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Novel Biodegradable Molecularly Imprinted Polymers Based on Poly(3-hydroxybutyrate)

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Summary: Novel biodegradable molecularly imprinted polymers (MIP) based on poly(3-hydroxybutyrate) (PHB) were prepared and characterized. Low molecular weight PHB diol was prepared from bacterial PHB, and was used to synthesize acrylate end-capped PHB macromer. The synthesis of hydroxyl-telechelic PHB and acrylate end-capped macromer was confirmed using FT-IR and ^{1}H NMR. PHB macromer was used to prepare biodegradable crosslinked networks by photopolymerization with functional monomer (methacrylic acid) and a model template (theophylline). The theophylline-imprinted polymer showed higher binding capacity for theophylline than non-imprinted polymer (NIP). The data of theopylline binding into the theopylline-imprinted polymer correlated well with the Scatchard plot. The equilibrium binding constant (K_E) and the total number of bound sites of MIP ($[PS]_{total}$) were 7.1×10^3 M^{-1} and 18.5 μmol/g, respectively, which were estimated from the negative slope and intercept in the Scatchard plot.

Keywords: biodegradable; biomaterials; molecular recognition; molecularly imprinted polymer; poly(3-hydroxybutyrate)

Introduction

Poly(3-hydroxybutyrate) (PHB), a class of polyhydroxyalkanoates (PHA), is biodegradable thermoplastic polyester accumulated by many microorganisms as an intracellular carbon and energy storage material.[1] More than 100 HA units have been detected as constituents of PHA, which allows these materials to have various mechanical properties from hard crystalline polymer to elastic rubber depending on the incorporated monomer units. At present, PHA is considered for applications in the medical and pharmaceutical field,[2] rather than as substitutes for conventional plastics due to the high production cost of PHA compared with petrochemical derived plastics. Poly(4-hydroxybutyrate) has been successfully applied in the tissue engineering of trileaflet heart valve in a sheep model.[3]

Molecular imprinting has been established as a new technique which allows the creation of tailor-made binding sites for certain molecules such as chiral molecules.[4–7] Molecularly imprinted polymers (MIPs) are usually prepared by polymerizing a mixture of a target molecule (template), functional monomers and an excess of crosslinkers, and then removing the template from the crosslinked polymer network. MIPs have been used for analytical separation[8] and biosensor systems[9] to separate and detect molecules with very similar structures. Only recently have researchers applied these techniques to hydrogel systems, to biologically significant target molecules, and to controlled drug delivery systems.[10–12] We reported biodegradable MIPs which can be potentially applied to various biomedical applications by combining two important properties required for ideal biomaterials, biodegradability (with biocompatibility) and mole-

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cular recognition property.[13] In this study, we prepared biodegradable MIPs based on bacterial PHB using theophylline as a model template molecule and examined the molecular recognition performance of theophylline-imprinted polymer in comparison with non-imprinted polymer (NIP) by carrying out batch rebinding tests.

