



Kinetics of Enzyme Biodegradation of New Synthesized Copolymers

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Received: December 21, 2004

Accepted: April 19, 2004

Published: April 28, 2005

Abstract: Block copolymers of the poly-(hexanlactam)-co-block-poly-(δ -valerolactone) from ABA-type were synthesized via anionic polymerization of hexanlactam (HL) with the sodium salt of hexanlactam (Na-HL) as an initiator and polymeric activator (PAC). PAC, on the base of poly- δ -valerolactone (PVL), was used as a soft central block. Synthetic PVL is very attractive biomaterial – nontoxic, biocompatibility and biodegradable polyester^[5-8]. Modification of HL with PVL, renders these system biodegradable¹. Isolated copolymers were characterized by various spectroscopic techniques. The effect of the chemical and physical structure of the synthesized block copolymers on the biodegradation was investigated. Biodegradation of block copolyester amides was studied by means of lipase and involves the enzymatic hydrolysis of ester groups in PVL.

Keywords: Biodegradation, Lipase, Polyester amides, Polylactones

Introduction

Biodegradable polymers are subject of special interests of environmental, industrial and academic researchers [3, 4]. The biodegradable polymers could be degraded by several microbial strains. As it is well known the aliphatic polyesters are very important class of biodegradable polymers and they are widely investigated.

The ABA copolymers of poly-(hexanlactam)-co-block-poly-(δ -valerolactone), with flexible aliphatic polyester PVL as a central B-block were synthesized.

The aim of the present work was to study the kinetics of enzyme treatment of synthesized copolymers of poly-(hexanlactam)-co-block-poly-(δ -valerolactone) with lipase from *Rhizopus arrizus*.

Experimental part

Materials

As a starting material was used hexanlactam (BASF), dried in a desiccator over P₂O₅ at 60 °C in vacuum, for 3 days. The initiator Na-HL, which we utilize were synthesized and purified in advance. Bifunctional telechelic PVL was performed by a living anionic polymerization of δ -valerolactone with number average molecular weight [M_n of 3200 g/mol].



Lipase [E.C], isolated from *Rhizopus arrizus* was supplied by the plant of microbial preparation in Biovet LTD. (Peshtera, Bulgaria).

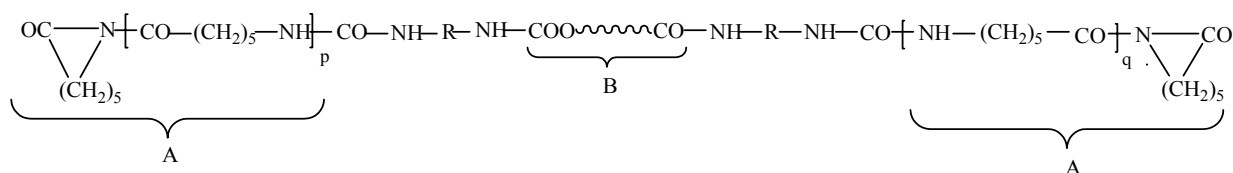
The activity of the lipase was determined using olive oil as a substrate, according [5, 2]. The residual activities of the enzyme was determined in water solutions (from the range of pH=7.4÷10 at 25°C and for the temperature range of 20÷50 °C at pH=8.0 and titrated with 0.1N NaOH.

The kinetic study was carried out at different polymer concentrations in the range 80÷170 mg in 15 cm³.

The ¹H-NMR spectra of the HL/PVL block copolymers were obtained on a Bruker apparatus, operating at 400 MHz. The samples were dissolved in a mixture of solution of HCOOH/CDCl₃=3:1. In addition HCOOH was used as an internal standard.

Results and discussion

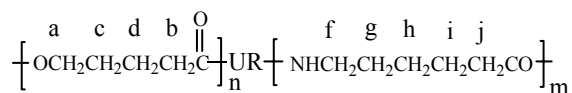
A-B-A-type copolymers of poly-(hexanlactam)-co-block-poly-(δ-valerolactone) were synthesized via bulk anionic polymerization of HL with the Na-HL as an initiator and PAC. PAC on the base of PVL was used as soft central block. The chemical structure of the synthesized ABA copolymers is demonstrate on /Scheme 1/.



Scheme 1.

