Chapter 16
Microbial Degradation of Plastics and Water-Soluble Polymers

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16.1 Introduction

Polymer chemistry began approximately 90 years ago, when Staudinger established the theoretical background from which commercial production of synthetic polymers arose. Synthetic polymers, especially solid ones known as plastics, have been at the forefront since World War II. Annual worldwide production of plastics amounts to more than 200 million tons. Synthetic polymers were originally designed to replace natural polymers, with the advantages of long life (no decay), better performance, plasticity of form, and low cost of production, dependent on cheap petroleum. However, public concern over the use of synthetic polymers has been increasing since the end of 1980s as plastic bags have been polluting the environment. Plastic bags can be found everywhere from the deep sea to the highest mountains and can cause serious environmental problems by threatening wildlife and destroying scenic areas.

Synthetic polymers include all kinds of polymerized compounds, including water-soluble, oily, and solid polymers. Plastics are solid synthetic polymers of various forms. They are not water-soluble or miscible. Since plastics are highly visible, their fate in the environment and their recyclability cause public concern. On the other hand, water-soluble synthetic polymers are often neglected because they are invisible, although their total production corresponds to that of plastics. Environmental problems first attracted worldwide public concern at the end of the 1980s, and studies of biodegradation and the production of biodegradable synthetic polymers were promoted in the 1990s to establish standards for biodegradation and biodegradable synthetic polymers. A number of previous studies have shown that

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biodegradability is determined by the chemical structures and physico-chemical properties of polymers and not by their origins, whether natural or artificial. The biodegradability of many xenobiotic polymers has been verified, as described below. More recently, a paradigm shift from oil-based production to bio-based production has caused concern and attracted increasing interest in this area, with greater focus on bio-based production of existing monomers presently produced from oil to form polyolefins, polyesters and polyamides. At the same time, more light has been shed on microbial polyesters [poly(hydroxyalkanoate) (PHA)] and chemobiopolyesters [poly(lactic acid) (PLA)]. In the near future, significant ranges of xenobiotic polymers might be replaced with microbial polyesters and chemobiopolymers, which include not only PLA, but also other polymers from bio-based monomers, using environmentally friendly technology.

The biodegradability of xenobiotic polymers is highly dependent on their chemical structures and physical properties. The chemical structures of the major polymers are shown in Fig. 16.1. Most carbon-backbone polymers, barring polyvinyl alcohol (PVA), are generally recalcitrant to biodegradation, but many hetero-backbone polymers, such as polyethers, polyesters, and polyamides are biodegradable. Based on existing information on the degradability of synthetic polymers, copolymers consisting of different types of polymer chains or monomers have been designed, some of which are industrially produced as biodegradable polymers.

16.2 Microbial Degradation of Xenobiotic Polymers

What does "biodegradability of polymers" mean? The first criterion for biodegradation is enzymatic processing. In particular, hydrolyzable polymers such as polyesters and polyamides are enzymatically degraded into monomers (depolymerization process) that can easily enter central metabolic processes unless they are xenobiotic compounds. Most monomers are naturally occurring compounds, such as organic acids, alcohols/glycols, and amide compounds. Proteases, lipases, and esterases originating from animals, plants, and microbes can hydrolyze xenobiotic polymers such as polyamides and polyesters, and PLA, although their original substrates are proteinaceous compounds, lipids, and esters. This is well explained by the fact that enzymes cannot discriminate between original substrates and substrates analogous to them. For example, proteinase K can degrade PLA because lactic acid is analogous to alanine. The second and most important criterion for biodegradation is microbial degradation of target polymers, which are often assimilated by microorganisms as sole carbon and energy sources.

The biodegradability of many polymers has been investigated. The results are summarized in Table 16.1 (Kawai 2010b). Previous studies have revealed that depolymerization proceeds by two processes, exogenous and endogenous. Hydrolyzable polymers are readily depolymerized endogenously by hydrolytic enzymes to yield momoner units that are metabolized by central metabolic

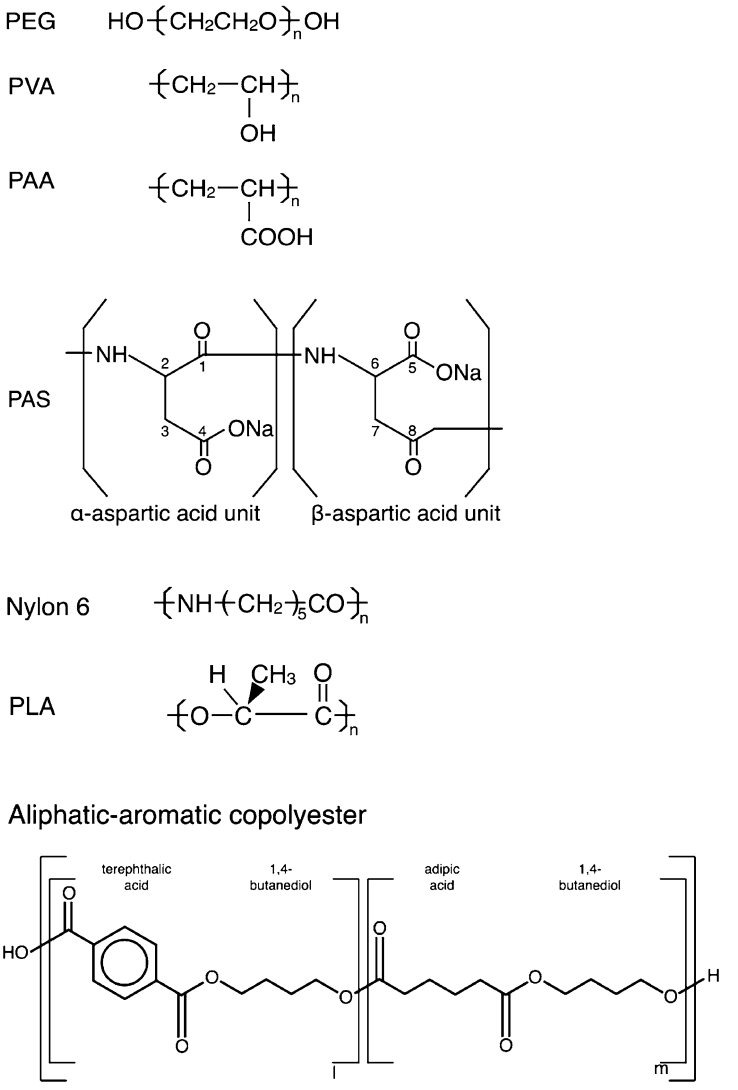

Fig. 16.1 Chemical structures of xenobiotic polymers discussed in this article

pathways. Generally, esterases/lipases contribute to the hydrolysis of synthetic polyesters. PHA- and PLA depolymerases are also categorized as polyester-degrading enzymes, but their enantioselectivities and substrate specificities indicate that there are three categories of polyester-degrading enzymes, general lipases, PHA depolymerases, and PLA-degrading enzymes (Tokiwa and Calabia 2004), although some cross these boundaries. PHA- and PLA-degrading microorganisms have different specific depolymerases for PHA and PLA, respectively.

