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Mechanism of Enzymatic Degradation of Poly(butylene succinate)

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Abstract: Poly(butylene succiate) (PBS), poly(butylene succinate-*co*-L-lactate) (PBSL), and poly(butylene succinate-*co*-6-hydroxycaproate) (PBSCL) polymers were degraded by lipase PS®, and the enzymatic degradation mechanism of PBS was analyzed in detail. The enzymatic degradation of PBS gave 4-hydroxybutyl succinate (4HBS) as the main product. An exo-type hydrolysis mechanism was proposed based on this observation. The terminal chain of PBS had conformational similarity to ordinary tri- and diglycerides and could be incorporated as a substrate in the active site of this lipase. The surface adsorption of the lipase was much larger on PBS and its copolymer films than on the other polyester films because the lipase adhered quite strongly to the polymer terminal through a specific adsorption mechanism. Kinetic analysis showed that the total number of surface adsorption points per unit area of PBSL and PBSCL copolymers was larger than that of the PBS homopolymer.

Keywords: lipase PS®, enzymatic degradation, hydrolysis mechanism, terminal chain, surface adsorption, homopolymer.

Introduction

Poly(butylene succinate) (PBS) and its copolymers have been developed for use as biodegradable plastics. Their biodegradability has been confirmed by the field tests in soil and sea water.[1] However, the degradation mechanism has not yet been well understood because few enzymes have been isolated from the microorganisms that degrade PBS. Considering the responsibility of the enzymes that are liberated from microorganisms for the biodegradation, the enzymatic degradation of various biodegradable polymers has been a subject of study. For example, the enzymatic degradability of polycaprolactone (PCL) and poly(3-hydroxybutyrate) (PHB) has been studied with various types of lipases that are either conventionally available or newly isolated from the specific bacteria.[2-4] Their degradation products have also been isolated and identified to clarify the mechanism of enzymatic degradation. Unfortunately, no specific enzyme for the hydrolysis of PBS has been discovered thus far, and even the commercially available hydrases are inactive. Because of this absence of the specific enzyme, the mechanistic study of the biodegradation of PBS has been difficult, even though PBS is quickly degraded in natural environment, particularly in soil.[1] Based on this background, we tried to analyze the mechanism of enzymatic degradation by use of lipase PS® originated from *Pseudomonas cepacia* that was discovered to be highly active for the degradation of PBS and its copolymers.

We also analyzed the surface adsorption of the lipase on several aliphatic polyester films, which is to be the initiation step of the enzymatic reaction of insoluble polymeric substrates. Furthermore, we succeeded in isolating a hydrolysis product from the enzyme solution. Based on identification of this product, an exe-type scission of the polymer chain is proposed. The kinetic analysis was also carried out for the enzymatic degradation by tracing the hydrolysis products by UV spectroscopy.

Experimental

Materials. PBSL was supplied in pellet form by Mitsubishi Chemical Industries Co., Ltd. (Tokyo, Japan). It was thoroughly dried in a vacuum oven at 85 °C for 10 h. PBSCL was supplied in pellet form by Daicel Chemical Industries Co., Ltd. (Tokyo, Japan). It was thoroughly dried in a vacuum oven at 85 °C for 10 h. Chemically synthesized poly(3-

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[RS]-hydroxybutyrate) (RS-PHB) was supplied by Takasago Koryo Co., Ltd. (Tokyo, Japan). Polymer films of poly(L-lactide) (PLLA) were supplied by Mitsui Chemical Co., Ltd. (Tokyo, Japan). Polymer films of PBS (pressing condition: 144 °C, 3 min), PBSL (141 °C, 3 min), and PBSCL (139 °C, 3 min) were prepared by hot pressing at temperature 30 °C higher than T_m, using a pressing machine TCU-BSN-2 (Gonno Htdraulic Press Manufacturing Co., Osaka, Japan). The lipase originated from *Pseudomonas cepacia* (lipase PS®) was supplied by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan).

Measurements. The molecular weights (M_n and M_w) and its distribution (M_w/M_n) were determined by gel permeation chromatography (GPC). The analyzer was composed of a Shimadzu LC-10Ad pump, a Shodex RI SE-31 RI detectorm and a Shimadzu C-R7A Chromatopac data processor. A combination of two polystyrene gel columns of Tosoho TSK gel G4000H and G2500H (7.5 mm I.d.×300 mm, each) was used with chloroform as the eluent at 35 °C. Its limited exclusion molecular weight was 7×10^5 Da. The molecular weight was calibrated according to polystyrene standards. Differential Scanning Calorimetry (DSC) was carried out on a Shimadzu DSC-50 thermal analyzer under a nitrogen flow of 20 mL/min at heating and cooling rates of 10 °C/min for about 2.0 mg of samples. T_m was taken as the peak temperature of the melting endotherm (the first run). T_g was taken as the inflection point of the specific heat decrement at the glass transition of the samples quenched in liquid nitrogen(the second run). Ultraviolet Spectroscopy (UV) was measured on a Shimadzu-1600 PC spectrophotometer. 200 MHz ^{1}H NMR spectra were measured on a Varian Gemini-200 spectrometer in $CDCl_3$ and D_2O with tetramethylsilane (TMS) and sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) as the internal references, respectively. HPLC was conducted with a system composed of two Shimadzu LC-10A pumps and a Shimadzu RID-10A refractive index detector using a reversed phase column of COSMOSIL $5C_{18}$-MS (4.6 mm and 150 mm length; Nacalai Tesque). 0.02 M phosphoric acid (pH=2.0)/methanol (=90/10) was used as the mobile phase whose flowing rate was 1.0 mL/min.

