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KINETICS AND MECHANISM OF POLY(3-HYDROXYBUTYRATE) DEGRADATION

Keywords: Bacterial poly(3-hydroxybutyrate), copolymer PHBV, polylactide, PHB-PLA blend, non-enzymatic hydrolysis, role of molecular weight, crystallinity, total weight loss, WAXS, AMF.

This work is designed to be an informative source for biodegradable poly(3-hydroxybutyrate) and its derivatives' research. We focuses on hydrolytic degradation kinetics at 37 and 70°C in phosphate buffer to compare PLA and PHB kinetic profiles. Besides, we reveal the kinetic behavior for copolymer PHBV (20% of 3-hydroxyvalerate) and the blend PHB-PLA. The intensity of biopolymer hydrolysis characterized by total weight lost and the viscosity-averaged molecular weight (MW) decrement. The degradation is enhanced in the series PHBV < PHB < PHB-PLA blend < PLA. Characterization of PHB and PHBV includes MW and crystallinity evolution (x-ray diffraction) as well as AFM analysis of PHB film surfaces before and after aggressive medium exposition. The important impact of MW on the biopolymer hydrolysis is shown.

Ключевые слова: бактериальный поли(3-гидроксибутират), сополимер ПГБВ, полилактид, смесь ПГБ-ПЛЛ, без-ферментативный гидролиз, роль молекулярной массы, кристалличность, общая потеря массы, метод WAXS, АСМ.

Работа посвящена биоразлагаемому поли(3-гидроксибутирату) и исследованию его производных. Исследована кинетика гидролитического разложения при 37 и 70°C в фосфатном буфере для сравнения кинетических профилей ПЛЛ и ПГБ. Кроме того, изучена кинетика для сополимера ПГБВ (20% 3-гидроксивалерата) и смеси ПГБ-ПЛЛ. Интенсивность гидролиза биополимера характеризуется общей потерей массы и уменьшением усредненной молекулярной массы (ММ). Разложение усиливается в серии ПГБВ <ПГБ< смесь ПГБ-ПЛЛ <PLA. Характеристики ПГБ и ПГБВ включают ММ и изменение кристалличности (дифракции рентгеновских лучей), а также АСМ-анализ поверхности ПГБ-пленок до и после экспозиции в агрессивной среде. Показано влияние ММ на гидролиз биополимера.

Introduction

The bacterial polyhydroxyalkanoates (**PHA**)s and their principal representative - poly(3-R-hydroxybutyrate) (PHB) create a competitive option to conventional synthetic polymers such as polypropylene, polyethylene, polyesters et al. These polymers are nontoxic and renewable. Their biotechnology output does not depend on hydrocarbon production as well as their biodegradation intermediates and resulting products (water and carbon dioxide) do not provoke the adverse actions in environmental media or living systems [1-3]. Being friendly environmental [4], the PHB and its derivatives are used as the alternative packaging materials, which are biodegradable in the soil or different humid media [5,6].

The copolymerization of 3-hydroxybutyrate entities with 3-hydroxyoctanoate (HO), 3-hydroxyheptanoate (HH) or 3-hydroxyvalerate (HV) monomers modifies the physical and mechanical characteristics of the parent PHB, such as ductility and toughness to depress its processing temperature and embrittlement. Besides, copolymers PHB-HV [7], PHB-HH [8] or PHB-HO [9] et al. have improved thermophysical and/or mechanical properties and hence they expand the spectrum of constructional and medical materials/items. For predicting the behavior of PHB and its copolymers in a aqueous media e.g. in vitro, in a living body or in a wet soil, it is essential to study kinetics and mechanism of hydrolytic destruction.

Despite the history of such-like investigations reckons about 25 years, the problem of (bio)degradation in semicrystalline biopolymers is too far from a final resolution. Moreover, in the literature the description of hydrolytic degradation kinetics during long-term period is comparatively uncommon [10-14]. Therefore, the main object of this paper is the comparison of long-term degradation kinetics for the PLA, PHB and its derivatives, namely its copolymer with 3-oxyvalerate (PHBV) and the blend PHB/PLA. The contrast between degradation profiles for PHB and PLA makes possible to compare the degradation behavior for two most prevalent biodegradable polymers. Besides, a significant attention is devoted to the impact of molecular weight (MW) for above polymer systems upon hydrolytic degradation and morphology (crystallinity and surface roughness) at physiological (37°C) and elevated (70°C) temperatures.

Experimental

Materials

In this work we have used poly-L-lactide (PLA) with different molecular weights: 67, 152, and 400 kDa (Fluka Germany); chloroform (ZAO EKOS-1, RF), sodium valerate (Sigma-Aldrich, USA), and mono-substituted sodium phosphate (NaH_2PO_4, ChimMed, RF).

