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Biological degradation of synthetic polyesters—Enzymes as potential catalysts for polyester recycling

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Abstract

The depolymerization of polymers by enzymes is of great interest for biodegradable plastics, a group of materials which has been developed as an answer to increasing problems in plastics waste management. Polyesters play the dominant role in biodegradable polymers and recently a model of polyester degradation by hydrolyses (lipases) has been published. The chain mobility of the polymer chains proved to be the most relevant factor controlling polyester biodegradability, usually excluding many aromatic polyesters such as PET from biodegradation. Recently a new thermophilic hydrolase (activity optimum at 65 °C) from *Thermobifida fusca* (TfH) was isolated, characterized and expressed in recombinant *Escherichia coli*. This enzyme is especially active in degrading polyesters containing aromatic constituents, and exhibits a 65% sequence similarity to a lipase from *Streptomyces albus* and combines characteristics of lipases and esterases. TfH is even capable to degrade commercial PET from beverage bottles. Specific modification of the active site of enzymes like TfH may open the door for enzymatic PET recycling in the future.

Keywords: Polyester; Biodegradation; Lipase; Polymer recycling; PET; Actinomycetes

1. Introduction

More than half a century ago synthetic polymers started to substitute natural materials in almost every area and nowadays plastics became a indispensable part of our life. With time stability and durability of plastics have been improved continuously, and hence this group of materials is now considered as a synonym for materials being resistant to many environmental influences. As xenobiotics plastics proved to be especially resistant against microbial attack, since during their short time of presence in nature evolution could not design new enzyme structures capable to degrade synthetic polymers.

The dramatically increasing production of plastics during the last decades in combination with their durability lead to increasing problems with littering and waste management of these bulk materials. Therefore, about twenty years ago scientists started to look if plastics could designed to become susceptible to microbial attack making them degradable in a microbial active environment, but still maintaining their favorable use properties—biodegradable plastics started to be developed.

It early turned out that especially polymers with heteroatoms in the main chain such as polyesters, polyethers, polyamides or polyurethanes can be degraded by microorganisms and from the early stage of development biodegradable plastics were dominated predominately by polyester-based materials, e.g. by the microbially produced polyester poly(β-hydroxybutyrate) (PHB) [1] or aliphatic polyesters such as poly(ε-caprolactone) [2].

Already in 1977 Tokiwa and Suzuki [3] reported that isolated enzymes also can attack synthetic, man made polymers. Various lipases, usually cleaving fats and oils in nature, proved to be able to hydrolyze ester bonds in various aliphatic polyesters.

However, biodegradability seemed to be limited to aliphatic polyesters, which only provide very limited use properties and therefore, excluding biodegradable plastics from many applications. An improvement could be achieved when it was discovered, that also aliphatic–aromatic copolyesters in a certain range of composition were degraded by microorganisms [4]. Aromatic polyesters such as polyethyleneterephthalate (PET) or polybutyleneterephthalate (PBT) in contrast were still considered as inert against any biological attack.

Since the factors controlling the ability of enzymes to cleave ester bond in one polyester but not another one were not well

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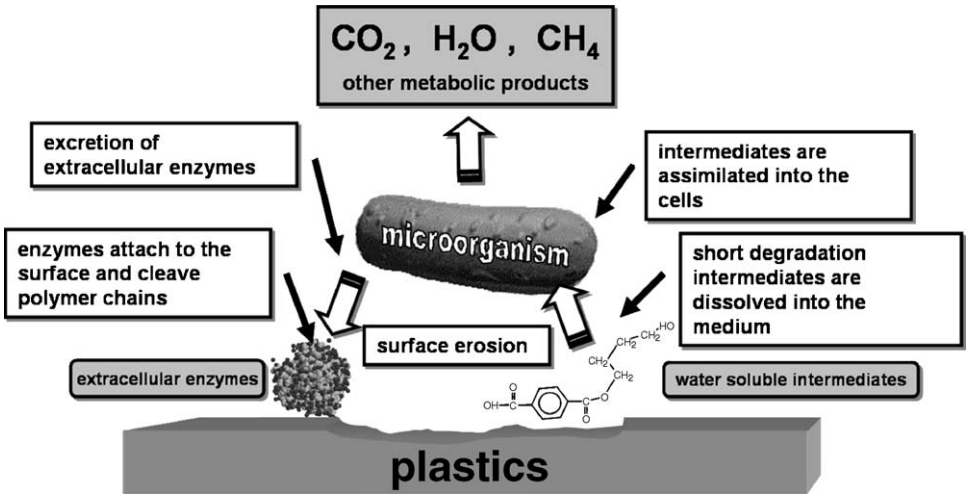

Fig. 1. Scheme of general mechanism of enzymatic catalyzed hydrolytic polymer degradation.

understood, intensive work was done to elucidate the mechanism of enzymatic polyester degradation. Aim of this effort was to become possibly able to design tailor made new biodegradable plastics but also the perspective to use new genetically engineered enzymes as biocatalysts for recycling of bulk polyesters such as PET was in the scope of research.