The isolated polymers were characterized by H-NMR spectroscopy (Fig. 1).

The ¹H NMR spectra confirm that the PACs are incorporated in the polymer chain. All characteristics for poly-(hexanlactam) (PHL) and PVL /Scheme 2/ are observed. The peaks at h- 1.38ppm, i-1.57ppm, g-1,97ppm, j-2.40ppm, f-3.30ppm, NH-CO-7,46ppm were characterized the PHL, the peaks at a-3.90ppm, b-2.30ppm, c,d-1.69ppm PVL segments.



Scheme 2.

The copolymer chains included the ester bonds of PVL which are responsible for biodegradation. The enzyme activity was established toward new synthesized block copolymers and was compared with the activity toward olive oil Table 1.

Table 1.

Specific activity toward olive oil, [U/mg]	Specific activity toward block copolymers, [U/mg]
1.35	1.08

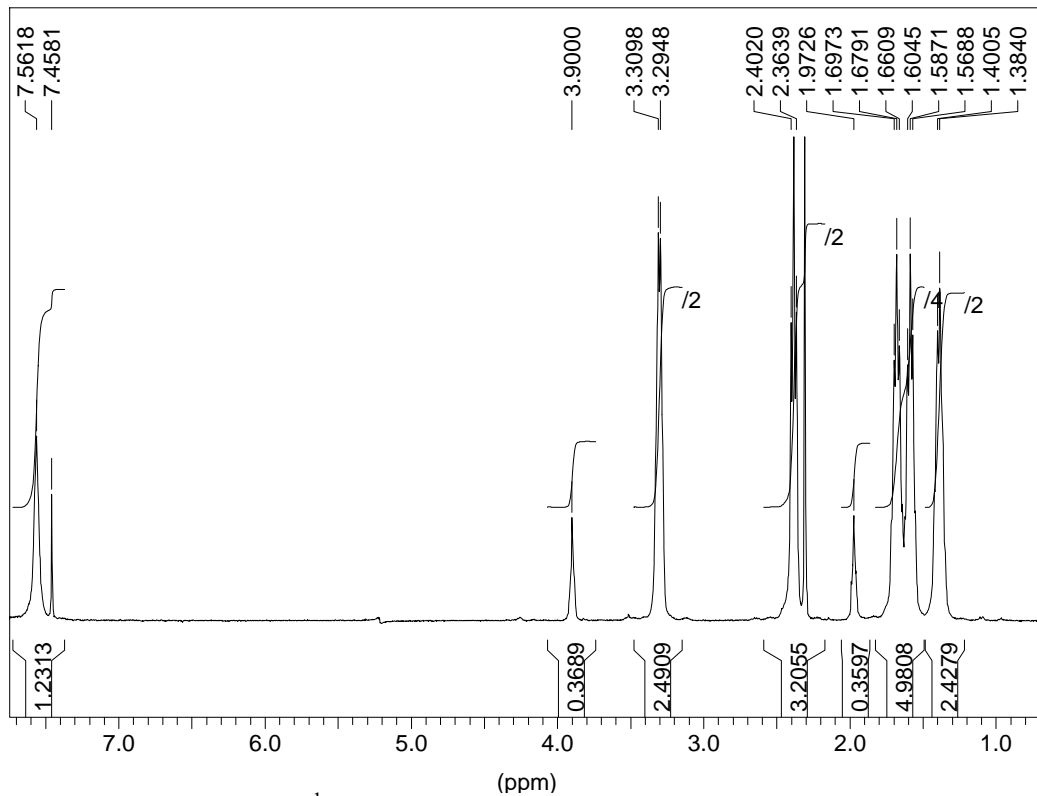


Fig. 1 ^1H NMR spectra of N-6/PVL copolymer

As shown on the table the enzyme manifested very little change in the activity. The results can be interpreted on the base of copolymers chemical structure and the relatively low number ester bonds.

The temperature optimum for the enzyme toward block copolymer substrate is found and the results are shown on Fig. 2.

