Natural Thermoresponsive Rice Granules as Biocompatible Drug Carriers

Xue Liu,‡,† Pongpat Oungeun,§ Wijit Banlunara,¶ Asada Leelahavanichkul,⊥ and Supason Wanichwecharungruang‡,*

†Nanoscience and Technology Program, Graduate School, ‡Center of Excellence on Petrochemical and Materials Technology, §Nanotec-CU Center of Excellence on Food and Agriculture, Department of Chemistry, Faculty of Science, ¶Department of Pathology, Faculty of Veterinary Science, ©Division of Immunology, Department of Microbiology, Faculty of Medicine, and †Center of Excellence in Materials and Bio-Interfaces, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand

ABSTRACT: Through thermal gravimetric, X-ray diffraction, infrared spectroscopy, and scanning electron microscopic analyses, here we reveal that the 4–5 μm diameter pentagonal shaped rice granules are surprisingly stable against α-amylase, trypsin, lipase, diluted acids, and diluted bases. Some papain-sensitive proteins play an important role in the granular shape stabilization. We employ the reversible thermoresponsive expansion/shrinking character in aqueous medium of this biopolymeric pentagonal granular assembly to encapsulate the antibiotic vancomycin into the granule at the drug loading content of 80% drug mass with only 20% rice granule mass. The obtained drug-loaded granules display no-burst but steady sustained release of the water-soluble vancomycin in an aqueous environment for more than 24 h.

1. INTRODUCTION

Starch is one of the most widely and longest experienced edible materials in human history. It is the main energy supply for humanity worldwide and is a common form of carbohydrate reserve found in various rhizomes, cereals, and roots and tubers of diverse plants. Starch is generated in amyloplasts as discrete granules with various distinguishing shapes such as oval, round, ogival or elongated to flat, and polyhedral or lenticular and sizes from submicron to more than 100 μm in diameter.1 Starch granules contain two major polysaccharides, amylose and amylopectin. Although amylose is regarded as a linear polymer chain of α-(1,4)-linked D-glucose residues whereas amylopectin is considered as a chain of α-(1,4)-linked D-glucose residues with several branches along the chain through α-(1,6)-glycosidic linkages, amylose with few branches also exists.2 Usually, amylose possesses a long linear chain of several hundred to thousand glucosyl units,3 whereas amylopectin is extensively branched and possesses a comparatively shorter length of the main chain.4 Most starches in nature contain amylose and amylopectin with such well-distiguishing structures. Nevertheless, some starches, from genetically modified or from mutant plants, contain the so-named intermediate materials.6–8 As the name indicates, structures of these polyglucans are in between amylopectin and amylose, regarding the branches and the lengths of the main chains. Normally, amylopectin in starch granules constitutes the major component by weight, whereas amylose occupies around 15–30%, but some genetically modified starches contain 50–80% amylose.9–11 Amylopectins usually exist as double helical structures with crystallinity, which contribute to the semi-crystalline structure of the starch granules.12 Crystallite parts in starch granules are arrangements of double helixes of amylopectin chains into specific patterns such as monoclinic or hexagonal unit cells.13,14

Proteins are minor constituents in starch granules; rice granules contain about 1% by weight of protein. It has been assumed that they are mostly for storage, biosynthesis, and starch digestion functions.15 Lipids are rare in root and tuber starches but more abundant in cereal starches, and they are mostly in the forms of free fatty acids or phospholipids. Rice granules contain approximately 1–2% by weight of lipids.16 Their functional or structural role is unknown. To the best of our knowledge, the molecular assembly of biopolymers into the pentagonal granular structure is still far from complete understanding.

Research over the utilization of natural materials and renewable resources has expanded nowadays, ranging from chitosan, cellulose, dextrin, gums to starch. In terms of applied research, bioplastics, natural polymer blends, etc. are remarkable and concreate.17,18 Moreover, renewable resources forms to catalyst is also achievable.19 Rice granules, another natural material, have been used mostly in the food industry. The scientific community has witnessed endless efforts in chemical syntheses and assemblies of nano- and microsized carriers using various processes and materials for various applications such as drugs, cosmetics, and paints. Nevertheless, to date, sustaining the release of water-soluble drugs in aqueous environments is still a challenge. The biocompatibility of starch granules is improved by various treatments, such as heat, pressure, and chemical modification, so that the drug can be released in a sustained manner.20–22 Various methods of drug loading into starch granules, such as gelatinization,23–27 microencapsulation,28,29超临界流体提取30–33 and chemical modification,34–36 have been developed. Since the natural material can be used in various applications, including biomedical, pharmaceutical, and food sectors,28,37–39 the study of drug delivery from natural starches has increased interest.

