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Molecular dynamics of the spin probes in dry and wet poly(3-hydroxybutyrate) films with different morphology

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Abstract

The molecular mobilities of the spin probes TEMPO and TEMPOL in microbial poly(3-hydroxybutyrate) (PHB) have been studied by the ESR method in a range of temperatures from 293 to 363 K. The spectra of the dry and wet films with isotropic and textured morphology are analyzed as a spectral superposition of the “fast-” and “slow-rotating” fractions. Nonpolar TEMPO is sensitive to structural heterogeneity and practically nonsensitive to water sorption. The polar TEMPOL can interact with the carbonyl groups of PHB and the ESR spectra are affected by water molecules. The differences of spectral characteristics between the textured and isotropic PHB films are manifested in the rotation mobility (rotation correlation times) and the mole fractions of the “fast-” and “slow-rotating” ESR probes.

Comparison of ESR data with water diffusivities in the textured and isotropic samples reveals the relationship between transport phenomena and morphology of the novel biopolymer material. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Molecular dynamics; Spin probes; Poly(3-hydroxybutyrate)

1. Introduction

Poly(3-hydroxybutyrate) (PHB)—the most important representative of bacterially synthesized polyhydroxyalkanoates [1,2] has been intensively explored because of extending fields of its applications. PHB has found applications in forms of films, fibers and construction materials as environmentally friendly packages [3,4], in agriculture for incrustation of seeds, in biotechnology and surgery under materials designing for implantation in human organism [5,6]. PHB is a biodegradable polymer [7–9], possessing thromboresistance [10,11] and is compatible with biological media [12,13]. Materials based on PHB have fields of application as surgical threats and are used for designing of drug delivery systems in medicine [14,15], in particularly, against the oncology diseases [16].

Despite much attention in the past, the problems of molecular dynamics of PHB macromolecules and its relationship with structural [17,18] and transport [19,20] features remain unsolved. However, the search for such relations presents immense potential for designing new materials with regulated transport properties. The main goal of the presented work is the investigation of molecular dynamics films with different structural organizations of PHB [21] by means of ESR. Moreover, we will try to understand how water, the main component of bioactive and corrosive media, effects the PHB molecular mobility.

2. Experimental

The films of PHB with different structural organization, textured and isotropic, were investigated. The textured films were prepared according to the following procedure: The solubility of the initial polymer powder of PHB (Biomer®) in boiling chloroform was about 10^{-2} g/ml. The soluble fraction of the initial powder of PHB was extracted with boiling chloroform using a Schott glass filter. The polymer film was cast from the extracted polymer using a 3% solution in chloroform. The solution was poured into a Petri dish, closed with a glass plate, and allowed to sand at 293 K until the solvent was evaporated.

The isotropic films of PHB were prepared by the dissolution of the initial polymer power in dioxane by heating the solution to the boiling point; a 5% solution of the polymer was prepared. After the evaporation of dioxane, the as-received polymer was dissolved in chloroform on heating and a 3% solution was obtained. This solution was filtered through the Schott glass filter, poured into the Petri dish, closed with a glass plate, and allowed to stand at 293 K until the solvent was evaporated.

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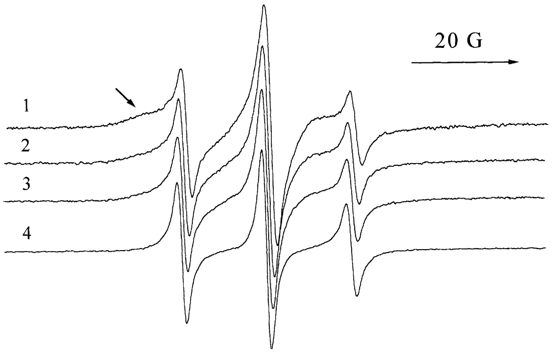

Fig. 1. ESR spectra for probe TEMPO in the textured and dry samples of PHB: (1) 296 K; (2) 313 K; (3) 333 K; (4) 353 K.

