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A heterogeneous catalytic kinetics for enzymatic biodegradation of poly(ϵ-caprolactone) nanoparticles in aqueous solution

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Abstract

Water insoluble poly(ϵ-caprolactone) (PCL) was micronized into narrowly distributed stable nanoparticles. The biodegradation of such PCL nanoparticles in the presence of the enzyme, Lipase PS, was monitored by using laser light scattering because the scattering intensity is directly related to the particle concentration. The PCL and enzyme concentration dependence of the biodegradation rate supports a heterogeneous catalytic kinetics in which we have introduced an additional equilibrium between the inactive and active enzyme/substrate complexes. The initial rate equation derived on the basis of this mechanism was used to successfully explain the influence of surfactant, pH and temperature on the enzymatic biodegradation. Our results confirmed that both the adsorption and the enzymatic catalysis were important for the biodegradation of the PCL nanoparticles.

Keywords: Poly(ϵ-caprolactone) nanoparticles; Enzyme/substrate complexes; Lipase PS

1. Introduction

Biodegradable polymeric colloidal nanoparticles have recently attracted some attention because of their potential applications in controlled drug delivery [1]. It has been shown that the polymeric nanoparticles can be used, orally or intravenously, to administer peptides and other drugs. Using polymeric nanoparticles could increase the availability, decrease possible associated adverse effects, and avoid surgical implantation in some cases [2,3]. Synthetic aliphatic polyesters, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(ϵ-caprolactone) (PCL), are often used in biomedical applications because they are biocompatible and non-toxic [4–7]. The pharmacologically inactive biodegradation products, such as lactic acid from PLA and 6-hydroxycaproic acid from PCL, can be absorbed by body or removed by metabolism [8,9], so that the removal of these polymer devices becomes unnecessary. Lemoine et al. [10] studied the biodegradation of PCL, PLA and PLA/PGA in aqueous solution.

It has been known that certain enzymes can catalyze the hydrolysis of aliphatic polyesters. For example, poly(β-hydroxybutyrate) (PHB), a natural polymer with a structure of aliphatic polyesters, can be hydrolyzed into metobolizable, small molecules in the presence of extracellular PHB depolymerases [11]. The classic model was used to describe the enzymatic biodegradation kinetics [12], i.e.

$$E + S \rightleftharpoons ES \rightarrow P + E \tag{1}$$

where E, S, ES, and P represent enzyme, substrate, the enzyme/substrate complex, and the biodegradation product, respectively. Most biodegradable polymers, such as PHB, PCL and PLA, are insoluble in water [13]. Thus Mukai et al. [14] modified Eq. (1) after considering that each enzyme molecule has two discrete domains: the hydrophobic adsorbing site and the catalytic site [15]; namely, the enzymatic hydrolysis involves the binding of the enzyme onto the substrate at one point and the hydrolytic scission at another point. The modification resulted in

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \qquad ES + S \xrightarrow{k_2} ES + P \tag{2}$$

which leads to a rate equation of

$$\nu_0 = \frac{(k_1 k_2 / k_{-1})[E]_0}{\{1 + (k_1/k_{-1})[E]_0\}^2} \tag{3}$$

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where $[E]_0$ is the initial enzyme concentration. In both Eqs. (1) and (2), the adsorption of the enzyme onto the substrate is the first and essential step. Our previous studies showed that the enzyme, Lipase Pseudomonas (PS), could speed up the biodegradation of PCL [16]. The micronization of PCL into the PCL nanoparticles stable in water not only made the enzymatic biodegradation $\sim 10^3$ times more faster, but also led a novel, reliable and fast laser light scattering method to monitor the biodegradation kinetics [17]. We also studied the biodegradation of block PEO-*b*-PCL copolymers and its potential biomedical applications [18]. In this study, we will concentrate on the catalytic kinetics of the enzymatic biodegradation of polymeric nanoparticles.