PHAs production

The samples of PHB and copolymer of hydroxybutyrate and hydroxyvalerate (PHBV) have been produced in A.N.Bach's Institue of Biochemistry. A highly efficient strain-producer (80 wt.% PHB in the dry weght of cells), *Azotobacter chroococcum 7*Б, has been isolated from rhizosphere of wheat (the sod-podzol soil). Details of PHB biosynthesis have been published in [15]. Under conditions of PHBV synthesis, the sucrose concentration was decreased till 30 g/L in

medium and, after 10 h incubation, 20mM sodium valerate was added. Isolation and purification of the biopolymers were performed via centrifugation, washing and drying at 60°C subsequently. Chloroform extraction of BPHB or BPHBV from the dry biomass and precipitation, filtration, washing again and drying have been described in our previous work [15]. The monomer-content (HB/HV ratio) in PHBV has been determined by nuclear magnetic resonance in accordance with procedure described previously in [16]]. The percent concentration of HV moiety in the copolymer was calculated as the ratio between the integral intensity of methyl group of HV (0,89 ppm) and total integral intensity the same group and HB group (1,27 ppm). This value is 21 mol.%.

Molecular weight determination.

The viscosity-averaged molecular weight (MW) was determined by the viscosity (η) measurement in chloroform solution at 30°C. The calculations of MW have been made in accordance with Mark-Houwink equation [17]:

$$[\eta] = 7{,}7 \cdot 10^{-5} \cdot M^{0{,}82}$$

Film preparations of PHAs, PLA and their blends

The films of parent polymers (PHB, PHBV and PLA) and their blends with the thickness about 40μm were cast on a fat-free glass surface. We obtained the set of films with different MW = 169±9 (defined as PHB 170), 349±12 (defined as PHB 350), 510±15 kDa (defined as PHB 500) and 950±25 kDa (defined as PHB 1000) as well as the copolymer PHBV with MW=1056±27 kDa (defined as PHBV). Additionally we prepared the set of films on the base of PLA with same thickness 40μm and MW=67 (defined as PLA 70), MW=150 and 400kDa. Along with them we obtained the blend PHB/PLA with weight ratio 1:1 and MW = 950 kDa for PHB, and MW = 67 kDa for PLA (defined as PHB+PLA blend). Both components mixed and dissolved in common solvent, chloroform and then cast conventionally on the glass plate. All films were thoroughly vacuum-processed for removing of solvent at 40°C.

Hydrolytic degradation in vitro experiments

Measurement of hydrolytic destruction of the PHB, PLA, PHBV films and the PHB-PLA composite was performed as follows. The films were incubated in 15 ml 25 mM phosphate buffer, pH 7.4, at 37°C or 70°C in a ES 1/80 thermostat (SPU, Russia) for 91 days; pH was controlled using an Orion 420+ pH-meter (Thermo Electron Corporation, USA). For polymer weight measurements films were taken from the buffer solution every three day, dried, placed into a thermostat for 1 h at 40°C and then weighed with a balance. The film samples weighed 50–70 mg each. The loss of polymer weight due to degradation was determined gravimetrically using a AL-64 balance (Acculab, USA). Every three days the buffer was replaced by the fresh one.

Wide angle X-ray diffraction

The PHB and PHBV chemical structure, the type of crystal lattice and crystallinity was analyzed by wide angle X-ray scattering (WAXS) technique. X-ray scattering study was performed on device on the basis of 12 kW generator with rotating copper anode RU-200 Rotaflex (Rigaku, Japan) using CuK radiation (wavelength λ = 0.1542 nm) operated at 40 kV and 140 mA. To obtain pictures of wide angle X-ray diffraction of polymers two-dimentional position-sensitive X-ray detector GADDS (Bruker AXS, Germany) with flat graphite monochromator installed on the primary beam was used. Collimator diameter was 0.5 mm [18]

Atomic force microscopy of PHB films

Microphotographs of the surface of PHB films were obtained be means of atomic force microscopy (AFM). The AFM imaging was performed with Solver PRO-M (Zelenograd, Russia). For AFM imaging a piece of the PHB film (~2 × 2 mm^2) was fixed on a sample holder by double-side adhesive tape. Silicon cantilevers NSG11 (NT-MDT, Russia) with typical spring constant of 5.1 N/m were used. The images were recorded in semi-contact mode, scanning frequency of 1—3 Hz, scanning areas from 3 × 3 to 20 × 20 μm^2, topography and phase signals were captured during each scan. The images were captured with 512x512 pixels. Image processing was carried out using Image Analysis (NT-MDT, Russia) and FemtoScan Online (Advanced technologies center) software.