Received: March 7, 2019
Accepted: April 18, 2019
Published: May 1, 2019

DOI: 10.1021/acsomega.9b00642
ACS Omega 2019, 4, 7911–7918

ACS Publications © 2019 American Chemical Society

7911

http://pubs.acs.org/journal/acsonf
of rice granules witnessed through the history of human beings and their naturally stable micron-sized granular structure contrive us to hypothesize the possibility of using this natural granule as a reservoir to sustain the release of oral drug molecules. We have exercised our curiosity through the use of the water-soluble antibiotic vancomycin as a model drug. Vancomycin, a tricyclic glycosylated nonribosomal antibiotic peptide, has been used in the treatment of chronic osteomyelitis. Different carrier systems such as chitosan aerogel particles, poly(lactide-co-glycolide) nanoparticles, and N-trimethyl chitosan nanoparticles have been experimented for controlling the release of vancomycin, and most systems still possess low loading and burst release characteristics. Here, we show the use of the natural rice granule as a carrier to sustain the release of water-soluble vancomycin. We demonstrate the exceptionally high stability and extremely high loading capacity of this natural carrier, together with the surprisingly steady sustained drug-release character of the obtained vancomycin-loaded rice granules. This paper also covers molecular interactions among biopolymers that result in granular structure stabilization and those between vancomycin and biopolymers in the granules that result in sustained release of the encapsulated drug molecules. Finally, reaction and degradability of the granules in tissues were investigated in rats and are reported here.

2. EXPERIMENTAL SECTION

2.1. Thermoresponsive Swelling and Shrinking of Rice Granules. Rice granules (50 mg, ground from Oryza sativa grains, Thai Flour Industry, Bangkok, Thailand) were dispersed in 20 mL of water, and the mixture was heated in a closed round-bottomed flask at 80 °C for 30 min. The obtained granules were immediately (1) subjected to scanning electron microscopic (SEM, JEOL JSM-6480LV) and confocal microscopic analyses (Confocal Laser Scanning Microscope, Eclipse, Ti series microscope, Nikon, Japan) and (2) kept at 4 °C for 24 h and then subjected to both SEM and microscopic analyses. A similar experiment was carried out but changing the temperature from 80 to 100 °C.

2.2. Rice Granule Stability. The rice granules were subjected to incubation with bases, acids, and various enzymes, e.g., α-amylase, trypsin, lipase, and papain.

2.2.1. Alkaline Treatment. Rice granules (1 g) were put into 5.0 mL of 0.05 M aqueous NaOH solution, and the mixture was stirred for 6 h at room temperature. Then, the obtained granules were subjected to SEM analysis directly.

2.2.2. Acid Treatment. Rice granules (1 g) were put into 5.0 mL of 0.05 M aqueous HCl solution, and the mixture was stirred for 6 h at room temperature. Then, the obtained granules were immediately observed by SEM.

2.2.3. α-Amylase Treatment. α-Amylase (240 000 units of the α-amylase enzyme from Bacillus subtilis, Sigma-Aldrich, Singapore) was added into the suspension containing 0.01 M phosphate buffer with pH 7.4 (5 mL) and rice granules (1 g) and stirred at 37 °C; the treated granules were sampled at 1 and 3 h and then observed by SEM. In addition, 50 mg of rice granules was dispersed in 5 mL of 0.01 M phosphate buffer with pH 7.4 and 400 μL of fresh saliva was added and the mixture was incubated at 37 °C with gentle shaking for 1 and 3 h. In addition, 50 mg of rice granules was dispersed in 5 mL of water and 400 μL of fresh saliva was added and the mixture was incubated at 37 °C with gentle shaking for 1 and 3 h. A similar experiment with saliva was carried out using 0.01 M phosphate buffer with pH 7.4 in place of water.