2. Experimental

2.1. Materials

Poly(ϵ-caprolactone) (PCL) ($M_w = 1.43 \times 10^5$) was synthesized by ring-opening polymerization with a catalyst composed of yttrium trifluoroacetate, $Y(CF_3COO)_3$, and triisobutylaluminum, Al(*i*-Bu) [18]. Lipase PS from *Pseudomones cepacia* (courtesy of Amano Pharmaceutical Co.) was purified by freeze drying. Cationic hexadecyltrimethylammonium bromide (HTAB) (from Eastman Kodak Co.) and anionic sodium lauryl sulfate (SDS) (from BDH Chemical Ltd) as stabilizers were used without further purification. Dilute hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used in the pH dependent study. The PCL nanoparticles in the size (diameter) range 190–250 nm were prepared by adding the dilute PCL acetone solution (2.50×10^{-3} g/ml) dropwise into an excess of water containing either cationic HTAB or anionic SDS. The surfactant concentration was 1 CMC (critical micelle concentration) except stated otherwise. The nanoparticles have a core–shell structure with the core made of collapsed PCL chains and the shell made of an adsorbed surfactant layer. The final PCL concentration in the dispersion used was in the range 2–20 μg/ml. The trace amount of acetone in the resultant dispersion was removed by distillation under a reduced pressure. The detail of the micronization was reported elsewhere [17]. Hereafter, we will denote the PCL nanoparticles stabilized by HTAB and SDS, respectively, as PCL-HTAB and PCL-SDS.

2.2. Laser light scattering

A modified commercial LLS spectrometer (ALV/SP-125) equipped with an ALV-5000 multi-τ digital time corrector and a solid state laser (ADLAS DPY 425II, output power is ~400 mV at $\lambda = 532$ nm) was used. In static LLS, the angular dependence of the excess absolute time-average scattered intensity, i.e. Rayleigh ratio $R_{vv}(\theta)$, was measured. For a very dilute polymer solution, $R_{vv}(\theta)$ at a small scattering angle θ can be approximately related to both the nanoparticle concentration (C) and weight-average molar mass (M_w) as [19]

$$R_{vv}(\theta) \approx KM_wC \tag{4}$$

where K is a constant. In dynamic LLS, the cumulant analysis of the measured intensity–intensity time correlation function $G^{(2)}(t, q)$ led to the average translational diffusion coefficient ($\langle D \rangle$) or the average hydrodynamic radius ($\langle R_h \rangle$) of the PCL nanoparticles. The details of the LLS instrumentation and theory can be found elsewhere [20,21].

2.3. Enzymatic biodegradation

The PCL nanoparticle dispersion and the Lipase aqueous solution were, respectively, clarified by 0.8 and 0.5 μm Millipore filters to remove dust. In each enzymatic biodegradation experiment, a proper amount of dust-free Lipase PS aqueous solution was added into 2 ml dust-free PCL nanoparticles suspension in the light scattering cell. Both $R_{vv}(\theta)$ and $G^{(2)}(t, q)$ were in situ and simultaneously measured during the enzymatic biodegradation. Note that the scattered light intensity from the nanoparticles was so strong that the weak scattering from the enzyme, water, and small biodegradation products could be ignored, i.e. in LLS, we can only "see" the *remaining* PCL nanoparticles in the dispersion during the biodegradation. Our results showed that during the biodegradation, the size of the remaining nanoparticles [22], i.e. M_w, is a constant. Therefore, the change of the scattered light intensity was directly related to the nanoparticle concentration on the basis of Eq. (4). By measuring the change of $R_{vv}(\theta)$ in a dilute dispersion, we were able to monitor the change of the PCL nanoparticle concentration (Δ[PCL]), by

$$\Delta[\mathrm{PCL}] = [\mathrm{PCL}]_0 - [\mathrm{PCL}]_t$$

$$= [\mathrm{PCL}]_0\{1 - R_{vv}(\theta, t)/R_{vv}(\theta, 0)\}$$

where the subscripts "0" and "t" denote time 0 and t, respectively.