Results and discussion

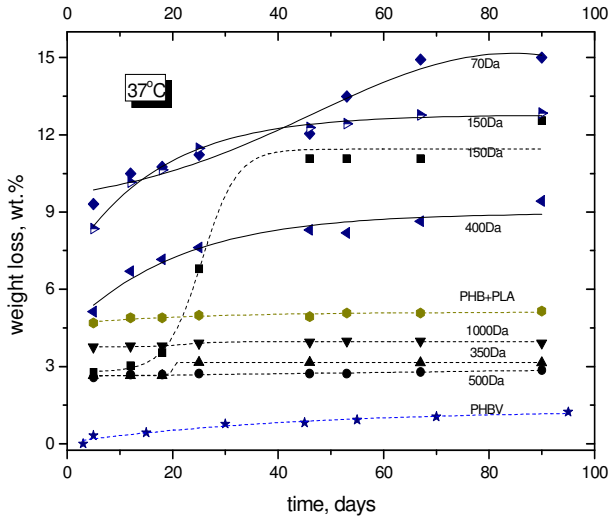
The in vitro degradation of PHB with different molecular weight (MW) and its derivatives (PHBV, blend PHB/PLA) prepared as films was observed by the changes of total weight loss, MW, and morphologies (AFM, XRD) during the period of 91 days.

1. The hydrolysis kinetics of PLA, PHB, and its derivatives

The hydrolytic degradation of the biopolymer and the derivatives (the copolymer PHBV, and the blend PHB/PLA 1:1) has been monitored for 3 months under condition, which is realistically approximated to physiological conditions, namely, *in vitro*: phosphate buffer, pH=7.4, temperature 37°C. The analysis of kinetic curves for all samples shows that the highest rate of weight loss is observed for PLA with the smallest MW ≈ 70 kDa and for PHB with relatively low MW ≈ 150 kDa (Fig.1). On the base of the data in this figure it is possible to compare the weight-loss increment for the polymers with different initial MW. Here, we clearly see that the samples with the higher MWs (300 - 1000 кДа) are much stabler against hydrolytic degradation than the samples of the lowest MW. The total weight of PHB films with MW=150 kDa decreases faster compared to the weight reduction of the other PHB samples with higher MW's = 300 and 450 or 1000 kDa. Additionally, by the 91st day of buffer exposition the residual weight of the low-MW sample reaches 10,5% weight loss that it is essentially higher than the weight loss for the other PHB samples (see Fig.1 again).

After establishing the impact of MW upon the hydrolysis, we have compared the weight-loss kinetic curves for PLA and PHB films with the relatively comparative MW = 400 and 350 kDa respectively and the same film thickness. For the PLA films one can see the

weight depletion with the higher rate than the analogous samples of PHB. The results obtained here are in line with the preceding literature data [8,12,19-21].



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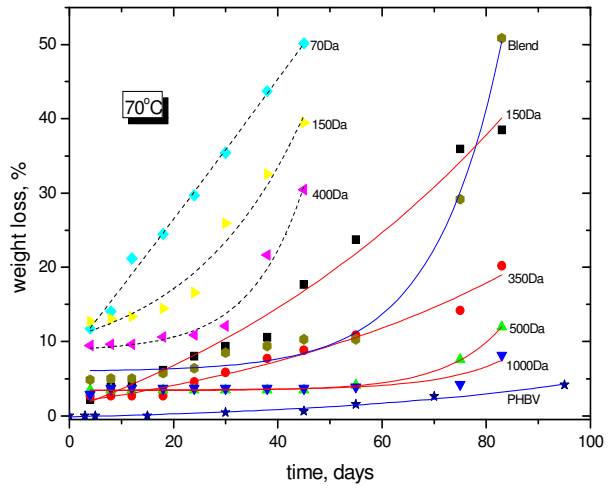

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Fig. 1 - Weight loss in the phosphate buffer for PHB and its derivatives with different MW (shown on the curves in kDa). 37°C , 70°C: ♦,▶, and◀ are PLA films with MW=70, 150, and 400kDa respectively; ■,▲,●, and ▼ are PHB samples with 170, 350, 500, and 1000 kDa, respectively; PHBV 1050 (★); and PHB-PLA blend (⬢)

Having compare destruction behavior of the homopolymer PHB and the copolymer PHBV, we can see that the introduction of hydrophobic entity (HV) into the PHB molecule via copolymerization reveals the hydrolytic stability of PHBV molecules. For PHBV an hydrolysis induction time is the longest among the other polymer systems and over a period of 70 days its weight loss is minimal (<1% wt) and possibly related with desorption of low-molecular fraction of PHBV presented initially in the samples after biosynthesis and isolation. The kinetic curves in Fig.1 show also that the conversion the parent polymers to their blend PHB-PLA decreases the hydrolysis rate compared to PHB (MW=1000 kDa) even if the second component is a readily hydrolysable polymer: PLA (MW=70 kDa).