In order to remove the traces of the solvents, the films were placed into a thermostatted chamber and kept for 2–3 h at 353 K in vacuum. The completion of solvent evaporation was controlled by means of FTIR spectroscopy. The FTIR spectra of the films were recorded with the FTIR spectrometer IFS-48 Bruker at a resolution of $2\ \mathrm{cm}^{-1}$. During the evaporation procedure we observed a decrease in the intensity of the following bands for the PHB samples: at 873–876 and $2855\ \mathrm{cm}^{-1}$ for dioxane and at 756, 3012–3040, and $2976–2992\ \mathrm{cm}^{-1}$ for chloroform [22,23].

As estimated by X-ray measurements, the crystallinity of the above mentioned polymer films was about 70% [18]. According to our viscosity measurements [21], M_η was equal to $(110 \pm 26) \times 10^3$ and $(193 \pm 32) \times 10^3$ g/mol for the samples prepared by the first and second procedures, respectively.

The stable nitroxides 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPOL) or 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) were added to the polymer solution before being poured into a Petri dish. The spin-probe concentration was about 10^{-4} M to avoid dipolar broadening of the ESR lines.

Films with a length of 2 cm and a width of 2 mm were cut out from the PHB films after the evaporation procedure. The thickness of the films was about $50 \pm 5\ \mu$m. A stack of the plates was placed in a glassy ampoule of diameter 4 mm and then the ESR spectra were recorded.

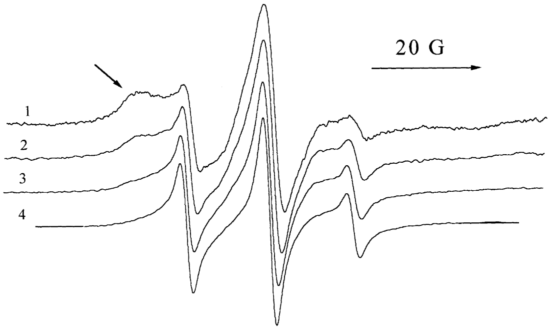

Fig. 2. ESR spectra for probe TEMPOL in the textured and dry sample of PHB: (1) 293 K; (2) 313 K; (3) 333 K; (4) 353 K.

The wet samples of the PHB films were also studied. The PHB films were kept in the saturated water vapors at 296 K for 15–17 h. It was shown recently by our sorption experiments [21,24] that a period of time was necessary for equilibrium saturation of the polymeric films by the water vapors. Then the PHB samples were placed in the glassy ampoules, the ampoules closed and the ESR spectra recorded. According to the sorption data [21,24], the water concentration in the PHB films was about $5 \times 10^{-3}\ \mathrm{g/cm}^3$ (recalculated for the amorphous fraction of the polymer) in the stated conditions.

The EPR spectra were recorded with an EPR-spectrometer of x-diapason "Radiopan" SE/X-2544, $\omega_0 = 9$ MHz (Poland). The attenuation power and modulation amplitudes were adjusted well below the saturation and distortion of the spectra. The spectra were registered both while heating and cooling the samples in the temperature range from 293 to 363 K. The speed of heating and cooling of the samples was 10 K/min.

3. Results and discussion

The high-crystalline PHB films, cast from organic solutions, form the crystalline entities where the polymer chains reside in folded conformations [2,25]. The orthorhombical crystalline cell of PHB with parameters: $a = 0.58$ nm, $b = 1.3$ nm and $c = 0.60$ nm [17,18], was arranged by two macromolecule fragments in antiparallel spiral forms. In textured films the axes of the PHB crystallites are normally aligned to the film surface. Besides, the crystallites in the films form ordered fields, stacks, where they are situated perpendicular to the film surface. In isotropic films the stacks are much more disordered and the crystallites are distributed more randomly in comparison with the textured films [18,21].