2.2.4. Trypsin Treatment. Trypsin (5 mg, 65 000 units, from porcine pancreas, Type IX-S, lyophilized powder, Sigma-Aldrich) was dissolved in 1 mM HCl solution (50 mL), and then 1 g of vancomycin-loaded rice granules was added and the mixture was stirred for 6 h. Afterward, samples were subjected to SEM analysis immediately.

2.2.5. Lipase Treatment. Lipase (5 mg, 2000 units, from porcine pancreas, Type III, lyophilized cake, Sigma-Aldrich) was dissolved in 0.05 M sodium carbonate solution (7 mL), and then 50 mg of vancomycin-loaded rice granules was introduced and gently shaken at 37 °C. Samples were collected at 6 and 8 h post incubation for immediate SEM analysis.

2.2.6. Papain Treatment. Papain (1000 mg, 10 000 units, from papaya latex, lyophilized powder, Sigma-Aldrich, Singapore) was put into the suspension of 0.1 M phosphate buffer saline (50 mL, pH = 7.4) containing vancomycin-loaded rice granules (1.0 g) and stirred for 6 h at room temperature. The treated granules were then subjected to SEM analysis. Papain (50 mg, 500 units) was added to the rice granule suspension (50 mg granules in 20 mL water), and the mixture was stirred at room temperature for 6 h. Then, α-amylase suspension (2 mg, 760 units, in 2 mL water) was added and the mixture was stirred at 37 °C for 1 h.

2.3. Vancomycin Encapsulation. Rice granules (50 mg) were put into an aqueous solution of vancomycin (Vancocin-S, Siam Pharmaceutical Co. Ltd., Thailand; 20 mg of vancomycin in 20 mL of water). The mixture was stirred at 80 °C for 3 h. The granules were separated from the supernatant by centrifugation, washed with water, and then kept at 4 °C. We also carried out the similar experiments with different initial concentrations of vancomycin: 1000; 3000; 5000; 10 000; and 20 000 ppm.

The quantification of the amount of unencapsulated vancomycin in the supernatant was carried out by (1) centrifugal filtering (Amicon Ultra 0.5 mL Filters, with the molecular weight cutoff of 10 000) of the encapsulating mixture (a mixture of granules and drug solution after heating at 80 °C for 3 h) to separate granules from the supernatant and (2) quantifying the amount of drug in the obtained supernatant using UV–visible spectrophotometry (Optizen Pop Series, Korea) with the aid of a calibration curve constructed from vancomycin standard solutions prepared in water. The encapsulated vancomycin content, encapsulation efficiency (EE%), and loading capacity (loading%) were calculated using below equations.

\[
EE = \frac{\text{wt of drug in the granules}}{\text{wt of drug originally used}} \times 100\%
\]

\[
\text{Loading%} = \frac{\text{wt of drug in the granules}}{\text{wt of drug in the granules + wt of the granules}} \times 100\%
\]

Vancomycin-loaded granules with 80% loading content were subjected to papain digestion for 3 h using the same protocol as that for the rice granules. After digestion, the granules were then subjected to release experiment as described in Section 2.4.

2.4. Release of Vancomycin from the Granules. The release was carried out by dispersing 25 mg of the vancomycin-loaded granules (80% vancomycin loading content) in 10 mL of the release medium, which was 0.01 M phosphate-buffered...
saline (0.137 M of NaCl, pH 7.4), and placing this suspension into the 12 kDa MW cutoff dialysis bag (Sigma-Aldrich) and dialyzed against 40 mL of the same release medium. The setup was done using the jacketed container in which the temperature of the water jacket was controlled at 37 °C. At each sampling time point, 2.0 mL of the release medium outside the dialysis bag was collected with the replacement of the same volume of the fresh release medium into the system to maintain the volume. The collected aliquot was centrifuged at 10,000 rpm for 3 min, and the supernatant was then subjected to vancomycin quantification by UV–visible absorption spectroscopic analysis at 282 nm with the aid of a calibration curve obtained from vancomycin standards freshly prepared in the same buffer. To make sure that no other entities from the granules would interfere with the absorption of vancomycin at 282 nm, the blank used in the UV absorption measurement was prepared in a way similar to that of the samples, except that drug-free rice granules were used in place of the vancomycin-loaded granules.

