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MINI-REVIEW

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Microbial degradation of polyurethane, polyester polyurethanes and polyether polyurethanes

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Abstract Polyurethane (PUR) is a polymer derived from the condensation of polyisocyanate and polyol and it is widely used as a base material in various industries. PUR, in particular, polyester PUR, is known to be vulnerable to microbial attack. Recently, environmental pollution by plastic wastes has become a serious issue and polyester PUR had attracted attention because of its biodegradability. There are many reports on the degradation of polyester PUR by microorganisms, especially by fungi. Microbial degradation of polyester PUR is thought to be mainly due to the hydrolysis of ester bonds by esterases. Recently, polyester-PUR-degrading enzymes have been purified and their characteristics reported. Among them, a solid-polyester-PUR-degrading enzyme (PUR esterase) derived from *Comamonas acidovorans* TB-35 had unique characteristics. This enzyme has a hydrophobic PUR-surface-binding domain and a catalytic domain, and the surface-binding domain was considered as being essential for PUR degradation. This hydrophobic surface-binding domain is also observed in other solid-polyester-degrading enzymes such as poly(hydroxyalkanoate) (PHA) depolymerases. There was no significant homology between the amino acid sequence of PUR esterase and that of PHA depolymerases, except in the hydrophobic surface-binding region. Thus, PUR esterase and PHA depolymerase are probably different in terms of their evolutionary origin and it is possible that PUR esterases come to be classified as a new solid-polyester-degrading enzyme family.

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Introduction

Polyurethane (PUR) is the general term used for a polymer derived from the condensation of polyisocyanates and polyols having intramolecular urethane bonds (carbamate ester bond,–NHCOO–) (Fig. 1). PUR has been in use since the 1940s and is now widely used as a base material in various industries. PUR can adopt various forms (from soft to hard) depending on the chemical structures of the polyisocyanates and polyols (functional group number or molecular mass), the raw materials of PUR. PUR can be used as a foam, elastomer, paint, adhesive, elastic fiber or artificial leather. Among these, the demand for foams is the greatest, their production amounting to approximately 2%–3% of the total amount of plastic produced. PUR foams are widely used as padding, heat insulation and structural materials.

Although, in general, synthetic plastics (e.g., polyethylene or polystyrene) are not biodegradable, PUR, in particular, polyester PUR, is known to be vulnerable to microbial attack (Morton and Surman 1994). For this reason, polyester PUR has not been considered as being very useful. But recently, environmental pollution by plastic wastes has become a serious issue and polyester PUR has attracted attention because of its biodegradability.

For the utilization of polyester PUR as biodegradable plastic, studies on the degrading microorganisms, degradation pathways, and the enzymes involved are thought to be important in addition to studies on the material chemistry of PUR. Information about PUR-degrading enzymes is helpful for the synthesis and evaluation of new biodegradable PUR, and an investigation of the genes involved (by the polymerase chain reaction) is required for the assessment of the distribution of indigenous PUR-degrading microorganisms in the disposal site. Unfortunately, studies on the microbial degradation of PUR have been performed from the viewpoint of materials engineering to prevent biodete-

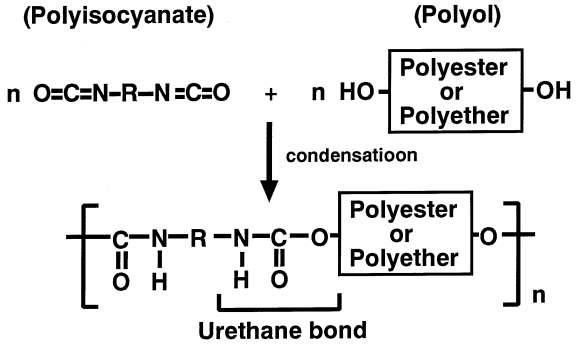

Fig. 1 Structure of polyurethane

rioration and not from that of the biochemical and physiological mechanisms involved in the degradation.

