10.2 An Intelligent Biopolymer Gel with Pendant L-proline Methyl Ester

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Abstract: Linear poly(acryloyl-L-proline methyl ester, A-ProOMe), obtained by radiation-induced polymerization of its monomer in ethanol, exhibits a lower critical solution temperature (LCST) at 14°C. A-ProOMe was copolymerized with a minor amount of 2-hydroxypropyl methacrylate (HPMA) or 2-hydroxyethyl methacrytlate (HEMA), to obtain intelligent biopolymer gels for application in drug delivery systems. The poly(A-ProOMe/HPMA) gel was characterized by an initial rapid shrinkage at the surface in the swollen state, as resulting in formation of a rigid membrane barrier devoid of micropores. This gel is called a surface regulated matrix. In the case of poly(A-ProOMe/HEMA), no such a barrier formed, instead, the whole matrix shrunk without the disappearance of micropores. This gel is called a matrix pumping gel. Testosterone (T) was incorporated into the poly(A-ProOMe/HPMA) gel, and it was found that the daily dose of T released *in vivo* from this formulation remained constant at approximately 30 μg/day throughout an experimental period of 54 weeks. On the other hand, 9-β-D-arabinofuranosyladenine (Ara-A) was incorporated into the poly(A-ProOMe/HEMA) gel to evaluate the pulsatile drug release when cycled at 10 and 37°C. The *in vitro* release rate of Ara-A was found to be 11 ng/h at 10°C and 33 ng/h at 37°C.

INTRODUCTION

As an investigastion direction for the development of functional polymers, many researchers have actively prepared polymer gels which are responsive to external stimuli such as temperature, pH, electric field, and chemicals (Alhaique *et al.*, 1981, Bae *et al.*. 1989, Ito *et al.*, 1989,Tanaka *et al.*, 1982, Winnik *et al.*, 1990). It is well-known that living organisms contain various sensory systems with response function for external stimuli, and that α-amino acids, nucleic acids, and lipids play essential roles in the manifestation of responsive functions to physiological metabolism. Accordingly, as the first step, we decided to synthesize N-acryloyl and methacryloyl derivatives with pendant α-amino acids, and to investigate their polymerization and intelligent function. We found that the resulting polymer gel based on L-alanine showed reversible swelling-deswelling behavior when cycled in solution between different temperatures (Ding *et al.*, 1994, Safranj *et al.*, 1993). We now describe the synthesis and properties of poly(A-ProOMe). For this L-proline based polymer, there was a drastic volume collapse when the temperature was changed. We describe the reversible shrinkage and swelling kinetics of the gels containing A-ProOMe and also report the *in vivo* release of T from the poly(A-ProOMe/HPMA) gel and *in vitro* release of Ara-A from the poly(A-ProOMe/

HEMA) gel responsive to change in temperature.

EPERIMENTAL

A-ProOMe of Rf = 0.54 (ethyl acetate, Merck kieselgel 60F254) and $[\alpha]_D$ = 133.7° (c = 1, methanol) was synthesized by a coupling reaction of HCl•H-ProOMe and acryloylchloride (Yoshida *et al.*, 1993). HEMA and HPMA was purchased from Shin-Nakamura Chemical Co.

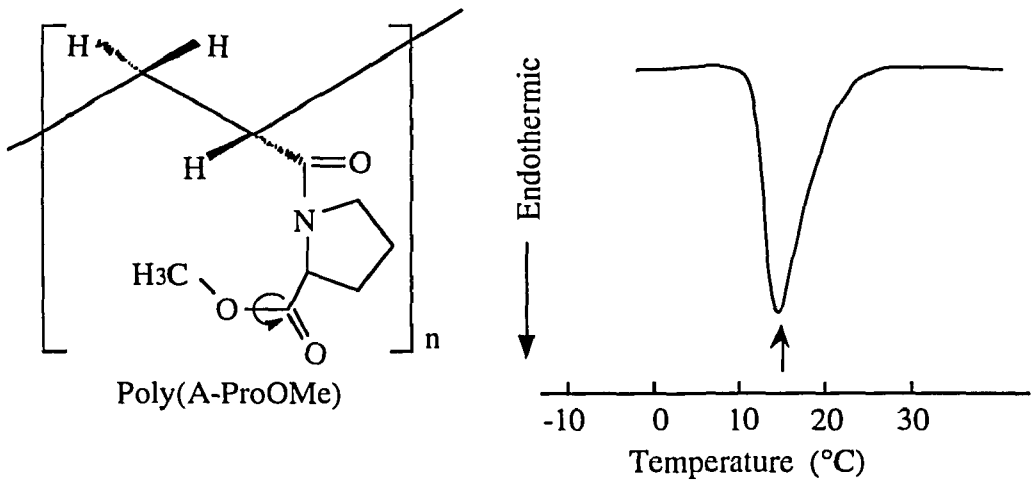

Fig. 1 DSC curve of poly(A-ProOMe) in 10 % (V/w) aqueous solution.

