The synthesis of poly(lactide)-vitamin E TPGS (PLA-TPGS) copolymer and its utilization to formulate a curcumin nanocarrier

Phuong Thu Ha, Thi Minh Nguyet Tran, Hong Duong Pham, Quang Huan Nguyen and Xuan Phuc Nguyen

Institute of Materials Science, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

E-mail: haphuongthu@yahoo.com

Received 18 March 2010
Accepted for publication 23 April 2010
Published 2 July 2010
Online at stacks.iop.org/ANSN/1/015012

Abstract
Curcumin is a natural substance that exhibits the ability to inhibit and/or treat carcinogenesis in a variety of cell lines, but because of its poor solubility in water the treatment efficacy is limited. In this paper we report on the fabrication of self-assembled micelle nanoparticles loaded with a curcumin drug by use of a biocompatible copolymer of PLA-TPGS (d-a-tocopheryl polyethylene glycol 1000 succinate—vitamin E TPGS) conjugate. The polylactide (PLA)-TPGS copolymer synthesized by ring-opening polymerization was characterized by Fourier transform infrared spectroscopy (FTIR) and $^1$H nuclear magnetic resonance ($^1$H NMR) techniques. The surface morphology of PLA-TPGS and curcumin loaded PLA-TPGS was determined by field emission scanning electron microscopy (FE-SEM). The absorption and fluorescence examinations indicated that due to micellar capsulation the intensity of both types of spectra increased by about 4 times in comparison with those of the free curcumin sample.

Keywords: biomedical materials, nanocarrier, copolymer, curcumin, cancer drug

Classification numbers: 5.08, 5.09, 5.10

1. Introduction

The roots of turmeric have long been used in Indian cuisine and in traditional Asian medicine to treat gastrointestinal upset and arthritic pain. Curcuminoids are the major substances that give turmeric its yellow color. The major curcuminoids are curcumin (the chemical structure is shown in figure 1), demethoxycurcumin and bisdemethoxycurcumin. Curcumin extract is not only a powerful anti-oxidant and anti-inflammatory compound but also a cancer medicine and chemopreventive agent. Many of the products from curcumin have been made to treat cancer (colon, skin, breast, gastric, ovarian, prostate, stomach, duodenal ulcers, etc) [1–4]. Some laboratory also studies suggest that curcumin may be active against HIV [5].

However, at high doses or with prolonged use, curcumin may actually further irritate or upset the stomach. To improve its bioavailability and clinical efficacy, the solubility of curcumin in aqueous solutions needs to be increased. In order to solve this problem, curcumin has been encapsulated in various materials such as liposome [6], porous silica [7] and polymeric nanoparticles [8–10]. Polymeric micelles act as nanocarriers with many advantages, such as low toxicity,
high stability and small size. Several drugs formulated in polymeric micelles are in clinical trial development for the treatment of various cancers [9, 10]. As indicated in [8], nanocurcumin particles less than 100 nm in size could be synthesized using a cross-linked and random copolymer of N-isopropyl crylamid (NIPAAM) with N-vinyl-2-pyrrolidone (VP) and poly(ethyleneglicol)monoacrylate (PEG-A), which demonstrate superior efficacy compared to free (bulk) curcumin in human cancer cell line models.

Polymeric nanoparticles have attracted significant attention in the study of drug delivery systems as they offer a means for localized or targeted delivery systems of a drug to specific tissue/organ sites of interest with an optimal release rate [11]. Such drug delivery systems are usually restricted by the biocompatibility of the polymeric matrix material and the surfactant used in the formulation process. Poly (lactide) (PLA), poly (dl-lactide-co-glygolide) (PLGA), and poly (caprolactone) (PCL) are FDA (US Food and Drug Administration)-approved biodegradable polymers, which are most often used in studies of drug delivery due to their very low toxicity. D-α-tocopheryl polyethylene glycol 1000 succinate, derived from natural vitamin E (α-tocopheryl), is a safe and effective form of vitamin E for reversing or preventing vitamin E deficiency due to its good oral bioavailability. It has many other potential applications, such as solubilizer, absorption enhancer and vehicle for lipid-based drug delivery formulations. TPGS is readily absorbed in the gastrointestinal tracts, and inhibits P-glycoprotein, the multidrug transporter, in the intestine to enhance the cytotoxicity of anticancer agents such as doxorubicin, vinblastine, paclitaxel and curcumin [12, 13]. Significant potential has been identified for TPGS in application to nanoparticle and lipid-based technologies for drug delivery. The chemical structure of vitamin E TPGS comprises both lipophilicity and hydrophilicity, resulting in amphiphilic properties. Moreover, its lipophilic alkyl tail and hydrophilic polar head portion are bulky and have a large surface area. Such characteristics makes it a good emulsifier, able to emulsify a wide range of water-oil immiscible systems.