Recently, characteristics of a solid-polyester-PUR-degrading enzyme, the gene encoding this enzyme and their unique structures were reported (Akutsu et al. 1998; Nomura et al. 1998). This PUR-degrading enzyme possesses a special structure that enables it to degrade hydrophobic solid substrates and a similar structure is observed in other solid polyester-degrading enzymes.

Structure of polyurethane

The raw materials used in the synthesis of PUR are classified into polyisocyanates, polyols, catalysts, and auxiliary materials (Table 1). Some of the auxiliary materials used are chain-extension agents (e.g. short-chain diols), crosslinking agents (e.g. short-chain polyols with three or more hydroxyl groups) that react with isocyanate groups, addition agents for the PUR manufacture process and improvement agents (e.g. silicone compounds used as antifoams and aromatic esters as flexibilizers).

Table 1 Raw materials of polyurethane

Polyisocyanate	
	2,4-Tolylene diisocyanate (*2,4-TDI*)
	2,4-TDI/2,6-TDI (80/20 mixture)
	4-4′-Diphenylmethane diisocyanate
	1,3-Xylylene diisocyanate
	Hexamethylene diisocyanate
	1,5-Naphthalene diisocyanate
Polyol	
Polyester-type	
	Poly(butylene adipate)
	Poly(ethylene butylene adipate)
	Poly(ethylene adipate)
	Polycaprolactone
	Poly(propylene adipate)
	Poly(ethylene propylene adipate)
Polyether-type	
	Poly(oxytetramethylene) glycol
	Poly(oxypropylene) glycol
	Poly(oxypropylene)-poly(oxyethylene) glycol
Chain extension or crosslinking agent	
	1,4-Butanediol
	Ethylene glycol
	1,3-Butanediol
	2,2-Dimethyl-1,3-propanediol
	Trimethylolpropane
	Glycerol
	1,2,6-Hexanetriol

As polyols, polyether and polyester polyols are generally used. PUR synthesized from polyester polyol is termed polyester PUR, and that synthesized from polyether polyol is termed polyether PUR. Although most PUR used at present is polyether PUR, polyester PUR has recently become the focus of attention because of its biodegradability and therefore its advantages from the viewpoint of waste treatment.

Polyisocyanates include aromatic and aliphatic compounds. Among these, tolylene diisocyanate and diphenylmethane diisocyanate are the most commonly used. Since an isocyanate group generates carbon dioxide on reacting with water, foam-type PUR is synthesized by the addition of a small quantity of water during the synthetic process.

Microbial degradation of polyurethanes

It is well known that PUR is susceptible to microbial deterioration. There are many reports on the degradation of PUR by microorganisms, especially by fungi (Table 2). Unfortunately, most studies on the microbial degradation of PUR have been performed with the aim of preventing microbial hazards, and not much research has been conducted on the enzymes or biochemical mechanisms involved in PUR degradation by microorganisms. PUR differs from other macromolecules in that its physicochemical characteristics depend on the kinds of polyols and polyisocyanates used as raw materials for its synthesis. Therefore, it is difficult to compare the degradation activities of different PUR-degrading microorganisms, since the type of PUR selected to test biodegradation by each researcher is different.

The microbial degradation process can roughly be divided into the degradation of urethane bonds and the degradation of polyol segments, which are the major constituents of PUR, and PUR degradability is largely influenced by the chemical structure of the polyol segment (polyester type or polyether type).