Identification of the Hydrolysis Product. Lipase PS® was dissolved in 0.1 M phosphate buffer in a concentration of 0.176 U/mL. A 7.6 mL portion of the solution was ultrafltered through a membrane with a pore size of 0.2 μm (MILLIPORE Filter Model 8050) whose exclusion molecular weight was 3,000 Da. As the supplementary solvent for this filtration, 0.1 M phosphate buffer (pH=6.0) was used. The resultant supernatant of this filtration was diluted to a volume of 7.6 mL. This solution (7.6 mL) was found to retain an enzymatic activity of 0.092 U/mL, which was 52% of the original. Then, a PBS film (size: 5×60 mm) was dipped in this enzyme solution to conduct the hydrolysis at 50 °C for 7 days. Finally, the enzyme solution was again ultra-filtered through the same membrane by using distilled water as the supplementary solvent to remove the lipase. The filtrate was evaporated to dryness, and the residue was subjected to analysis by ^{1}H NMR spectroscopy and HPLC.[5]

End-Capping Reactions of PBS. A 6.0 g amount of PBS was dissolved in 60 mL chloroform, and 4.8 g of acetic anhydride was added to it. After refluxing at 60 °C for 7 h, the solution was poured into an excess of methanol to recover the polymeric product quantitatively.[6] The acetylation of the hydroxyl terminals was supported by the 200 MHz ^{1}H NMR spectroscopy; i.e., the whole signal due to the hydroxylmethyl terminals of the original PBS (detected at δ 3.4 ppm) shifted to a lower magnetic field (around δ 4.2 ppm) in the acetylated PBS.

Similarly, 6.0 g of PBS was dissolved in 60 mL chloroform, and 50 mg of 9-anthryldiazomethane (ADAM, purchased from Funakoshi Co., Tokyo) was added to it. After this solution had been kept at room temperature for 1 h, the polymeric product was isolated by the reprecipitation as above.[7,8] In the 200 MHz ^{1}H NMR spectrum of the 9-anthryldiazomethyl PBS, the signals due to the anthryl groups were detected at δ 7.1-7.8 ppm in addition to the methyene signal at δ 5.1 ppm, while no change was observed in the signals due to PBS. Both end-capped PBS were processed to polymer films (thickness: 200 μm) for hydrolysis study.

Surface Adsorption of Enzyme.[9] A sample film (5×40 mm) was dipped in 7.6 mL phosphate buffer containing lipase PS® for a pre-determined time. Then the film was taken out, washed with distilled water three times, immersed in 2.5 mL of an aqueous solution of 3 wt% sodium dodecylsulfate (SDS) for 25 min, and finally sonicated for 5 min to completely detach the enzyme adsorbed on the surface. The protein concentration in the SDS solution was determined by the conventional bicinchonic acid (BCA) method by using a reagent kit for micro BCA protein assay (Pierce).[10,11] The reaction with the BCA reagent was conducted at 60 °C for 1 h, and the absorbance at 562 nm was measured for determination of the protein concentration.

Kinetic Analysis of the Enzymatic Degradation.[12,13] A sample film (0.5×0.5 cm) was first dipped in a 0.1 M phosphate buffer (pH=6.0) containing lipase PS® (10 μg/mL) at 50 °C for 16 h to remove various artifacts due to primary difference in the surface roughness and the bread-out of contaminants. Then, the film was taken out, washed with distilled water, immersed in an aqueous SDS solution (3 wt%) for 1 h and finally sonicated in it for 10 min to completely detach the enzyme adsorbed on the surface. Then the film was again incubated in 16 mL of a buffer solution containing lipase PS® in a certain concentration at 50 °C for 3 days. From time to time a portion of the solution was taken out to measure the UV spectrum for moni-

Table I. Properties of PBS Derivatives

	$M_n \times 10^4$ Da	M_w/M_n	T_m (°C)	T_g (°C)
PBS	5.3	1.9	114	-34
PBSL	4.9	2.0	111	-33
PBSCL	5.9	2.0	109	-36
RS-PHB	13.8	3.4	52	4
PLLA	10.0	1.6	161	71

toring the formation of the degradation product, 4-hydroxybutyl succinate (4HBS) at 201 nm. The absorption coefficient at 201 nm for 4HBS was determined to be 0.953 mL mg^{-1} cm^{-1} at 50 °C.