2. General mechanism of polyester degradation

Biodegradation of polymers usually refers to the attack of microorganisms on water insoluble polymer-based materials (plastics) rather than to the cleavage of water-soluble polymers (e.g. polyacrylamides, polyethylenoxides, etc.). This implies, that biodegradation of plastics is usually a heterogeneous process. Because of water insolubility and the size of the polymer molecules, microorganisms are not able to pick up the polymers directly into the cells where most of the biochemical processes take place, but first have to excrete extracellular enzymes which depolymerize the polymers outside the cells (Fig. 1). If the molar mass of the polymers is sufficiently reduced to generate water-soluble intermediates, these can be transported into the microorganisms and introduced there into the metabolic pathways. As a final result of these processes microbial metabolic end-products such as water, carbon dioxide, methane (in the case of anaerobic degradation), etc. and new biomass are produced. In many cases the first step in the degradation process, the reduction of molar mass, is the rate-limiting factor of plastics biodegradation.

Since extracellular enzymes are too large to penetrate deeper into the polymer material they can only act on the polymer surface making biodegradation of plastics a classical surface erosion process as demonstrated with a degradation experiment of poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) in an anaerobic environment (Fig. 2).

The electron micrograph of the native PHBV film shows a smooth surface. After exposure of the film to an anaerobic microorganism for some weeks, star shaped structures appear at the surface. These structures can be identified as spherolites of the semi-crystalline polyester which degrade slower than the intermediate amorphous material. This part of the polyester has been ‘‘etched’’ layer by layer from the surface uncovering finally the crystal structures. These degradation characteristics could be recently be confirmed by Herzog et al. for the enzymatic depolymerization of polyesters nanoparticles with lipases from *Candida cylindracea* and *Pseudomonas* sp. and described in a mathematical model [6].

3. Factors controlling biodegradability of polyesters

Enzymes are known in many cases as catalysts with a high substrate specificity, that means that a distinct enzyme only

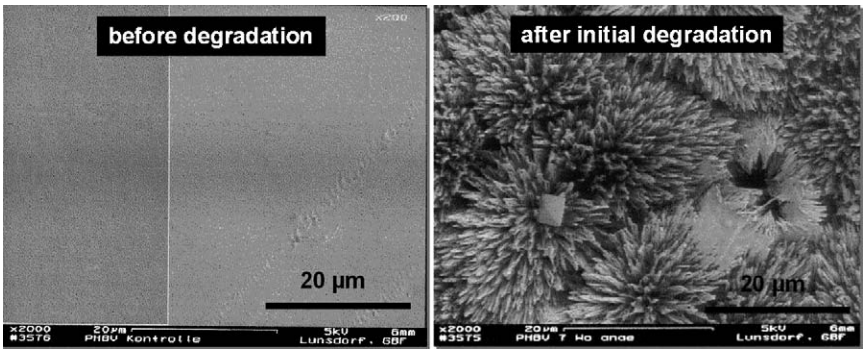

Fig. 2. Surface erosion at a PHBV-film incubated with an anaerobic microorganism (Clostridium, Strain 5a) on mineral salt agar for 7 weeks at 35 °C [5].