Table 16.1 Biodegradability of xenobiotic polymers and oligomers by enzymes and microorganisms

Chemical structure	Biodegradable molecular size	Degradation mechanism	Relevant enzymes/ microorganism
A. Polyethers			
Polyethylene glycol (PEG)	MW: 20,000 (MW: 40,000)	Exogenous	Dehydrogenases; aerobic bacteria (anaerobic bacteria)
Polypropylene glycol (PPG)	MW: 3,000–4,000	Exogenous	Dehydrogenase: bacteria
Polytetramethylene glycol (PTMG)	Dimer–octamer	Exogenous	Dehydrogenase: bacteria
Polyputylene oxide (PBO)	MW: 2,000	Exogenous	Dehydrogenase; bacteria
B. Vinyl polymers			
PEwax	MW: 3,000	Exogenous	Bacteria and fungi
Polyvinyl alcohol (PVA)	MW: ca.100,000	Endogenous	Oxidase/dehydrogenase and hydrolase: bacteria and fungi
Polyacrylate (PAA)	MW: 4,500	Exogenous	Bacteria
Polyisoprene	MW: 1,000		Bacteria
Butadiene	Tetramer–decamer		Baceria
Styrene	Dimer		Bacteria
Acrylonitril	Trimer		Fungus
C. Polyamides			
Polyasparatate (PAS)	MW: ca. 20,000	Endogenous/exogenous	Hydrolases; bacteria
Nylon	Dimer–hexamer	Endogenous/exogenous	Hydrolases; bacteria
D. Polyesters			
Polycaprolactone (PCL)		Endogenous	Lipases and cutinases: bacteria and fungi
Polylactic acid (PLA)	Up to a few hundreds of thousands	Endogenous	Proteases and lipases (cutinases): actinomycetes and *Bacillus*

(continued)

Table 16.1 (continued)

Chemical structure	Biodegradable molecular size	Degradation mechanism	Relevant enzymes/ microorganism
Aliphatic		Endogenous	Lipases and cutianases: bacteria and fungi
Aromatic		Endogenous	Cutinases
Aliphatic-*co*- aromatic		Endogenous	Hydrolase; actinomycetes and *Bacillus*-related species
E. Polyurethane			
Ether type		?	?
Ester type		Endogenous	Lipases: bacteria and fungi

Thus the biodegradability of a given polymer is not uniform, and its evaluation requires substantial insight, even if the basic chemical structures and underlying mechanisms are the same.

On the other hand, some synthetic polymers are oxidized repeatedly and are cleaved exogenously by one terminal monomer unit that is assimilated as a carbon source into the central metabolic pathway. Typical examples of exogenous depolymerization are the oxidative degradation of polyethers, PAA, and probably polyolefins by a series of oxidative steps for polyethers, and β-oxidation-like processes for PAA and polyolefins. PVA is degraded by a combination of primary oxidation and hydrolysis of oxidized PVA, as described below. Except for PVA, high molecular weight carbon-backbone polymers are recalcitrant to biodegradation, although their oligomers, including polyethylene wax (PEwax), isoprene oligomers, styrene oligomers, and triacrylonitrile, are biodegradable (Kawai 1995). Hetero-backbone polymers are more susceptible to biodegradation than carbon-backbone ones. Examples of the former are polyethers, such as PEG, most polyesters, including PHA and PLA, and polyamides, such as PAS (polyaspartate).

16.3 Microbial Degradation of Synthetic Water-Soluble Polymers

The scale of the industrial production of synthetic water-soluble polymers is approximately the same as that of plastics. Examples of synthetic water-soluble polymers are polyethers, PVA, and PAA. Polyethers and PVA are often used in synthesizing copolymers to be used as biodegradable segments or to improve performance. Although these polymers are believed to be nontoxic to organisms, they have strong surface activity and produce large amounts of foam, and thus inhibit oxygen recovery in water. This poses a serious threat to water-borne organisms as well as to humans. Water-soluble polymers can be neither recycled nor incinerated after use, and eventually enter streams. Therefore, microbial degradation of these polymers is of great importance worldwide, since it is the only means to remove them from water systems.

16.3.1 Polyethers

Poly(alkylene glycol)s have a common structural formula: $HO[R–O]_nH$ [R=CH_2CH_2 for PEG, CH_3CHCH_2 for polypropylene glycol (PPG), a polymer of 1,2-propylene oxide, $(CH_2)_4$ for polytetramethylene glycol (PTMG), and $C_2H_5(CHCH_2)$ for polybutylene oxide (PBO), a polymer of 1,2-butylene oxide], where n represents the average range of units. The physical properties of PEGs vary from viscous liquids to waxy solids based on their molecular sizes, although

every PEG from oligomers up to polymers with a molecular weight (MW) of a few million is completely water-soluble. Commercially available PPG can be divided into two groups, the diol and triol types, based on the straight or branched chain structure of the polymer. The water solubility of PPGs is lost when the MW is increased to more than approximately 700 (triol type) and 1,000 (diol type) due to the inclusion of a methyl group in each monomer unit. Therefore, copolymers of PEG and PPG are used as detergents, where PEG is a hydrophilic constituent and PPG a hydrophobic one. Another copolymer is also used as a water-soluble flame-resisting pressure liquid, where ethylene oxide and propylene oxide are randomly copolymerized. PBO is an oily polymer due to its pendant ethyl groups. In general, PTMG is a waxy substance, from which water-soluble oligomers have been removed as impurities. PEG was the first member of the polyether group to be manufactured in large quantities and to be used as a commodity chemical in various industrial fields. The most common hydrophilic moieties in the nonionic surfactants are ethylene oxide polymers. The majority of PEGs produced are used in the production of nonionic surfactants, very important groups of industrial products with applications from domestic detergents to agrochemicals, food emulsifiers and other industrial preparations. These products ultimately constitute a significant burden on domestic and industrial wastewater systems. Therefore, their biodegradability characteristics have been observed over the past 50 years, which were reviewed by Kawai (1987, 2002, 2010b). Because of low toxicity and skin irritation, PEGs are widely used in the pharmaceutical industry in the preparation of ointments, suppositories, tablets, and solvents for injection, and also for the preparation of cosmetics, such as creams, lotions, powders, cakes, and lipstick. They are also used as intermediates in the production of resins, such as alkyd resin and polyurethane resin, and as components in the manufacture of lubricants, antifreeze agents, wetting agents, printing inks, adhesives, shoe polish, softening agents, sizing agents, and plasticizers. Furthermore, this material has been used in making resin gels to immobilize enzymes or microbial cells and in the chemical modification of enzymes. Although PEGs appear to be metabolically inert and nontoxic, they are sulfated in vitro by the rat and guinea pig liver (Roy et al. 1987), and repeated topical application of a PEG-based antimicrobial cream to open wounds in rabbits and burn patients has been found to cause a syndrome related to the metabolism of PEGs to various compounds, including mono- and diacids (Herald et al. 1989). Furthermore, the possibility, that PEG 400 and PEG oligomers are toxic, has also been suggested (Gordienko and Kudokotseva 1980). Biodegradation of PEG might pose an additional risk due to metabolite production. Chemically unsubstituted PPG is used in solvents for drugs and in paints, lubricants, inks, and cosmetics, but is mostly transformed to polyurethanes or surface-active agents. PBO is an oily material used in sizing agents, cleaning agents, and dispersants. PTMG is used exclusively as a constituent of polyurethane.

PEGs with different MWs have been produced and have been used in industrial and domestic applications for more than 60 years. Some of them are included as non-toxic and biodegradable segments of copolymers, and their larger parts are transformed into neutral detergents and liberated into streams after use.

