Lipase-catalyzed synthesis of hydrophobically modified dextrans: Activity and regioselectivity of lipase from Candida rugosa

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Vinyl decanoate-modified dextran macromolecules (DexT40-VD) were synthesized in dimethyl sulfoxide at 50°C using lipase AY from Candida rugosa for catalyzing transesterification between polysaccharide and vinyl fatty esters. The extent of dextran modification (quantified by the molar ratio of attached alkyl tails to sugar repeat units) with native-, pH-adjusted-, 18-crown-6 ether pretreated pH-adjusted-, and stepwise addition of pretreated lipase AY yielded ~3%, 49%, 64% and 96% modified dextran respectively. Lipase AY accelerated the transesterification of DexT40 from 2- to 63-fold higher than the non-catalyzed system. This procedure was extended to other acyl donors showing that modification pattern exhibited regioselectivity depending on acyl donor structure. Regioselectivity equaled between 2- and 3-OH with saturated fatty acyl donors. The 2-OH was favored for unsaturated fatty acyl donors, while sterically hindered acyl donors oriented modification toward 3-OH position. DexT40-VD at 96% modification was a water-insoluble polymer forming 150 nm diameter nanoparticles in water which can be used as drug carrier systems.

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Key words: Drug carrier system; Lipase; Transesterification; Dextran; Vinyl ester; Regioselectivity

A tailored functionalization of existing biomaterials to create new drug carrier systems has been the subject of extensive studies over the past years. In that context, dextran, bacterial polysaccharide consisting essentially of α-1,6 linked α-D-glucopyranoside residues with a small percentage of α-1,3 linked side chains represents one of the most promising target of modification to provide a wide range of properties appropriated for drug release application such as degradability, colloidal stability and the stealthiness of resulting nanoparticles in biological environment (1,2). In fact, it is possible to obtain such nanoparticles by attachment of certain amount of the appropriated hydrophobic group onto a specific hydroxyl group of dextran. This concept was proven recently from the elaboration of a series of dextran derivatives (esters) in a single step under mild reaction detail (3). The use of other types of dextran derivatives such as dextrans (esters) to prepare the core of nanoparticles, in contrast, has never been reported in the literature. This might be a consequence of the difficulty to obtain regioselective modification of sugar ring with such functional substituents.

Enzymatic catalysis in organic solvents allows the preparation of dextran derivatives (esters) in a single step under mild reaction conditions. The advantages of using enzymes in organic solvents are well recognized, e.g. synthesis is favored over hydrolysis, tunable enzyme selectivity via reaction condition engineering, and obviously increased substrate and product solubility. Recently lipase-catalyzed transesterifications of polysaccharides in organic solvents such as dimethyl sulfoxide (DMSO) or dimethylformamide (DMF) were reported. Many polysaccharides have been studied including inulin (4), starch (5), and dextran (6–9). Although several studies were undertaken (6–9), no detailed report dealt with effect of acyl donor in enzymatic modification of dextran and the percent modification of dextran with vinyl ester higher than 52% has never been achieved (8).

Previously, we reported an improved activity of lipase AY from Candida rugosa for modification of dextran T40 (DexT40) with vinyl decanoate (VD) by pH adjustment in a phosphate buffer at pH 7.5 prior to DMSO addition (8). The transesterification percentage at 50°C, 25 h reach 22% and 49% when molar ratio of dextran to VD was 1:2, and 1:4 respectively, compared to 2.95% conversion when the
non-pH adjusted lipase AY at the same condition was used. Although
the pH-adjusted lipase AY showed its high activity resulting in high
percent modification, unfortunately it lost its activity after 11 h of
reaction (8).

Herein we describe a novel strategy to improve the activity of the
enzyme in DMSO resulting in the highest extent of modification
obtained by biocatalyst which has never been reported. The strategy
included the stepwise addition of a new active enzyme to replace
inactive enzyme in DMSO (Fig. 1). We also improved the activity of
lipase AY by co-lyophilization of pH-adjusted lipase AY together with
18-crown ether, as well as the type of acyl donors that had an effect on
extent of modification and their regioselectivity. Acyl donors were
compared among saturated acyl donor with short alkyl chain (i.e., vinyl
acetate, vinyl propionate), long alkyl chain (i.e., vinyl decanoate, vinyl
laurate), unsaturated acyl donor (i.e., vinyl acrylate, vinyl crotonate) and
highly sterically-hindered acyl donor (i.e., vinyl pivalate) (Fig. 2). The
tunable degree of modification of dextran catalyzed by lipase AY
obtained in this study, consequently leads to a novel functional material
in which cannot be synthesized using traditional chemical technique,
and can form a nanoparticle which is potential in drug carrier systems.