Results and Discussion

Synthesis of PBS and Properties of PBS Derivatives. PBS homopolymer was prepared by the ordinary melt-polycondensation of dimethyl succinate and 1,4-butanediol by using zinc acetatedihydrate (0.06 wt%) as the catalyst. Table I shows a typical resultant of PBS derivatives. PBS had a number-average molecular weight (M_n) of 53,000 Da with a polydispersity in weight/number average molecular weight ratio of M_w/M_n=1.9. Its melting (T_m) and glass transition temperatures (T_g) were 114 °C and -34 °C, respectively. PBSL with a unit composition of butylene succinate/L-lactate=97/3 (mol/mol) was prepared in pellet form that thoroughly dried in a vacuum oven at 85 °C for 10 h. Its T_m and T_g were 111 °C and -33 °C, respectively, and its M_n was 49,000 Da with M_w/M_n=2.0. PBSCL with a unit composition of butylene succinate/6-hydroxycaproate=95/5(mol/mol) was prepared in pellet form. Its T_m and T_g were 109 °C and -36 °C, respectively, while its M_n was 59,000 Da with M_w/M_n=2.0. Chemically synthesized poly(3-[RS]-hydroxybutyrate) (RS-PHB) was T_m and T_g were 52 °C and 4 °C, respectively, while its M_n was 138,000 Da with M_w/M_n=3.4. Polymer films of poly(L-lactide) (PLLA) was T_m and T_g were 161 °C and 71 °C, respectively, and its M_n was 100,000 Da with M_w/M_n=1.6. Polymer films of PBS, PBSL, and PBSCL were prepared by hot pressing at temperature 30 °C higher than T_m. The activity of this *Pseudomonas cepacia* (lipase PS®) guaranteed the company was 30,000 U/g where 1 U corresponds to releasing 1.0 μmol of aliphtic acids from an olive oil in 1 min at the optimum conditions (pH=6.0, 50 °C).

Hydrolysis Mechanism of PBS. Figure 1 shows a typical HPLC chromatogram of the crude hydrolysis products that were obtained from the solution of lipase PS® after the enzymatic treatment of a PBS film. In addition to the small peaks due to 1,4-butanediol and succinic acid, a large peak is shown at a long retention time. This peak can reasonably be assigned to 4HBS in reference to the chromatogram of its model compound that was readily prepared by the equimolar reaction of succinic anhydride and 1,4-butanediol (Scheme I). Figure 2 shows a typical ^1H NMR spectrum recorded for the crude hydrolysis product in D_2O. The main signals are assigned to 4HBS, i.e., 4.15 (m,-COOCH_2-,2H), 3.61 (m, -CH_2OH-, 2H), 2.6 (m, -COCH_2CH_2CO-, 4H), 1.5-1.8 (m, -C-CH_2CH_2-C-, 4H). The failed detection of the signals of succinic acid and 1,4-butanediol suggests that the crude hydrolysis product consisted almost of 4HBS (>90%). It is therefore indicated that the enzymatic hydrolysis preferentially gives 4HBS as the degradation product.

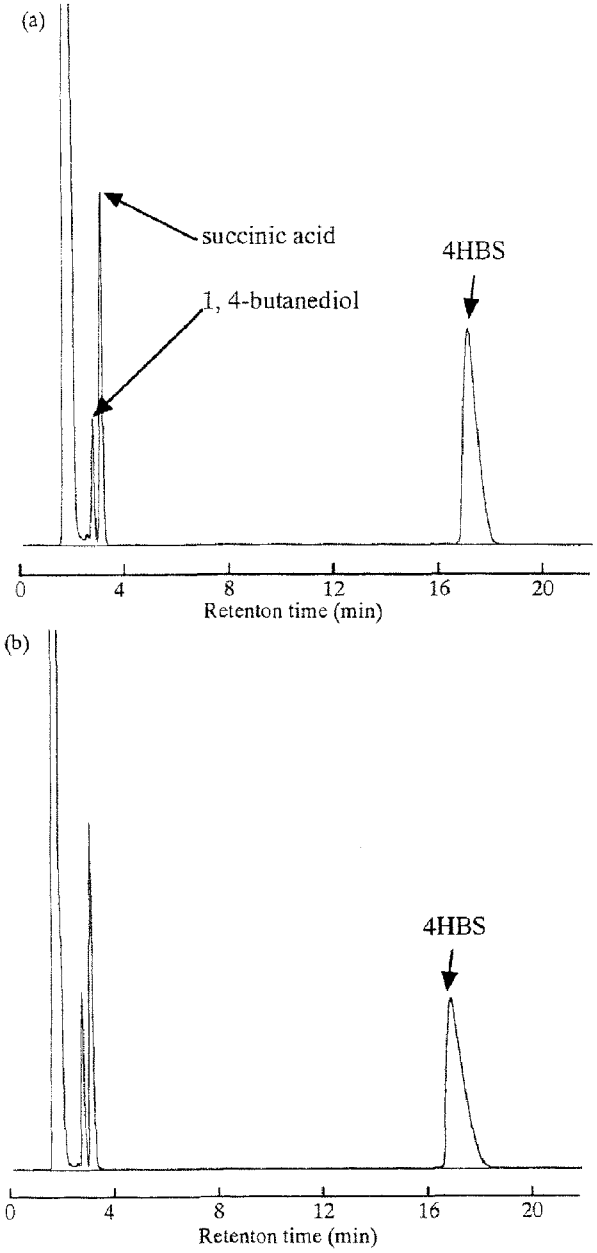
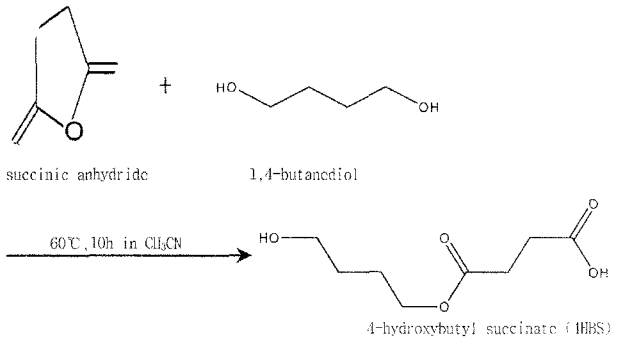