Both TEMPO and TEMPOL spin probes have linear sizes more than 0.5 nm [26–28] and cannot penetrate into the intracrystalline areas. Most probable these probes are located in intracrystalline areas, the average sizes of which are about 1.8 nm [18]. At any rate, in semicrystalline polymers the spin probes are localized conventionally in the nonordered microfields of the polymers and the segmental mobility of the proper noncrystalline polymer areas is characterized by the spin rotational mobility of the ESR probes [26,27].

The ESR spectra of TEMPO and TEMPOL in the PHB films (Figs. 1 and 2, respectively) are presented as composite triplets featuring the superposition of the two probe populations, and are characterized by the different rotational correlation times for fast motion and slow motion. The superposition manifested as additional peaks on the background of the origin triplet. As illustrated in Figs. 1 and 2, the most pronounced features of spectra superposition are present for TEMPOL. Any angle dependence for the spectra is absent.

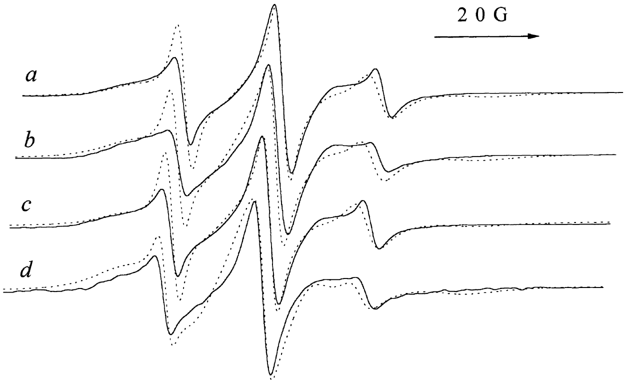

Fig. 3. Experimental (solid line) and simulated (dotted line) ESR spectra at 293 K for probe TEMPO in the textured (a, c) and isotropic (b, d) samples. Spectra of dry (a, b) and wet (c, d) samples. Simulation parameters are presented in Table 2.

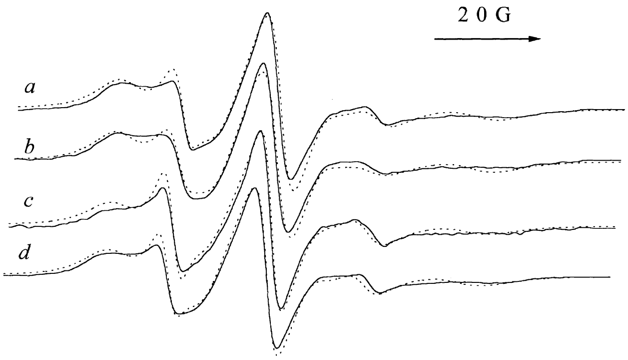

Fig. 4. Experimental (solid line) and simulated (dotted line) ESR spectra at 293 K for probe TEMPOL in the textured (a, c) and isotropic (b, d) samples. Spectra of dry (a, b) and wet (c, d) samples. Simulation parameters are presented in Table 1.

With increasing temperature the intensity of segmental motion is increased and, hence, the rotational correlation times are decreased. Thus, the evidences for superposition do not appear as clearly as they do at room temperature. In the interval 293–363 K, the heating of dry samples at any temperature and the subsequent cooling to the same temperature gives the reusable spectra, it allows us to suggest that irreversible structure transitions in the experimental temperature interval do not occur.

We have analyzed the radical spectra in the framework of the isotropic rotational model of diffusion [28]. It was suggested that each observed spectrum is a superpositional spectrum of rotation for two spin-probe populations (motionally narrowed and broadened components) with τ_1 and τ_2 correlation times and ω_1, ω_2, mole fractions, respectively. For spectrum modeling for TEMPO and TEMPOL, the values of the electron–spin parameters of the $\tilde{g}$ and $\tilde{A}$ tensors: $g_{xx} = 2.00241$; $g_{yy} = 2.00601$; $g_{zz} = 2.00901$; $A_{xx} = 6.8$ G s; $A_{yy} = 6.2$ G s; $A_{zz} = 34.3$ G s [28] are used in the framework of the spectral program designed by Timofeev [29].