Biodegradation of the urethane bond

Urethane bonds exist in both polyester and polyether PUR. It is well known that urethane compounds of low molecular mass can be hydrolyzed by some microorganisms and that the hydrolysis is catalyzed by an esterase (Matsumura et al. 1985a, b; Marty and Vouges 1987; Pohlenz et al. 1992; Owen et al. 1996; Ohshiro et al. 1997). On the other hand, little is known about the degradation of urethane bonds in PUR. Although there are some reports of urethane bonds being hydrolyzed

Table 2 Polyurethane (*PUR*)-degrading microorganisms. *PE* polyether PUR, *PS* polyester PUR

Microorganisms	PUR	Putative degrading enzymes	Reference
Fungi			
Aspergillus niger[b]	PS, PE	Unknown	Darby and Kaplan 1968
A. flavus[b]	PS, PE	Unknown	Darby and Kaplan 1968
A. fumigatus	PS	Esterase	Pathirana and Seal 1984a
A. versicolor[b]	PS, PE	Unknown	Darby and Kaplan 1968
Aureobasidium pullulans[b]	PS, PE	Unknown	Darby and Kaplan 1968
	PS	Unknown	Crabbe et al. 1994
Chaetomium globosum[b]	PS, PE	Unknown	Darby and Kaplan 1968
	PS	Esterase, protease, urease	Pathirana and Seal 1984a
Cladosporium sp.	PS	Unknown	Crabbe et al. 1994
Curvularia senegalensis	PS	Esterase[a]	Crabbe et al. 1994
Fusarium solani	PS	Unknown	Crabbe et al. 1994
Gliocladium roseum	PS	Esterase, protease, urease	Pathirana and Seal 1984a
Penicillium citrinum	PS	Esterase, protease, urease	Pathirana and Seal 1984a
P. funiculosum[b]	PS, PE	Unknown	Darby and Kaplan 1968
Trichoderma sp.[b]	PS, PE	Unknown	Darby and Kaplan 1968
Bacteria			
Comamonas acidovorans	PS	Esterase[a]	Nakajima-Kambe et al. 1991
Corynebacterium sp.	PS	Esterase	Kay et al. 1991, 1993
Enterobacter agglomerans	PS	Unknown	Kay et al. 1991
Serratia rubidaea	PS	Unknown	Kay et al. 1991
Pseudomonas aeruginosa	PS	Unknown	Kay et al. 1991
Staphylococcus epidermidis	PE	Unknown	Jansen et al. 1991

[a] Enzymes that have been purified
[b] All of these fungal strains were tested as a mixture

during its microbial degradation (Filip 1978; Jansen et al. 1991), it is still unclear whether urethane bonds in PUR are hydrolyzed directly or following breakdown into low-molecular-mass compounds.

Biodegradation of polyester polyurethane

Polyester PUR possesses many ester bonds that are vulnerable to hydrolysis. It is therefore thought that degradation of polyester PUR is mainly due to the hydrolysis of ester bonds. In regard to the biodegradation of polyester PUR, fungi are well-known effectors. In order to evaluate the microbial degradability of PUR, Darby et al. synthesized about 100 kinds of PUR and tested their degradability by seven kinds of fungi (*Aspergillus niger*, *A. flavus*, *A. versicolor*, *Penicillium funiculosum*, *Aureobasidium pullulans*, *Trichoderma* sp., and *Chaetomium globosum*) (Darby and Kaplan 1968). This was the first systematic study on PUR degradation by microorganisms. They reported that PUR is resistant to degradation when a side-chain is located in its polyester polyol segment and that degradation is also affected by the specific isocyanate used in the synthesis of PUR. Pathirana et al. also screened certain polyester-PUR-degrading fungi (*Gliocladium roseum*, *Chaetomium globosum*, *Penicillium citrinum*, *Aspergillus fumigatus*) (Pathirana and Seal 1984a, b, 1985a, b). Crabbe et al. selected *Fusarium solani*, *Curvularia senegalensis*, *Aureobasidium pullulans*, and *Cladosporium* sp. for the degradation of a colloidal type of polyester PUR (Crabbe et al. 1994). PUR degradation by these fungi requires the addition of several organic nutrients (e.g., gelatin). At present, there are no reports on PUR-degrading fungi that can utilize PUR as the sole carbon source.