Figure 1. Typical HPLC chromatograms of (a) the hydrolysis product obtained after the enzymatic hydrolysis of PBS and (b) a model compound prepared from succinic anhydride and 1,4-butanediol (4HBS).



Scheme I. Synthesis of 4-hydroxybutyl succinate (4HBS).

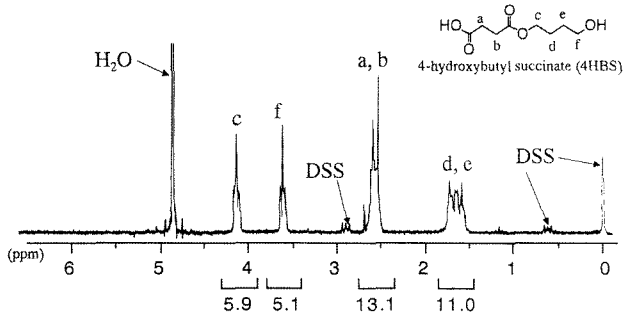
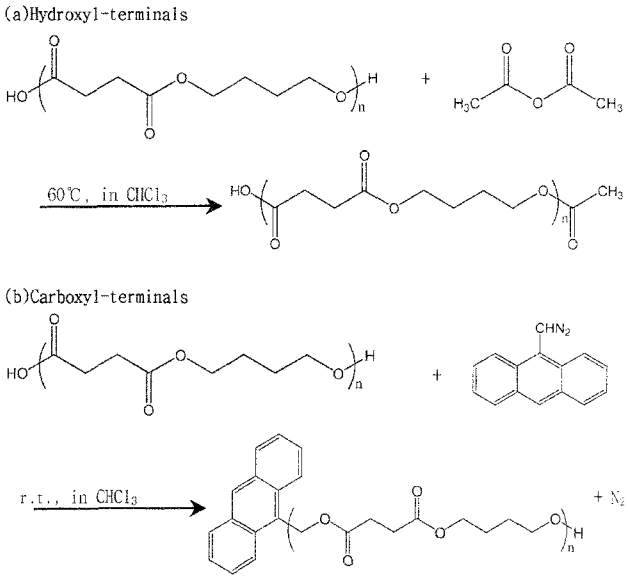
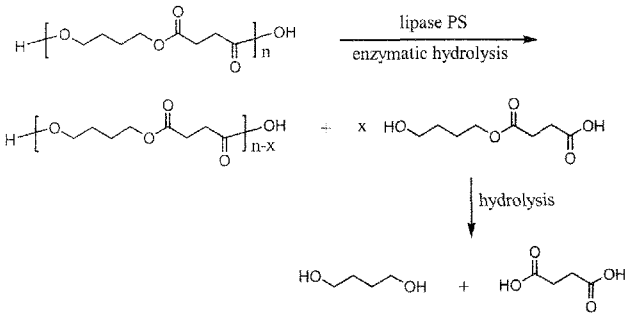


Figure 2. A typical 200 MHz ^{1}H NMR spectrum of the degradation product obtained by the enzymatic degradation of PBS for 7 days (in D_2O). The chemical sifts are in ppm from sodium 2,2-dimetyl-2-silapentane-5-sulfonate (DSS).



Scheme II. The end-capping reactions of PBS.

PBS was subject to the end-capping reaction and hydrolysis according to Schemes II and III. The capping yields were almost quantitative in both the acetylation of the hydroxyl terminals and the anthracenemethylation of the carboxyl terminals as described in Experimental. The resulting polymers were processed to thin films and incubated in



Scheme III. The hydrolysis of PBS.

Table II. Degradabilities of End-Capped PBS[a]

PBS Modified	Weight Decrease [mg/cm$_2$]	
	after 1 day	after 2 days
Control	3.1	4.9-5.8[b]
Acetylated(OH)	3.2	5.0
Anthracenemethylated(COOH)	1.6	5.5

[a]For a PBS film of 6×0.5 cm in size by the action of lipase PS® (0.176 U/mL) in phosphate buffer (pH=6.0) at 50 °C. [b]Fluctuated depending on the film samples.

the lipase solution at 50 °C for 1 day. The results of the enzymatic hydrolyses of PBS and its two types of end-capped derivatives are compared in Table II. It is known that the initial weight decrease is much lower for the carboxyl end-capped PBS in comparison with that of the control PBS or the hydroxyl end-capped PBS. This fact suggests that the carboxyl end-capping with bulky group efficiently retards the enzymatic reaction, while the hydroxyl end-capping has no effect. If no carboxyl group should be produced by the non-specific hydrolysis of PBS, the enzymatic hydrolysis could completely be terminated. However, even if the initial carboxyl content had been zero in the carboxyl end-capped PBS, the non-specific main chain hydrolysis gave the carboxyl terminals to receive the enzymatic hydrolysis. Within 3 days after the enzymatic hydrolysis the weight decrease of the carboxyl end-capped PBS became comparable with that of PBS and the hydroxyl end-capped PBS. No inhibition was therefore observed by the presence of the anthracenemethanal residue itself.