For the sake of hydrolysis amplification and its exploration simultaneously, an polymer exposition in aqueous media has usually been carried out at elevated temperature [11,19]. To find out a temperature impact on degradation and intensify this process, we have elevated the temperature in phosphate buffer to 70°C. This value of temperature is often used as the standard in other publications see e.g. [11]. As one should expect, under such condition the hydrolysis acceleration is fairly visible that is presented in Fig.1b. By the 45th day of PLA incubation its films turned into fine-grinding dust with the weight-loss equaled 50% (MW=70 kDa) or 40% (MW=350 kDa). Simultaneously the PHB with the lowest MW=170 kDa has the weight loss = 38 wt.% and the film was markedly fragmented while the PHB samples with higher MWs 350, 500 and 1000 kDa have lost the less percent of the initial weight, namely 20, 15 and 10% respectively. Additionally, for 83 days the weight drop in the PHB-PLA blend films is about 51 wt.% and, hence, hydrolytic stability of the blend polymer system is essentially declined (cf. Figures 1a and 1b).

At elevated temperature of polymer hydrolysis (70°C) as well as at physiological temperature 37°C we have demonstrated again that the PHBV films are the stablest because by 95th day they lost only 4 wt %. The enhanced stability of PHBV relative to the PHB has been confirmed by other literature data [21]. Here it is worth to remark that during biosynthesis of the PHBV two opposite effects of water sorption acting reversely each other occur. On the one side, while the methyl groups are replaced by ethyl groups, the total hydrophobicity of the copolymer is enhanced, on the other side, this replacement leads to decrease of crystallinity in the copolymer [22]. The interplay between two processes determines a total water concentration in the copolymer and hence the rate of hydrolytic degradation. Generally, in the case of PHBV copolymer (HB/HV = 4:1 mol. ratio) the hydrophobization of its chain predominates the effect of crystallinity decrease from 75% for PHB to ~60% for PHBV.

2. Change of molecular weight for PHB and PHBV

On exposure of PHB and PHBV films to buffer medium at physiological (37°C) or elevated (70°C) temperatures, we have measured both their total weight loss (Section 1) and the change of their MW simultaneously. In particular, we have shown the temperature impact on the MW decrease that will be much clear if we compare the MW decrements for the samples at 37^0 and 70^0C. At 70°C the above biopolymers have a more intensive reduction of MW compared to the reduction at 37°C (see Fig.2). In particular, at elevated temperature the initial MW (= 350 kDa) has the decrement by 7 times more than the MW decrement at physiological condition. Generally, the final MW loss is nearly proportional to the initial MW of sample that is correct especially at 70°C. As an example, after the 83-days incubation of PHB films, the initial MW= 170 kDa dropped as much as 18 wt.% and the initial MW= 350 kDa has the 9.1 wt.% decrease.

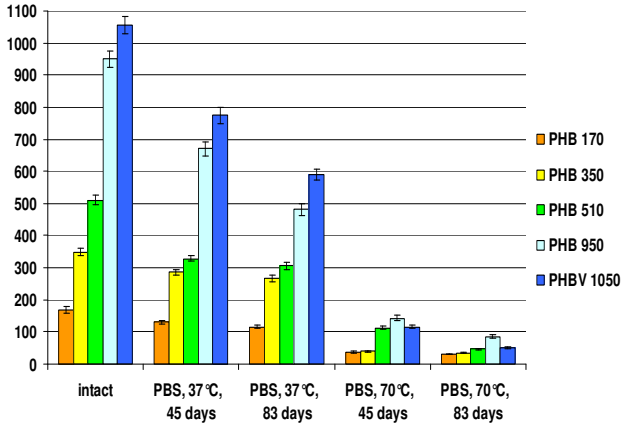

Fig. 2 - The molecular weight conversion of PHB and PHBV films during hydrolysis in phosphate buffer (PBS), pH = 7.4 , 37°C and 70°C

The diagrams in Fig.2 shows that the sharp reduction of MW takes place for the first 45 days of incubation and after this time the MW change becomes slow. Combining the weight-loss (Section 1) and the MW depletion, it is possible to present the biopolymer hydrolysis as the two-stage process. On the initial stage, the random cleavage of macromolecules and the MW decrease without an significant weight-loss occur. Within this time the mean length of PHB intermediates is fairly large and the molar ratio of the terminal hydrophilic groups to the basic functional groups in a biodegradable fragment is too small to provide the solubility in aqueous media. This situation is true for the PHB samples with middle and high MW (350, 500, and 1000 kDa) when at 37°C their total weight remains stable during all time of observation but the MW values are decreased till 76, 61, and 51 wt.% respectively. On the second stage of degradation, when the MW of the intermediate molecules attains the some "critical" value and the products of hydrolysis become hydrophilic to provide dissolution and diffusion into water medium, the weight reduction is clearly observed at 70°C. This stage is accompanied by the changes of physical-chemical, mechanical and structural characteristics and a geometry alteration. A similar 2-stage mechanism of PHB degradation has been described in the other publications [23,24]. Furthermore, in the classical work of Reush [25] she showed that hydrophilization of PHB intermediates occurs at relatively low MW namely, at several decades of kDa. Our results provide evidences that the reduction of MW till "critical" values to be equal about 30 kDa leads to the expansion of the second stage, namely, to the intensive weight loss.