Although there are relatively few reports on PUR-degrading bacteria, both gram-positive and gram-negative bacteria have been reported as PUR degraders. Kay et al. isolated 15 kinds of bacteria from polyester PUR pieces following their burial in soil for 28 days. Among these, *Corynebacterium* sp.B6, B12, and *Enterobacter agglomerans* B7 showed the highest PUR-degradation activities and thus were selected for further study (Kay et al. 1991). Following a 2-week cultivation, weight loss and a decrease in the tensile strength of the tested PUR were observed. PUR degradation by these bacteria required the addition of supplementary organic nutrients such as yeast extract, and the organisms could not utilize PUR as the sole carbon source. Kay et al. also analyzed the PUR breakdown products by FT-IR using *Corynebacterium* sp., and reported that PUR degradation was caused mainly by the hydrolysis of ester bonds. They concluded that PUR was not being utilized as a nutrient but was degraded as a result of co-metabolism (Kay et al. 1993).

Comamonas acidovorans strain TB-35, isolated by Nakajima-Kambe et al., is a gram-negative bacterium that has been reported to be capable of utilizing solid polyester PUR as the sole carbon source (Nakajima-Kambe et al. 1995). These researchers also investigated the metabolites produced by the degradation of PUR by strain TB-35, using GC-MS analysis, and concluded that the products were derived from the polyester segment of the PUR as a result of the hydrolytic cleavage of ester bonds (Nakajima-Kambe et al. 1997).

Biodegradation of polyether polyurethane

Although polyester PUR is relatively easily degraded by microorganisms, polyether PUR is relatively resistant to the microbial attack. Darby and Kaplan (1968) synthesized various kinds of polyether PUR in addition to polyester PUR, and examined their degradability. They reported that polyether PUR was scarcely susceptible to microbial degradation. Filip reported similar results from soil bacteria (Filip 1978). Jansen et al. reported that some kinds of polyether PUR (Biomer and Tuftane) were degraded by *Staphylococcus epidermidis* strain KH11 (Jansen et al. 1991) but that degradation by this strain progressed very slowly. The relative resistance of polyether PUR to microbial degradation is considered to be due to its degradation mechanism, which involves exo-type depolymerization (Kawai et al. 1978, 1985), whereas that of polyester PUR degradation involves endo-type depolymerization.

Polyester-polyurethane-degrading enzymes

Since there are few reports on microorganisms capable of degrading polyether PUR, little is known about the degradation enzymes or the genes involved, and much of what is known pertains to only polyester-PUR degraders.

In regard to polyester PUR degradation, it is thought that ester bonds in the polyester segment of PUR are hydrolyzed, because aliphatic polyesters such as poly(ethylene glycol adipate) or poly(caprolactone), which are used as raw materials for the synthesis of PUR, are known to be easily degraded by microorganisms or esterolytic enzymes such as lipase (Tokiwa and Suzuki 1974, 1977a, b; Tokiwa et al. 1976). The PUR synthesized using high-molecular-mass polyesters is degraded more easily than that synthesized using low-molecular-mass polyesters (Darby and Kaplan 1968).

As shown in Table 2, Pathirana et al. reported that some polyester-PUR-degrading fungi produce extracellular esterases, proteases or ureases in the presence of PUR (Pathirana and Seal 1984b, 1985a) but the relationship between these enzymes and PUR degradation is unclear. Crabbe et al. partially purified an extracellular-esterase-like enzyme produced by the colloidal-PUR-degrading fungus, *Curvularia senegalensis*, by ammonium sulfate precipitation, and observed the halo formed on a colloidal PUR plate by this enzyme (Crabbe et al. 1994). This enzyme had a molecular mass of 28 kDa and was stable even on heating at 100 °C for 10 min (Table 3). The enzyme was inhibited by phenylmethylsulfonyl fluoride, which is a specific inhibitor of the active serine residue of serine proteases and lipases.