Various types of PEG-degraders that are able to assimilate a variety of molecular sizes have been isolated since the first report of PEG 400 by Payne (1963). Although PEGs with MW higher than 1,000 were long considered to be bioresistant, those up to 20,000 or more have since been found to be biodegradable. PEGs with a high MW, from 4,000 to 20,000, are assimilated by a limited number of species: *Pseudomonas aeruginosa* (up to 20,000) (Haines and Alexander 1975), soil bacteria (up to 6,000) (Hosoya et al. 1978), *Pseudomonas stutzeri* (up to 13,500) (Obradors and Aguilar 1975), and *Sphingomonas* species (up to 20,000); the strains were originally identified as *Flavobacterium* species (Ogata et al. 1975). Sphingomonads include sphingolipids in their outer membranes instead of the lipopolysaccharides found in most Gram-negative bacteria. Various lipophilic xenobiotic-assimilating bacteria are included in this genus (Kawai 1999). Most recently, a Gram-positive actinomycete, *Pseudonocardia* sp. strain K1, originally isolated as a tetrahydrofuran degrader, was also found to grow on PEG 4,000 and 8,000 (Kohlweyer et al. 2000).

We have isolated various PEG-utilizing bacteria with various degradabilities towards PEG 400–20,000 (Ogata et al. 1975). Isolates able to degrade PEG 4,000 and 20,000 were identified as Sphingomonads, and based on the newest taxonomy, they have been renamed and designated type species of *Sphingopyxis macrogoltabida and Sphingopyxis terrae*, respectively (Takeuchi et al. 2001). Interestingly, *S. terrae* can grow on PEG as a symbiotic mixed culture with a concomitant associate (Kawai and Yamanaka 1986; Kawai 1996). Another focus of PEG degradation studies is the biochemical mechanism of degradation. Several reports have suggested different mechanisms (Kawai 2002), but the most probable metabolic pathway is an exogenous metabolic one based on repeated oxidation steps. PEG is oxidized by alcohol dehydrogenases linked with a dye or NAD. PEG-dehydrogenases (PEG-DHs) from PEG-utilizing Sphingomonads have been cloned and characterized as FAD-including alcohol dehydrogenases (Sugimoto et al. 2001; Ohta et al. 2006). PEG-aldehyde dehydrogenase was cloned from PEG-utilizing Sphingomonads and characterized as a NADP-containing nicotinoprotein PEG-aldehyde dehydrogenase (Ohta et al. 2005), the first report of a nicotinoprotein aldehyde dehydrogenase. The ether bond-splitting enzyme involved in the PEG metabolism was perhaps a glycolic acid oxidase or glycolic acid dehydrogenase active on carboxylated PEG (Yamanaka and Kawai 1991; Enokibra and Kawai 1997). All the metabolic enzymes included in PEG degradation have been localized in the membrane and are thought to work in the periplasm, in accordance with the fact that PEG and its metabolites were detected in the periplasmic fraction (unpublished data), suggesting that PEG is taken up into the periplasm and metabolized there. We cloned the genes involved in PEG degradation, and found that the *peg* operon consisted of five genes and was expressed by PEG through induction of an *araC*-type regulator (Charoenpanich et al. 2006; Tani et al. 2007, 2008), as shown in Fig. 16.2. This was the first report on the regulation of degradative genes by a macromolecule. Two genes coding PEG-DH and PEG-aldehyde dehydrogenase are involved in the *peg* operon. The role of other genes in the *peg* operon was suggested with regards to the PEG

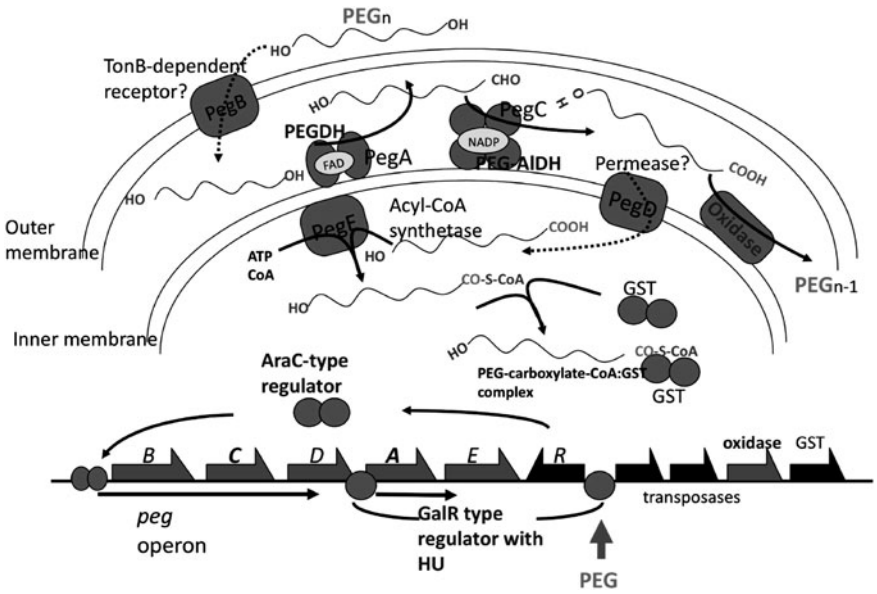

Fig. 16.2 The operonic structure of genes involved in PEG degradation and its regulation by PEG

metabolism, as summarized in Fig. 16.2 (Tani et al. 2007). A gene coding PEG-carboxylate dehydrogenase was detected in the downstream region of the *peg* operon, and was found to act as an ether bond-splitting enzyme (Somyoonsap et al. 2008). Anaerobic biodegradation of PEG has been well investigated as compared to other polymers. Schink's group reported that higher MW PEG (up to PEG 40,000) is degraded by anaerobes rather than by aerobes (Strass and Schink 1986; Schink and Stieb 1983). They proposed an anaerobic metabolic route for PEG, and suggested that acetaldehyde is produced from PEG by a diol dehydrorase-like enzyme (PEG acetaldehyde lyase), but they failed to purify the enzyme (Strass and Schink 1986; Frings et al. 1992). Schink's group also demonstrated conversion of 2-phenoxyethanol to phenol and acetaldehyde, in a way similar to a diol hydratase reaction, by a strictly anaerobic Gram-positive bacterium, *Acetobacterium* strain LuPhet1, but could not rule out an alternative pathway for the production of acetaldehyde (Speranza et al. 2002). Dwyer and Tiedje (1986) obtained a methanogenic consortia from sewage sludge that degraded ethylene glycol to PED 20,000. In addition, *Alcaligenes faecalis* var. *denitrificans* TEG-5, the first PEG-degrader able to degrade PEG under aerobic conditions, displayed PEG degradation under anaerobic nitrate-reducing conditions (Grant and Payne 1983).

In parallel with PEG degrading bacteria, we isolated PPG- and PTMG-utilizing bacteria (Kawai et al. 1977; Kawai and Moriya 1991). PPG with an MW of up to 4,000 was assimilated. Distinct degradation of PTMG was limited to oligomers up to octamer, since PTMG is insoluble in water and is used exclusively as a

constituent of polyurethanes, but oligomers up to octamer can be washed out with water as impurities from polymers, and are found in wastewater. The first attack on PPG and PTMG was considered to be dependent on dehydrogenases (Kawai and Moriya 1991; Tachibana et al. 2002). The presence of several different PPG dehydrogenases (PPG-DHs), localized in the membrane, the periplasm, and the cytoplasm respectively, has been suggested for PPG-utilizing *Stenotrophomonal maltophilia* (Tachibana et al. 2002) from which pyrroloquinoline quinone (PQQ)-dependent PPG-DH was purified and characterized as a type-I quinoprotein dehydrogenase, localized in the periplasm (Tachibana et al. 2003). Later, cytoplasmic NAD-dependent PPG-DH was characterized and hypothesized to work on low molecular sizes of PPG in the cytoplasm (Tachibana et al. 2008). This is different from the only membrane-bound PEG-DH suggested for PEG-degrading Sphingomonads. PPG might have more affinity with phospholipids, which are the main constituents of the cytoplasmic membrane, and oligomeric PPGs probably can traverse the cytoplasmic membrane and are metabolized in the cytoplasm (Kawai et al. 1985; Hu et al. 2008a). Oligomeric PPG might express genes related to PPG metabolism.

