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Mechanism and kinetics of the enzymatic hydrolysis of polyester nanoparticles by lipases

K. Herzog, R.-J. Müller*, W.-D. Deckwer

Gesellschaft für Biotechnologische Forschung (GBF) mbH, TU-BCE, Mascheroder Weg 1, D-38124 Braunschweig, Germany

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Abstract

The degradation of several aliphatic and aromatic polyesters with lipases from *Candida cylindracea* (CcL) and *Pseudomonas* species (PsL) was investigated applying nanoparticles of the polymers. Nanoparticles (diameters 50 nm to 250 nm) of a particle concentration up to 6 mg/ml could be prepared by a precipitation technique without adding any stabilizing agents in the aqueous solutions. Using a titration system to monitor ester cleavage, enzymatic degradation experiments could be performed in the time scale of some minutes. A kinetic model is proposed which is based on a surface erosion process dependent on molar ester bond density and enzyme loading. Experimental evidence provided that degradation of the particles occurs uniformly at the surface after a Langmuir type adsorption of the enzyme. Rate constants and the maximal enzyme loadings of enzyme were estimated from the kinetic model for different polyesters and the rate constants correlate well with the length of the diacid component of the polyester. Comparison of degradation rates of polyester films and nanoparticles revealed that nanoparticles of aliphatic polyesters are in the amorphous state. Hence, differences of the rate constants reflect the direct influence of the polymer structure on the enzymatic hydrolysis not overlaid by effects of crystallinity.

Keywords: Biodegradable polyesters; Lipases; Kinetics; Nanoparticles

1. Introduction

Polymeric materials have gained a wide influence on our every-day life due to their excellent mechanical and thermal properties. In the first instance the high stability of the polymers made them so important. However, this stability becomes more and more a problem. Especially "short live" products like packaging materials encumber the environment. Biodegradable polymers could be a useful alternative for such applications as they could be disposed to the municipal biowaste composting plants. One essential prerequisite prior to the disposal of these plastics is the evaluation of their biodegradability. In order to prove whether or not a plastic is biodegradable, a fast and reliable test system is essential [1]. Field tests as well as simulation tests (e.g. in water, soil or compost) reflect natural degradation conditions but they are time consuming (up to months and years) and the scope of analytical monitoring is quite limited (e.g. only visual observations or weight loss) in these complex and undefined environments. In contrast, defined laboratory tests e.g. with known microorganisms allow the precise measurement of degradation phenomena in an acceptable time scale. Both type of test methods are used for the evaluation of biodegradability in standardized tests (e.g. from DIN, CEN, ASTM or ISO).

However, especially for extended systematic investigations e.g. to correlate biodegradability with structural properties of the polymeric material, much faster and simpler tests than for instance the Sturm-test (detection of CO_2 during the degradation process) are necessary. Instead tests with isolated polymer degrading enzymes, e.g. hydrolases for degradable polyesters can be used. Usually plastics are not water soluble and thus are not directly bio-available for microorganisms.

* Corresponding author. Tel.: +49 5316181610; fax: +49 531 6181175.
E-mail address: rmu@gbf.de (R.-J. Müller).

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Therefore extracellular enzymes are secreted by the organisms performing the first step in the degradation process.

In this case, enzymes (PHB-depolymerases, lipases) catalyse the hydrolysis of the ester bonds in a polyester, thus leading to a decrease in the chain length and finally ending up in water soluble intermediates. These can be transported into microbial cells and metabolized there. The first step, the depolymerization of the polymers, is often rate limiting in the whole biodegradation process.

First investigations on the enzymatic attack on synthetic polyesters with lipases were done already by Tokiwa and Suzuki [2]. Detailed and systematic studies on the influence of polyester-specific parameters on the biodegradation with lipases were reported by Marten et al. [3,4]. However, even using enzymes under optimised conditions, test with polyester films need hours to some days for one experiment.

Since, biodegradation of plastics is a surface erosion process and the degradation rate depends on the surface area of the polymer, it should be feasible to accelerate the degradation rates in enzyme tests by increasing the surface area e.g. by using polyester nanoparticles.