### 2.5. Thermal Gravimetric Analyses

Various granule samples were subjected to thermal gravimetric analyses (TGA, YRIS Diamond TG-DTA, High Temp 115 PerkinElmer instruments, S II Seiko instruments, Japan). The analyzed granules included: (1) original untreated rice granules, (2) vancomycin-loaded granules with 80% drug loading content, (3) vancomycin-loaded granules (with 80% drug loading content) that had already been incubated in the released medium for 72 h, and (4) pure vancomycin.

### 2.6. X-ray Diffraction Analysis

One way to probe the chemical interactions within the rice granules is to examine their X-ray diffraction (XRD) patterns. Here, rice granules, heat-treated rice granules (in water at 80 °C for 3 h), vancomycin-loaded rice granules (vancomycin loading content of 80%), and pure vancomycin were analyzed by XRD (Rikaku Ultima and 2000 X-ray Diffraction, Japan).

### 2.7. Tissue Interaction

To find out whether the vancomycin-loaded rice granules could be used as drug reservoirs in the tissue, we tested an inflammatory reaction in mice. The animal care and use protocol following the National Institutes of Health (NIH; #85-23, revised 1985) was approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Male, C57BL/6 mice, 8-week-old, were purchased from the National Laboratory Animal Center, Nakhornpathom, Thailand. The subcutaneous injection of rice granules was performed under isoflurane anesthesia under the sterile condition. In short, the hair above the loose skin between shoulders was removed and cleaned with 70% alcohol. Then, 0.375 mg of rice granules at the concentration of 2.5 mg/mL dispersed in PBS or PBS control (150 μL) (n = 5/group, group I: injected with PBS only; group II: injected with rice granules in PBS; group III: injected with vancomycin-loaded at 80% loading% rice granules in PBS) was administered subcutaneously. The inflammatory skin reaction was observed daily. Mice were sacrificed at 7 days post injection, and the skin lesion was fixed in 10% formalin, paraffin-embedded, and stained with periodic acid-Schiff reagent Hematoxylin and Eosin stain (Sigma-Aldrich, St. Louis, MO). The inflammatory cells were observed from the lesion.

### 3. RESULTS AND DISCUSSION

#### 3.1. Thermoresponsive Size Change of the Rice Granules

The original rice granules possess an average size of around 4.5 ± 0.7 (average ± SD) μm as estimated from their SEM images (Figure 1A-1). After being heated in water for 30 min at either 80 or 100 °C, the shape of the granules...
remained the same but the average size increased to 8.5 ± 0.66 μm as witnessed by both the confocal microscopic analyses (result not shown) and the SEM images (Figure 1A-2). It should be noted here that 30 min heating of the granules at 70 °C or lower temperatures in water did not produce significant size changes and heating in water at 100 °C could not destroy the granular structure (data not shown). The size of the heated granules, which had been cooled down to 4 °C for 24 h, was 4.4 ± 0.7 μm (Figure 1A-3). It should be noted that if the heated granules were left at room temperature of 30 °C their size did not change back to the original size. These results indicate that (1) the size of the rice granules can be expanded by heating in water and can be shrunk back upon cooling at 4 °C and (2) the pentagonal shape of the granules is preserved during the expansion. It should be noted here that some of the polymers, probably mostly amylose, leaked out from the granules during the heat treatment. Usually, the leak accounted for less than 29 ± 6% of the polymer mass lost and this agrees well with the previous report.

The swelling and shrinking ability of rice granules implies that these thermoresponsive granules do not possess a rigid membrane structure. The molecular packing inside the granules is likely to be one of the major factors determining their morphology. We speculate that partial hydration/dehydration of biopolymers is responsible for the granule expansion/shrinking character. We explain the expansion/shrinking of the material as follows. During the heating process, thermal energy is used to break the strong polymer–polymer interactions in the granules so that water–polymer interactions can take place. Although water–polymer interaction probably gives less negative enthalpy (ΔH) than that from the strong polymer–polymer binding, the increase in entropy upon polymer swelling at a higher temperature (larger TΔS term) helps propel the swelling process forward as the more negative Gibbs free energy will be released. Therefore, the granules expand at high temperature together with water absorption into their interior. Upon cooling down, the entropy term becomes less prominent at lower temperatures; thus, the system adapts itself to achieve a more negative Gibbs free energy by allowing the polymer–polymer interactions to take place so that larger heat release (comparing to that resulted from polymer–water interaction) can be obtained. This makes the shrinking process spontaneous at lower temperatures. It should be noted here that water draining out from the particles was clearly observed when the centrifuged heated granules were kept at 4 °C.