The preferential formation of 4HBS by the enzymatic hydrolysis and the retarded hydrolysis of the carboxyl-terminated PBS strongly suggests a hydrolysis mechanism by exe-type scission. Lipase PS® is known to catalyze the hydrolysis of tri- and diglycerides consisting of fatty acyl chains with a number of carbon atoms from 8 to 12.[14] Recent reports on the lipase PS® structure revealed that the enzyme has an ovoid cleft with an opening of 10 Å×25 Å and a depth of 15 Å and that the serine active site lies at the bottom of this cleft.[15-19] The substrate can bind in this cleft through hydrophobic interaction with the cleft wall to

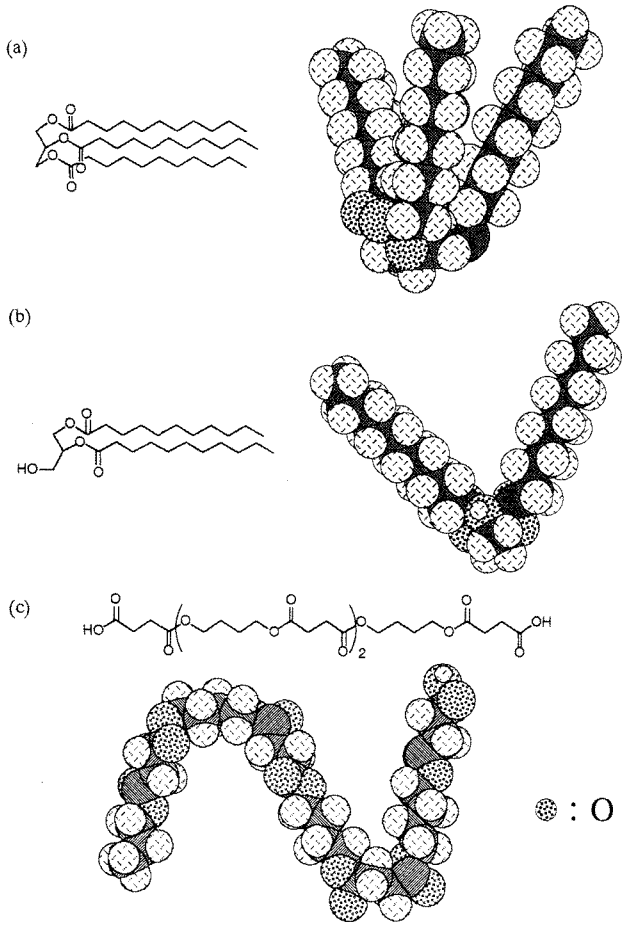
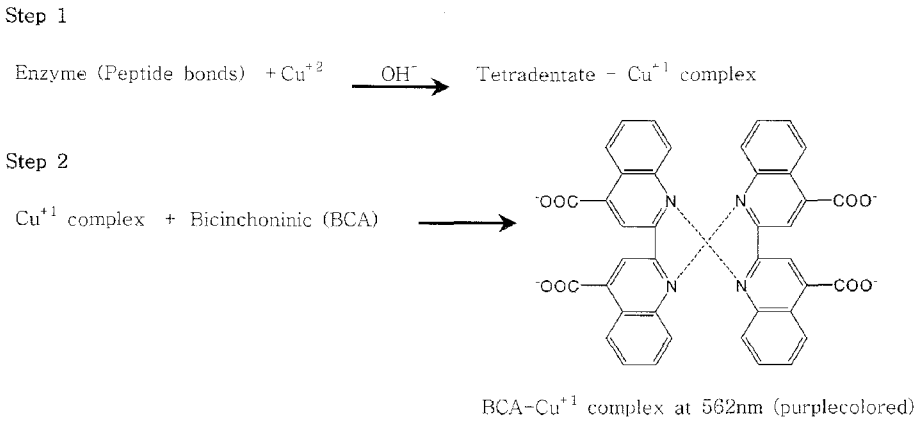


Figure 3. Possible molecular structures of typical substrates of lipase PS®: (a) tridecanoyl-glyceride, (b) didecanoyl-glyceride, and (c) a PBS chain at the carboxyl end.