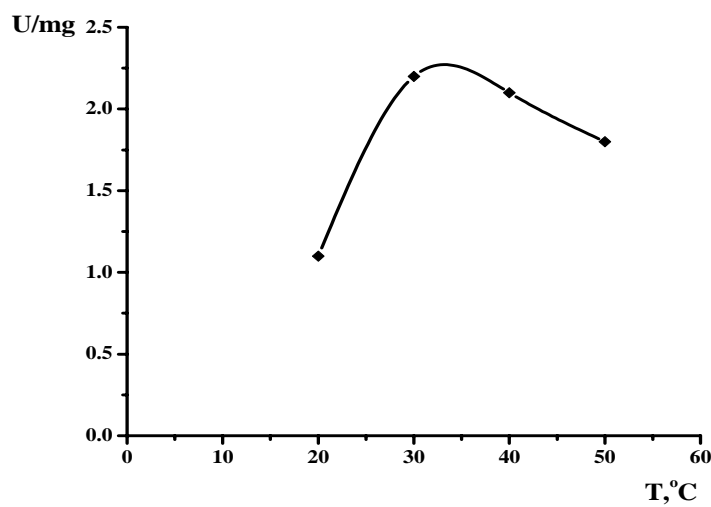


Fig. 2 The enzyme activity toward block copolymer substrate vs. different temperature

The enzyme was characterized with the respect to its pH maximum (Fig. 3).

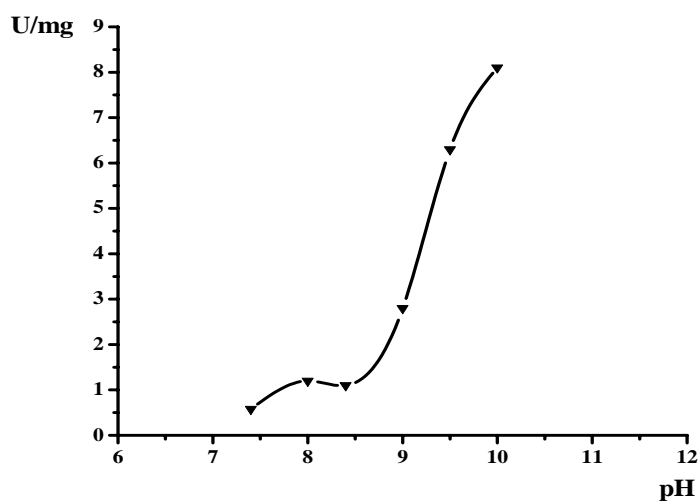


Fig. 3 The enzyme activity toward block copolymer substrate vs. different pH

It can be seen from the figures the enzyme has demonstrated its maximum activity at pH=8 and $T=30^{\circ}\text{C}$. The higher activity at pH>9 is probably due to problems to the stability of the copolymers.

The data reviews that the highest degree of hydrolyses of the ester bonds in copolymer was obtain at different time of incubation and 150 mg synthetic substrate in the reaction mixture Fig. 4.

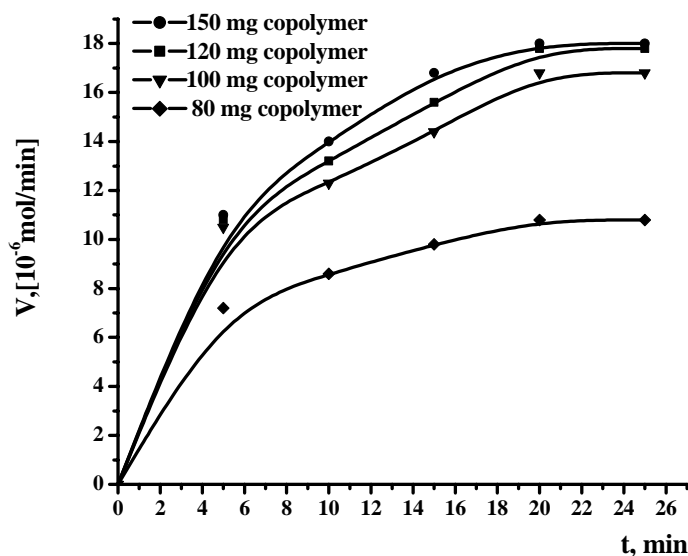


Fig. 4 V versus time (t) at different substrate concentration

Conclusions:

The results from our experiments show that the proposed method of treatment by block copolymers containing ester bonds is possible for the future application.



This method opens up prospect for various processes of bioconversion of over similar synthetic polymers.

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