With regard to bacteria, Kay et al. determined an esterase activity in the culture supernatant supplemented with yeast extract and PUR, of *Corynebacterium* sp. (Kay et al. 1993). This enzyme is thought to be responsible for PUR degradation, because degradation of PUR by this strain mainly depends on the hydrolysis of ester bonds. Nakajima-Kambe et al. also examined the relationship between PUR degradation and the secretion of extracellular esterase in *Comamonas acidovorans* TB-35 (Nakajima-Kambe et al. 1997). Strain TB-35 produced two kinds of esterase, one secreted into the culture broth and the other bound to the cell surface. Of the two, only the cell-surface-bound esterase catalyzes the degradation of polyester PUR. The extracellular esterase was considered to be bound to the cell surface by hydrophobic bonds and not secreted into the culture broth because of its high hydrophobicity.

The physicochemical properties of this enzyme were markedly different from those of the colloidal-PUR-degrading enzymes described above (Table 3). This dissimilarity seemed to depend on the differences in the type of PUR substrate (solid or colloidal), in addition to the type of organism (bacterium or fungus).

Characteristics of the solid polyester-PUR-degrading enzyme of *C. acidovorans* TB-35

Enzymatic reactions against water-soluble substrates proceed rapidly because enzyme molecules can easily come in contact with the substrates. On the other hand,

Table 3 Properties of purified polyester-PUR-degrading enzymes

Property	Source	
	Curvularia senegalensis	*Comamonas acidovorans*
Substrate PUR	Polyester PUR (colloidal)	Polyester PUR (solid)
Localization	Extracellular (culure-broth-secreted)	Extracellular (cell-surface-bound)
Molecular mass (Da)	28000 (monomer)	62000[a] (monomer)
pH optimum	Not determined	6.5
Temperature optimum	Not determined	45 °C
Thermal stability	100 °C, 10 min	55 °C, 30 min
Isoelectric point	5.1	5.9[b]
Reference	Crabbe et al. 1994	Akutsu et al. 1998

[a] Estimated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis
[b] Calculated from its amino acid sequence

solid substrates such as PUR are thought to have extremely inefficient contact with enzyme molecules. In order to overcome this problem, enzymes that degrade solid substrates are considered to possess some characteristics that enable them to be adsorbed onto the surfaces of the solid substrates (van Tilbeurgh et al. 1986; Fukui et al. 1988; Hansen 1992).

Akutsu et al. purified the polyester-PUR-degrading enzyme, PUR esterase, derived from *C. acidovorans* TB-35, and reported its unique characteristics (Akutsu et al. 1998). This enzyme reacted with solid polyester PUR to hydrolyze the ester bonds of PUR. PUR degradation by the PUR esterase was strongly inhibited by the addition of 0.04% deoxy-BIGCHAP, a kind of surfactant. On the other hand, deoxy-BIGCHAP did not inhibit the activity when *p*-nitrophenyl acetate, a water-soluble compound, was used as a substrate. These observations indicated that this enzyme degrades PUR in a two-step reaction: hydrophobic adsorption onto the PUR surface followed by hydrolysis of the ester bonds of PUR. Thus, the PUR esterase was considered as having a hydrophobic-PUR-surface-binding domain and a catalytic domain, and the surface-binding domain was considered to be essential for PUR degradation.

This unique structure observed in PUR esterase has also been reported in poly(hydroxyalkanoate) (PHA) depolymerase, which degrades PHA, a kind of solid polyester of natural origin. In PHA depolymerase, the existence of a hydrophobic PHA-binding domain has already been determined by the analysis of its amino acid sequence and its various physicochemical and biological characteristics (Fukui et al. 1988; Behrends et al. 1996; Shinomiya et al. 1997). Mukai et al. proposed a two-step kinetic model for PHA degradation (Mukai et al. 1993). PUR esterase and PHA depolymerase are similar in that both enzymes have a hydrophobic surface-binding domain in addition to their catalytic domain, but their three-dimensional structures are considered to be different. The PHA-binding domain in most PHA depolymerases is located at the C terminus of the polypeptide chain, and the active and surface-binding domains are linked by a flexible linker, which is a threonine-rich region (Jendrossek et al. 1996), or a module resembling fibronectin type III (Hansen 1992). Owing to this structure, the degradation activity of PHA depolymerase is decreased in the presence of an excess of the enzyme (Fig. 2A).