16.3.2 Polyvinyl Alcohol

Historically, PVA has been produced on an industrial scale by the hydrolysis of poly(vinyl acetate), since a vinyl alcohol monomer cannot exist due to tautomerization into acetoaldehyde. PVA are widely used due to its excellent physico-chemical properties, especially for fabric and paper sizing, fiber coating, adhesives, emulsion polymerization, films for packing and farming, and the production of poly(vinyl butyral). Maximum production of PVA amounted to about 1,250 kt in 2007 (http://www.sriconsulting.com/CEH/Public/Reports/580,1810), the top volume in the total volume of synthetic water-soluble polymers produced in the world, and consumption is expected to increase annually. Large quantities of PVA are poured into water systems each year, especially when used in paper and textile mills. PVA might be the only polyvinyl-type synthetic polymer that is biodegradable. The biodegradation of PVA has been reviewed by Matsumura (2002), Chiellini et al. (2003) and Kawai and Hu (2009). The history of PVA biodegradation goes back over 70 years, since the first report of degradation by *Fusarium lini* B (Nord 1936). Suzuki et al. (1973) reinitiated extensive studies of PVA biodegradation, followed by Watanabe et al. (1975). A variety of microorganisms with the ability to assimilate PVA have been reported. Most PVA-degraders are Pseudomonads or Sphingomonads, but they range across Gram-negative and Gram-positive bacteria and fungi (Kawai and Hu 2009). Some of them can degrade PVA as a mixed culture, due to different underlying mechanisms (Kawai 2010b). No anaerobic PVA-degrading microbe has yet been isolated, but river sediments and anaerobically preincubated-activated sludge have been found to degrade PVA (Matsumura et al. 1993). The anaerobic biodegradation rate of

PVA is low and is influenced by its MW, unlike the biodegradation of PVA under aerobic conditions.

The main metabolic route is based on two steps. The first is either (i) oxidation of two adjacent hydroxyl groups leading to β-diketone structures, or (ii) the oxidation of one hydroxyl group, yielding monoketone structures. Based on the products of the first step of PVA degradation, there are two possible pathways for the second step: either hydrolysis of β-diketone structures of oxidized PVA (oxiPVA) by a β-diketone hydrolase (oxiPVA hydrolase) or the aldolase reaction of the monoketone structures of oxiPVA. We cannot rule out the possibility of an aldolase reaction, but diketone structures are surely the main products of PVA degradation (Kawai and Hu 2009). Since diketone structures are non-enzymatically hydrolyzed, the oxidation process for the degradation of PVA is the most important. *Penicillium* sp. appears to utilize the metabolic pathway proposed above in the bacterial degradation of PVA (Qian et al. 2004).

The two-step degradation of PVA was confirmed by the genetic structure of the *pva* operon in *Sphingopyxis* sp. 113P3 (Klomklang et al. 2005), which consisted of three genes encoding oxiPVA hydrolase, PVA dehydrogenase (PVA-DH), and cytochrome *c*, as shown in Fig. 16.3. Cytochrome *c* has been suggested to be a natural electron acceptor for PVA-DH in vivo (Mamoto et al. 2008). PVA-DH is a member of the type-II quinohemoprotein alcohol dehydrogenases (Hirota-Mamoto et al. 2006), but the position of the amino acid sequences for the heme-binding domain and superbarrel domain found in this family are the reverse of those of the other members. This is considered significant in terms of the ability of PVA-DH to react with a macromolecule such as PVA. Unfortunately, a non-quinohemoprotein PVA oxidase has not yet been cloned. PVA-DH was reported to be constitutive, as opposed to PVA oxidase, which was PVA-inducible, but we found that the *pva* operon was constitutively expressed, although its expression was promoted with PVA (Hu et al. 2008b). We confirmed that PVA was taken up through the outer membrane (Hu et al. 2007b) and accumulated in the periplasm (unpublished data), where the three enzymes are located. How PVA regulates the expression of the *pva* operon and the size required for induction remains to be determined. In a megaplasmid, the presence of the *pva* operon is similar to that of a *peg* operon (Tani et al. 2007), which promotes the acquisition of degradation ability, resulting in a wide distribution of the operon among different species. In fact, the widely distributed PEG-DH gene (AB196775) of *S. macrogoltabida* strain 103 has been detected in newly isolated PEG-degraders, including *S. macrogoltabida*, *Stenotrophomonas maltohilia*, *Pseudomonas* sp., and *Sphingobium* sp., and shows 99% identity with the original (Hu et al. 2007a).

Although the TonB-dependent receptor-like gene and the permease-like gene are included in the *peg* operon, no gene that might be related to translocation of a polymeric compound is included in the *pva* operon (Hu et al. 2008b), although its expression is clearly promoted by the presence of PVA, which necessitates regulation of the *pva* operon by PVA or its metabolite. On the other hand, morphological changes in the cell surface occur upon exposure to PVA (Hu et al. 2007b). Hence, on the basis of PEG and PVA degradation studies,

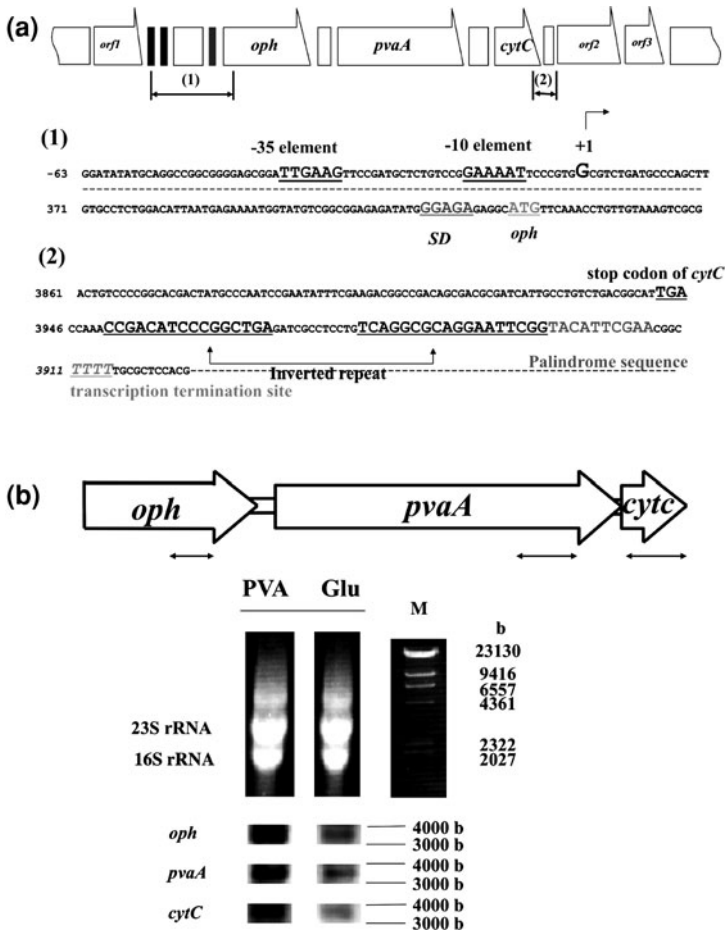

Fig. 16.3 The operonic structure of genes involved in PVA degradation and its expression. **a** The operonic structure of three genes; **b** the expression of three genes in PVA and glucose media. Symbols: *oph* oxiPVA hydrolase gene; *pvaA* PVA-DH gene; *cytC* cytochrome *c* gene

we can conclude that these operons are regulated by macromolecules and require a specific transport system for inducer macromolecules.