In order to study drug uptake and distribution, a fluorescent agent is normally used to link with the drug for optical microscope and/or confocal laser scanning microscope observation. In contrast to other reported fluorescent agents such as dye substance or quantum dots, the curcumin drug is itself a natural fluorescent agent, so that while being soluble into micelles in a surfactant the photoluminescence intensity is increased by many orders (16 times in cetyltrimethylammonium—CTAB [14] or even 55 times in a mixture of CTAB with sodium dodecyl benzene sulfate—SDBS), so that its linear dependence on the curcumin concentration can be used for quantitative release analysis [14, 15] and also for effective fluorescence intracellular imaging [16].

In this study, we describe a synthesis method for a polymeric amphiphile based on PLA and TPGS, and its application to formulate a micellar structure of curcumin. As will be shown, the PLA-TPGS conjugate forms self-assembled micelle nanoparticles with the hydrophobic curcumin drug at the core.

2. Experimental

2.1. Materials

The used lactide (3,6-dimethyl-1,4-dioxane, C₆H₈O₄) was purchased from Aldrich, while vitamin E TPGS (d-α-tocopheryl polyethylene glycol 1000 succinate) and C₃₃H₅₄O₁₂₃ (CH₂CH₂O) were from Merck. Stannous octoate
Figure 3. FTIR spectra of TPGS (a) and PLA-TPGS (b) copolymer.

(Sn(OOCC$_7$H$_{15}$)$_2$) was purchased from Sigma. The solvents dichloromethane, toluene and methanol were of analytical grade and obtained from Merck. The curcumin was from Mumbai, India. Phosphate buffered saline (PBS) was purchased from Sigma.

2.2. The synthesis of PLA-TPGS copolymer

PLA-TPGS copolymer was synthesized by ring-opening bulk polymerization of lactide monomer with vitamin E TPGS in the presence of stannous octoate as catalyst. The synthesis of the PLA-TPGS is shown schematically in figure 2. PLA-TPGS of component ratio PLA/TPGS = 50 : 50 w/w was dissolved in distilled toluene. PLA, TPGS (in toluene) and stannous octoate were added in the ampoule. The mixture was stirred for 10 h at 130 °C under nitrogen flow in a silicone oil bath. After polymerization, the resultant sample was gently stirred overnight to evaporate the organic solvent. The product was dissolved in dichloromethane and then precipitated in cold.
Figure 4. $^1$H-NMR spectra of PLA (a), TPGS (b) and PLA-TPGS (c).

Figure 5. The molecular structure of PLA-TPGS copolymer.

methanol in excess to remove unreacted lactide monomers and TPGS. The final product was obtained by filtration.

2.3. Encapsulation of curcumin in micelles

Curcumin solution in methanol was added to the solution of PLA-TPGS in dichloromethane (DCM) in a polymer ratio of 1:100. Next the solvents were evaporated under a vacuum to produce the PLA-TPGS/curcumin mixture. Micelles were formed by extensively vortexing the mixture in PBS. Non-encapsulated curcumin was finally separated by centrifugation of the micelle suspension.