Rice thus happens to be a commonly known thermoresponsive material. Nevertheless, it should be mentioned here that the reports and study on a stimuli-responsive material that is 100% natural are rare. In contrast, there are many reported synthetic materials that are specifically designed and assembled to be stimuli responsive, e.g., the light-responsive triblock copolymers, the optically active and thermoresponsive star block copolymers, the redox-responsive micelles, etc.

3.2. Stability of Rice Granules. The rice granules are surprisingly stable against various physical and chemical treatment processes. Normal grinding of the dry granules could not change their size and shape (data not shown). Neither 6 h soaking in 0.05 M sodium hydroxide solution nor in 0.05 M hydrochloric acid could destroy the granules (Figure 1A-4,B-1). The results indicate that granules well tolerate acids and bases to a certain level. Interestingly, although amylose and amylopectin are the main constituents of rice granules, incubating the granules with α-amylase, an enzyme that can digest amylose and amylopectin, did not produce granule disintegration; the granular structure of the material was well preserved. Only some changes on the surface smoothness could be observed (Figure 1B-2). The surface-eroded granules observed from α-amylase digestion help confirm our above hypothesis that the preservation of the granular structure does not involve any membrane structure. This surprising result also implies a limited amount of accessible amylose and amylopectin at the surface of the granules that can be digested by α-amylase. Incubation of rice granules with fresh saliva also did not produce any disintegration of the granules (data not shown).

Trypsin treatment was carried out to investigate whether the enzymes in the human stomach act over the rice granules. No change on granules was observed after trypsin treatment (Figure 1B-3). Combined trypsin and α-amylase treatments, either trypsin first or α-amylase first, also produced no significant change to the granules (SEM data not shown). Last, lipase-treated granules also showed no significant change from the original polyhedral granules with 4.5 ± 0.68 μm diameter (Figure 1B-4). This implies that a lipid is not likely to be involved in granular shape stabilization. Using these enzymes on heat-treated granules also gave similar results.

Remarkably, papain, a protease with endopeptidase, aminopeptidase, and dipeptidyl peptidase activities, could effectively destroy the rice granule structure. The papain-treated granules mostly lost granular shape, and disintegration of the granules clearly took place (Figure 1C-1). Adversely, when α-amylase treatment was carried out on the papain-treated granules, complete destruction of the granules could be observed (Figure 1C-2).

Above observations lead us to hypothesize that the granules contain amylose and amylopectin packed with some specific proteins; without destroying the papain-sensitive proteins first, amylose and amylopectin could not be digested by α-amylase. These papain-sensitive proteins probably comprise an insignificant amount of arginine and lysine amino acid residues since they cannot be destroyed by trypsin. As mentioned above, partial hydration is responsible for the granule swelling. Since papain-sensitive proteins are the components responsible for the granular shape stabilization, it is also possible that these protein components might be the unswellable components of the granules.

The above results contradict the long-time assumption of the complete digestion of rice granules in the mouth and the stomach where α-amylase and trypsin exist, respectively. However, the experimental results here match the fact that eating enough rice usually produces long-lasting energy and good bulk for the colon. It is very likely that rice granules are not fully digested and absorbed through mouth/stomach/small intestines but are also being digested by some microorganisms in the colon. Their resistance to complete digestion may contribute to the necessary bulk required for a healthy environment for microorganisms in the colon. Traditional Thai northeastern cuisine of raw papaya (sauce of papain) with rice has been known to supply people with high energy for labor load. Eating rice with papain likely helps to release more carbohydrates from the granules.

3.3. Drug Encapsulation. Here, the drug encapsulation was carried out by incubating the thermoresponsive rice granules in vancomycin solution at 80 °C. We obtained the
amounts of drug molecules taken up into the granules by quantifying the amount of drug left in the solution after separating out the granules by filtration centrifugation. We also performed a washing of the drug-loaded granules with water and took into account the amount of drug in the washed solutions as the unloaded drug. The result clearly indicates that vancomycin could be effectively loaded into the granules at various drug loading contents depending on the concentration of vancomycin solution used during the loading process. The vancomycin-loaded granules (Figure 1C-3) possess morphology similar to those of the heat-treated granules but with the size of 5.7 ± 0.95 μm (after being placed at 4 °C).