First experiments concerning the enzymatic degradation of polyester nanoparticles were published by Wu and co-workers [5–8]. They investigated the enzymatic degradation of poly(ε-caprolactone) nanoparticles with a lipase of *Pseudomonas cepacia* following the degradation process using laser light scattering. An increase of the degradation rate of about a factor of 1000 compared to a polymer film was achieved. However, from the results reported in theses papers it is not possible to gain detailed information about the degradation process and the kinetics of enzymatic degradation of nanoparticles since only limited information about the influence of the polyester surface area and the enzyme concentration can be extracted from these experiments.

The intention of this work is to describe in detail and quantitatively the kinetics and the mechanism of the enzymatic hydrolysis of polyester nanoparticles. Particles with different and known diameters were hydrolysed by lipases to investigate the influence of the particle diameter and the polymer surface on the degradation rates. As surfactants influence the enzymatic degradation [6,9,10] the nanoparticles used in this work were prepared without any surfactants, in contrast to the experiments performed by Wu and co-workers. As test system a titration method was used, which directly monitors the cleavage of the ester bonds. For comparison the degradation of polyester films and larger particles was also studied.

2. Materials und methods

2.1. Preparation and characterization of the polyester

The polyesters were prepared by the method described by Witt et al. [11]. The relative molecular weights of the polyesters were measured by gel permeation chromatography (GPC) using polystyrene standards for calibration. The measurements were carried out at room temperature with chloroform as an eluant at a flow rate of 1 ml/min. The thermal properties of the polyester (melting point T_m, melt enthalpy ΔH_m) were studied by differential scanning calorimetry (DSC, Mettler Toledo DSC 12 E, Mettler Toledo, Switzerland). The results are listed in Table 1.

2.2. Preparation of polyester films

Polyester films were made with a hydraulic press (Perkin–Elmer, Germany) using elevated temperatures and appropriate distance rings which determine the film thickness. The pressing temperature was always set to 5 °C below the melting point of the polyester.

As the polyester films should be used at test temperatures also higher than the melting point of the materials, the films were pressed on a filter paper (Schleicher und Schüll, Dassel, Germany) as shape-stabilizing matrix. Defined circular films (diameter 0.9 cm) were punched out from the pressed films.

2.3. Preparation of polyester spheres

To prepare polyester spheres with defined diameters, 2 g of the polyester were dissolved in 50 ml of acetone. This solution was dropped into a 2000 ml beaker filled with distilled water without stirring. The polyester spheres obtained were filtered using a sieve with a mesh size of 50 μm (Retsch, Haan, Germany) and dried for 48 h at room temperature in the vacuum. Afterwards the spheres were separated into different fractions using appropriate sieves with varying mesh sizes (50–1000 μm).

2.4. Preparation and characterization of polyester nanoparticles

All polyester nanoparticles were prepared by a precipitation technique. Polyester (50 mg) was dissolved in 5 ml of acetone

Table 1
Characteristic data of polyesters used

Polyester	Monomers		M_w (g mol^{-1})	M_n (g mol^{-1})	T_m (°C)	ΔH_m (J g^{-1})
	Diole	Dicarbonic acid				
SP3/6	1,3-Propanediol	Adipic acid	20,800	11,000	42	53
SP3/10	1,3-Propanediol	Sebacic acid	15,400	7800	55	67
SP3/12	1,3-Propanediol	Dodecanic acid	16,200	7200	65	93
SP4/4	1,4-Butanediol	Succinic acid	41,000	23,500	114	72
SP4/6	1,4-Butanediol	Adipic acid	54,500	26,600	56	66
SP4/8	1,4-Butanediol	Suberic acid	39,700	19,600	60	80
SP4/10	1,4-Butanediol	Sebacic acid	52,200	23,400	66	89
SP4/12	1,4-Butanediol	Dodecanic acid	33,700	21,700	73	90
SP5/6	1,5-Pentanediol	Adipic acid	40,400	21,700	42	60
SP5/7	1,5-Pentanediol	Pimelic acid	37,600	15,000	42	69
SP6/4	1,6-Hexanediol	Succinic acid	19,700	8600	51	77
SP6/6	1,6-Hexanediol	Adipic acid	28,000	12,400	58	89
PCL	ε-Caprolactone		50,000	n.d.[a]	60	n.d.[a]
PBI	1,4-Butanediol	Isophthalic acid	23,500	13,200	147	45
PPeT	1,5-Pentanediol	Terephthalic acid	22,200	10,800	130	40

[a] Not determined.