16.3.3 Polyacrylate

The biodegradation of polyacrylate was reviewed by Kawai and Hayashi (2002). This "acrylic polymer" commonly includes a variety of homo- and copolymers of acrylic and methacrylic acids and their esters. After World War II, the demand for

this family of acrylic polymers expanded rapidly, and numerous applications depended on the specific polymer structure, molecular weight, and composition. Among acrylic polymers, PAA is a linear homopolymer of acrylic acid and its salts. Due to the presence of a large number of carboxyl groups, PAA dissolves in water and has a large share of the water-soluble speciality polymer market. The polymer is currently used in detergent builders, pigment and filler dispersants, and flocculants in water treatment. After use, PAA is usually disposed off in sewage and water bodies, e.g., streams, rivers, and lakes. As has been described for PEG and PVA, molecular size significantly affects its susceptibility to biodegradation. The MW of commercially available PAA ranges from approximately 100 to several million. High MW PAA is predicted to be either non- or slightly biodegradable, but when practically applied, it is removed by precipitation and disposed off in its solid form by incineration or in a landfill. In addition, when exposed to UV light, high MW PAA in a dilute solution (less than 1%) is photochemically decomposed into smaller fragments that are biodegradable. Ozonization can also lower the MW of PAA. Hayashi (1998) concluded that even high MW PAA can be finally biodegraded after physico-chemical treatment. What, then, does MW have to do with the environment? By adsorption to sewage sludge and precipitation in the form of polymer-calcium complexes or by coagulation–flocculation with aluminium chloride or ferric chloride, more than 90% of the polymers with MWs of tens of thousands are removed from the water and 45–60% of the polymers with MWs of 3,000–4,000 are removed. Sand-column tests have shown that PAA with a MW greater than about 3,500 is strongly adsorbed to soil particles (Rittmann et al. 1992b). Therefore, biodegradability is a prerequisite for PAA with a MW less than about 3,500.

Matsumura et al. (1988) did the first study of the biodegradability of acrylic oligomers using activated sludge. They suggested that partial mineralization of PAA with an average MW of 2,000 or 4,500 occurs within 90 days. Hayashi et al. (1993) first reported the isolation of a bacterium (*Arthrobacter* sp.) from soil samples that is capable of assimilating the acrylic oligomers up to a heptamer. Kawai (1993) also reported three bacterial strains that assimilated acrylic trimer, and showed metabolic activity towards PAA (MW 1,000–4,500); they proposed an aerobic metabolic pathway for PAA degradation based on a β-oxidation form of metabolism (Kawai et al. 1994). PAA with a MW of less than 1,000 can be biodegraded to a considerable extent by activated sludges (Larson et al. 1997). Hayashi et al. (1994) isolated aerobic bacteria capable of degrading PAA of MW 4,000 from soils, including a single bacterium (*Alcaligenes* sp.) and a consortium of three bacteria (*Alcaligenes* sp., *Sphingomonas* sp., and *Mycoplana* sp.), and suggested that effective decomposition of high MW PAA can be achieved by a combination of physico-chemical treatment and microbial degradation. Rittmann et al. (1992a) found that soluble PAA with a MW of 1–100 kD could be mineralized to CO_2 with an anaerobic biomass formed in a sand column. More recently, Doser et al. (1997) reported that *Pseudomonas* sp. isolated from activated sludge was capable of utilizing high MW PAA (MW, 100 kD) as a carbon source. Iwahashi et al. (2003) found that a microbial consortium of several bacterial

species degraded PAA with an average MW of 2,100, and they proposed a metabolic pathway similar to the β-oxidation pathway identified by Kawai et al. (1994) with different metabolites. Hence, PAA of a MW lower than 3,500 is considered biodegradable.

16.3.4 Poly(amino acid)s and Polyamides

The degradation of poly(amino acid)s and polyamides was reviewed by Obst and Steinbüchel (2004). In nature, proteins and poly(amino acid)s exist as two different types of amino acid polymers. The most critical differences are (i) that the former is a random polymerization of 20 amino acids and the latter a polymer of a single amino acid, and (ii) the synthesis of proteins is ribosome-dependent and forms an α-amino-α-carboxyl peptide linkage while that of poly(amino acid)s is ribosome-independent and catalyzed by peptide synthetases (EC 5.3.2.-), and forms other linkages, including β- and γ-carboxyl groups as well as ε-amino groups. Theoretically, poly(amino acid)s can be biosynthesized from acidic and basic amino acids with a free amino or carboxyl group that is not used in peptide bonds, such as glutamic acid, aspartic acid, lysine, histidine, and arginine. Naturally occurring poly(amino acid)s include poly(γ-glutamic acid) (γ-PGA), poly(ε-L-lysine) (ε-PL), and cyanophycin (cyanophycin granule polypeptide (CGP)). The CGP molecular structure is related to that of PAS, and unlike synthetic PAS, it is a comb-like polymer with α-amino-α-carboxyl-linked L-aspartic acid residues representing the poly(α-L-aspartic acid) backbone and L-arginine residues bound to the β-carboxylic groups of aspartic acids. A charged polypeptide composed of repeated oligomeric arginine and histidine units has been identified in the ergot fungus *Verticillium kibiense* (Nishikawa and Ogawa 2004). Poly(amino acid)s occur in various molecular sizes and are generally polydisperse, whereas proteins are monodisperse. These poly(amino acid)s have a poly(ionic) nature and are generally water soluble; they can be used in many applications. PAS and nylons have been commercialized as synthetic polyamides. The former is used as a biodegradable detergent builder substituting for hardly biodegradable PAA. Nylon is a solid polymer used in films and fabrics, for example, and is non-biodegradable. However, oligomeric by-products from a factory producing nylon can be biodegraded, as described below.

PAS is not a naturally occurring poly(amino acid) and is chemically synthesized, principally by thermal polymerization. This produces a branched PAS comprising α and β-carboxy-linked poly(D/L-aspartic acid). PAS has the same chelating ability as PAA due to the presence of the same carboxyl groups on the polymer chain, and thus it is expected to be a suitable substitute for PAA due to its higher susceptibility to microbial degradation. Since branched PAS is less biodegradable than straight-chain PAS, development of it with little or no branching is expected. Recently, Soeda et al. (2003) found that α-poly(D/L-aspartic acid) with molecular weight of up to 3,700 Da can be synthesized from diethyl L-aspartate in

organic solvents using a *Bacillus subtilis* protease as catalyst. In nature, only aspartic acid-rich polypeptide sequences have been found. For example, these regulate the formation of calcite crystals in sea shells (Rusenko et al. 1991). In view of the structural similarity between CGP and PAS, alternative degradation mechanisms for CGP initiated by hydrolytic β-cleavage leading to the release of free arginine from CGP are desirable for the formation of straight-chain PAS, for which many technical applications are known, but this has not been reported. A PAS peptide comprising 20 aspartic acids is secreted by an engineered *B. subtilis*, WB600/pBE92 (Ornek et al. 2002).