T (17β-hydroxy-4-androsten-3-one) was obtained from Sigma Chemical Company.

In the case of the preparation of thermo-responsive gel with T, A-ProOMe/HPMA monomer (80/20 vol. %, 0.25 ml) was mixed with T (30 mg) to give the homogeneous solution, and the solution was charged into an 8 mm internal diameter glass ampoule with a flat bottom. The ampoule was sealed under nitrogen and then irradiated for 3 hours at a dose rate of 10 kGy/h at 25°C using γ-rays from a ^{60}Co source. After the irradiation, a solid and transparent copolymer matrix with drug was obtained in tablet form by separating the product from the mold. It was washed with excess ethanol at 10°C to remove unreacted monomer. The polymer gel was allowed to swell in distilled-deionized water at 10°C just before use. The content of T in the swollen gel immediately before its implantation was found to be 27.85 ± 0.01 mg (n = 50). Drug concentration was assayed at 238 nm using a Hitachi U-3210 spectrophotometer. The poly(A-ProOMe/HPMA) gel with T was implanted under the back skins of male Wistar strain rats weighing 300 ~ 350 g at 3rd week from castration (one tablet/rat, five rats/group).

On the other hand, Ara-A was loaded into a lyophilized polymer gel consisting of A-ProOMe, HEMA, and a minor amount of crosslinker nonamethylene glycol dimethacrylate (Miyajima *et al.*, 1993). The weight of drug loaded in the gel was estimated from the weights of gels before and after loading. The *in vitro* release of Ara-A from thermo-responsive gels was determined in water by repeating measurements between 10 and 37°C at 60-minute intervals. The amount of drug released was assayed spectrophotometrically at 259 nm.

RESULTS AND DISCUSSION

The linear poly(A-ProOMe) with a chemical structure as shown in Fig. 1 was obtained by radiation-induced polymerization in the presence of ethanol at a dose rate of 10 kGy/h (a total

dose of 30 kGy) at 25°C, under nitrogen atmosphere. The LCST temperature of linear polymer was determined by DSC measurements, from the maximum of the endotherm, which gave us 14°C as a result (Fig. 1). Below the LCST, poly(A-ProOMe) exhibits the extended coil structure with strong interaction between the ProOMe group and water, in which the radius of

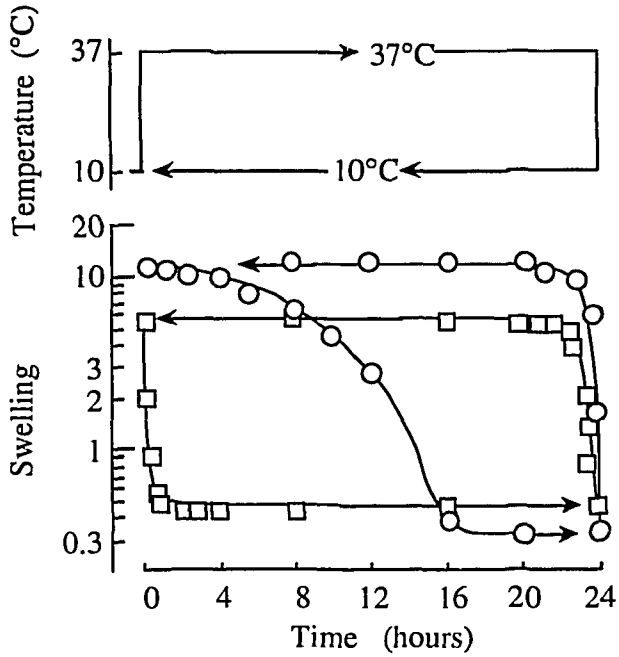

Fig. 2 Swelling-deswelling profiles of (O) poly(A-ProOMe/HPMA) gel and (□) poly(A-ProOMe/HEMA) gel between 10 and 37°C in water.

gyration $\langle S \rangle^{1/2}$ and apparent volume were found to be 40 nm and 270,000 nm^3 at 10°C, respectively. With increasing temperature, the hydrophobic interactions between proline groups become dominant and, at temperatures above the LCST, lead to inter- and intramolecular interactions forming compact clusters with a radius of gyration $\langle S \rangle^{1/2} = 8$ nm and apparent volume of 2,100 nm^3 at 50°C.