Here A_{xx}, A_{yy}, A_{zz} are the principal components of the nitroxide hyperfine interaction tensor, which is the tensor of the hyperfine interaction of the electron spin with the nuclear one; g_{xx}, g_{yy}, g_{zz} are the principal components of the **g** tensor, which characterizes the interaction of an external magnetic field with the spin of an unpaired electron which is localized at a radical fragment.

The comparison of the experimental and simulated spectra in the wet and dry samples of PHB are presented in Figs. 3 and 4. It is seen that the simulated spectra image all the main features of the experimental spectra for both probes TEMPO and TEMPOL.

The existence of two spin probe population may be explained by the existence of microfields in the PHB matrix with different densities and segmental mobilities. The distance of the microfields from the surface of the PHB crystallities may be one of the possible causes of microfields' differentiation. In that case segmental motion is restricted near the crystalline surface and the PHB macromolecules have more ordered structural organization in comparison with macromolecules localized further away from the crystalline surface. It is possible that the density of the former exceeds the average density in the intercrystalline areas of the polymer. In short, we shall call such microfields as "solid". On the contrary, for segments that are localized quite distantly from the crystalline surface, the intensity of segmental mobility is higher, the average density is lower and the density is close to the amorphous

Table 1
The rotation correlation times (τ) of probe TEMPOL in the dry and wet PHB samples with different structural organization (τ_1 and τ_2—the rotation correlation times of spin probes, located in the films' microfields of fast and restricted segmental motions, respectively; the molar contents of the probes, located in the fast (ω_1) and restricted (ω_2) microfields are presented in brackets)

PHB sample	Sample condition	Temperature			
		293 K		313 K	
		$\tau_1 \times 10^9$ (s)	$\tau_2 \times 10^9$ (s)	$\tau_1 \times 10^9$ (s)	$\tau_2 \times 10^9$ (s)
Textured	Dry	1.5 (16%)	9.5 (84%)	1.1 (17%)	7.0 (83%)
	Wet	1.5 (24%)	9.5 (76%)	1.1 (22%)	6.0 (78%)
Isotropic	Dry	1.9 (16%)	9.5 (84%)	1.5 (18%)	7.0 (82%)
	Wet	1.3 (16%)	8.5 (84%)	1.4 (30%)	6.8 (70%)

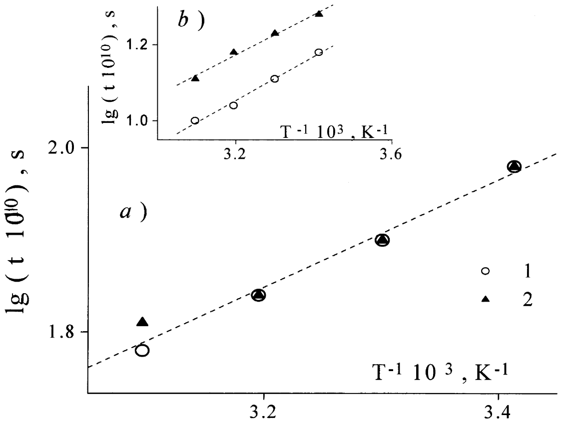

Fig. 5. Temperature dependences of the rotation correlation times for probe TEMPOL in the textured (1) and isotropic (2) PHB samples. The spin probes of "slow" (a) and "fast-rotating" (b) populations.

polymer density. This suggestion allows to classify such microfields as "amorphous".

The values of the rotation correlation times and mole fractions of probes TEMPOL and TEMPO in the PHB films are presented in Tables 1 and 2, respectively. The correlation times and the mole fractions of the ESR probes with different rotation characteristics are estimated from spectral simulations. The following results may be noted. Firstly, there are no differences between the rotational correlation times of the TEMPOL population characterized by slow rotational motion ($\tau_2 = 9.1 \times 10^{-9}$ s) in the isotropic and textured PHB films. The rotational mobility of the "fast" TEMPOL population in textured films is slightly higher than that in isotropic films. Secondly, the distribution between the "slow" and "fast" populations of TEMPOL in the textured and isotropic samples is the same.