receive hydrolysis. For fitting the substrate molecules into this cleft, the tri- and diglycerides should take an arrowhead conformation as shown in Figures 3(a) and (b), respectively. For the hydrolysis of PBS and its copolymers, the polymer chain should be incorporated in part into the active-site cleft. It has been known that PBS takes a highly extended helical chain conformation in the crystalline state. In the amorphous state, however, the PBS chains should bend to form complex coil conformations. For fitting the PBS in the enzyme active-site cleft, the polymer chain is rendered to take a bent structure shown in Figure 3(c) where every other succinate unit has a coil conformation. This bent conformation is very similar to the arrowhead conformation of diglycerides. One 4HBS unit has a chain length comparable to decanoic acid whose tri- and diglycerides can be good substrates of this lipase and is fully hydrophobic to bind in the active site cleft. If the enzymatic hydrolysis should occur from the carboxyl terminal, only one of the two succinate ester groups lying on the active site should receive hydrolysis preferentially to give 4HBS. That is the one near the carboxyl terminal. In the hydroxyl terminals, on the other hand, the hydrolyzable succinate unit is to be connected to either a short 4-hydroxybutyl chain or a long di(4-hydroxybutyl) succinate chain for which the fitting with the active-site becomes worse. There may be a possibility of hydrolysis by the so-called endo mechanism if the arrowhead conformation is allowed in loop on the surface. However, the probability of forming such conformation is not very high, particularly on the crystal-containing surface of PBS, and the exo hydrolysis from the carboxyl terminals is much faster than the endo hydrolysis from the middle chain.

Surface Adsorption of Enzyme. In the hydrolysis of insoluble solid polymers, surface adsorption of the enzyme is the primary advent of the reaction. However, little has been known about it. In the present study, we evaluated the amount of enzyme adsorbed on a PBSL film by detaching it from the surface and measuring the detached protein concentration by the BCA method (Scheme IV). Figure 4 shows typical changes in surface adsorption of lipase PS® with incubation time at various temperatures. The hydrophobic enzyme lipase PS® is adsorbed on the film surface very quickly probably through hydrophobic interaction. The adsorption reaches a plateau within a few minutes. Above 50 °C, where the enzymatic hydrolysis is very fast, the amount of adsorbed enzyme reaches 9 μg/cm^2 and slightly



Scheme IV. The outline of the BCA method.

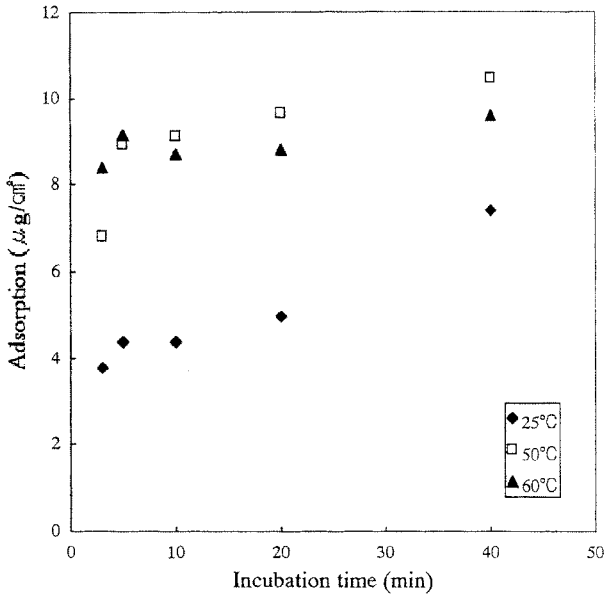


Figure 4. Surface adsorption of lipase PS® as a function of incubation time at different temperatures.

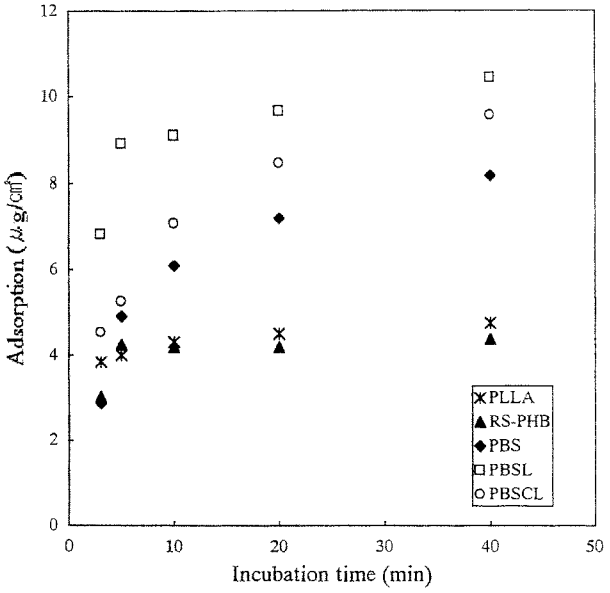


Figure 5. Surface adsorption of lipase PS® as a function of incubation time for different films.