MATERIALS AND METHODS

Materials Lipase AY (EC 3.1.1.3) from Candida rugosa was a generous gift from
Amano Enzyme Co. (Nagoya, Japan), with reported activity of 32,800 U/g (Lot No.
LAYE151016). DexT40 (Mn 26,000, Mw 40,000) from Leuconostoc mesenteriodes was
purchased from Pharmacia (Uppsala, Sweden). VD, 18-crown-6 ether (1,4,7,10,13,16-
Hexaoxacyclooctadecane), vinyl acetate, vinyl propionate, vinyl laurate, vinyl acrylate,
viny crotonate, vinyl pivalate and other chemicals were of analytical grade and
purchased from Sigma Aldrich (Buchs, Switzerland). The dialysis membrane with
MWCO 6–8000 was purchased from Spectrum Laboratories Inc. (CA, USA).

Lipase-catalyzed transesterification of DexT40 with fatty acid vinyl ester
DexT40 (81 mg, 0.5 mmol eq. to glucopyranosyl unit of dextran) was dissolved in DMSO (5 ml) at
room temperature. After VD (396 mg, 2.0 mmol) was added, the reaction mixture was
heated to 50°C for 5 min before the addition of pH-adjusted lipase AY (26–133 mg). The
preparation of pH-adjusted lipase AY was described elsewhere (8). The water content of the
reaction system determined by Karl Fischer titration (C30 Compact Karl Fischer Coulometer,
Mettler Toledo, Germany) was equal to 0.33 ± 0.01% (see Table S1 in supporting
information). The reaction was vigorous stirred at 50°C for 32 h, and cooled down to stop
the enzymatic reaction. The reaction mixture was dialyzed for 2 days each against 13% (v/v)
water in ethanol, 40% (v/v) water in ethanol, and followed by 100% water with several
changes. The obtained aqueous solution was lyophilized for 48 h, and their structure were
then analyzed by 1H NMR, 13C NMR, 1H-1H correlation spectroscopy (COSY) using a BRUKER
600 MHz spectrometer (see in the supporting information; Figs. S1, S2, Tables S2, S3 for NMR
spectrums and signals, respectively) and FTIR using BRUKER IFS 25 spectrometer (see Fig. S3
and Table S4). It is worth noting that the separation of lipases remaining in the obtained
modified DexT40 was not necessary since they were filtrated out simply during the
solubilization of predetermined amount of modified DexT40 in THF. In fact, modified DexT40
was completely soluble in THF in which lipases were insoluble and precipitated out (see

FIG. 1. (A) Schematic representation of lipase AY-catalyzed transesterification of dextran with vinyl decanoate. Regioselectivity toward the 2-OH (above) or 3-OH (below) in the
glucopyranosyl unit of dextran. (B) Comparison of percent modification of native lipase, pH-adjusted lipase, pH-adjusted lipase co-lyophilized with 18-crown-6 ether, and the lipase
stepwise addition reaction. All reactions were performed in DMSO, 50°C.

FIG. 2. Structure of vinyl esters used as acyl donor.
Table S5 in supporting information). The obtained product was white solid with yield of 125 mg ca. 90%. The effect of acyl donor structure on the extent of modification and regioselectivity was also investigated by replacing VD with either vinyl acetate, vinyl propionate, vinyl laurate, vinyl acrylate, vinyl crotonate or vinyl pivalate using similar procedure.

Co-hydrophosphilization of pH-adjusted lipase AY with 18-crown-6 ether One gram of lipase AY was added to the solution of 18-crown-6 ether (6 mg, 23 μmol) in 20 mM phosphate buffer pH 7.5 (20 ml). The mixture was stirred at room temperature for 1 h, then the solution was flash-frozen in liquid nitrogen followed by lyophilization for 48 h. The obtained product was white solid with yield of 1.202 g ca. 79%. Amount of 18-crown-6 ether added was varied from 11, 23, 46 and 92 μmol.