3. Results and discussion

Fig. 1 shows that for a fixed PCL/enzyme ratio, the rate and extent of the biodegradation increase as the PCL and enzyme concentrations increase. Note that Fig. 1 was confirmed by repeated experiments. The initial slope leads to the initial reaction rate ν_0 defined as $\{d[PCL]_t/dt\}_{t\to 0}$. Fig. 2 reveals the initial PCL concentration dependence of $\nu_0/[\mathrm{Lipase}]_0$ which is a normalized initial reaction rate per unit concentration (μg/ml). It is clear that the initial biodegradation rate (ν_0) increases linearly as both the initial PCL concentration and the initial Lipase PS concentration, i.e. $\nu_0 \propto [PCL]_0[E]_0$. Considering that the enzymatic biodegradation involves both the adsorption of Lipase PS onto the PCL nanoparticles and the enzymatic hydrolysis [14], we adopted the biodegradation reaction mechanism suggested

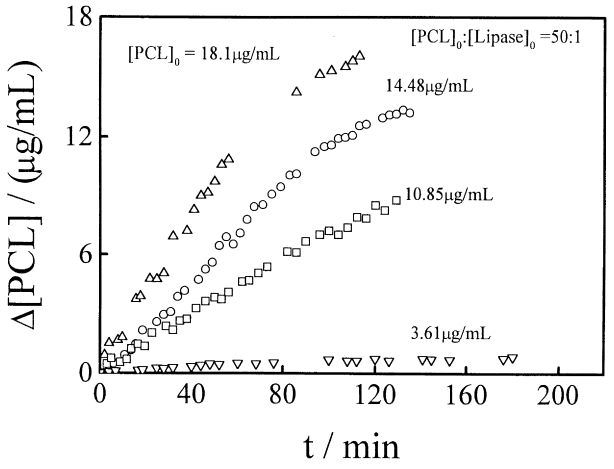

Fig. 1. Enzymatic biodegradation kinetics of the PCL-HTAB nanoparticles, where $T = 25$°C, pH = 6.2, and $\Delta[\mathrm{PCL}] = [\mathrm{PCL}]_0 - [\mathrm{PCL}]_t$.

by Mukai et al. [14] and formulated it as

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (\mathrm{E}\cdots\mathrm{S}) \qquad (\mathrm{E}\cdots\mathrm{S}) \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (\mathrm{E} :: \mathrm{S})^* \qquad (5)$$

$$(\mathrm{E} :: \mathrm{S})^* \xrightarrow{k_3} \mathrm{P} + \mathrm{E}$$

where (E···S) represents the inactive enzyme/PCL complex in which only the adsorption site is attached to the substrate, while $(\mathrm{E} :: \mathrm{S})^*$ represents the active enzyme/PCL complex in which both the adsorption and catalysis sites are attached to the substrate. In comparison with Eq. (2), we have introduced an additional equilibrium between (E···S) and $(\mathrm{E} :: \mathrm{S})^*$, so that we have

$$\nu_0 = -\left[\frac{\mathrm{d}[S]}{\mathrm{d}t}\right]_{t\to 0} = k_1\left(1 - \frac{k_{-1}}{k_{-1} + k_2 - \dfrac{k_2 k_{-2}}{k_{-2} + k_3}}\right)[\mathrm{E}]_0[\mathrm{S}]_0 = k[\mathrm{E}]_0[\mathrm{S}]_0 \qquad (6)$$

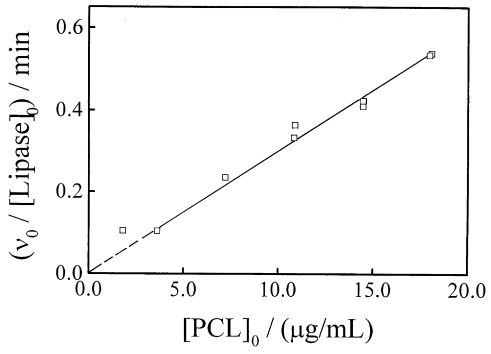

Fig. 2. Initial PCL concentration dependence of the initial biodegradation rate (ν_0) normalized by the initial Lipase concentration, where ν_0 is defined as $\{\mathrm{d}[\mathrm{PCL}]_t/\mathrm{d}t\}_{t\to 0}$.