Since a TEMPOL molecule contains a hydroxyl group, one may suggest that in the intercrystalline the microfields being responsible for the slow rotation are associated by the oxygen-containing carbonyl groups of PHB. These groups encourage the immobilization of the TEMPOL radicals via hydrogen bond formation and, thereby, restrict the probe rotational mobility. Here it is worthwhile to note that for the ESR probe TEMPOL both its rotation correlation times and its "fast"/"slow" molar fraction are changed after water sorption in PHB.

The above distinctions of rotation behavior for TEMPOL and TEMPO in the dry and wet samples of PHB could be connected with a different chemical structure of the ESR probes. Owing to the OH group of TEMPOL and its immobilization on the >C=O group in accordance with reaction (A), the 'slow' population of the hydrophilic probe appears. Water sorption in PHB leads to the liberation of the probe in accordance with reaction (B):

$$^{*}\underset{\text{(TEMPOL)}}{\text{ONROH}} + {>}\text{C=O} = {>}\text{C} = \text{O}\cdots\text{H–ORNO}^{*} \qquad \text{(A)}$$

$$\begin{aligned} &{>}\text{C} = \text{O}\cdots\text{H}\cdots\text{ORNO}^{*} \\ &\quad + \text{H}_2\text{O} = {>}\text{C} = \text{O}\cdots\text{H–OH} + {}^{*}\underset{\text{(TEMPOL)}}{\text{ONROH}} \end{aligned} \qquad \text{(B)}$$

and, as a consequence, increases the fraction of the 'fast' rotation probe. A qualitative comparison of the TEMPOL spectra for dry and humid samples (Fig. 4a and c for textured samples or Fig. 4b and d for isotropic samples, respectively) shows the remarkable distinction of the low field components of the spectra. After numerical simulations, such distinction could be interpreted as a consequence of both the increase of rotation mobility (decrease of time correlation from 1.9×10 to 1.3×10^{-9} s after hydration) in the isotropic samples and the change of the fast-rotating population fraction of the ESR probe in the textured samples from 0.16 to 0.24, as shown in Table 1. The modeling of the above complex spectra will be continued.

In contrast to the TEMPOL probe, the TEMPO spectra of the humid and dry samples in PHB are similar (Fig. 3) and gives the constant parameters of the probe rotation before and after water sorption (Table 2). For the textured PHB films it can be emphasized that the molar fraction of the probes, localized in the "amorphous" microfields of the polymer matrix, is higher than for case of isotropic films.

The temperature dependence of rotational correlation times of the probe TMEPOL in the PHB samples are presented in Fig. 5. The rotational correlation times of the slow population in textured and isotropic samples are the same in the overall temperature interval. But the τ_1 values of "fast" population in textured samples are less than the τ_1 values in isotropic samples.

The activation energy for the rotation of probe TEMPOL in the dry films was estimated as 10 kJ/mol for all populations in the isotropic and textured samples. The values of the preexponential multiplier for "slow" rotation is 10.5×10^{-11} s in the textured and isotropic samples; for "fast" rotation it is 1.8×10^{-11} and 3.2×10^{-11} s for the textured and isotropic samples, respectively.