We hypothesize that during the swelling of the granules in hot vancomycin solution the drug together with water got into the interior of the granules and was trapped in the granules (Figure 2). The increase in encapsulation efficiency (EE) along with the increase in initial drug concentration agrees well with our proposed encapsulation mechanism that drug molecules occupy the interior of the granules during the heat-induced granule swelling as the solution penetrates the granules. The higher drug concentration means the larger numbers of molecules at a certain volume of solution. Swelling capacity limits the volume of aqueous solution getting into the granules. Therefore, granules that are swollen in solution with high drug concentrations should have higher numbers of drug molecules getting into their interior, compared to those that are swelled in solution with lower drug concentrations. After getting in, vancomycin molecules likely bind to polymer chains in the granules, probably through multiple hydrogen bonding (Figure 3), and are retained in the granules even after the granules are cooled and water molecules migrate out from the granules.

3.4. Release of Vancomycin from Granules. Vancomycin-loaded granules with 80% vancomycin loading content showed no-burst release of the drug. Steady sustained release of 60% of the loaded drug during the 24 h incubation in aqueous release medium was observed. The release of approximately 10% of the loaded drug then followed on the next day. Explicitly, the release then stopped afterward (Figure 4). The experiments were repeated many times along with the replacement of the release medium, which made sure that the stop was not caused by the high concentration of the drug in the release medium. The same results were still obtained. The result indicated that approximately 30% of the loaded vancomycin possess strong interaction with components of the granules and could not be released. Adding papain to the system did not produce more release of vancomycin from the granules (data not shown). This result rules out the physical barrier as the drug-sustaining factor but confirms that the molecular interaction of vancomycin with biopolymers, likely amylose/amylpectin, is responsible for the drug retention. Heating the granules did not help in releasing 30% drug left in the granules. Therefore, we concluded that strong interaction

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Efficiencies of the encapsulation process and loading contents of the obtained vancomycin-loaded granules at various initial drug concentrations.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Model of vancomycin loading inside a rice granule. Hydrogen-bond interactions among amylose and amylopectin chains are disrupted in the presence of vancomycin molecules, which can effectively form hydrogen bonding with the amylose and amylopectin chains.}
\end{figure}
between vancomycin and biopolymers probably accounted for the 30% unreleased vancomycin. It should be noted here that, to make sure that the 30% unreleasable vancomycin was not misinterpreted from drug degradation, we also encapsulated the bright purple anthocyanin into the granules (data not shown). The release experiment of anthocyanin-loaded rice granules gave a similar result to that of the vancomycin-loaded granules; however, this time, the left-over anthocyanin in the granules could be confirmed easily as the granule were purple.

3.5. TGA Analysis. The thermogram of the original rice granules displays a relatively sharp endothermic peak at around 295–315 °C with the maximum at 305 °C (Figure 5), agreeing well with their semicrystalline structure. Heat-treated granules and alkaline-treated granules showed similar thermograms to those of the original granules. Acid-treated granules also showed a thermogram similar to that of the original granules (data not shown). These data indicate that heating in water (100 °C or less) and alkaline/acid treatment could not disrupt interactions among biopolymer chains within the granules. In contrast, papain-treated granules showed a much broader endothermic peak with the peak maximum at around 260–330 °C with the maximum at 285 °C. The information agreed with the SEM images (Figure 1), which explicitly showed that papain could effectively destroy the granular assembly of the biopolymers.