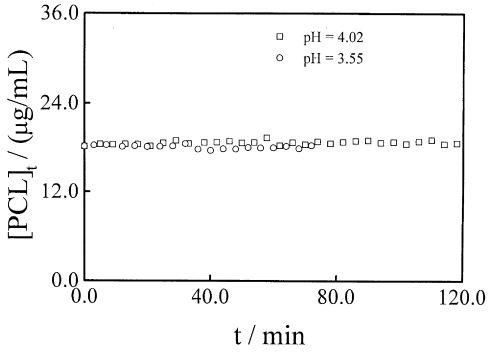

Fig. 3. Enzymatic biodegradation kinetics of the PCL-SDS nanoparticle at two different pH values, where $[\mathrm{PCL}]_0 = 1.81 \times 10^{-5}$ g/ml, $[\text{Lipase PS}]_0 = 3.57 \times 10^{-7}$ g/ml, and $T = 25$°C.

where S represents PCL in this case and $k = k_1\{1 - k_{-1}/[k_{-1} + k_2 - k_2 k_{-2}/(k_{-2} + k_3)]\}$. The results in Fig. 2 supports Eq. (6). However, it should be noted that Fig. 2 also supports the classic model as shown in Eq. (1). We have to seek other evidence to distinguish them.

On the basis of Eq. (6), if the desorption is much fast, i.e. $k_{-1} \gg k_2, k \to 0$, so that $\nu_0 \to 0$. Our studies showed that if the anionic SDS instead of the cationic HTAB was adsorbed on the PCL nanoparticles to stabilize the PCL nanoparticles, no enzymatic biodegradation could be observed because of the repulsion between the negatively charged enzyme and anionic SDS, i.e. a larger k_{-1}. Eq. (6) also slows that when $k_{-2} \gg k_3, k \to 0$, so that $\nu_0 \to 0$, independent of k_{-1}, which means that even for a strong adsorption, there could still be no biodegradation. For example, when pH < 5, there was no biodegradation in the pH range of 3.55–4.02 even though the positively charged Lipase PS was strongly adsorbed on the negatively charged surface of the PCL-SDS nanoparticles in the acidic condition, as shown in Fig. 3.

Fig. 4 shows that the initial rate (ν_0) and the maximum extent ($\{\Delta[\mathrm{PCL}]/[\mathrm{PCL}]_0\}_{t\to\infty}$) of the biodegradation decrease as the HTAB concentration increases. It has been known that when the HTAB concentration was higher than its critical micelle concentration (CMC), many HTAB micelles were formed in the dispersion. These small positively charged HTAB micelles competed with the PCL nanoparticles for the adsorption of enzyme, resulting in a less number of effective Lipase PS molecules, i.e. a smaller $[\mathrm{E}]_0$ in Eq. (6). This is why the rate and the maximum extent of the biodegradation were lower at higher HTAB concentrations.

Fig. 5 shows that both the initial rate (ν_0) and the maximum biodegradation extant ($\{\Delta[\mathrm{PCL}]/[\mathrm{PCL}]_0\}_{t\to\infty}$) increase as the pH increases due to a stronger adsorption and a fast catalytic reaction at higher pH values. There was a cut-off pH (~5) value for the enzymatic biodegradation. When pH < 5, the enzyme has an overall negative charge and the nanoparticles are also negatively charged so that electrostatic repulsion leads to a decrease of the adsorption. Moreover, the enzyme activity becomes very low when pH is lower than 5. Therefore, as pH decreases, k_{-1}

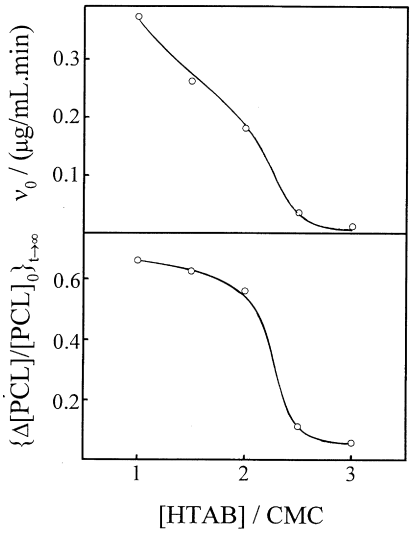

Fig. 4. Surfactant concentration dependence of initial rate (ν_0) and maximum extent ($\{\Delta[PCL]/[PCL]_0\}_{t\to\infty}$) of biodegradation of PCL-HTAB nanoparticles, where $[PCL]_0 = 1.08\times10^{-5}$ g/ml, $[\text{Lipase PS}]_0 = 2.13\times10^{-7}$ g/ml, and $T = 25$°C.

increases and k_3 decreases, which resulted in $k \to 0$ on the basis of Eq. (6).