3. Crystallinity of PHB and PHBV

We have above revealed that during hydrolytic degradation, PHB and PHBV show the MW reduction (Section 2) and the total weight decrease (Section 1). Additionally, by the X-ray diffraction technique (XRD) we have measured the crystallinity degree of PHB and PHBV that varied depending on time in the interval of values 60 - 80% (see Fig.3A).

We have noted that on the initial stage of polymer exposition to the aqueous buffer solution (at 37°C for 45 days) the crystallinity degree has slightly

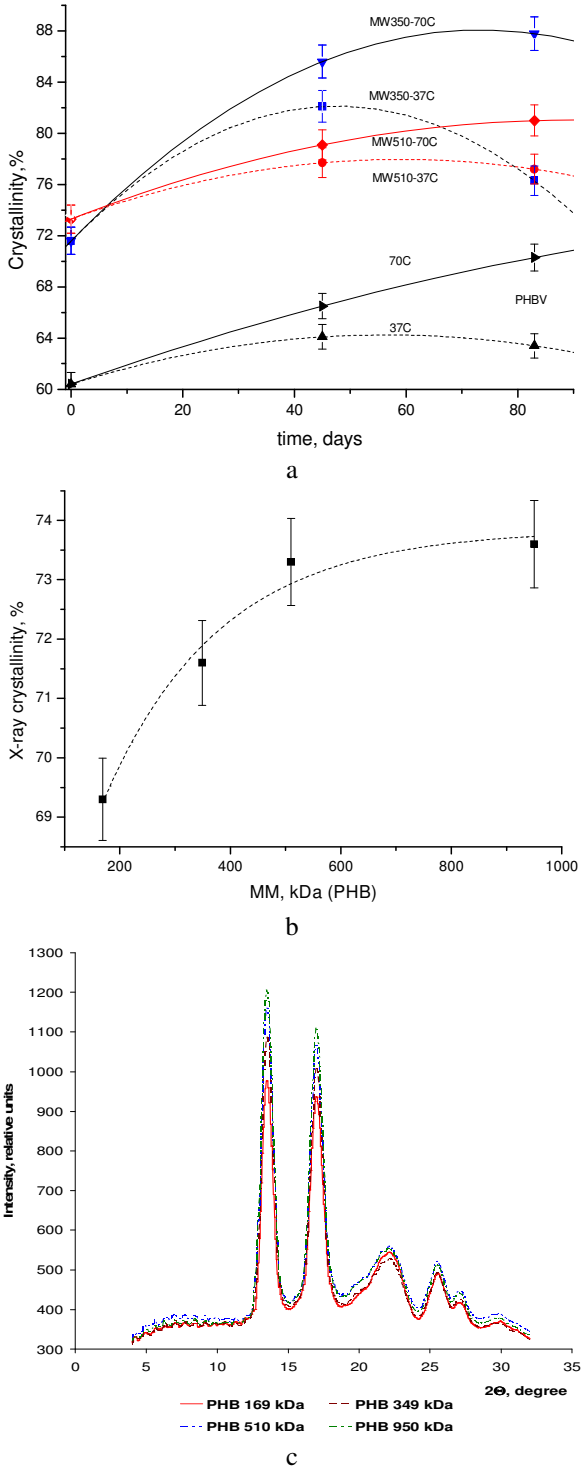

Fig. 3 - A: Crystallinity evolution during the hydrolysis for PHB and PHBV films (denoted values of temperature and MW). B: Crystallinity as function of initial MW for PHB films prepared by cast method. C: X-ray diffractograms for PHB films with different molecular weight given under x-axis

increased and then, under following exposition to the buffer, this characteristic is constant or even slightly decreased showing a weak maximum. When taken into account that at 37°C the total weight for the PHB films with MWs equal 350, 500 and 1000 kDa and the PHBV film with MW equals 1050 are invariable, a possible reason of the small increase in crystallinity is

recrystallisation described earlier for PLA [26]. Recrystallization (or additional crystallization) happens in semicrystalline polymers where the crystallite portion can increase using polymer chains in adjoining amorphous phase [22].