Having established that the A-ProOMe gel is characterized by a thermo-responsive function, the swelling-deswelling kinetics of the gel between two fixed temperatures with the passage of time was next studied using two types of gels such as A-ProOMe/HPMA and A-ProOMe/ HEMA. The swelling-deswelling profile between 10°C and 37°C at 24-hour intervals is shown in Fig. 2. The water temperature was first kept at 10°C to swell the gel, adjusted to 37°C for a period of 24 hours, and then re-adjusted to 10°C for 24 hours. The shrinkage of poly(A-ProOMe/HPMA) gel occured gradually throughout the experimental period of 24 hours, in contrast to a rapid shrinkage in the first 2 hours for the poly(A-ProOMe/HEMA) gel. On the other hand, reswelling equilibrium was reached within 2 hours in both cases. The results following repeat of the swelling-deswelling procedure at this temperature cycle show that the thermo-response of this gel is reversible. In order to clarify the difference in the swelling-deswelling kinetics, the appearance and cross sectional structure of this gel were investigated microscopically. Both gels were translucent in the swollen state at 10°C, but in the deswollen state their appearances differed markedly. The deswollen poly(A-ProOMe/HPMA) consisted of two layers, a transparent layer at the surface and opaque layer inside the matrix. In contrast, no such phase separation was seen in the case of deswollen poly(A-ProOMe/HEMA). The

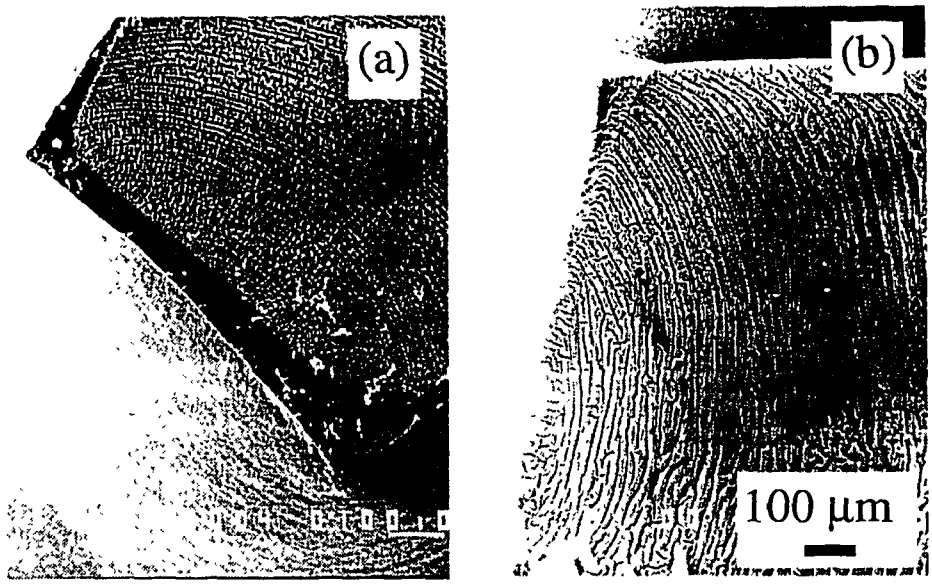

Fig. 3 SEM photographs of cross-sectional structures of poly(A-ProOMe/HPMA) gel (a) and poly(A-ProOMe/HEMA) gel (b) after deswelling for 8 hours at 37°C (both saturated previously with water at 10°C).

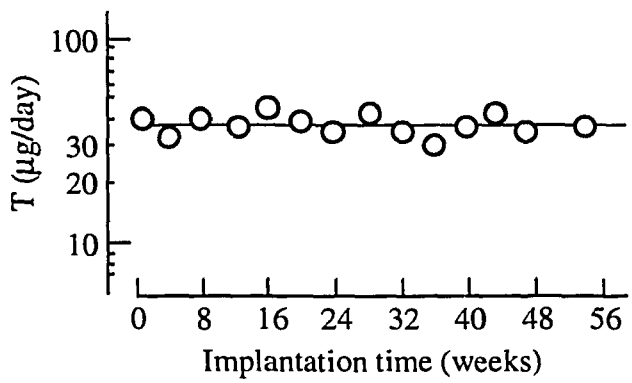

Fig. 4 Daily dose of T released *in vivo* from poly(A-ProOMe/HPMA) gel with a surface regulated function.