In a previous work [21] we have shown that the translation diffusion coefficients of water (D_w) in the textured samples of PHB exceeded the analogous coefficient in the isotropic ones (see Table 3). When using the rotational correlation times of the "fast" population of the ESR

Table 2
The rotation correlation times (τ) of probe TEMPO in the dry and wet PHB samples with different structural organization at 293 K (τ_1 and τ_2—the rotation correlation times of spin probes, located in the films' microfields of fast and restricted segmental motions, respectively; the molar contents of probes, located in the fast (ω_1) and restricted (ω_2) microfields are presented in brackets)

PHB sample	Sample condition	$\tau_1 \times 10^9$ (s)	$\tau_2 \times 10^9$ (s)
Textured	Dry	1.00 (0.40)	8.00 (0.60)
	Wet	1.00 (0.40)	8.00 (0.60)
Isotropic	Dry	1.00 (0.15)	7.00 (0.85)
	Wet	1.00 (0.15)	5.50 (0.85)

Table 3
The values of coefficients of translational diffusion of water molecules and coefficients of rotational diffusion of “fast” populations of probe TEMPOL in the isotropic and textured PHB matrices

Temperature (K)	Sample			
	Textured		Isotropic	
	$D_w \times 10^9$ (cm^2/s)	$D_r \times 10^{-9}$ (s^{-1})	$D_w \times 10^9$ (cm^2/s)	$D_w \times 10^{-9}$ (s^{-1})
303	36	0.13	4.0	0.10
318	68	0.15	8.5	0.11
325	183	0.17	18.5	0.13
333	219	0.22	26.4	0.16

probe TEMPOL (τ_1), we calculated the rotation diffusion coefficients (D_r) of the probe in both the isotropic and textured matrices [27]:

$$D_r = 1/6\tau_1.$$

The comparison of diffusion coefficients for water, D_w, and the ESR probe, D_r, in matrices with different morphology (Table 3) shows that in the temperature range 303–332 K both of these characteristics tend to increase not only with temperature, but with a change from isotropic to textured matrices of PHB as well. Two reasons provide, at least, an explanation for the diffusion behavior in matrices with different crystalline organization. Firstly, on the micro-level the segmental mobility in the intercrystalline areas of PHB depends on the distribution of the crystallites and their orientation in the polymer matrix [21]. Secondly, on the morphological level the different organization of the crystallites in the polymer can have an effect on the “solid”/“amorphous” microfield ratio (ω_2).

Within certain limits, the motion of the water molecule may be treated as a small hydrophilic probe motion that has good affinity to the carbonyl groups of PHB [30] and high accessibility in the intercrystalline areas. In spite of the difference in the mechanism of translation diffusion of water molecules as a specific probe and rotation diffusion of the polar ESR probe TEMPOL the dynamics and morphological factors affect their diffusion behaviors similarly.

4. Conclusions

Thus, the present experiments for the ESR probe, TEMPO, indicated that in the intercrystalline area of PHB there are microfields with different molecular mobilities: dynamic microheterogeneity. The existence of such microfields is likely to be dictated by structural heterogeneity for both isotropic and textured (crystalline organization) samples. The sorption of water molecules in the PHB matrices did not change practically the forms for ESR spectra and, hence, did not effect the rotation mobilities (τ) and the mole fraction of ‘fast-rotating’ and ‘slow-rotating’ populations of TEMPO, see Table 2. Consequently, here we did not observe the plasticizing of the polymer by the water molecules that would be reflected by the increase of amplitude and frequency of segmental mobility.

Another scenario is seen for the experiments with the probe, TEMPOL, the molecules of which have, in contrast to TEMPO, one hydroxyl group. For TEMPOL the interaction between the hydroxyl group and the carbonyl group of PHB should be taken into account. In that event, it is quite clear that the values of τ_2 for the isotropic and textured samples are equal (Fig. 5a), so the slow rotation is determined predominantly by reaction A. As we know from TEMPO experiments, sorption of water molecules did not plasticize the PHB, but it promotes only the delivery of the free probe population of TEMPOL in accordance with reaction B. In this case one finds the increase of the mole fraction of the fast-rotating population in textured samples at 293 K and the isotropic samples at 313 K, as well as the rotating frequency in isotropic samples at 293 K, see Table 1.

The differences of molecular mobilities in the textured/isotropic matrices for the dry and wet samples of PHB as well as the careful comparison of the ESR data with water translation mobility will give the next important impact in the fundamental understanding of the relationship between morphology and transport properties of the novel polymer materials.

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