Experimental Part

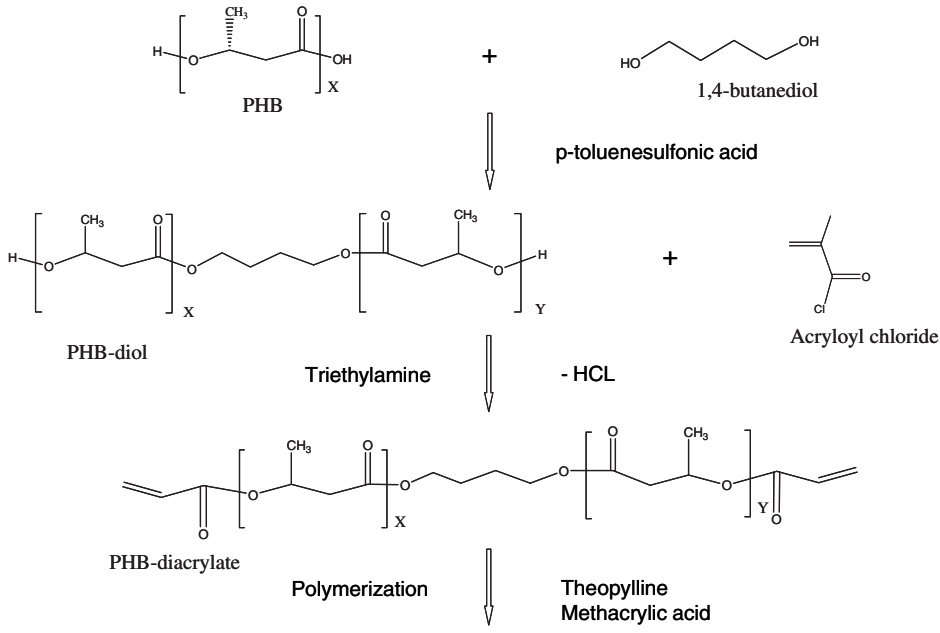
Materials

PHB, p-toluenesulfonic acid, 1,4-butandiol, acrylic acid, triethylamine, methacrylic acid, theopylline, and UV initiator 2,2-dimethoxy-2-phenylacetophenone (DMPA) were obtained from Aldrich. All other chemicals used were of reagent grade and were used without further purification.

Synthesis and Characterization of PHB Diol and PHB Diacrylate

The overall reaction for the synthesis of PHB diol and PHB diacrylate is shown in Scheme 1. PHB diol was prepared by the method described previously.[14] Briefly, PHB (20 g) was dissolved in 150 ml of chloroform, to which p-toluenesulfornic acid (7 g) and 1,4-butanediol (28 g) were added. The reaction was carried out at 60 °C under nitrogen atomsphere. The reaction time was varied from 17 to 30 h to control the molecular weight. The product was obtained by pouring the filtrate in a large excess of methanol. The solid precipitate was washed with methanol, acetone and distilled water and dried under vacuum for 24 h.

The PHB diol were end-capped with acrylate groups to form a polymerizable macromer. Typically, 4.0 g (2 mmol) of PHB diol (molecular weight 2000) was dissolved in 40 ml of 1,2-dichloroethane in a 100 ml of round-bottomed flask. And then, 0.63 ml (4.5 mmol) of triethylamine and 0.37 ml (4.5 mmol) of acryloyl chloride were added to the flask. The reaction mixture was stirred for 3 h at 80 °C. After the reaction, it was filtered to remove



Scheme 1.
Overall reaction for the synthesis of PHB diol, PHB diacrylate, and crosslinked MIP network.

triethylamine hydrochloride. The diacrylated macromer was obtained by pouring the filtrate in a large excess of methanol. The solid precipitate was dried under vacuum for 24 h.

IR spectra were measured using a Bomem MB100 spectrophotometer. The ^{1}H NMR spectra were measured with a Bruker Advance-500 MHz spectrometer in $CDCl_3$.

Preparation and Characterization of MIPs

MIPs were formed by UV photopolymerization of the resulting macromer as a crosslinking agent. Two milliliter of a theophylline solution (10 μmol/ml chloroform) and 0.085 ml (1 mmol) of acrylic acid were added in a 25 ml multi-injection vial and stirred. After 5 min incubation, 0.6 g (0.3 mmol) of PHB macromer and 0.05 ml of the initiator solution (0.1 g of DMPA dissolved in 1 ml of 1-vinylpyrrolidinone) were added to the reaction mixture and polymerized for 20 min to ensure thorough gelation using a low-intensity UV lamp at 366 nm with a light intensity of approximately 50 mW/cm^2 under air. MIPs were prepared as a disc-shaped thin film with a diameter of 5 cm and a thickness of a 80 μm. Theophylline was removed by washing three times with a 90 ml chloroform/10 ml acetic acid solution for 24 h and then washing twice with a 100 ml chloroform for 24 h. Control, non-imprinted polymer was prepared without theophylline.

The rebinding experiment was carried out by incubating MIP and NIP in a 50 ml theophylline solution (5–20 μg/ml chloroform) with shaking at 200 rpm at 30 °C. Theophylline concentrations were determined by measuring the UV absorbance at 275 nm with spectrophotometer (UV-1650PC, Shimadzu). The binding capacity, amount of theopylline bound to the MIP, was calculated by the following equation:[15,16]

$$[S_a] = (C_0 - C_t)V/W$$

Here, $[S_a]$ is binding capacity (μmol/g), C_0 and C_t are the theophylline concentration (μM) measured at initial and after interval time, V and W are the volume of the theophylline solution and the weight of dry polymer used for the binding experiment, respectively.

