, $\overline{M}_w/\overline{M}_n$ increased with time but no appreciable difference among these protein solutions was detected. It was also found that the rate of increase in $\overline{M}_w/\overline{M}_n$ is reduced by the addition of 0.1 *M* urea to the solution of albumin and fibrinogen. Such effects of urea, added to albumin and fibrinogen solutions, on the decrease in $\overline{M}_w$ and

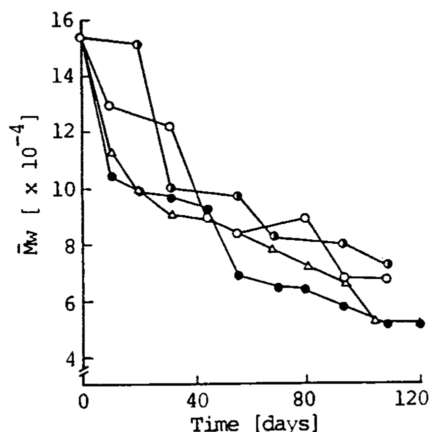

Fig. 4. Effect of plasma proteins on the decrease in weight-averaged molecular weight ($\overline{M}_w$) of poly(L-lactide) in the degradation of poly(L-lactide) microcapsules dispersed in a 0.1 *M* urea solution. (○) Albumin (30 mg/ml); (◑) γ-globulins (30 mg/ml); (●) fibrinogen (5 mg/ml); (△) no protein.

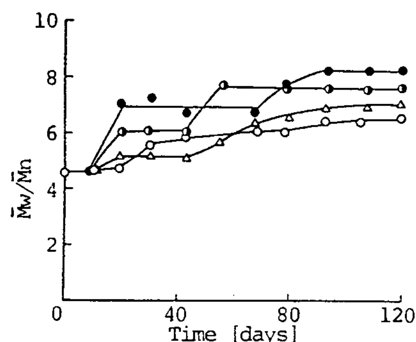

Fig. 5. Effect of plasma proteins on $\overline{M}_w/\overline{M}_n$ in a 0.1 *M* urea solution. Symbols are the same as in Fig. 4.

on the increase in $\overline{M}_w/\overline{M}_n$ can be explained as follows. For albumin solutions, such cleavage of poly(L-lactide) chains that is effective on the reduction of $\overline{M}_w$ may occur less frequently in the solution containing 0.1 *M* urea than in that containing no urea. Therefore, the increase in $\overline{M}_w/\overline{M}_n$ is suppressed by the addition of 0.1 *M* urea, while the decrease in $\overline{M}_w$ is not affected. For fibrinogen solutions, the frequency of the attack of water molecules to cleave poly(L-lactide) chains that reduces M_w effectively may be increased by the addition of urea. Also, poly(L-lactide) molecules of high molecular weight can be rapidly broken down to molecules of lower molecular weight.

Figure 3c shows that the adsorbed amount of fibrinogen on the poly(L-lactide) microcapsule surface is affected by the addition of 0.1 *M* urea. This is probably because the hydrophobic interaction between adsorbed fibrinogen molecules and the microcapsule surface is weakened by the addition of urea, which destroys the water structure. However, the adsorption of albumin or γ-globulins was little affected by the presence of urea (Figs. 3a and b). The rate of weight loss was accelerated in γ-globulin solutions by the addition of urea, whereas no effect of urea was observed in albumin solutions. In fibrogen solutions, the addition of urea caused a weight loss of poly(L-lactide), which was not seen in the absence of urea. These results suggests that urea affects the mobility of water molecules structured and located around the hydrophobic area of proteins and increases the reactivity of water molecules to attack the ester bonds of poly(L-lactide).

It was found that the lactic acid concentration in albumin solutions in the presence of urea was greater than that observed in the absence of urea. This implies that a large amount of water-soluble poly(L-lactide) with an intermediate molecular weight exists in the bulk solution phase. The rate of conversion from the water-soluble poly(L-lactide) to lactic acid is considered to be accelerated by the increase in mobility of water molecules. However, no appreciable difference in the amount of lactic acid was detected for the other proteins with and without urea.

From these results, destruction of water structure on and in the poly(L-lactide) microcapsule membrane and also in and around the proteins seems to be an important factor to accelerate the cleavage of ester bonds of poly(L-lactide) except in the case of γ-globulin solutions. This may confirm the hypothesis that the adsorption of proteins makes the electric potential of the microcapsule membrane in contact with the hydrophobic region of adsorbed proteins highly negative and that the solubility of poly(L-lactide) is increased by the addition of proteins to the aqueous phase, followed by the accelerated degradation of poly(L-lactide) microcapsules. In the case of γ-globulins, the interaction of poly(L-lactide) microcapsules with the protein could not be well evaluated, because the protein was denatured so that the orientation of adsorption was completely affected as was expected from the measurement of the zeta potential of the microcapsules covered with γ-globulins (results are not shown) by the addition of urea.

REFERENCES

1. K. Makino, M. Arakawa, and T. Kondo. *Chem. Pharm. Bull.* 33:1195–1201 (1985).
2. K. Makino, H. Ohshima, and T. Kondo. *J. Microencapsul.* 3:203–212 (1986).
3. K. Makino, H. Ohshima, and T. Kondo. *J. Colloid Interface Sci.* (in press).
4. H. Maeda, N. Ishida, H. Kawaguchi, and K. Tuzimura. *J. Biochem.* 65:777–783 (1969).
5. K. Makino, H. Ohshima, and T. Kondo. *Colloid Polymer Sci.* (in press).
6. T. Okubo and N. Ise. *J. Phys. Chem.* 73:1488–1494 (1969).