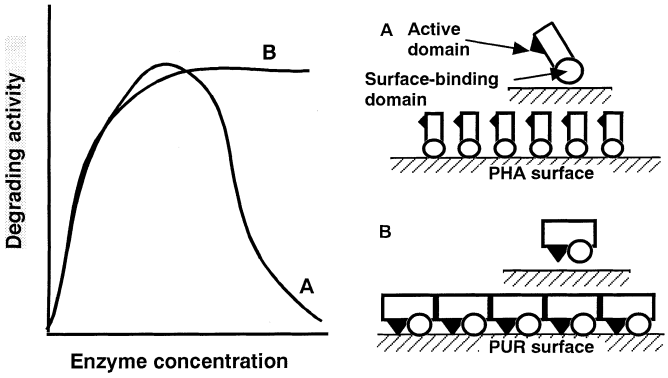

Fig. 2A, B Effects of poly(hydroxyalkanoate) (*PHA*) depolymerase and polyurethane (*PUR*) esterase concentrations on the degradation of solid substrates, and kinetic models of these enzymes. **A** PHA depolymerase; **B** PUR esterase

On the other hand, in the case of PUR esterase, the degradation activity did not decrease but rather remained constant when an excess of the enzyme was present (Fig. 2B). On the basis of this observation it was inferred that the surface-binding site and the catalytic site of the PUR esterase existed in three-dimensionally close positions, unlike those in PHA depolymerase. In this model, the catalytic domain can gain access to the PUR surface even if the PUR surface is saturated with enzyme molecules. However, since the number of adsorbable enzyme molecules per unit surface area of the PUR is fixed, the PUR-degradation activity also remains constant.

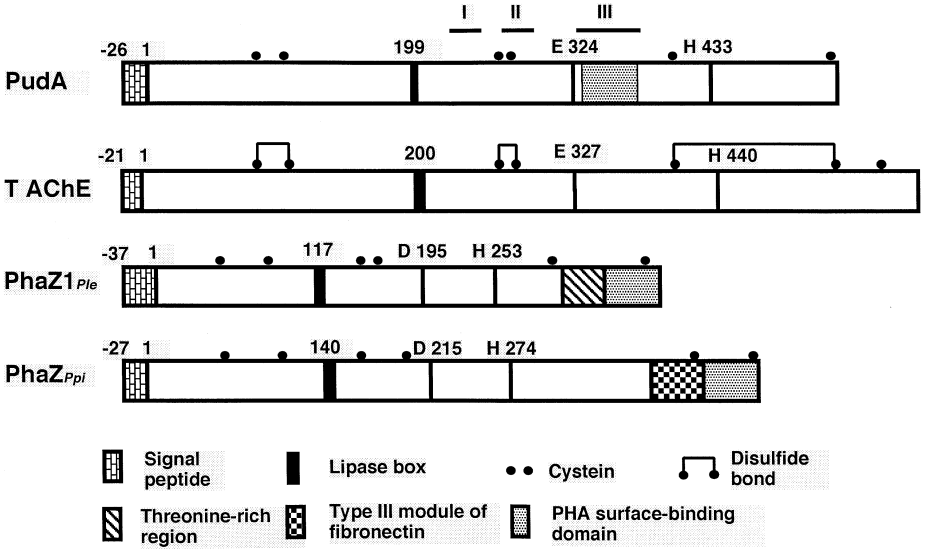

Fig. 3 Comparison of the domain structures. *Comamonas acidovorans* PUR esterase (*PudA*), *Torpedo californica* acetylcholinesterase (*T AChE*) (Sussuman et al. 1991), and PHA depolymerase ($PhaZ_{ple}$ and $PhaZ_{ppi}$) (Jendrossek et al. 1995a, b)