Results and Discussion

The FT-IR spectra of the representative PHB, PHB diol, and PHB diacrylate are shown in Figure 1. An absorption band at 3455 cm^{-1} shown in PHB diol is due to the terminal hydroxyl group, which was almost disappeared in the PHB diacrylate due to

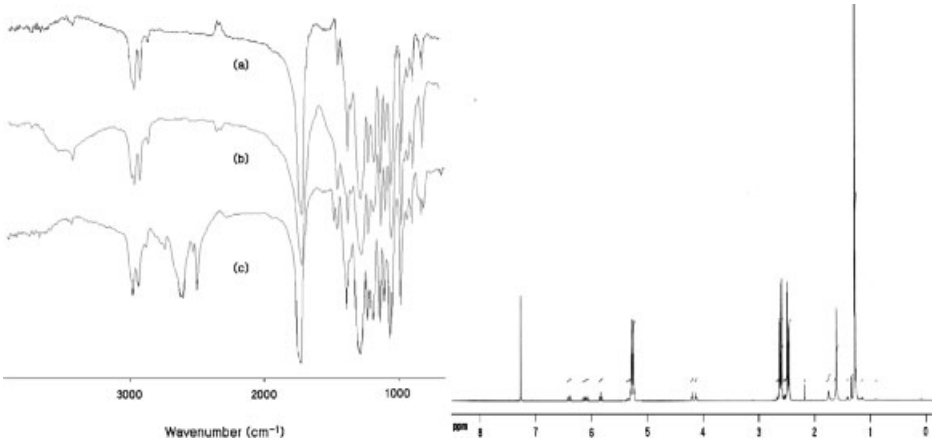


Figure 1.
Synthesis of PHB macromers. Left: FT-IR spectra of (a) natural-origin PHB, (b) PHB diol, and (c) PHB diacrylate. Right: ^{1}H-NMR spectrum of PHB diacrylate.

acrylation. The peaks at 1635 cm^{-1} in the PHB diacrylate are ascribed to the double bond signals due to acrylate functionalities.[17–19] The formation of PHB diacrylate was also confirmed through ^1H NMR spectrum as shown in Figure 1. The vinyl groups of the PHB diacrylate appeared in the 5.79–6.43 ppm range. The molecular weights of PHB diol prepared from bacterial PHB could be controlled and decreased with increasing the reaction time at 60 °C. The number average molecular weights of three PHB diol determined by GPC were 5000, 3500, and 2000 when the reaction time was 17, 22, and 30 h, respectively. The conversions of PHB diol to PHB diacrylate were calculated from the ratio of the peak integral of the three acrylate protons to those of the methylene protons adjacent to the ester bond in the PHB backbone, and were in the range of 65.1–83.1%.

Figure 2(a) shows representative binding profiles of theophylline to the theophylline-imprinted polymer (MIP) prepared from PHB diol of molecular weight 2000 in comparison with theophylline binding to the control, non-imprinted polymer (NIP). The initial concentration of theophylline solution was 20 μg/ml. Binding amounts of templates to the polymer increased with time and became constant. The saturation behavior of the template binding indicates that the binding sites of the imprinted polymer were filled with the template. The saturated binding capacity of MIP for theophylline, defined as the binding amount at 5 h, was 6.8 μmol/g, which was 2.3 times higher than that of NIP (3.0 μmol/g).

The binding experiments were performed with various theopylline concentrations in the range of 5–20 μg/ml. The saturated binding capacity increased as the theopylline concentration was increased. It was known that equilibrium binding contant (K_E) can give a quantitative information about the substrate binding into the polymer.[15,16] Thus, the theopylline binding into the theopylline-imprinted polymer was evaluated by the following equation of Scatchard relationship.

$$\begin{aligned}\frac{[S_a]_b}{[S_a]_f} &= K_E[PS]_f \\ &= K_E\{[PS]_{total} - [PS]_b\} \\ &= K_E\{[PS]_{total} - [S_a]_b\}\end{aligned}$$