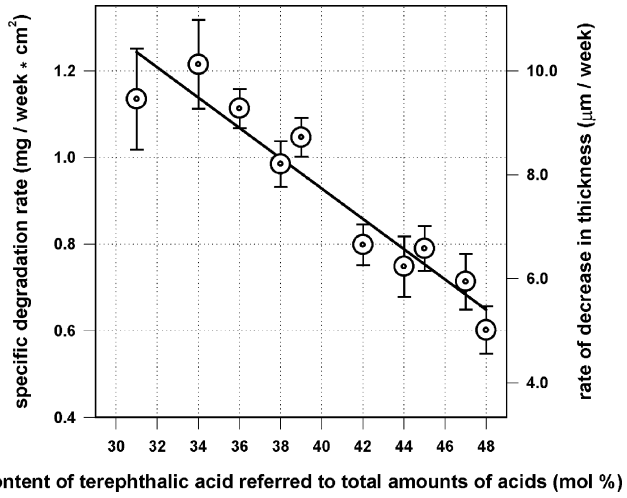

Fig. 3. Decrease in degradation rate (monitored as weight loss) of films of aliphatic–aromatic copolyesters (BTA, copolyesters composed from adipic acid, terephthalic acid and 1,4-butanediol). Incubation of films on a mineral medium agar plate inoculated with a pre-screened microbial culture from compost at 60 °C. Test duration 6 weeks [14].

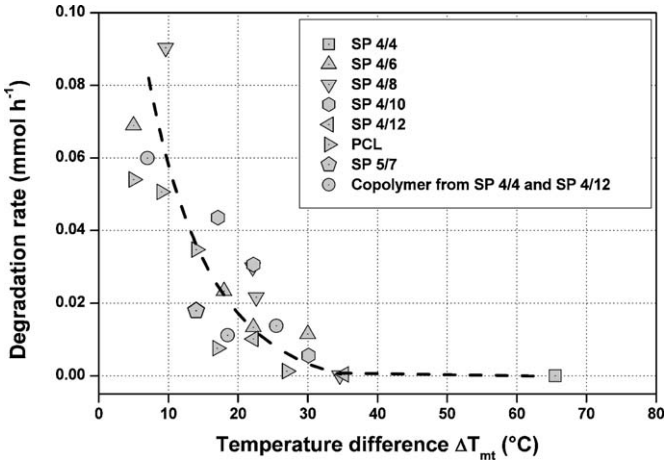

Fig. 4. Dependence of the degradation rate of aliphatic polyesters (SP refers to ''saturated polyester'') from the difference between the degradation temperature and the melting temperature of the polyester (ΔT_{mt}). Degradation with lipase from *Pseudomonas* sp. at pH 7, monitored as cleavage of ester bonds [15].

catalyses a special reaction with high efficiency. Only this characteristics makes it possible to have many different reactions running in parallel in a biological cell at the same time. Also lipases exhibit a specific behavior when cleaving esters in oils or fats with different chemical composition [7].

For polyesters the same question arose: why some polyesters could be depolymerized by lipases and why others were resistant. Many factors such as hydrophobicity of the surface, molar mass of the polymer or the chemical composition of the polymer chains have been discussed to be responsible for controlling the biodegradability of polyesters [8–11], but non of these polymer characteristics could really explain the degradation behavior of polyesters.

Generally it seemed that many polyesters composed of aliphatic monomers were degradable by lipases, while most aromatic polyesters were characterized as biologically inert. In aliphatic–aromatic copolyesters the tendency was found that biodegradability decreases with the content on aromatic constituents [4] (Fig. 3) For copolyesters composed from adipic acid, terephthalic acid and 1,4-butanediol (BTA-polymers) a maximum content of about 50–60% terephthalic acid in the diacid component was reported to be the limit for biodegradability [12,13].

It could be supposed that ester bonds in vicinity to bulky aromatic groups are less accessible for the lipases to catalyze hydrolytic cleavage due to steric hindrance and polyesters with many of such ester bonds loose degradability.

However, first systematic investigations of Marten et al. [15] showed, that a number of low molecular weight model esters containing aromatic components representing structure elements in non-degradable aromatic polyesters could be hydrolyzed by lipases. From these results it could be concluded that not the chemical structure around the ester bond itself was the major factor controlling degradability of aromatic polyesters, but a quantity related to the polymeric nature of the material. In the same work it could be demonstrated that the biodegradation rate of various aliphatic polyesters with a commercial lipase from *Pseudomonas* sp. corresponded well with ΔT_{mt} the difference between the temperature at which the degradation takes place and the melting point of the polyester (Fig. 4). Already in 1979 Tokiwa et al. observed a correlation of polyester degradability with the melting point, but did not postulate a general rule at this time.

Since the melting point of a semi-crystalline polymer is related to its crystalline fraction, ΔT_{mt} was interpreted as a measure for the ability of the polymer chains to temporarily leave the stringent order in the polymer crystals and to temporarily form a kind of loop able to penetrate into the active site of the lipase which is located at the surface of the material. ΔT_{mt} was defined as a measure for ''chain mobility'' of the polyesters.