The thermogram of pure vancomycin shows a broad endothermic peak at around 210–290 °C with the maximum at 237 °C, which corresponds to the noncrystalline structure of vancomycin (Figure 5). Compared to unloaded granules, vancomycin-loaded granules with 80% drug loading content possess a much broader endothermic peak at 250–320 °C. The disappearance of the peak maxima of both the original granules at 305 °C and the vancomycin at 237 °C together with the new peak maxima at 265 and 302 °C clearly illustrates molecular interactions between vancomycin molecules and the polymer constituents inside the rice granules and disruption of the original molecular interactions among polymer chains in the original granules and interactions among vancomycin molecules. The result implies that the vancomycin molecules and biopolymers in the granules are in the state of solid solution with well-dispersed vancomycin molecules in the granules. Unfortunately, the interaction between vancomycin and polymer constituents in the granules could not be witnessed from the IR spectrum of the vancomycin-loaded granules (Figure 6). Nevertheless, the IR spectrum of the drug-loaded granule confirms the high drug loading content of drug molecules in the granules as the spectrum of the drug-loaded granules resembles the spectrum of vancomycin.

After incubating the vancomycin-loaded granules (80% drug loading content) in the release medium for 72 h and approximately 70% of the loaded drug released from the granules, the thermogram of the granules still showed roughly the broad peak at 260–300 °C, not much different in peak shape from that of the vancomycin-loaded granules (before the release), only lower in intensity. This also confirms the presence of unreleasable vancomycin in the granules.

3.6. XRD Analysis. To further understand the molecular packing inside the granules, XRD analysis was carried out. The noncrystallinity detected by the XRD pattern of vancomycin (Figure 7) correlates well with its broad TGA peak (Figure 5) shown earlier. Original rice granules with characters of semicrystallinity show three semisharp peaks at 2θ of 17, 18, and 23°. The XRD pattern of the heat-treated granules was similar to that of the original granules, except that the peaks

![Figure 4](image-url)  
**Figure 4.** Accumulative release of vancomycin from vancomycin-loaded granules with 80% vancomycin loading content. The release was carried out by dispersing 25 mg of the vancomycin-loaded granules (80% vancomycin loading content) in 10 mL of 0.01 M phosphate-buffered saline (0.137 M NaCl, pH 7.4).

![Figure 5](image-url)  
**Figure 5.** Thermal gravimetric analysis of granules with 80% vancomycin loading content; vancomycin-loaded granules after incubation in release medium for 72 h (70% of the loaded vancomycin released out and thus left with granules containing 55% vancomycin loading content); original granules; pure vancomycin; heat-treated granules; and alkaline-treated granules.

![Figure 6](image-url)  
**Figure 6.** IR spectra of rice granules, vancomycin, and vancomycin-loaded rice granules (with 80% drug loading content).
were somewhat less sharp, which means that the crystallinity of the granules was slightly destroyed by heating in water. Vancomycin-loaded rice granules were significantly less crystalline than the unloaded granules, and the XRD pattern of the material showed a broad peak at 2θ of 20°. The disappearance of granule’s crystallinity upon drug encapsulation illustrates the change in the molecular packing within the granules; vancomycin interference into the original ordered packing of the polymers plays the role. Both the XRD and TGA results prove that, inside the granules, the direct interaction between vancomycin molecules and biopolymeric chains disrupts the molecular packing and crystallinity of the granules. We also investigated the XRD pattern of the drug-loaded granules after drug release. As mentioned above (Section 3.4), complete release can never be achieved; the granules possess approximately 30% drug loading after the release. The XRD information agrees well with the TGA result. This confirms that the remaining vancomycin molecules bind to the biopolymers. The original packing of the granules was not restored with partial drug release.

3.7. Tissue Interaction. In addition to showing above that rice granules are indigestible by amylase in the mouth, trypsin in the stomach, and lipase in the small intestine, here we have also observed that the granules are nonallergic to the tissue and also undigestible by any enzyme of the skin reaction. Both the granule injection and the PBS injection showed no inflammatory reaction. In the histology at 7 day post injection, the granules were still intact with no significant inflammatory reaction (data not shown). In addition, there were no eosinophils and basophils in the lesion, implying the nonallergenic reaction of the granules in the mice.