At higher temperature of hydrolysis, 70°C, the crystallinity increment is strongly marked and has a progressive trend. The plausible explanation of this effect includes the hydrolysis progress in amorphous area of biopolymers. It is well known that the matrices of PHB and PHBV are formed by alternative crystalline and noncrystalline regions, which determine both polymer morphologies and transport of aggressive medium. Additionally, we have revealed recently by H-D exchange FTIR technique that the functional groups in the PHB crystallites are practically not accessible to water attack. Therefore, the hydrolytic destruction and the weight decrease are predominantly developed in the amorphous part of polymer [22,27]. Hence, the crystalline fraction becomes larger through polymer fragment desorption from amorphous phase. This effect takes place under the strong aggressive conditions (70°C) and does not appear under the physiological conditions (37°C) when the samples have invariable weight. Owing to the longer lateral chains in PHBV, copolymerization modifies essentially the parent characteristics of PHB such as decreasing in crystallinity, the depression of melting and glass temperatures and, hence, enhancing ductility and improvement of processing characteristics [14,28,29]. Additionally, we have founded out that the initial crystallinity of PHB films is a monotonically increased function of initial MW (see Fig 3B). For samples with relatively low molecular weight it is difficult to compose the perfect crystalline entities because of a relatively high concentration of terminal groups performing as crystalline defects.

Thus, at physiological temperature the crystallinity, measured during degradation by XRD technique, has an slightly extreme character. On the initial stage of PHB degradation the crystalline / amorphous ratio is increased owing to additional crystallization through involvement of polymer molecules situated in amorphous fields. In contrast, at 70°C after reaching the critical MW values (see section 2), the following desorption of water-soluble intermediates occurs. On the following stage, as the degradation is developed till film disintegration, the crystallinity drop must takes place as result of crystallite disruption.

4. The analysis of film surfaces for PHB by AFM technique

Morphology and surface roughness of PHB film exposed to corrosive medium (phosphate buffer) have been studied by the AFM technique. This experiment is important for surface characterization because the state of implant surface determines not only mechanism of degradation but the protein adsorption and cell adhesion which are responsible for polymer biocompatibility [30]. As the standard sample we have used the PHB film with relatively low MW=170 kDa. The film casting procedure may lead to distinction in morphology between two surfaces when the one plane of the polymer film was adjacent with glass plate and the other one was exposed to air. Really, as it is shown in Fig. 4 the surface exposed to air has a roughness formed by a plenty of pores with the length of 500-700 nm.

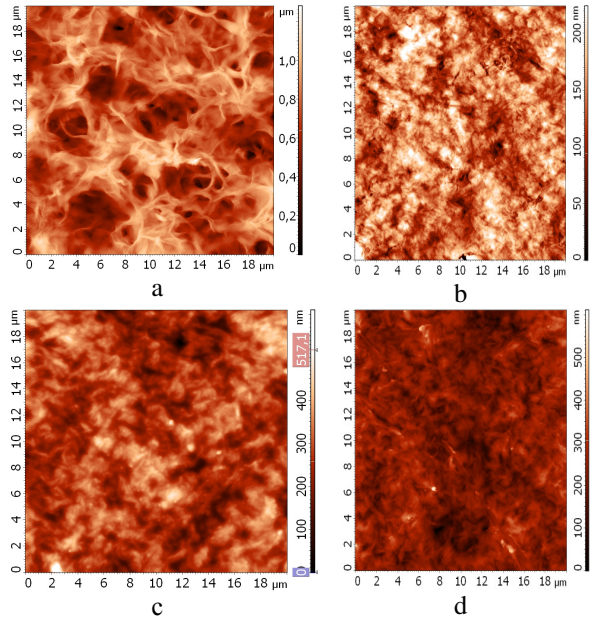

Fig.4 - AFM topographic images of PHB films (170 kDa) with a scan size of 18x18 µm: the rough surface of fresh-prepared sample (exposed to air) - a; the smooth surface of fresh-prepared sample (exposed to glass) - b; the sample exposed to phosphate buffer at 37°C for 83 days - c; the sample exposed to phosphate buffer at 70°C for 83 days - d. General magnificence is 300

The opposite side of the film contacted with glass (Fig.4B) is characterized by minor texture and by the pores with the less length as small as 100 nm. At higher magnification (here not presented) in certain localities it can see the stacks of polymer crystallites with width about 100 nm and length 500-800 nm.

Summarizing the AMF data we can conclude that during degradation the air-exposed, rough surface remained stable that probably related with the volume mechanism of degradation (V-mechanism [31,32]). The pores on the surface provide the fast water diffusion into the bulk of PHB. However, under the same environmental conditions, the change of surface porosity (roughness) for glass-exposed surface is remarkable showing the engagement of surface into degradation process (S-mechanism [31,32]). Last findings show that along with the volume processes of polymer degradation the surface hydrolysis can proceed. Several authors [20,21] have recently reported on surface mechanism of PHB destruction but traditional point of view states a volume mechanism of degradation [12]. Here, using an advanced method of surface investigation (AMF) we have shown that for the same film under the same exterior conditions the mechanism of degradation could be changed depending on the prehistory of polymer preparation.