Here, $[S_a]_f$ and $[S_a]_b$ are the concentrations of the substrates free and bound to the polymer sites, respectively. $[PS]_f$ is the number of free binding sites of polymer, which equals $[PS]_{total}$ minus $[PS]_b$. $[PS]_b$ equals $[S_a]_b$ and $[PS]_{total}$ is the total number of bound sites of polymer. Figure 2(b) shows Scatchard plot obtained by binding

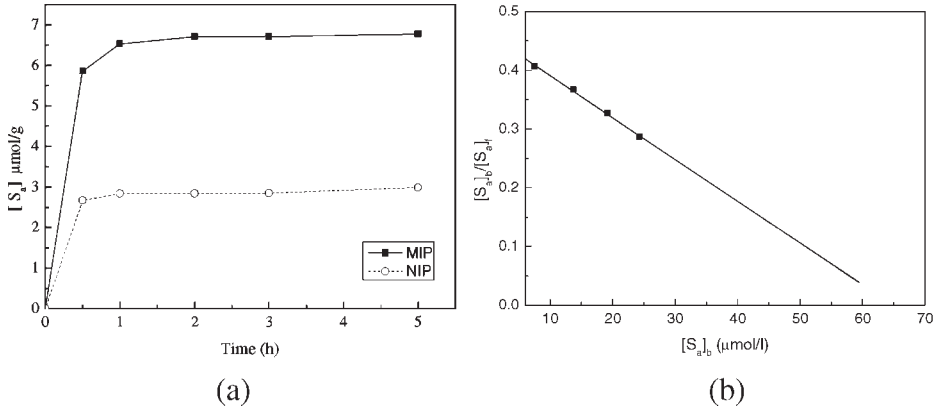


Figure 2.
Binding properties of theophylline-imprinted polymer (MIP) and non-imprinted polymer (NIP). (a) Binding profiles of MIP and NIP with time. (b) Scatchard plot of the binding of theophylline to the theophylline-imprinted polymer.

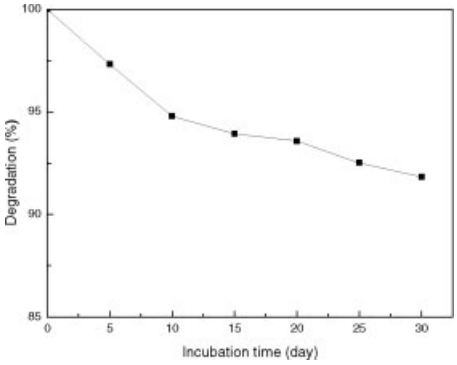


Figure 3.
Degradation curve of PHB-based MIP in vitro (pH 7.4 and 37 °C).

experiments of theopylline for the theopylline-imprinted polymer system. Since the plot showed a linear relationship between $[S_a]_b$ versus $[S_a]_b/[S_a]_f$, negative slope and intercept gave each value of K_E and $[PS]_{total}$. The resulted K_E and $[PS]_{total}$ were 7.1×10^3 M^{-1} and 18.5 μmol/g, respectively. In comparison with the data of Reddy et al.,[16] the theophylline binding to the PHB-based MIP showed higher total binding sites than the dibenzofuran binding to the MIPs made of non-degradable common polymers such as polyvinyl chloride, polysulfone, polystyrene, and polyacrylonitrile. The equilibrium binding constant of the PHB-based MIP was similar to the values of imprinted polystyrene and polyacrylonitrile.

The in vitro degradation characteristics of the PHB-based MIPs were investigated in PBS and 1 N NaOH at 37 °C. These MIPs degraded very fast in 1 N NaOH (over 90% within 24 h) and degraded about 10% of the initial weights in 30 days in PBS (Figure 3). The in vivo degradation products are presumably 3-hydroxybutyric acid and oligo (acrylic acid). They are nontoxic and can be excreted directly or after entry and exit from various metabolic pathways.[20]

Conclusions

We described novel biodegradable MIPs based on bacterial PHB which can be potentially applied to biomedical applications such as targeted drug delivery system. The theophylline-imprinted polymer prepared from the PHB macromer showed higher binding capacity for theophylline than NIP. The degradation rate of biodegradable MIPs can be controlled by changing the crosslinking density (molecular weight of macromer and ratio of functional monomer to crosslinking agent) and hydrophilicity/hydrophobicity (type of polymer backbone).

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