increases thereafter. The maximum amount is a little higher at the enzyme optimum temperature of 50 °C, than at 60 °C. At 25 °C, where little polymer hydrolysis is induced, the amount of adsorbed enzyme is almost 4 μg/cm^2 at the initial plateau region, but gradually increases up to a level comparable to that observed above 50 °C. With the other polyester films such as PLLA and PHB, the amount of the adsorbed enzyme was found to be in a level of 4 μg/cm^2 as shown in Figure 5, which is similar to the initial plateau level observed at 25 °C. It is therefore considered that the enzyme is initially adsorbed on the polymer surface by non-specific manner in which the amount of adsorbed enzyme is in a level of 4 μg/cm^2. The enzyme adsorption is also possible by a specific mechanism, i.e., by the interlocking bond between the substrate and the enzyme active-site. The amount of enzyme adsorbed through the latter process corresponds to the difference between the plateaus levels of the adsorption at 50 and 25 °C, which is about 4 μg/cm^2. This quantity is much larger than expected if the enzyme is only specifically adsorbed on the terminal carboxyls in the polymer surface. There might be a secondary accumulation mechanism between the adsorbed enzyme and free enzyme, because a large quantity of enzyme was used for the present adsorption test. These enzymes may be adsorbed excessively on sample films. The adsorption behavior of the enzyme was slightly different among the three PBS derivatives (Figure 5). The maximum amount of the adsorbed enzyme was larger in the order of PBSL > PBSCL > PBS. This may correspondent to the increased order of surface concentration of carboxylterminals of PBS. The lactate units of PBSL should have high non-specific hydrolyzability to produce the PBS carboxylterminals in a fast rate. The caprolactate units in PBSCL should also be non-specifically hydrolyzed to increase the enzyme adsorption site, but in a slow rate. Although the comonomer ratio of PBSCL is higher than that of PBSL, the carboxylterminals by the hydrolysis of the comonomer units is slightly smaller in PBSL. The increase of the adsorption in the PBSCL case seems to still continue even after 40 min. The adsorption on PBS was the lowest because the carboxyl-terminals can not be additionally formed from the comonomer units.

Kinetic Analysis of the Enzymatic Degradation.[12,13] Figure 6 shows a relationship between the maximum

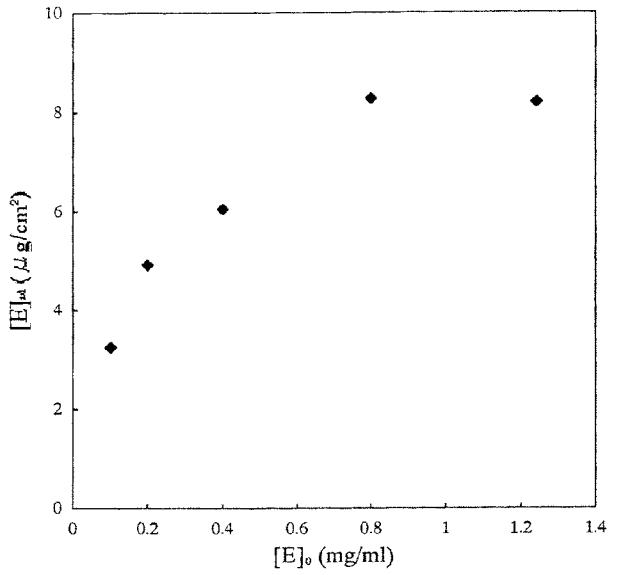


Figure 6. Amount of adsorbed enzyme$[E]_{ad}$ vs. concentration of lipase solution $[E]_0$ in the enzymatic hydrolysis of PBS.

amount of the adsorbed enzyme $[E]_{ad}$ and the enzyme concentration $[E]$ when a PBS film was incubated in a solution of lipase PS® at 50 °C for 40 min. This result supports the idea that the enzyme is adsorbed on the film surface by the Langmuir mechanism. The Langmuir adsorption of enzyme is generally expressed by eq. (1).[20]

$$[E]_{ad} = [E]_{max}\{K_e[E]/(1+K_e[E])\} \quad (1)$$

where $[E]_{max}$ and K_e are the maximum amount of enzyme adsorbed on the surface and the adsorption equilibrium constant of the enzyme, respectively, and $[E]$ is the enzyme concentration used for the incubation. Because the plots of reciprocal numbers eq. (1) gave a linear relation, the Langmuir-type adsorption was supported in the present case. From this result, we lead the following simple kinetic equation for the hydrolysis of the PBS film. As mentioned earlier, the enzymatic hydrolysis of PBS gives water-soluble product 4HBS as the main product. The formation of 4HBS could readily be monitored by the increase in UV absorption at 201 nm to obtain the time course of the degradation of PBS and PBS copolymers. The data were analyzed by the ordinary simple kinetic model, in which the enzyme is first adsorbed by the Langmuir-type mechanism, and the adsorbed enzyme hydrolyzes PBS to 4HBS by the exo mechanism. The whole reactions are shown in eq. (2),

$$E + S^* \xrightarrow{K} ES^* \xrightarrow{k'} P + E \quad (2)$$

where E, S^*, ES^*, and P denote enzyme, substrate, enzyme/substrate complex, and hydrolysis product, respectively, and K and k' represent the adsorption equilibrium constant and the hydrolysis rate constant of the respective reactions. Here, the total number of adsorption points per unit area of the film surface is represented by N_o, and the number of enzymes adsorbed per unit area by N.

