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Kinetic-mechanistic studies of lipase-polymer micelle binding and catalytic degradation: Enzyme interfacial activation

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ABSTRACT

Relatively small and uniformly sized block copolymer micelles from low polydispersity poly (ethylene glycol) (PEG) block poly (ε-caprolactone) (PCL) (PEG_{45}-*b*-PCL_{60}) give ^{1}H NMR spectra useful for direct micelle characterization and kinetic-mechanistic studies. *P. cepacia* lipase catalyzed PEG_{45}-*b*-PCL_{60} micelle degradations were followed by ^{1}H NMR and GPC to obtain simultaneous evaluation of the micelle composition, degradation kinetics and appearance of the water soluble hydrolysis products. Analysis and simulation of the concentration versus time profiles for *P. cepacia* lipase catalyzed PEG_{45}-*b*-PCL_{60} micelle degradation show that the process conforms to a Michaelis–Menten mechanism ($E + M \rightleftarrows EM \rightarrow P + E$) with enzyme-micelle complex product inhibition ($EM + P \rightleftarrows EMP$). Formation and tight binding of the lipase enzyme-micelle complex, activation of lipase catalysis and sequential micelle degradation are characteristics of PEG_{45}-*b*-PCL_{60} micelle degradation which parallel features of enzyme interfacial activation associated with lipase catalyzed hydrolysis of lipids in membranes.

1. Introduction

Carboxyl ester hydrolases are categorized as either esterases or lipases where both have activity with water soluble esters, but only lipases have the capability to catalyze the hydrolysis of lipid ester groups in water insoluble aggregates like membranes and vesicles [1–3]. Aggregates of synthetic polyesters in aqueous suspensions [4], thin films [5], crystals [6] and nanoparticles [7] can also function as substrates for lipase enzymes. Poly (ethylene glycol) (PEG) block poly (ε-caprolactone) (PCL) [8–11] are prototypical amphiphilic block copolymers [12,13] that self-assemble into core–shell micelles [14–17] in water where the hydrolytically non-degradable hydrophilic segment (PEG) forms the exterior corona and the core contains the hydrolytically degradable hydrophobic block (PCL). Block copolymer micelles have a variety of biomedical applications [18–20], and are particularly prominent as drug and gene delivery vehicles [21–23] that function through their capability for loading, transport and release of lipophilic substances incorporated in the hydrophobic core. Development of strategies for selective release of micelle transported therapeutics motivate a growing effort to explore the mechanistic origins of micelle degradation [10,24–28].

Several lipase enzymes have been observed to catalyze polyester hydrolysis and degradation of micelles derived from water insoluble PEG-*b*-PCL block copolymers [8,9,29,30]. Techniques previously applied in studying micelle degradations have typically utilized bulk properties of the system such as changes in light scattering [8,9,31] that do not directly identify and quantify the micelle composition and concentration. This article reports on advancing mechanistic understanding of the *P. cepacia* lipase [32,33] catalyzed PEG_{45}-*b*-PCL_{60} micelle degradation primarily through using ^{1}H NMR observations of micelle dispersions. Application of ^{1}H NMR provides a comprehensive direct experiment to evaluate relative micelle concentrations, composition, and degradation kinetics, as well as identification and quantification of the water soluble hydrolysis products. Complementary GPC studies give the relative concentrations of intact block copolymers in the micelles and the products from lipase catalyzed polyester hydrolysis. The small spherical micelle particles dispersed in aqueous media are treated as pseudo-molecules that conform to the law of mass action in equilibrium and rate expressions for the lipase-micelle reaction system. Analysis and simulation of the concentration versus time profiles for *P. cepacia* lipase catalyzed PEG_{45}-*b*-PCL_{60} micelle degradation show that the process conforms to a Michaelis–Menten mechanism ($E + M \rightleftarrows EM \rightarrow P + E$) with enzyme-micelle complex product inhibition ($EM + P \rightleftarrows EMP$). A new model for lipase catalyzed micelle degradation is proposed based on analysis and interpretation of the experimental results.

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Formation and tight binding of the lipase enzyme-micelle complex, activation of lipase catalysis and sequential micelle degradation are characteristics of PEG_{45}-*b*-PCL_{60} micelle degradation which parallel features of enzyme interfacial activation [34–37] associated with native lipase catalyzed lipid membrane hydrolysis [38–43].