Tabata et al. (2000) isolated *Pedobacter* sp. KP-2 and *Sphingomonas* sp. KT-1, which degrade high MW linear PAS completely to low MW products in their mixed culture. The former endogenously hydrolyzes PAS of masses between 5 and 15 kDa to aspartic oligomers, whereas the latter completely degrades exogenously PAS with masses below 5 kDa to aspartic acid monomers. PAS-hydrolyzing enzymes (hydrolase-1 and hydrolase-2) have been purified from cell extracts of strain KT-1 (Tabata et al. 2001; Hiraishi et al. 2003a, b). Hydrolase-1 specifically cleaves the bonds between the β, β-amide units of thermally synthesized PAS endogenously to aspartic oligomers. Hydrolase-2 exogenously hydrolyzes α-oligo(L-aspartic acid) to aspartic acid and shows similarity to a putative peptidase (Hiraishi et al. 2003a, 2004).

Nylons, synthetic polyamides, are one of the most successful commercialized plastics; they are widely used in producing stockings, fibers, carpeting, ropes, fishnets, and so on. They are barely biodegradable, but nylon oligomers occurring as by-products during the synthesis of nylon can be biodegraded by microorganisms. Linear and cyclic oligomers of ε-aminocaproic acid (by-products of nylon-6 manufacturing) are assimilated by *Flavobacterium* sp. KI72 and *Pseudomonas* sp. NK87 (Kinoshita et al. 1975; Kanagawa et al. 1989), which utilize them as their sole carbon and nitrogen sources. Three enzymes are associated with the degradation of the oligomers: (i) 6-aminohexanoate-cyclic-dimer hydrolase (NylA) (Kinoshita et al. 1977), (ii) 6-aminohexanoate-linear-dimer hydrolase (exo-type), which degrades the dimer-hexamer to 6-aminohexanoate (Nyl B) (Kinoshita et al. 1981), and (iii) an aminohexanoate-oligomer hydrolase (endo-type) (NylC), which is responsible for the cleavage of cyclic and linear oligomers with more than 3 subunits into linear dimers (Negoro et al. 1992; Kakudo et al. 1993, 1995). Genes for three hydrolases were encoded on one of the plasmids in strain K172 (Negoro et al. 1992; Negoro 2000). Negoro and co-workers have confirmed that nylon oligomer-degrading enzymes can be obtained through experimental evolution from a non-degrader (Prijambada et al. 1995), and they have found that a mutant of a carboxyesterase with a β-lactamase fold with weak activity has acquired greater affinity and catalytic efficiency for the substrate (Kawashima et al. 2009). They isolated novel alkanophilic nylon oligomer-degrading bacteria, *Agromyces* sp. KY5R and *Kocuria* sp. KY2, and found that the genetic organization of the nylon oligomer-degrading enzymes is similar to that of strain K172, albeit with some rearrangements (Yashuhira et al. 2007a, b). These results indicate that microorganisms rapidly evolve by random mutagenesis of existing genes, and that these

occasionally cause variations in the catalytic properties of the respective encoded enzymes and make possible adaptation to synthetic polymers newly introduced into the environment. However, the mutation rate is not the same for all synthetic polymers, since polyethylene or polypropylene are only slightly biodegradable, although they have been used for approximately the same period of time as nylon. Besides, a thermophilic bacterium, *Geobacillus thermocatenulatus*, has been suggested as a possible degrader of nylon-12 and 66 but not nylon-6 (Tomita et al. 2003a), but its enzymes have yet to be characterized.

16.4 Biodegradation of Plastics

Plastics are generally very resistant to environmental influences such as humidity and microbial attack (Müller et al. 2001), but during the past two decades several biodegradable plastics have been developed, and their uses have been gradually expanded and commercialized, for example, Apexa® (DuPont), Ecoflex® (BASF), EasterBio® (Eastman Chemicals), Bionole® (Showa Highpolymer), Matabee® (The Nippon Synthetic Chemical Industry), LACEA® (Mitsui Chemicals), Runale® (Nippon Shokubai), Novon® (Warner–Lanbert), and Nature Works® (Cargill Dow Polymers). Biodegradable plastics can be applied in single-use articles that can be disposed of by biological waste treatment such as composting or anaerobic digestion (Baere et al. 1994). It is notable that a large number of biodegradable plastics are categorized as polyesters. Many examples of plastic degradation have been documented. Here I introduce our work on the biodegradability of PEwax, aliphatic–aromatic-*co*-polyester, and PLA.

16.4.1 Polyethylene Wax

Polyethylene (PE) is regarded as a chemically inert polymer due to various factors, such as its long degradation time. Early studies of the biodegradation of PE indicated that the biodegradation of PE is affected by various factors: preliminary irradiation from a UV source, the presence of photodegradative enhancers, morphology and surface area, additives, and MW (Albertsson et al. 1987). By measuring $^{14}CO_2$ generation, they showed that the degradation of PE proceeded very slowly. Scot (1975) had concluded that an attack on PE by microorganisms is a secondary process. The first process in the degradation of PE is an oxidation process that reduces the MW of the molecule to the level required for biodegradation to occur. Based on this theory, he developed the so-called Scott-Gilead process (Scott and Gilead 1978) to enhance the oxidation of PE molecules. Potts et al. (1973) found that linear paraffin molecules (approximately below MW 500) are utilized by several microorganisms. Otake et al. (1995) reported remarkable degradation of low density PE thin films buried under soil for over 32 years,

but reported no data on molecular sizes. We have tested the microbial degradation of low-density PE capsules was enhanced by 3% w/w of Scott–Gilead system. The PE includes photoactivators, such as iron acetyl acetonate and nickel dibutyldithiocarbamate (Kawai et al. 1999). The capsules were kept outdoors for several years and were used as photodegraded PE (PDPE) that was fragmented perhaps through a pathway similar to that of the Norrish reactions (Al-Malaika et al. 1986). PDPE and commercial PEwax (MW = 1,290) were used as sole carbon and energy sources for the soil microorganisms (154 field soil samples) (Kawai et al. 1999). Several consortia grew on PDPE or PEwax, and were confirmed to have degraded by weight loss or gel permeation chromatography (GPC). Based on the GPC pattern, appreciable degradation was found in PEwax up to approximately 3,000 Da. On the other hand, manganese peroxidase produced by a white-rot fungus strain, IZU-154, also nonspecifically degraded PE as well as Nylon 66 (Deguchi et al. 1997).

16.4.2 Aliphatic–Aromatic Copolyester

Polyesters are classified into three groups; aliphatic, aromatic, and aliphatic-*co*-aromatic. There are many reports of enzymatic and microbial attack on aliphatic polyesters. The polyesters are gradually hydrolyzable in water and are susceptible to enzymatic attack by lipases in general, but their degradation rate is dependent on their chemical structure or the melting point. Aromatic polyesters, such as polyethylene terephthalate (PET), are practically non-biodegradable, although two papers reported that cutinases can hydrolyze PET with low crystallinity (Müller et al. 2005; Ronkvis et al. 2009). The addition of aliphatic groups to aromatic polyesters endows them with advantageous physico-chemical properties for practical use together with biodegradability characteristics. They can be used as a film or coating for disposable packaging of food (for example, bowls, plates, cups, sandwich wraps, and clamshell sandwich containers), and can be biodegraded in compost. Since the aromatic group is typically terephthalic acid, the physical properties of the plastics are determined by the type and content of the aliphatic groups. The biodegradation of aliphatic–aromatic copolyesters has been extensively studied by Müller et al. (2001). They used Ecoflex (a copolymer of 1,4-butane diol, dimethyl terephthalate, and adipic acid) as the target polyester. Biodegradation of Apexa® (formerly Biomax®, consisting of terephthalic acid, ethylene glycol, and an undisclosed component that ensures compostability) has also been studied in a bioreactor maintained at 58°C, inoculated with compost tea (Nagarajan et al. 2006) and in compost (Hu et al. 2008c).