Stepwise addition of lipase AY and VD to improve % modification of DexT40

DexT40 (81 mg, 0.5 mmol eq. to glucopyranosyl unit of dextran) was dissolved in DMSO (5 ml) at room temperature. After VD (396 mg, 2.0 mmol) addition, the reaction mixture was heated to 50°C for 5 min before adding pH-adjusted lipase AY (26 mg). The reaction was vigorously stirred at 50°C for 52 h, then lipase AY (26 mg) and VD (396 mg, 2.0 mmol) were added to start the second step of transesterification. The same amount of lipase AY and VD was added again after 96 h of reaction. The total amount of lipase AY and VD added after three consecutive steps were equal to 78 mg and 1.188 g, respectively. The purification and characterization procedures were similar to single enzyme addition method described above. The obtained product was white solid with yield of 170 mg ca. 87%.

Preparation of DexT40-VD nanoparticles Nanoparticles of DexT40-VD were prepared by solvent diffusion method (3). DexT40-VD (25 mg) dissolved in THF (5 ml) was added drop-wise to aqueous solution (10 ml) under vigorous magnetic stirring. The organic solvent was then evaporated at room temperature under vacuum. The size of nanoparticles was determined in 1.0 mM NaCl at 30°C by dynamic light scattering using a Malvern High Performance Particle Sizer and by SEM using a Hitachi S-2500 scanning electron microscope. The DLS and SEM images were provided in Fig. S4 of the supporting information.

### RESULTS AND DISCUSSION

#### Effect of acyl donor on lipase AY-catalyzed regioselective transesterification

Using 0.1 M DexT40 (calculated based on number of moles of glucopyranosyl repeating units) and 0.4 M of fatty acid vinyl ester, significant transesterification by pH-adjusted lipase AY were obtained in 32 h (Table 1). When vinyl acetate was used as acyl donor, percent modification of DexT40 increased from 31.8% to 60.5% in the presence of enzyme. While the enzyme accelerated the reaction with vinyl propionate up to 58.6% compared to 8.2% without enzyme. When saturated long hydrocarbon chain such as vinyl decanoate (C10) and vinyl laurate (C12) was employed, enzymatic modification of DexT40 caused notably high extent of modification between 40 and 50%, as compared to less than 3% without enzyme. Acylation with unsaturated fatty acid ester such as vinyl acrylate, vinyl methacrylate, and vinyl crotonate to DexT40 in DMSO demonstrated high extent of modification of 74.3%, 51.2%, and 25.0% with addition of enzyme, compared to 25.5%, 1.7% and 0% without biocatalyst. It should be noted that transesterification of DexT40 using vinyl crotonate as an acyl donor was not possible within 32 h reaction without pH adjusted-lipase AY. In case of vinyl pivalate which processes tert-butyl group, the highly sterically hindered structure resulted in the low percent modification at 13.4%, the lowest value observed in this study. By considering the substrate binding site of Candida rugosa lipase, the scissile fatty acyl chains is bound in a hydrophobic and narrow tunnel which is unique among lipases (10). The narrow binding site of lipase AY may not fit to the high sterically hindered fatty acyl donor such as vinyl pivalate, hence yielded the lowest extent of modification. However, our results clearly demonstrated that various types of acyl donor can be used to prepare hydrophobically modified dextrans with different degrees of modification. This is an important parameter to control the physico-chemical properties of the target amphiphilic dextran.

For oligosaccharides with only 2–4 sugar moieties, lipases usually acylate primary hydroxyl group at the 6-position of oligosaccharides due to its distinct reactivity and less sterically hindered compared to other hydroxyl groups at 2-, 3-, 4-positions of the glucopyranosyl ring. Ferrer et al. synthesized fatty acid esters of maltose using lipase from Thermomyces lanuginosus in a reaction medium containing 2-methyl-2-butanol/DMSO. They found that hydroxyl 6-OH in the non-reducing end of maltose was acylated in high yields of 72% in 24 h using 5% DMSO mixture (11). It was also reported that percent modification and regioselectivity of lipase-catalyzed acylation of oligosaccharides normally depend on the type of sugar units, acyl donors, reaction medium, and the support used for immobilized enzyme (12).