2. Results and discussion

2.1. ^1H NMR for PEG_{45}-*b*-PCL_{60} diblock copolymer micelles

Narrowly size dispersed 30 ± 1 nm (pH = 7.4, T = 37 °C) spherical micelles were prepared from diblock copolymer (PDI = 1.08) which has a much lower polydispersity than PEG-*b*-PCL diblock copolymers used in prior micelle degradation studies [8,9] (Figures S1–S4). The 30 ± 1 nm micelles form stable dispersions of particles with a mass of 2.0 ± 0.5 × 10^6 Da evaluated by Debye plots [44] from light scattering measurements. ^1H NMR spectra for the 30(1) nm micelles in aqueous PBS buffer dispersions are also similar to homogeneous solutions of molecules where the hydrogen resonances for the polymer chains in the micelle dispersions are broadened compared to water soluble small molecules, but all of the resonances are readily observed and fully assigned (Fig. 1, Figure S2). The line width for the resonance of the more mobile exterior shell PEG units (5.4 Hz) is smaller than the value for the less exposed and less mobile CH_2 groups for the PCL segment in the micelle core (~10 Hz). The relatively small micelle diameter (30 nm) and thermal motions of the polymer chains within the micelles are sufficient to give substantial averaging of the local dipolar magnetic interactions [45]. NMR is usually applied to solution species rather than dispersions of particles in fluid media, but there are precedents where NMR has been used effectively to study dispersions of small particles like liposomes and vesicles [46–48].

^1H NMR of the terminal CH_2OH (3.67 ppm) of the PCL oligomers shows that *P. cepacia* catalyzes the hydrolysis the PCL chains into a series of PCL fragments PCL_n (n = 1–6) (Fig. 2A) in which 6-hydroxycaproic acid ($HO(CH_2)_5CO_2H$) is effectively absent during the first hour of the PCL hydrolysis. Subsequent hydrolysis of the PCL oligomers which proceed in solution even after the micelles have fully reacted away produces 6-hydroxycaproic acid as the final product of hydrolysis of the PCL_{60} segment (Figure S7).

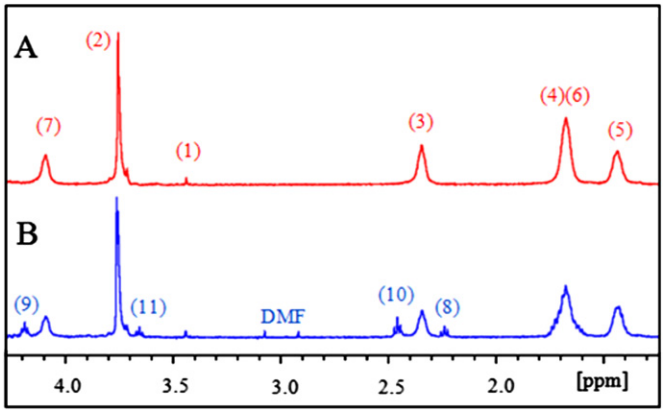

Fig. 1. ^1H NMR (500 MHz) of PEG_{45}-*b*-PCL_{60} block copolymer micelles (0.714 mg/mL) with PBS buffer (pH = 7.4; T = 37 °C): (A) ^1H NMR assignments for micelle peaks: $CH_3(1)O–[CH_2CH_2(2)–O–]_{45}[C(O)\ CH_2(3)CH_2(4)CH_2(5)CH_2(6)CH_2(7)–O]_{60}C(O)(CH_2)_5OH$. (B) Micelle with *P. cepacia* lipase at an activity of 0.714 U/mL, and a reaction time of 1.0 h ^1H NMR assignments for diagnostic hydrogens in PCL oligomers: $HOC(O)CH_2(8)(CH_2)_3CH_2(9)\ O[C(O)CH_2(10)\ (CH_2)_3CH_2(9)\ O]_nC(O)CH_2(10)\ (CH_2)_3\ CH_2(11)OH$, the chemical shifts in ppm for each assignment are as follow: $\delta(1)$ = 3.45; $\delta(2)$ = 3.76; $\delta(3)$ = 2.35; $\delta(4,6)$ = 1.70; $\delta(5)$ = 1.45; $\delta(7)$ = 4.10; $\delta(8)$ ~ 2.25; $\delta(9)$ ~ 4.19; $\delta(10)$ ~ 2.46; $\delta(11)$ ~ 3.67.