This model could soon be extended to aliphatic-aromatic copolyesters [16] proving ΔT_{mt} the major factor ruling biodegradability of all polyesters with this type of enzymes. The final check of the model could then be made by demonstrating that also ''non-biodegradable'' aromatic polyesters could be cleaved by lipases as long as they were maintained in the amorphous state where chain mobility was not limited by crystal structures (when the glass transition temperature T_g was low enough to guarantee sufficient chain mobility in the amorphous phase, too) [17,6]. Amorphous state of the polyesters was achieved by applying polyester nano-particles with a scale of some 100 nm, retarding crystallization for many polyesters. Test with this sophisticated polymer-enzyme system could be run in a scale of seconds to some minutes opening the possibility for extended systematic investigations.

The model of chain mobility can generally describe the degradation behavior of a series of polyesters with lipases such as lipase from *Pseudomonas* sp. including the missing degradability of polyesters like PET or PBT which exhibit very high melting points above 200 °C. Biodegradability of PET seemed, according to the model presented above, not feasible at all.

4. Hydrolase from *Thermobifida fusca*—a special hydrolase?

Parallel to the development of new biodegradable aliphatic–aromatic copolyesters efforts were made to identify microorganisms responsible for the degradation of this new class of biodegradable materials, especially in a compost environment. Thermophilic actinomycetes proved to be the group of microorganisms which is very active in degrading aliphatic–aromatic copolyesters. In a screening a strain of *Thermobifida fusca* (DSM 43793; former name *Thermomonospora fusca*) was found as the most potent organism in degrading BTA—copolyesters (copolyesters from 1,4-butanediol, terephthalic acid and adipic acid) and an extracellular hydrolase (TfH) responsible for the polyester cleavage could be isolated and characterized [18,19]. The hydrolase is an induced enzyme and is only expressed by *T. fusca* if an ester-containing substance (e.g. a BTA-copolyester) is present in the medium [20]. TfH is a thermophilic enzyme (temperature optimum at 65 °C), has a molar mass of about 28 kDa and its amino acid sequence shows a high similarity to a lipase from *Streptomyces albus* (65%). TfH exhibits a active site with a highly conserved amino acid sequence -**G**-X_1-**S**-X_2-**G**- which is typical for serine hydrolases. However, degradation characteristics of TfH differs significantly from conventional lipases. While lipases need a surface activation and are only able to cleave ester bonds at a hydrophobic surface, TfH can also hydrolyze dissolved esters which is usually done by esterases. That means TfH combines lipase and esterase characteristics (Fig. 5).

All hydrolases shown in Fig. 4 are able to degrade films of the aliphatic polyester poly(butylene adipate). However, AoL and PsL are not able to cleave all ester bonds available in the material since they need a hydrophobic surface to be active. Ester bonds which are located in water-soluble intermediates cannot be hydrolyzed by these enzymes. The intermediates diffuse in the surrounding medium and do not get in contact with the enzymes located at the surface of the plastic material.

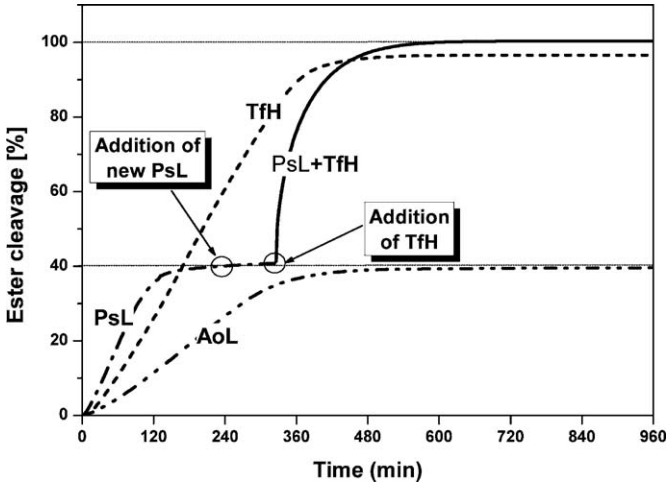

Fig. 5. Degradation of poly(butylene adipate) films with different hydrolases at 40 °C in 0.9 M NaCl solution. AoL: Lipase from *Aspergillus oryzae*, PsL: lipase *from Pseudomonas sp.*, TfH: hydrolase from *Thermobifida fusca* [19].