In case of dextran, its repeated glucopyranosyl rings are mainly linked to each other at 1- and 6-positions, therefore 1- and 6-OH group are not available. Our previous results showed that activity of Candida rugosa lipase was toward the more sterically hindered and less reactive 2- and 3-OH of dextran (8,9). In the absence of enzyme, regioselectivity of transesterification of DexT40 with saturated acyl donors of both short and long alkyl chains was comparatively higher in 3-position, while the substitution was distributed equally between 2-OH and 3-OH in enzymatic reaction. In contrast, Ge et al. reported the chemical structure of DexT40-VD with a substitution degree of 23% when reacted with nanogel encapsulated lipase L-1754, the regioisomer was favorable at 2-OH (ratio of 2- to 3-position was 7:3). Lipase AY and lipase L-1754 showed the different activity in catalyzing the transesterification between DexT40 and VD. The difference was probably due to the different isoforms of Candida rugosa lipase produced by different fermentation conditions (13). Therefore, it is possible that the percent modification and regioselectivity of lipase L-1754 and lipase AY toward glucose unit of dextran might be different.

When unsaturated fatty acyl donors were employed, the acylation of the glucopyranosyl ring occurred mainly at the 2-OH (77–85%) compared to 3-OH (15–23%). In contrast, Ferreira et al. reported that the regioisomer of DexT70-Vinyl acrylate at 3-position was highly favored with a ratio of 2- to 3-OH of 28.72 using pH-adjusted lipase AY (6). It should be noted, that Ferreira’s study 25% modification was reported in the lipase-catalyzed transesterification between DexT70 and vinyl acrylate at 50°C for 72 h. The results obtained suggest that lipase AY could not perfectly distinguish the 2-OH and 3-OH positions. In addition, its regioselectivity might be different or changing during the enzymatic transesterification step. The molecular recognition of DexT40 and DexT40-VD with low and high % modification to the substrate binding site of lipase AY might provoke different pattern. Since hydrophobic alkyl chain of the VD which attached to the glucopyranosyl ring can change the physico-chemical properties of the entire DexT40, resulting in the slight change at the position of binding in the active pocket site which showed an effect on the regioselectivity of acylation and regioselective transesterification.

### Table 1. Effect of acyl donors on the percent modification and regioselectivity of DexT40 catalyzed by pH-adjusted lipase AY.

<table>
<thead>
<tr>
<th>Type of acyl donors</th>
<th>Without enzyme</th>
<th>With enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% modification</td>
<td>Modification at 2-OH:3-OH</td>
</tr>
<tr>
<td>Saturated, short chain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinyl acetate</td>
<td>31.8</td>
<td>34:66</td>
</tr>
<tr>
<td>Vinyl propionate</td>
<td>8.2</td>
<td>36:64</td>
</tr>
<tr>
<td>Saturated, long chain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinyl decanoate</td>
<td>2.8</td>
<td>47:53</td>
</tr>
<tr>
<td>Vinyl laurate</td>
<td>0.7</td>
<td>Non detectable</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinyl acrylate</td>
<td>25.5</td>
<td>55:45</td>
</tr>
<tr>
<td>Vinyl methacrylate</td>
<td>1.7</td>
<td>Non detectable</td>
</tr>
<tr>
<td>Vinyl crotonate</td>
<td>0.0</td>
<td>Non detectable</td>
</tr>
<tr>
<td>Sterically-hindered</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinyl pivalate</td>
<td>1.5</td>
<td>Non detectable</td>
</tr>
</tbody>
</table>

DexT40:vinyl ester = 1:4 molar ratio. The reactions were performed at 50°C for 32 h in DMSO, % modifications and regioselectivities were determined by 1H NMR and 13C NMR, respectively. Values in parentheses show the ratio of biocatalyst conversion over the non-biocatalyst conversion.
of the DexT40-VD. Further investigations about the relationship between the extent of modification and regioselectivity are currently in progress in our laboratory.

In case of high sterically hindered acyl donors such as vinyl pivalate, a unique regioselectivity was obtained in which the reaction proceeded predominantly up to 71% at 3-OH. The results demonstrated again that lipase AY had the loose regioselectivity toward 2-OH and 3-OH in the glucose unit of dextran. The type of acyl donors showed a strong influence on the regioselectivity of the lipase AY. Our results are in consistent with lipase-catalyzed acylation of oligosaccharides system in which regioselectivity normally depends on the type of sugar units, acyl donors, reaction medium, and the support used for enzyme immobilization (12).