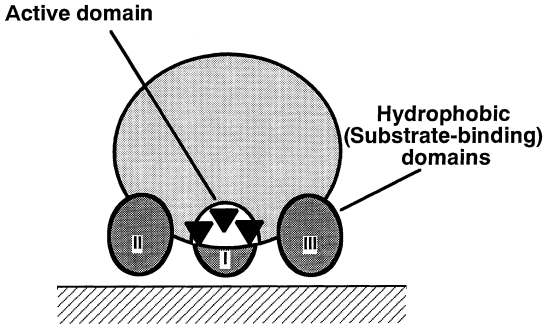

Fig. 4 Putative three-dimensional structure of PUR esterase

Characteristics of the PUR esterase gene

More recently, Nomura et al. cloned the structural gene of PUR esterase, *pudA*, from *C. acidovorans* TB-35 (Nomura et al. 1998). The open reading frame consists of 1644 bp with a putative ATG initiation codon, and encodes a 548-amino-acid enzyme. The amino acid sequence of this enzyme shows about 30% homology to that of the acetylcholinesterase (AChE) of *Torpedo californica* (Schumacher et al. 1986). In addition, a comparison of the secondary-structure motifs of the two enzymes revealed a strong conformational resemblance between PUR esterase and *Torpedo* AChE. *Torpedo* AChE is one of a newly recognized kind of esterases possessing a catalytic triad consisting of Ser, Glu and His, with the glutamate residue replacing the usual aspartate residue (Cygler et al. 1993; Zock et al. 1994; Brenner 1988). Since the locations of the putative catalytic triad and the primary structures near the active center of the PUR esterase closely resemble those in *Torpedo* AChE, PUR esterase is probably a type of Ser-Glu-His esterase (Fig. 3).

There are three regions containing many hydrophobic amino acid residues in the PUR esterase; corresponding hydrophobic regions do not exist in *Torpedo* AChE. Figure 4 shows the three-dimensional structure of PUR esterase as predicted from that of *Torpedo* AChE established by X-ray crystallography (Sussuman et al. 1991). It is estimated that these three hydrophobic regions surround the active center. This predicted three-dimensional structure is consistent with the biochemical characterization of this enzyme with respect to catalytic activity not being inhibited by its presence in excess. On the basis of this observation, it is inferred that these hydrophobic regions play the role of at least one of the binding domains of PUR esterase.

In addition, one of these hydrophobic regions exhibited significant homology to the substrate-binding domain of PHA depolymerase (Jendrossek et al. 1995a). On the other hand, no significant homology between PUR esterase and PHA depolymerase, except in this hydrophobic region, was observed (Fig. 3). Thus, PUR esterase and PHA depolymerase are probably different in terms of their evolutionary origin. Since PUR is a chemical product that does not exist in nature, and PHA is a natural product produced in microorganisms, it is not surprising that these degrading enzymes are different in terms of their evolutionary origin. Furthermore, it is of interest that both enzymes have a special structure facilitating their adsorption onto the substrate surface and that significant sequence homology is observed only in this region. It is possible that new solid-polyester-degrading enzymes can be created by adding these binding domains to esterases that cannot degrade solid substrates.

It is evident that the PUR-degradation enzyme of *C. acidovorans* TB-35 is a novel enzyme different from any of the polyester-degrading enzymes reported to date. Although studies on the microbial degradation of PUR have been conducted for a long time (Morton and Surman 1994), few report on the enzymes or genes involved in the degradation. It is possible that enzymes of this group will come to be classified as a new solid-polyester-degrading enzyme family, in addition to the PHA depolymerase family, in the near future when additional PUR-degrading enzymes are purified and characterized, and their genes cloned.

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