Conclusion

Analyzing all results related with hydrolytic degradation of PHB and its derivatives, the consecutive stages of such complicated process are presented as follows. During the initial stage, the total weight is invariable and the cleavage of biomolecules resulting in the MW decrease is observed. Within this time the PHB intermediates are too large and hydrophobic to provide solubility in aqueous media. Because the PHB crystallites stay stable, the crystallinity degree is constant as well and even it may grow up due to additional crystallization. On the second stage of hydrolysis, when the MW of intermediates attain the "critical" value, which is equal about 30 kDa, these intermediates can dissolve and diffuse from the polymer into buffer. Within this period the weight loss is clearly observed. The intensity of hydrolysis characterized by the weight loss and the MW decrement is enhanced in the series PHBV < PHB < PHB-PLA blend < PLA.

The growth of initial MW (a terminal group reducing) impacts on the hydrolysis stability probably due to the increase of crystallite perfection and crystallinity degree. The XRD data reflect this trend (see Fig. 3b). Moreover, the surface state of PHB films explored by AFM technique depends on the condition of film preparation. After cast processing, there is a great difference in morphologies of PHB film surfaces exposed to air and to glass plate. It is well known that the mechanism of hydrolysis could include two consecutive processes: a) volume degradation and b) surface degradation. Under essential pore formation (in the surface layer exposed to air) the volume mechanism prevails. The smooth surface of PHB film contacted during preparation with the glass plate is degraded much intensely than the opposite rough surface (Fig.4).

In conclusion, we have revealed that the biopolymer MW determines the form of a hydrolysis profile (see Fig.1). For acceleration of this process we have to use the small MW values of PHB. In this case we affect both the degradation rate and the crystalline degree (Fig 3b). By contrast, for prolongation of service-time in a living system it is preferable to use the high-MW PHB that is the most stable polymer against hydrolytic degradation.

References

1. Sudesh, K., Abe, H., Doi, Y. Synthesis, structure and properties of polyhy-droxyalkanoates: Biological polyesters. Progress in Polymer Science (Oxford), 2000, 25 (10): 1503-1555.
2. Lenz R.W., Marchessault R.H. Bacterial Polyesters: Biosynthesis, Biodegradable Plastics and Biotechnology. Biomacromolecules, 2005, 6(1): 1-8.
3. Bonartsev A.P., Iordanskii A.L., Bonartseva G.A. and Zaikov G.E. Biodegradation and Medical Application of Microbial Poly (3-Hydroxybutyrate). Polymers Research Journal, 2008, 2(2): 127-160
4. Kadouri, D., Jurkevitch, E., Okon, Y., Castro-Sowinski, S. Critical Reviews in Microbiology 31 (2), pp. 55-67 (2005) Ecological and agricultural significance of bacterial polyhydroxyalkanoates.
5. Jendrossek D., Handrick R. Microbial degradation of polyhydroxyalkanoates. Annu Rev Microbiol. 2002; 56: 403-432.
6. Steinbuchel, A. and Lutke-Eversloh, T. Metabolic engineering and pathway construction for biotechnological production of relevant poly-hydroxyalkanoates in microorganisms. Biochem. Eng. J. 2003;16: 81–96.
7. Miller N.D, Williams D.F. On the biodegradation of poly-beta-hydroxybutyrate (PHB) homopolymer and poly-beta-hydroxybutyrate-hydroxyvalerate copolymers. Biomaterials. 1987, 8(2):129-137.
8. Qu XH, Wu Q, Zhang KY, Chen GQ. In vivo studies of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) based polymers: biodegradation and tissue reactions. Biomaterials. 2006; 27(19):3540-3548
9. L.J.R. Fostera, V. Sanguanchaipaiwonga, C.L. Gabelisha, J. Hookc, M. Stenzel. A natural-synthetic hybrid copolymer of polyhydroxyoctanoate-diethylene glycol: biosynthesis and properties. Polymer 2005; 46: 6587–6594
10. Marois, Y., Zhang, Z., Vert, M., Deng, X., Lenz, R., Guidoin, R. Mechanism and rate of degradation of polyhydroxyoctanoate films in aqueous media: A long-term in vitro study. Journal of Biomedical Materials Research, 2000; 49 (2): 216-224.
11. Freier T., Kunze C., Nischan C., Kramer S., Sternberg K., Sass M., Hopt U.T., Schmitz K.-P. In vitro and in vivo degradation studies for development of a biodegradable patchbased on poly(3-hydroxybutyrate). Biomaterials, 2002, 23; 2649–2657.
12. Doi Y, Kanesawa Y, Kawaguchi Y, Kunioka M. Hydrolytic degradation of microbial poly(hydroxyalkanoates). Makrom Chem Rapid Commun 1989;10:227–230.
13. Renstadt R, Karlsson S, Albertsson A.C. The influence of processing conditions on the properties and the degradation of poly(3-hydroxybutyrate-co-3-hydroxyvalerate). Macromol Symp. 1998; 127: 241–249.
14. Cheng Mei-Ling, Chen Po-Ya, Lan Chin-Hung, Sun Yi-Ming. Structure, mechanical properties and degradation behaviors of the electrospun fibrous blends of PHBHHx/PDLLA. Polymer 2011, doi:10.1016/j.polymer.2011.01.039 in press.
15. Myshkina V.L., Nikolaeva D.A., Makhina T.K., Bonartsev A.P., and Bonartseva G.A. Effect of Growth conditions on the Molecular weight of poly-3-hydroxybutyrate produced by Azotobacter chroococcum 7B. Applied Biochemistry and Microbiology, 2008, 44(5): 482-486.
16. Myshkina V. L., Ivanov E. A., Nikolaeva D. A., Makhina T. K., Bonartsev A. P., Filatova E. V., Ruzhitsky A. O., and Bonartseva G. A. Biosynthesis of Poly-3-Hydroxybutyrate–3-Hydroxyvalerate Copolymer by Azotobacter chroococcum Strain 7B. Applied Biochemistry and Microbiology, 2010, 46(3): 289–296.
17. Akita S., Einaga Y., Miyaki Y., Fujita H. Solution Properties of Poly(D-β-hydroxybutyrate). 1. Biosynthesis and Characterization. Macromolecules. 1976: 9: 774–780.
18. Rebrov A.V., Dubinskii V.A., Nekrasov Y.P., Bonartseva G.A., Shtamm M., Antipov E.M. [Structure phenomena at elastic deformation of highly oriented polyhydroxybutyrate. Polymer Science (Russian) 2002, 44A, 347–351
19. Koyama N. and Doi Y. Morphology and biodegradability of a binary blend of poly((R)-3-hydroxybutyric acid) and poly((R,S)-lactic acid). Can. J. Microbiol., 1995, 41(Suppl. 1): 316-322.
20. Majid M.I.A., Ismail J., Few L.L. and Tan C.F. The degradation kinetics of poly(3-hydroxybutyrate) under non-aqueous and aqueous conditions. European Polymer Journal. 2002; 38(4): 837-839.
21. Choi G.G., Kim H.W., Rhee Y.H. Enzymatic and non-enzymatic degradation of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolyesters produced by Alcaligenes sp. MT-16. The Journal of Microbiology, 2004, 42(4): 346-352.