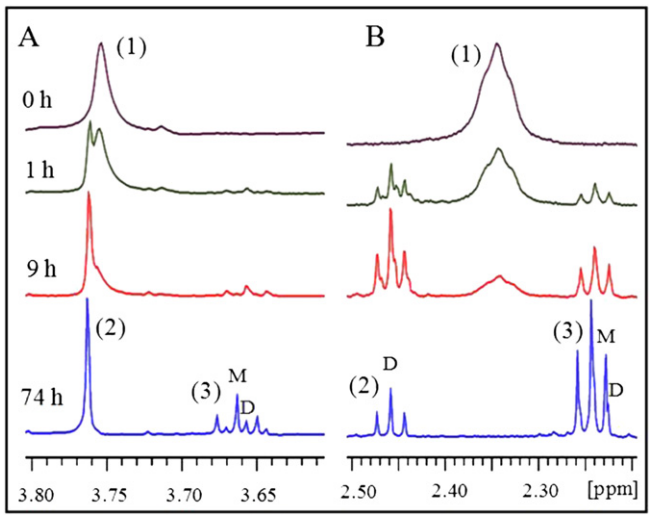

Fig. 2. ^1H NMR (500 MHz) of PEG_{45}-*b*-PCL_{60} block copolymer micelles (0.714 mg/mL) in PBS buffer(pH = 7.4) at T = 37 °C with *P. cepacia* lipase at an activity of 0.714 U/mL at a series of reaction times (M = $HO(CH_2)_5–CO_2H$; D = $HO(CH_2)_5–C(O)O(CH_2)_5–CO_2H$); (A) PEG segment in the micelle (1A) and water soluble PEG fragment from hydrolysis ($PEG_{45}–PCL_n$ (n = 0–6)) (2A) and the $–CH_2OH$ terminal groups for the PCL oligomers (3A). (B) Ester $–CH_2–C(O)–O$-hydrogens of PEG-*b*-PCL diblock copolymer micelles (1B) and ester$–CH_2–C(O)–O–$ (2B) and terminal CH_2CO_2H (3B) for water soluble PCL oligomers.

GPC observations of lipase catalyzed micelle degradation GPC traces at a series of reaction times for *P. cepacia* lipase catalyzed PEG_{45}-*b*-PCL_{60} micelle (0.714 mg/mL) degradation that are obtained after freeze drying the PBS reaction solution and dissolving in DMF are shown in Fig. 3. The DMF solutions used for the GPC studies contain all of the constituents that are present during the enzyme catalyzed micelle degradation. GPC traces obtained by this procedure with DMF as the solvent reflect the constituents of the entire sample consisting of the enzyme, residual unreacted diblock PEG_{45}-*b*-PCL_{60} and all of the PEG_{45}-*b*-PCL_n and PCL_n units produced in the hydrolysis of PEG_{45}-*b*-PCL_{60} micelle. The GPC traces corresponding to the PEG_{45}-*b*-PCL_{60} diblock copolymer decrease in area as the reaction proceeds, but the position and width are unchanged (Fig. 3). Appearance of new peaks in the GPC corresponding to PEG_{45}-*b*-PCL_n

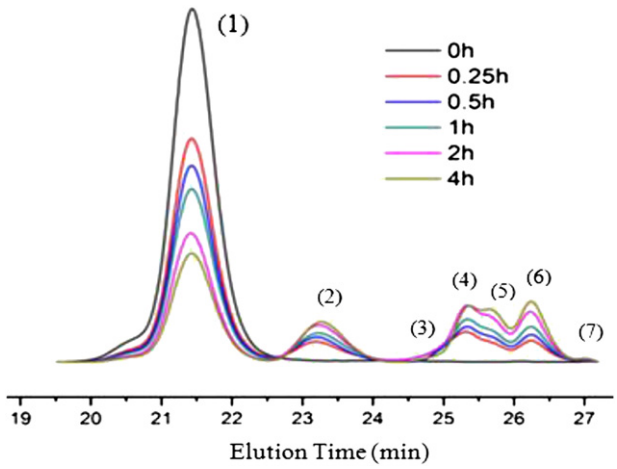

Fig. 3. GPC traces for DMF solutions containing all the constituents from the *P. cepacia* lipase (2.86 U/mL) catalyzed hydrolysis of PEG_{45}-*b*-PCL_{60} and micelle degradation in water at 37 °C and pH = 7.4. (1) PEG_{45}-*b*-PCL_{60}; (2) PEG_{45}-*b*-$PCL_{(1–6)}$; (3) $PCL_{(5–6)}$; (4) PCL_4; (5)PCL_3; (6) $HO(CH_2)_5–C(O)O(CH_2)_5–CO_2H$; (7) $HO(CH_2)_5–CO_2H$.