Co-lyophilization of pH-adjusted lipase AY with 18-crown-6 ether The important drawback of the use of organic solvents for enzyme reactions is that its activity is generally several orders of magnitude lower when compared to its activity in aqueous solution. Prior lyophilization of the enzyme from an aqueous solution which is buffered at the pH of optimal aqueous enzyme activity (pH-adjusted enzyme) improved the activity in organic solvent (8,14). The pH-adjusted lipase AY employed in this study increased the activity to 49% compared to less than 3% when native lipase AY was used (Fig. 1). It has been reported that when 18-crown-6 was added to enzyme solution before lyophilization, transesterification activity of sulcatol with vinyl acetate in toluene by Burkholderia cepacia lipase and Candida antarctica lipase B increased up to 2.5- and 1.4-fold, respectively (15). Moreover, addition of crown ethers to α-chymotrypsin, subtilisin and other proteases considerably enhanced the activity of these enzymes in transesterification reactions of N-acetyl-alanine and N-phenylalanine esters in organic solvents. Up to 640-fold of activity enhancement were observed by prior lyophilization of the enzymes in the presence of 18-crown-6 ether (14). In our study, we aimed to improve lipase AY activity by prior co-lyophilization of the enzyme with 18-crown-6 ether. Different molar ratio (25–200 mol 18-crown-6 compared to 1 mol of lipase) was dissolved in 20 mM phosphate buffer pH 7.5, then lipase AY (1 g) was dissolved and lyophilized.

The degree of substitution increased with increasing concentration of crown ether and at 50 molar equivalents, it reached the highest value with 64% modification (Fig. 3A). From the results crown ether enhanced degree of substitution up to 1.4-fold compared to pH-adjusted enzyme alone, but increased 22.7-fold when compared to native lipase AY (Fig. 1). It is important to be aware of the water content in the reaction mixture that has been reported to affect the degree of substitution (16). To that the water content of reaction mixtures containing different enzyme preparations was determined. It appears that the water content of reaction mixtures containing different enzyme preparations were approximately 0.34 wt.% in all cases (see Table S1 in supporting information). Thus water activity cannot be responsible for the observed differences. Several complexation processes resulting from crown ether may contribute to the enzyme activation such as buffer cations present in the enzyme preparation, charged residues on the enzyme surface, and water at the active site of the enzyme (14). Crown ether was reported to induce activation of enzymes in non-aqueous media, and the increased $V_{max}$ values of crown ether-treated enzyme most probably originated from conformational changes, which after kcat as well as the amount of catalytically active enzyme (17).

It has been reported previously that crown ether could play role as activator or inhibitor depending on the polarity of solvent. Indeed, the activation of crown ether to enzyme solution before lyophilization does strongly enhance the transesterification activity of α-chymotrypsin and lipases in non-polar organic solvent and the activation level decreased with the increase of polarity of organic solvent (15,18). However, the activation by 18-crown-6 added before lyophilization was observed in strong polar solvent as DMSO when molar ratios of 18-crown-6 to lipase were more than 50. Fig. 3A clearly revealed that the presence of more than 50 equivalents of 18-crown-6 ether showed the reverse effect to the enzyme activity. No activation effect was observed when 18-crown-6 was added to reaction medium (Fig. 3A). These results agree with the previous study of transesterification of sulcatol with vinyl acetate by lipases in toluene reported by Secundo et al. It has been suggested that the decrease of activity in organic solvent at the higher molar ratios of 18-crown-6 to lipase relates to the inactivation of lipase in water when concentration of 18-crown-6 increased. The enzyme activity becomes deleterious when enzyme is lyophilized (15). Moreover, reequilibration of water may probably be the explanation of this behavior. When crown ether is in excess, it will be slowly released from the enzyme molecule into the organic solvent. Due to an increased solvent polarity, the solvent becomes more “water-demanding”, therefore water will redistribute between the crown ether containing organic solvent, and the enzyme. As a result, the enzyme becomes dehydrated and will consequently become inactive (17). Due to this phenomenon, the optimum is shifted towards 50 equivalents of 18-crown-6 ether.