The ratio of occupation of enzyme molecules (θ) is expressed by eq. (3),

$$\theta = N/N_o \quad (3)$$

The rate of the enzymatic hydrolysis (R) is depicted by eq. (4), and substitution of eqs. (3) to (4) gives (4′),

$$R = dP/dt = k'[ES^*] = k'N \quad (4)$$
$$= k'N_o\theta \quad (4')$$

The adsorption equilibrium is expressed as (5), which is converted to (5′) and (5″),

$$K = [ES^*]/[E][S^*] \quad (5)$$
$$= N/[E](N_o\text{-}N) \quad (5')$$
$$= \theta N_o/(1\text{-}\theta)N_o \quad (5'')$$

From (5″), the following eq. (6) is derived.

$$\theta = K[E]/(1 + K[E]) \quad (6)$$

$[E]$ is approximated to the initial enzyme concentration, if an excess amount of enzyme is used respective to N_0.

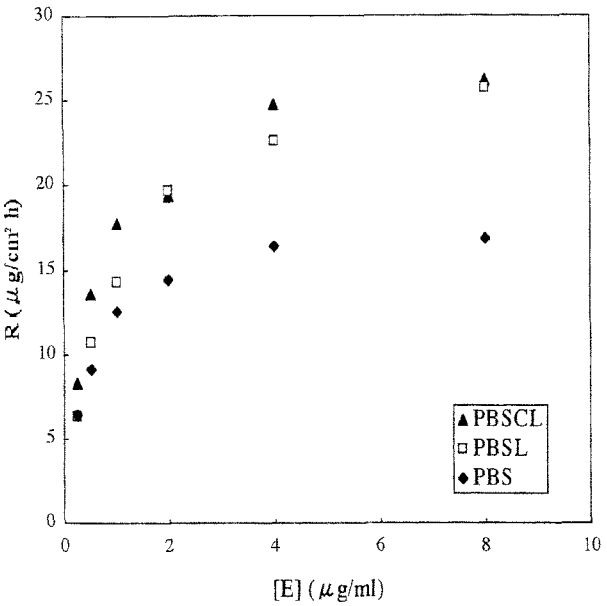


Figure 7. Effects of the concentration of lipase PS®[E] on the rate of 4HBS liberation (after 3 days).

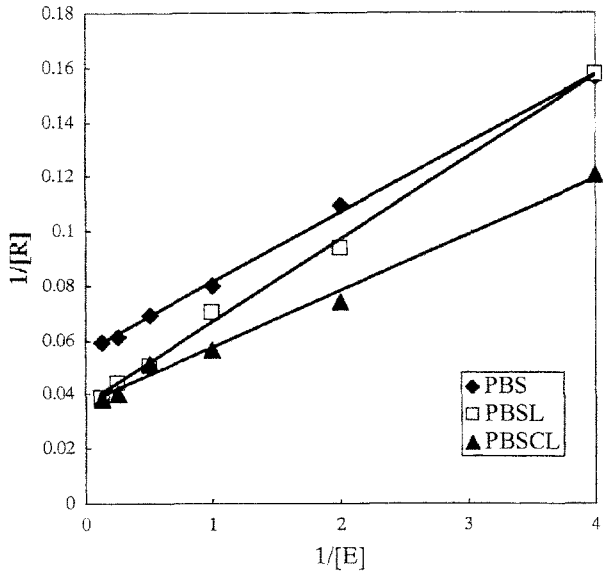


Figure 8. Linear plots of 1/[R] against 1/[E].

From eqs. (4) and (6), R is expressed by

$$R = k' N_o K[E]/(1+K[E]) \quad (7)$$

whose linear form is shown as follows,

$$1/R = (1/Kk)(1/[E]) + 1/k \quad (8)$$

where $k = k'N_o$.

From the linear plot according to eq. (8), both k and K can be obtained. k is related with the hydrolysis rate (k'), and K is the adsorption equilibrium constant. Figure 7 shows the plots of R as a function of $[E]$ for the three films. These

Table III. Reaction Constants K and k in the Enzymatic Degradation

Polymers	K(mL/μg)	k(μg/cm^2 h)
PBS	2.2	18
PBSL	1.2	28
PBSCL	1.8	28

plots can be transformed to the linear plots shown in Figure 8, when their reciprocal values are plotted. Table III summarizes the k and K values. The k values of PBSL and PBSCL are equal and much lower than that of PBS. Because the k value depends on the total number of adsorption points per unit area of the film surface (N_o) and k' is thought to be identical among the three samples, the difference of k may corresponds to that of N_o. N_o of PBS homopolymer is thought to be smaller than that of PBSL and PBSCL copolymers. The K value increases in the order of PBSL < PBSCL < PBS. The order is not compatible with the aforementioned order of the surface adsorption of the enzyme PBS < PBSCL < PBS. In the former experiment the incubation time was much short; 40 min instead of the 3 days in the latter. The difference in K may be affected by L-lactate and 6-hydroxycaproate units.

Conclusions

The enzymatic hydrolysis of PBS was shown to proceed by the surface etching mechanism to give 4HBS as the main product with traces of succinic acid and 1,4-butanediol. An exo-type hydrolysis mechanism was reasonably proposed. The terminal chain of PBS was exhibited to possess a conformational similarity to the ordinary tri- and diglycerides and to be incorporated in the active site of this lipase as the substrate. Much higher surface adsorption of the lipase was observed on the fllms of PBS and its copolymers than on the films of other polyesters. From the kinetic measurement, the total number of surface adsorption points per unit area of PBS homopolymer is smaller than that of PBSL and PBSCL copolymers.

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