Microorganisms able to colonize on Luria–Bertani agar plates containing the polyester (Apexa® 4026) at 50°C were directly isolated from composted films (Hu et al. 2010). Actinomycetes accounted for approximately 70% of the total isolates, and were categorized into the genera *Streptomyces*, *Thermobifida*, *Saccharomonospora*, and *Thermoactinomyces*. Members of the *Bacillus* group

accounted for approximately 30% of the total isolates, and were categorized into the genera *Bacillus*, *Ureibacillus*, and *Aneurinibacillus*, We selected *Thermobifida alba* AHK119 for further work, since it was the strongest degrader of the copolyester and polycaprolactone (PCL). *T. alba* AHK119 degraded the particles of Apexa 4026 and 4027 and produced terephthalic acid in the culture supernatant. We cloned a gene coding a 300-amino acid protein, Est119, which belongs to an esterase-lipase superfamily (serine hydrolase). The highly conserved -G-X-S-X-G- serine hydrolase sequence was defined as -G-H–S-M-G- in Est119, and Ser129, His207, and Asp175 were identified as a catalytic triad. The mature protein is a single polypeptide chain made up of 266 amino acids. The sequence encoding the mature Est119 protein was cloned into pQE80L to create expression vector pQE80L-*est119*. The recombinant protein harboring an N-terminal hexahistidine tag was expressed in *Eschericia coli* Rosetta-gami B(DE3). The transformed cells produced an approximately 30 kDa protein when induced with 0.1 mM isopropyl-*β*-D-thiogalactopyranoside. The purified Est119 is a monomeric protein with a molecular mass of 30 kDa. The enzyme shares 84% identity with a hydrolase for an aliphatic–aromatic copolyester from *Thermomonospora fusca* (now *Thermobifida fusca*) DSM43793 (Chen et al. 2008; Kleeberg et al. 2005). There are only two enzymes of the genus *Thermobifida* that work on aliphatic–aromatic copolyesters. Both *Thermobifida* strains are major Actinomycetes components within composts and might contribute to the biological recycling of aliphatic–aromatic copolyesters.

16.4.3 Poly(lactic acid)

Chemical synthesis of PLA dates back to 1932, when Carothers first synthesized PLA of approximately 3,000 Da. In the 1960s, PLA was found use in the medical field as a bio-absorbable material. Since the latter half of the 1980s, plastic waste has caused public concern due to its negative impact on the environment, and recently rising costs and the limited availability of crude oil has turned more attention towards alternative sources. This trend has returned attention to PLA as a bio-based material capable of replacing oil-based materials. PLA is chemically synthesized from lactic acid, a representative of fermentation products from plant resources, and hence is defined as a biomass plastic. The biodegradability of PLA has been established since the first report on enzymatic hydrolysis of PLA by William (1981), who described its feasibility for proteases and unfeasibility for esterases. Later, certain lipases and esterases were reported to be able to hydrolyze PLA, but they appear to be active only for low molecular weight polymers or poly(DL-lactate) (DL-PLA). There have also been many reports on the microbial assimilation of PLA, since Pranamuda et al. (1997) first isolated PLA-assimilating *Amycolatopsis* sp. strain HT-32. Tokiwa and Calabia (2006) concluded that most PLA-degrading microorganisms belong phylogenetically to the family *Pseudonocardiaceae* and related genera, such as *Amycolatopsis* and *Lentzea*, in which

proteinaceous materials promote the production of the PLA-degrading enzyme. PLA-degrading enzymes of PLA-assimilating microorganisms were purified from different strains of *Amycolatopsis* at about the same time by two groups (Pranamuda et al. 2001; Nakamura et al. 2001) and were characterized as proteases. Later, both groups cloned the genes (Matsuda et al. 2005; Tokiwa et al. 2003). It is notable that almost all the degradation tests have been carried out using poly(L-lactic acid) (PLLA), and that no information regarding the biodegradability of poly(D-lactic acid) (PDLA) is available, except for the fact that proteinase K hydrolyzed PLLA, but not PDLA (Reeve et al. 1994). Tomita et al. (2003b) isolated a thermophile, *Bacillus stearothermophilus*, which grew at 60°C on PLA as sole carbon source.

Actinomycetes, *Bacillus*, *Brevibacillus*, and *Geobacillus*, have been reported to be thermophilic degraders (Kawai 2010a). Since PLA is hydrolyzed at a relatively high rate at high temperatures (>50°C), the question whether the strain excretes a PLA degrading enzyme or utilizes hydrolyzed products depends on future characterization of their PDLA degrading enzymes. Mayumi et al. (2008) recently cloned three genes encoding PLA depolymerases based on a metagenome derived from the compost. One of them coded for a thermostable esterase homologous to *Bacillus* lipase and showed an ability to bind to DL-PLA powders with molecular masses lower than 20,000. Since the expressed enzyme had no activity on PLLA with molecular masses of approximately 130,000, the enzyme might be able to degrade depolymerized PLA products. It can be surmised that the same mechanism exists in other thermophilic enzymes. A fungus, *Tritirachium album* ATCC22563, also shows an ability to degrade PLLA, silk fibroin, and elastin; degradation is inducible with gelatin, suggesting the induction of a protease (Jarerat and Tokiwa 2001). However, the role of the fungus in degrading PLA in nature is doubtful, since the enzyme was not induced at all in the absence of gelatin.

The degradation of PLA is possible at 30°C, far lower than the glass transition temperature (Tg: approximately 55°C), and is difficult to explain based on the flexibility of the substrate molecular chain, but is understandable because PLA absorbs water and collapses the polymer block, which then becomes available for attack by microbes or enzymes.

Masaki et al. (2005) isolated *Cryptococcus* sp. S-2 for use in wastewater treatment, and found that the strain displayed strong lipase activity. They cloned the gene for a lipase and found that it had higher homology with cutinases (EC 3.1.1.74) than with lipases, which showed stronger degradation ability toward PLA than proteinase K.

Matsuda et al. (2005) confirmed that a recombinant PLA depolymerase from *Amycolatopsis* did not work on PCL or PHB. PHB depolymerase does not act on PLLA, a kind of hydroxyalkanoate (PHA), due to differences in the optical activities of the two substrates and in the carbon chain lengths of 2-hydroxyalkanoate and 3-hydroxyalkanoate. Commercially available lipases (esterases) act on DL-PLA, but not on optically active PLLA or PHB, poly(D-hydroxybutyrate) (Tokiwa and Jarerat 2004). Thus polyester-degrading enzymes are categorized into

three groups: PLA depolymerase, PHB depolymerase, and general polyester-degrading enzymes, which also have a variety of substrate specificities towards aliphatic and aliphatic-*co*-aromatic polyesters. PLA is considered to be one of the third type of polyesters, following synthetic polyesters and PHA, including PHB.