Enhancement of pH-adjusted lipase AY activity via crown ether treatment, as well as the effect of various types of fatty acid vinyl ester employed as acyl donors were clearly demonstrated in our studies. Using regioselectivity information provided in this study, one can design the well-defined structure of hydrophobically modified dextran with tunable degree of modification, which will lead to controllable physico-chemical properties of the target polymeric surfactants.

**Tunable degree of modification by stepwise addition of lipase AY and vinyl ester** We reported previously that lipase AY from Candida rugosa lost its activity in DMSO with the extent of modification being less than 3% (Fig. 1) (8). When lipase AY was pH-adjusted in the presence of 20 mM phosphate buffer at pH 7.5
before starting reaction, the activity of catalyzing transesterification was increased to yield 49% modification (8). In this study, the crown ether treatment lipase AY increased % modification of the DexT40-VD to 64%. Unfortunately, pretreatment had no effect on lipase stability in DMSO (8). Therefore, the enzyme lost almost all of transesterification activity within 11 h at 50°C in DMSO (Fig. 3B). In order to improve the percent modification of DexT40, the direct addition of a new active pretreated-lipase AY was proposed. Our new strategy will open the way to boost up the percent modification of DexT40 after the enzyme lost its activity in DMSO. Our newly proposed idea was confirmed by addition of two or three consecutive lots of pretreated-enzyme (26 mg) and VD (396 mg, 2.0 mmol) to the solution of DexT40 (81 mg, 0.5 mmol) in DMSO (5 mL). As expected, the increase in degree modification of DexT40 was observed when a new active enzyme and VD was added at both second and third step (Fig. 4).

The extent of modification of glucopyranosyl units of dextran was approximately 60% within the first 24 h, after the plateau was attained at 52 h the freshly active pretreated lipase AY was added followed by an immediate increase of the percent modification of DexT40. The third step of enzyme addition was performed at 96 h, when previously added enzyme completely lost its activity. Finally, 96% degree of substitution was achieved after 112 h of reaction. Normally the 100% extent of modification of DexT40 cannot be reached because DexT40 contains about 5% of α-1,3 linked side chains (19) which will not be reactive with lipase AY. Therefore, by stepwise addition of active enzyme and VD, the highest yield of DexT40-VD was obtained in this study. It was reported that all the glucopyranosyl moieties of dextran are mono-substituted (6,9). Excess amount of VD was maintained in the reaction by stepwise addition of VD in order to keep molar ratio of glucose unit in DexT40/VD higher than 1/4 to ensure the high percent conversion as clearly demonstrated in our previous report (8). Amount of lipase AY added was in excess since the amount was able to vary from 26 to 133 mg without any influence to the % modification (data not shown). Addition of lipase AY less than 26 mg resulted in the significantly decrease in percent yield and % modification of the DexT40-VD.

Our attempts to obtain the highest degree of modification (96%) of dextran were successful by using the stepwise addition of the new active pretreated-lipase AY. This simple method can increase the yield of enzyme-catalyzed reactions in organic solvent.

Preparation of DexT40-VD nanoparticles The 96% modified DexT40-VD obtained in our study, led to a novel and soft functional material which can form nanoparticles useful as potential drug carriers. DexT40-VD nanoparticles prepared by solvent diffusion method (3) were observed under dynamic light scattering in the NaCl aqueous solution, resulting in the average size of 150 nm (See Fig. S4, SEM and DLS result in Supplementary material). It is notable that DexT40-VD with % modification less than 50% did not form any stable nanoparticles in an aqueous solution. The 96% modified DexT40-VD derived in our study, led to a unique polymeric material and formed nanoparticles which are promising for application in drug delivery systems. Physico-chemical properties, biodegradability, drug upload and release experiments are underway to further develop this unique and potent candidate. Experiments for applying our successful stepwise addition method to gain higher yield of other enzymatic reactions in organic solvent are ongoing to confirm its generality. Physico-chemical properties, biocompatibility and biodegradability, drug upload and release experiments of this unique and promising material were planned to expand its application in drug delivery systems (Complete description will be reported in forthcoming paper).

Supplementary materials related to this article can be found online at doi:10.1016/j.jbiosc.2011.04.004.

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