SEM photographs of cross sectional structures of the gels in the deswollen state are in Fig. 3. Deswelling of the poly(A-ProOMe/HPMA) gel leads to the formation of a rigid membrane barrier at the surface which lacks pores, and a number of pores in the interior of the matrix. The thickness of the barrier formed during the deswelling increased with time, and a small mass devoid of micropores was the eventual result. These finding imply that the dehydration of poly(A-ProOMe/HPMA) gel occurs from the surface of the matrix accompanying an initial rapid shrinkage, suggesting that the existence of a rigid barrier at the surface markedly retards the rate of passage of water from the interior of the gel. For reswelling , no such a barrier exists because of the regeneration of porous gel, suggesting that the microporous structure of the gel which can be freely changed in water by temperature cycle memorizes the event as a matrix, namely, a surface regulated matrix. In the case of poly(A-ProOMe/HEMA), no barrier

blocking the passage of water from the interior of the gel is present in the deswollen state; the micropores survive throughout the experimental period of 24 hours, despite a remarkable change in the shape of the gel and a marked decrease in the size of the micropore. In the

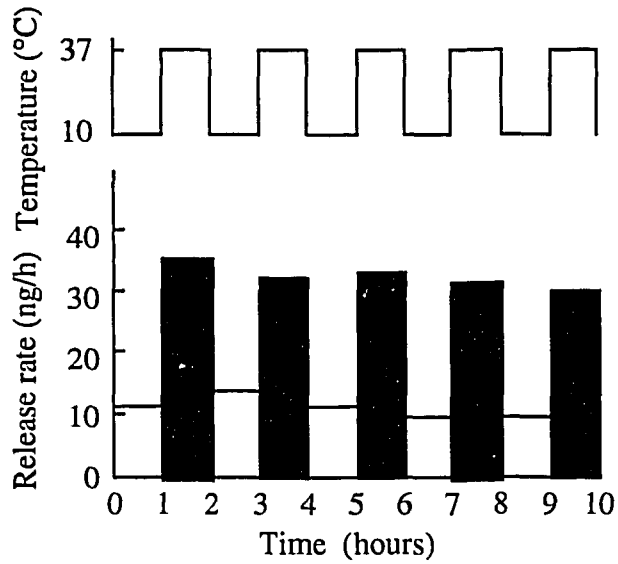

Fig. 5 *In vitro* release behavior of Ara-A from poly(A-ProOMe/HEMA) gel with a matrix pumping function

reswelling of this gel, both the pore size and the shape were restored to their initial states. We conclude from these results that deswelling of poly(A-ProOMe/HEMA) gel is accelerated by the passage of water from the inside matrix via pumping through the porous structure, namely a pumping matrix.

Testosterone (T) was incorporated into the gel by means of radiation-induced polymerization, to provide a T-loaded poly(A-ProOMe/HPMA) gel with 'surface regulating' function. In order to examine the thermo-response as a means for controlled drug release, tablet forms of the copolymer-drug gels were implanted subcutaneously into the backs of castrated rats. As can be seen clearly in Fig. 4, the *in vivo* daily dose of drug released from the gel was constant at approximately 39 μg/day throughout 54 weeks. Controlled drug release over a long period arises, we believe, because the gel blocks the initial rapid release of the drug owing to the formation of a rigid barrier at the surface of the matrix.

Ara-A was incorporated into a poly(A-ProOMe/HEMA/9G, 9/1/0.02 mmol) gel with a matrix pumping function to evaluate the *in vitro* release behavior. The gel employed, which has a size of 8 mm in diameter and 5 mm length, involves approximately 4.5 mg of Ara-A. The *in vitro* dose of Ara-A released from the gel was found to occur in the form of unstable release accompanying an initial burst during the first 24 hours period, followed by a constant release of approximately 11 ng/h at 10°C for 9 days from day 1 to 10. On the basis of this result, we decided to pretreat the drug-loaded gel in water at 10°C during the first 24 hours period in order to avoid the unstable period of release. Using this pretreated gel, the *in vitro* release behavior of Ara-A from the gel when cycled between 10 and 37°C at 60-minute intervals was examined and is depicted in Fig. 5. The *in vitro* release rate of Ara-A from the gel treated for 60 minutes at 10°C was approximately 11 ng/h, in contrast to approximately 33 ng/h at 37°C, suggesting that the release behavior of drug when cycled at different temperatures is reversible. The cause

of the high release rate of Ara-A at 37°C could readily be explained on the basis of the "matrix pumping" mechanism of the shrinking of the gel.

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