2.4. Characterization

The synthesized PLA-TPGS copolymer was characterized by $^1$H-NMR and FTIR. The molecular structure of the PLA-TPGS copolymer was investigated using a FTIR SHIMADZU spectrophotometer. The TPGS content and number-averaged molecular weight of the copolymer was
determined by $^1$H-NMR in CDCl$_3$ with a Bruker spectrometer of 500 MHz. The micelle-loaded curcumin was examined by absorption on a VARIAN spectrophotometer Cary 5000 UV-VIS-NIR while photoluminescence spectra were taken with a 442 nm excitation line. The surface morphology of PLA-TPGS and curcumin-loaded PLA-TPGS was determined by field emission scanning electron microscopy (FE-SEM) on a Hitachi S-4800 system.

3. Result and discussion

3.1. Effects of polymer matrix materials

Figure 3 shows FTIR spectra of the TPGS and PLA-TPGS copolymer. The carbonyl band of TPGS appears at 1745 cm$^{-1}$. For the synthesized copolymer, the carbonyl band was shifted to 1756 cm$^{-1}$. The stretching bands of PLA and of TPGS were observed at 2931 and 2926 cm$^{-1}$, respectively. In the PLA-TPGS copolymer, the CH stretching band decreased and was observed at 2924 cm$^{-1}$. The absorption band at 3426 cm$^{-1}$ is attributed to the terminal hydroxyl group. The absence of the peak at 3426 cm$^{-1}$ and the presence of the peak at 1756 cm$^{-1}$ confirms the appearance of an ester linkage in the conjugate.

The structure of the synthesized PLA-TPGS copolymer was detected by $^1$H-NMR in CDCl$_3$. Figure 4 shows $^1$H-NMR spectra of the PLA, TPGS and PLA-TPGS. In the PLA spectrum (figure 4(a)), the peaks at 5.043 and 1.630 ppm were assigned to the –CH protons and –CH$_3$ methyl protons, respectively. The signal at 3.641 ppm of the spectrum of TPGS (figure 4(b)) was assigned to the –CH$_2$CH$_2$– protons. These three peaks were, correspondingly, shifted to 5.166, 1.562 and 3.539 ppm in the spectrum of PLA-TPGS (figure 4(c)). This observation shows that PLA-TPGS was synthesized and the precipitation process in the treatment of the copolymer can remove the TPGS and PLA. Based on the FTIR and $^1$H-NMR
Figure 8. Photoluminescence images of curcumin particles (a) and polymeric particles of curcumin loaded in PLA-TPGS (b).

spectra, the structure of PLA-TPGS can be supposed to be as presented in figure 5.

The molecular weight of the PLA-TPGS was calculated by using the ratio between the peak areas at 5.166 and 3.639 ppm. The number-averaged molecular weight of the PLA-TPGS copolymer was estimated to be 13,000.

3.2. Effects of curcumin loading by PLA-TPGS

The micelle-encapsulated curcumin shows distinct photophysical properties. Figure 6 indicates that the curcumin in both the 30% aqueous-to-methanol and 100% methanol solution shows two absorption bands at 422 nm and 261 nm. The absorption intensity of the latter solution, however, is very much stronger (figure 6(b)), supposedly thanks to the higher solubility of curcumin in methanol. Meanwhile, the absorption of encapsulated curcumin increased sharply with maximum peaks at 415 nm and 232 nm. The enhancement of absorption intensity and the shift of maximum absorption of encapsulated curcumin might be due to its interaction with the amphiphilic PLA-TPGS to make the micelles more soluble in the solution [14, 15].

The photoluminescence spectra (PL) also exhibited similar behavior (figure 7). For methanol curcumin solution it shows maximum intensity at 557.2 nm, whereas the micelle-loaded curcumin shows maximum emission at 527.4 nm and with much higher PL intensity (four times more intensive compared to the free curcumin). These characteristics of fluorescence spectra suggest again that curcumin in PLA-TPGS is encapsulated in the hydrophobic core of the micelle.

A PL image of free (bulk) curcumin particles dispersed in methanol solution is shown in figure 8(a). The spherical shape of the particles was seen at sizes ranging from 1 to 10 µm. The contrast and resolution of this PL image is good enough for further investigation. Figure 8(b) shows a PL image of the micelle-encapsulated curcumin with large range of sizes similar to that of curcumin. As we have seen, curcumin micelle is a strong PL substance, thus when used to treat cancer, it could also act as a labeling material. Hence we

Figure 9. FE-SEM image of PLA-TPGS (a) and curcumin/PLA-TPGS (b and c).
can determine the efficiency of the drug transport process in different conditions.