22. Iordanskii A.L., Rudakova T.E., Zaikov G.E. Interaction of polymers with corrosive and bioactive media. 1984, VSP, New York -Tokyo
23. Wang H.T., Palmer H., Linhardt R.J., Flanagan D.R., Schmitt E. Degradation of poly(ester) microspheres. Biomaterials. 1990; 11(9):679-685.
24. Kurcok P., Kowalczuk M., Adamus G., Jedlinrski Z., Lenz R.W. Degradability of poly (b-hydroxybutyrate)s. Correlation with chemical microstucture. JMS-Pure Appl. Chem. 1995, A32: 875–880.
25. Reusch R.N. Biological complexes of poly-β-hydroxybutyrate. FEMS Microbiol. Rev., 1992, 103: 119–130.
26. Molnár K., J. Móczó, M. Murariu, Ph. Dubois, B. Pukánszky. Factors affecting the properties of PLA/CaSO4 composites: homogeneity and interactions. eXPRESS Polymer Letters Vol.3, No.1 (2009) 49–61
27. Spyros A., Kimmich R., Briese B., Jendrossek D. 1H NMR imaging study of enzymatic degradation in poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate). Evidence for preferential degradation of amorphous phase by PHB depolymerase B from Pseudomonas lemoignei. Macromolecules. 1997; (30): 8218–8225.
28. Luizier W. D. Materials derived from biomass/biodegradable materials. Proc. Natl. Acad. Sci. USA. 1992; (89): 839–842.
29. Gao Y, Kong L, Zhang L, Gong Y, Chen G, Zhao N, et al. Eur Polym J 2006; 42 (4):764-75.
30. Pompe T., Keller K., Mothes G., Nitschke M., Teese M., Zimmermann R., Werner C. Surface modification of poly(hydroxybutyrate) films to control cell-matrix adhesion. Biomaterials. 2007, 28(1): 28-37.
31. Siepmann J, Siepmann F. and Florence A.T. Local controlled drug delivery to the brain: Mathematical modeling of the underlying mass transport mechanisms. International Journal of Pharmaceutics, 2006; 314(2), 101-119.
32. Zhang T.C., Fu Y.C., Bishop P.L. et al. Transport and biodegradation of toxic organics in biofilms. Journal of Hazardous Materials, 1995; 41(2-3): 267-285.

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