Fig. 6 shows that the initial rate of the enzymatic biodegradation increases as the temperature increases until the maximum rate was reached at ~43°C. Further increase of the temperature slowed down the biodegradation because Lipase PS as a protein gradually loses its catalytic ability at higher temperatures [12,23]. The activation energy (E_A) estimated from the Arrhenius plot of the reaction rate constant (k) versus the reciprocal of the absolute reaction temperature (T^{-1}) shown in Fig. 7 was ~ 1.6×10^2 kJ/mol, comparable to those in literature [24].

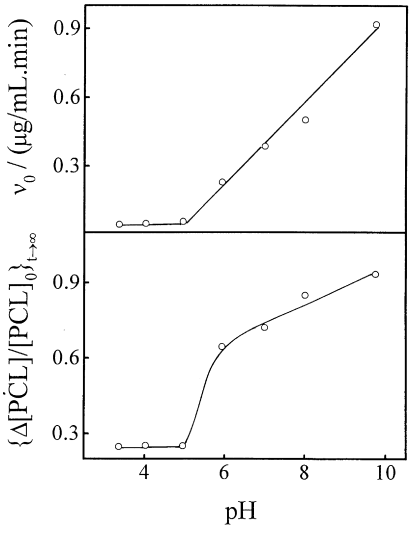

Fig. 5. pH dependence of initial rate (ν_0) and maximum extent ($\{\Delta[PCL]/[PCL]_0\}_{t\to\infty}$) of biodegradation of PCL-HTAB nanoparticles, where $[PCL]_0 = 1.81\times10^{-5}$ g/ml, $[\text{Lipase PS}]_0 = 3.57\times10^{-7}$ g/ml, and $T = 25$°C.

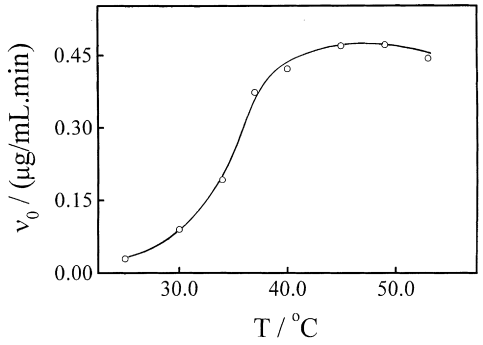

Fig. 6. Temperature dependence of initial biodegradation rate (ν_0) of PCL-HTAB nanoparticles, where $[PCL]_0 = 1.08\times10^{-5}$ g/ml and $[\text{Lipase PS}]_0 = 2.14\times10^{-7}$ g/ml.

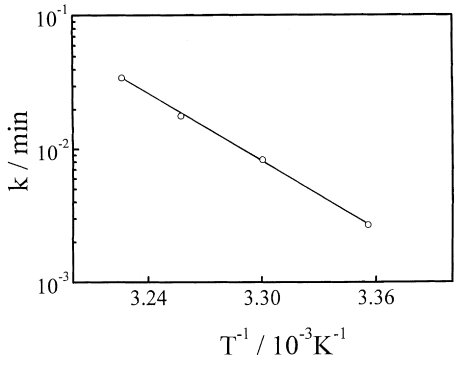

Fig. 7. Arrhenius plot of overall rate constant (k) versus reciprocal absolute temperature (T^{-1}) for the enzymatic biodegradation of PCL-HTAB nanoparticles, where $[PCL]_0 = 1.08\times10^{-5}$ g/ml and $[\text{Lipase PS}]_0 = 2.14\times10^{-7}$ g/ml.

In summary, the enzymatic biodegradation of the PCL nanoparticles in the presence of Lipase PS could be described by a modified heterogeneous kinetics, which consists of an adsorption equilibrium, an activation equilibrium and an enzymatic hydrolysis. The introducing of the activation equilibrium between the inactive (E···S) and active (E ∷ S)*. PCL/Lipase complexes were based on the fact that the biodegradation involves both the adsorption and enzymatic hydrolysis. The rate equation derived from this mechanism enabled us to explain the influence of pH, temperature, and the initial polymer, enzyme and surfactant concentrations on the biodegradation. One of the important features of this kinetics is that the reaction rate is controlled not only by the adsorption of Lipase PS, but also by the adsorption of the catalysis site onto the PCL nanoparticles.

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