3.3. Surface morphology

Figure 9 shows typical FE-SEM images of the pure copolymer PLA-TPGS (a) and two representative FE-SEM images of the curcumin loaded by PLA-TPGS (b) and (c). Figure 9(a) indicates that copolymer PLA-TPGS has a rough surface with size ranging from 100 to 400 nm. Figure 9(b) shows a micrometer-sized group of several clusters of much smaller particles. The small particles are 20–40 nm in size, and are shown in figure 9(c). From the FE-SEM images, one can suppose that the polymer is not required to exhibit sphere micelles, and accordingly it might even be better to use the polymer with rough morphology as shown in figure 9(a).

Regarding the particle size, we suppose that the particles shown by the PL images (figure 8(b)) correspond to the group/cluster of particles indicated by the FE-SEM images (figure 9(b)). So, although curcumin-loaded PLA-TPGS micelles of nanosized diameters have been fabricated, efforts still have to be made in future to solve two remaining problems, namely (i) to study the optimization of the encapsulation to obtain monodisperse micelles and/or (ii) to homogenize the size of the micelles themselves.

4. Conclusion

The copolymer PLA-TPGS, as amphiphilic conjugate, was successfully synthesized by ring-opening polymerization from polylactide (PLA) and d-a-tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS or TPGS) for the formation of nanostructured anticancer drugs.

Curcumin has been encapsulated by PLA-TPGS to form micelles of diameter ranging from several tens to hundreds of nanometers. The micellar curcumin showed 4 times enhancement of the intensity of absorption/fluorescence spectra, and it also exhibited strong fluorescence images for high resolution fluorescent microscope observation.

Acknowledgments

This work was financially supported by an IMS research grant and a MOST grant (No. 04/02/742/2009/HD-DTDL). The authors are thankful to Professor, Academician Nguyen Van Hieu for his encouragement and interest in this research.

References

[1] Aggarwal B B and Shishodia S 2006 Biochem. Pharmacol. 71 1379
[2] Choi H, Chun Y S, Kim S W, Kim M S and Park J W 2006 Mol. Pharmacol. 70 1664
[3] Srivastava K C, Bordia A and Verma S K 1995 Prostaglandins Leukot. Essent. Fatty Acids 52 223
[4] Aggarwal B B, Kumar A and Bharti A C 2003 Anticancer Res. 23 363
[5] Balasubramanyam K, Varier R A, Altaf M, Swaminathan V, Siddappa N B, Ranga U and Kundu T K 2004 J. Biol. Chem. 279 51163
[6] Li L, Ahmed B, Mehta K. and Kurzrock R 2007 Mol. Cancer Ther. 6 1276
[7] Clifford N W, Swamminathan Iyer K and Raston C L 2008 J. Mater. Chem. 18 162
[8] Bisht S, Feldmann G, Soni S, Ravi R, Karikar C, Amornath M and Amiraban M 2007 J. Nanobiotechnol. 5 3
[9] Sahu A, Bora U, Kasoju N and Goswami P 2008 Acta Biomater. 4 1752
[10] Matsumura, Hamaguchi Y T, Ura T, Muro K, Yamada Y, Shimada Y, Shirao K, Okusaka T, Ueno H, Ikeda M and Watanabe N 2004 Br. J. Cancer 91 1775
[11] Mu L and Seow P H 2006 Colloids Surf. B 47 90
[12] Zhang Z P and Feng S S 2006 Biomaterials 27 262
[13] Pan J and Feng S S 2008 Biomaterials 29 2663
[14] Iwunze M O 2003 Tenside Surf. Det. 40 96
[15] Wang F, Wu X, Wang F, Liu S F, Jia Z and Yang J H 2006 J. Fluoresc. 16 53
[16] Kunwar A, Barik A, Mishra B, Rathinasamy K, Pandey R and Priyadarsini K I 2008 Biochim. Biophys. Acta 1780 673