The addition of fresh PsL after reaching the plateau, did not induce a further ester cleavage and indicates that the incomplete ester cleavage is not a consequence of a possible enzyme deactivation with time. Both, PsL as well as AoL can only hydrolyze about 40% of the ester bonds in the polyester, demonstrating that this level is primary not depending on the enzyme but on the solubility of the intermediates. TfH, in contrast, reaches a level of almost 100% ester cleavage. The ability of TfH to cleave also dissolved esters is demonstrated by the addition of TfH to a solution were the polyester film has been totally depolymerized and solubilized by PsL before. Although no hydrophobic polymer surface is present anymore in the system, TfH cleaves the esters in the soluble intermediate totally.

Enzymes exhibiting the characteristics of TfH often are classified as cutinases in literature [21]. In deed, TfH also degrades the natural polyester cutin, but to classify TfH according to its ability to degrade the special substrate cutin seems to be questionable (e.g. the lipase PsL also exhibits catalytic activity against cutin). It is more feasible that in the group of hydrolases a continuous shift from pure lipases to pure esterases exists and TfH is located somewhere in the middle.

Recently TfH could successfully be expressed in recombinant *Escherichia coli*, purified and crystallized (publication of data in preparation). From its degradation behavior as well from its structure it seems to differ significantly from usual lipases making it a interesting enzyme for potential enzymatic treatment of polyesters.

5. Degradation of aromatic polyesters by *T. fusca* hydrolase

As mentioned above, aromatic polyesters usually are regarded as biologically inert. However, recently commercial PET from soft drink bottles could be depolymerized by the hydrolase from *T. fusca* (TfH) to an extent and at a rate which may open the door for a future biological recycling of such polyesters [22].

While lipases from *Candida antarctica* and *Pseudomonas* sp. did not significantly attack the PET films used in these experiments, TfH at a temperature of 55 °C causes erosion rates up to 17 μm/week—a 100 μm film would totally dissolve within 3 weeks. Compared to conventional chemical hydrolysis of such polyesters, which usually are performed at higher temperatures of some hundred degrees [23], enzymatic depolymerization with TfH probably would result in less side products and thus, higher purity of the monomers for following re-polymerization processes.

The surprising degradation of the commercial aromatic polyester PET is made possible by several factors. At first the degradation temperature of 55 °C, at which the thermophilic TfH is active and stable over a period of time sufficient for polyester degradation, leads to a somewhat smaller ΔT_{mt} and thus, to higher chain flexibility in the PET crystal domains. However, the difference of degradation temperature and melting temperature is still very high, resulting usually in a quite low degradation rate. The significant degradation

observed could only be achieved, since the crystallinity of the PET was low (estimated to be below 10%). The overall degradation rate depends on the rate limiting degradation rate of the crystalline domains (controlled by ΔT_{mt}) and additionally on the amount of crystals in the plastics, characterized as its crystallinity. As a consequence, enzymatic depolymerization on a technical scale seems only be feasible for low crystallinity PET. Such materials are commercially used in a large scale especially for beverage bottles because of their high transparency.

However, enzymes like TfH may not only be applicable as biocatalysts in polyester recycling, but also are of great interest for polyester surface modification, e.g. especially for fibers (enhancing hydrophylicity, Denier reduction, etc.) [24,25], where only a partial surface erosion in a scale of some nanometers is intended. TfH, as a robust and highly active enzyme in degrading aromatic polyesters is supposed to be of high commercial interest for environmentally friendly treatments of several polyesters.

6. Conclusion

Basically, the enzymatic depolymerization of polyesters and the ester cleavage of low molecular weight esters are ruled by different factors. The necessity of long polymer chains to penetrate into the active site of hydrolases which is often located in a more or less deep cavity makes the mobility of the polymers chains the most important factor controlling polyester degradability. Since this mobility is in principle correlated with the melting point of the material, it seems unlikely ever to be able to degrade high melting polyesters such as PET at a reasonable rate.

However the results obtained with the hydrolase from *T. fusca* indicate that also aromatic polyesters with melting points above 200 °C can be enzymatically depolymerized. From the preliminary data available on the three dimensional structure of TfH it can be supposed that the cavity of the active site of TfH is not so deep and protected as for other lipases. This structure leads to a lower demand on the polyester chain mobility and thus to higher degradation rates of high melting polyesters.

With the detailed knowledge of the TfH structure and the comparison with other lipases it might be possible to identify the crucial structural elements to enable an enzyme also to depolymerize polymers such as PET or PBT at technical relevant rates. Protein design may than open the door for enzymatic catalyzed PET or PBT recycling on a technical scale in the future.

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