Using the recombinant purified PLA-degrading enzyme from *Amycolatopsis* sp. K104-1 and the recombinant purified cutinase like enzyme (CLE) from *Cryptococcus* sp. S-2 (Masaki et al. 2005), we examined enantioselectivity towards PLLA and PDLA (Kawai 2010a). The PLA-degrading enzyme was PLLA-specific. Together with a report on the enatioselectivity of proteinase K (Reeve et al. 1994) and the fact that proteases originally recognize the polymer of L-amino acids, we concluded that protease-type PLA depolymerases are PLLA-specific. On the other hand, CLE acted on both PLLA and PDLA, but the activity was higher on PDLA than on PLLA (it was PDLA-preferential). The enantioselectivity of crude enzymes can be a good indicator in predicting the type of enzyme, either protease or cutinase, which leads to successful cloning of enzyme genes based on the conserved regions of the various groups.

Commercially available true lipases did not act on PLLA or PDLA. True lipases have a lid covering an active site that leads to interfacial activation (Schimid and Verger 1988), but some lipases, esterases, and cutinases have neither a lid nor interfacial activation. To cover an active site with a lid completely, the size of the active site inlet cannot be too big. On the other hand, the inlet of PLA depolymerase must be big enough to accommodate a macromolecular PLA. Accordingly, lipase-type PLA depolymerases are probably not typical true lipases, but esterases (cutinases) without a lid, useful for interfacial activation, and probably possesses an active cavity big enough to accommodate a polymer substrate. Cutin is a rather large molecule with a complex structure.

16.5 Non-Metabolic Degradation

The first report on the degradation of synthetic polymeric materials by lignin-degrading fungi described the degradation of nylon-6 and -66 by white-rot fungi, including an isolate (IZU-154) and stock cultures (Deguchi et al. 1997); nylon-degrading activity is based on oxidation by manganese peroxidase and is closely related to the lignolytic activity of fungi (Deguchi et al. 1998). Larking et al. (1999) found that the degradation of PVA was promoted by a combination of treatments with Fenton's reagent followed by biological degradation, probably by laccase produced in the culture supernatant of the white-rot fungus *Pycnoporus cinnabarinus.* Another lignin-degrading white-rot fungus, *Phanerochaete chrysoporium,* excreted lignin peroxidase, which promoted the degradation of PVA chains through the formation of carbonyl groups as well as double bonds (Mejia et al. 1999). A substantial decrease (approximately 80%) in average MW was observed. Since the carbon chain of oxidized PVA is readily cleaved through non-enzymatic processes, strong oxidation of PVA either by an oxidizing enzyme

or by a Fenton reaction leads to cleavage of the main carbon chain and yields oligomeric materials. Oligomers are generally biodegradable, as are oligomeric ethylene, styrene, and isoprene, although their polymers are non-biodegradable (Kawai 1995). On the other hand, the brown-rot fungus *Gloeophyllum trabeum* secretes quinones (Jensen et al. 2001) that reduce Fe^{3+} and produce H_2O_2, resulting in an extracellular Fenton reaction degrading PEG. Thus, brown- and white-rot fungi can play a significant role in the recycling of materials in the environment, and their degradation-related enzymes have potential applications in the treatment of polymer wastes and wastewater.

16.6 Conclusion

The degradation of polymers was at first expected to be catalyzed solely by extracellular enzymes, given the assumption that macromolecules are never incorporated into cells, but this was disproved by the periplasmic degradation of PEG and PVA, as described above. These are surely incorporated into the periplasm through the outer membranes of Sphingomonads and are metabolized by periplasmic enzymes, although the mechanism of macromolecule uptake has not been well characterized. Many enzymes related to the microbial degradation of polymers have been elucidated genetically. They show unique features: PEG-DH creates a small branch in GMC flavoprotein oxidoreductases (Zamocky et al. 2004) and PEG-aldehyde dehydrogenase was the first nicotinoprotein aldehyde dehydrogenase. Ether bond-splitting enzymes are not catalyzed by ether bond-specific enzymes, but by various divergent enzymes such as monooxygenase, oxidase, dehydrogenase, hydrolase, and lyase. PVA-DHs form a unique group of quinohemoprotein dehydrogenases. Research on nylon oligomer-degrading enzymes has verified that xenobiotic polymer-degrading enzymes can readily evolve by spontaneous mutation from ancestor enzymes that originally recognized natural compounds analogous to the target polymers. A PEG-DH gene from a Sphingomonad has been distributed and conserved among different genera in the 35 years since PEG-degrading Sphingomonads were isolated in 1975 and a gene for PEG-DH was cloned in 2001, suggesting a significant role for a large plasmid harboring a PEG-degradative gene cluster in circulation for degradation ability among microorganisms. Since a *pva* operon is on a large plasmid of *Sphingopyxis* sp. strain 113P3, the PVA-DH gene and the operon structure have probably been distributed among microorganisms. This strongly suggests that the short history of xenobiotic polymers to date has been sufficient for degrading enzymes to evolve since the prototype enzymes existed and the microorganisms had to adapt to an environment contaminated by xenobiotics. In addition, megaplasmids must have sped up the distribution rate of degrading ability. The existence of Sphingomonads that degrade various xenobiotic polymers, such as PEG and PVA, via intracellular enzymes suggest that they have a means of taking macromolecules into the periplasm as well as metabolic enzymes adapted to respond to these

macromolecules. Polymer-degrading microorganisms and their enzymes for polymer degradation have been well described. Many examples of symbiotic polymer degradation appear to suggest that symbiotic degradation occurs in an ecosystem when new artificial compounds are introduced. At the same time, non-metabolic polymer degradation suggests that the ecosystem has the disposal potential and versatility in terms of new artificial compounds.

However, information is still limited with regard to the operonic structures of the genes related to degradation and to the regulation of individual genes and operons, except for those involved in PEG and PVA degradation. The *peg* operon is expressed in the presence of PEG, but expression is triggered by oligomers having the size of more than tetramer. The *pva* operon is constitutively expressed, but expression is remarkably enhanced by PVA. The size of PVA that triggers promotion of the *pva* operon remains to be determined. Since PVA-DH and oxidized PVA-hydrolyzing enzymes are periplasmic enzymes, it is likely that depolymerized oligomeric PVA can be incorporated into the cytoplasm where it promotes the expression of the *pva* operon. Extracellular PLA-degrading proteases from PLA-degrading microorganisms have been induced with proteinaceous materials. Since it is probably impossible for hard, solid PLA to penetrate cells, the microorganisms must have selected another strategy for degradation, one that employs extracellular excretion of proteases induced by proteinous materials including alanine, analogously to lactate. An aliphatic–aromatic-*co*-polyester-degrading enzyme from *T. alba* strain AHK119 was expressed constitutively (unpublished data), as the target polyester can never be incorporated into cells. The polyester-degrading enzymes belong to the lipase family and are distinguished from true lipases in the sense that they have no lid covering the active site, which is prerequisite to the interfacial activation, characteristic of true lipases. They must have acquired the ability to recognize and hydrolyze solid macromolecules, but the details of the mechanism are still under study. Thus, microorganisms have employed a variety of adaptation strategies in relation to novel artificial compounds in a short time. Although oligomeric structures are biodegradable, some polymers, such as PE, polystylene, and polyacrylonitrile, are not practically biodegradable. This suggests limitations in the biodegradation of solid polymers with regard to their physico-chemical properties, such as MW, Tg, and mp. In other words, we can design biodegradable polymers and non-biodegradable polymers based on their intended uses.

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