4. CONCLUSIONS

Through SEM, confocal microscopic, TGA, and XRD analyses, here we have shown that the assembly of biopolymers into the pentagonal granular structure of the natural rice granules is surprisingly stable against aqueous heat treatment (at temperatures of less than 10 °C), diluted strong acids, diluted strong bases, and digestion by enzymes found in the gut system (α-amylase, trypsin, and lipase). The granule, however, can be effectively disintegrated by papain. The granules possess no allergic or inflammatory reaction to tissues of mice, but they are also indigestible and nonresorbable in the tissue. The granules show reversible thermoresponsive size changes in water with expansion at high temperature and size reduction when cooled. We have used this thermoresponsive size change character to encapsulate the water-soluble vancomycin into the granules and have easily obtained the vancomycin-loaded granules with the surprisingly high drug loading content of 80% (80% drug mass, 20% biopolymer mass of the granules). Through the TGA and XRD analyses, we have shown that vancomycin molecules interact with biopolymers. As expected from drug–biopolymer interactions, the vancomycin-loaded granules indeed show steady sustained release of vancomycin in an aqueous environment, with 60% of the loaded drug being steadily released out in 1 day with no-burst release and another 10% of the loaded drug being released the next day. The remaining 30% vancomycin could not be released from the granules. Other water-soluble compounds such as anthocyanin can also be encapsulated into rice granules, and the anthocyanin-loaded granules show similar sustained release characters with some unreleased anthocyanin remaining in the granules. Digestion with papain could not help releasing the nonreleasable vancomycin from the biopolymers. The stability of the rice granules against acids and digestive enzymes, their ability to encapsulate water-soluble drug molecules at high loading, and their sustained drug-release characters lead to not only a better understanding of this natural granular material but also the convincing application of rice granules in the oral drug delivery, especially for the colon-targeting drugs.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: psupason@chula.ac.th.

ORCID

Supason Wanichwecharungruang: 0000-0002-2802-4341

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the financial provisions of the following: Ratchadapiseksompot Fund (GCRSS8032301) from Chulalongkorn University (CU); the Center of Excellence in Materials and Bio-interfaces, CU; the Center of Excellence on Petrochemical and Materials Technology, CU; and the Nanotec-CU Center of Excellence on Food and Agriculture, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.

■ REFERENCES

(1) Jane, J.-L.; Kasemsuwon, T.; Leas, S.; Zobel, H.; Robyt, J. F. Anthology of starch granule morphology by scanning electron microscopy. Starch/Stärke 1994, 46, 121−129.
(2) Hizukuri, S.; Takeda, Y.; Yasuda, M.; Suzuki, A. Multi-branched nature of amylase and the action of de-branching enzyme. Carbohydr. Res. 1981, 94, 205−213.
(3) Hizukuri, S.; Takeda, Y.; Manuta, N.; Juliano, B.-O. Molecular structures of rice starch. Carbohydr. Res. 1989, 189, 227−235.
(4) Bertoft, E.; Piyachomkwan, K.; Chatakanonda, P.; Siroth, K. Internal unit chain composition in amylpectins. Carbohydr. Polym. 2008, 74, 527−543.
(5) Hanashiro, I.; Abe, J.-I.; Hizukuri, S. A periodic distribution of chain length of amylpectin as revealed by high-performance anion-exchange chromatography. Carbohydr. Res. 1996, 283, 151−159.
(6) Gérard, C.; Colonna, P.; Buleon, A.; Plancho, V. Order in maize mutant starches revealed by mild acid hydolysis. Carbohydr. Polym. 2002, 48, 131−141.

(7) Klucinec, J. D.; Thompson, D. B. Fractionation of high-amylose maize starches by differential alcohol precipitation and chromatography of the fractions. Cereal Chem. J. 1998, 75, 887−896.

(8) Li, L.; Jiang, H.; Campbell, M.; Jane, J.-L.; et al. Characterization of maize amylose-extender (ae) mutant starches. Part I: Relationship between resistant starch contents and molecular structures. Carbohydr. Polym. 2008, 74, 396−404.

(9) Colonna, P.; Mercier, C. Gelatinization and melting of maize and pea starches with normal and high-amylose genotypes. Phytochemistry 1985, 24, 1667−1674.

(10) Gérard, C.; Barron, C.; Colonna, P.; Plancho, V. Amylose determination in genetically modified starches. Carbohydr. Polym. 2001, 44, 19−27.

(11) Shi, Y.-C.; Capitani, T.; Trasko, P.; Jeffcoat, R. Molecular structure of a low-amylopectin starch and other high-amylose maize starches. J. Cereal Sci. 1998, 27, 289−299.

(12) Imberty, A.; Buleon, A.; Tran, V.; Perez, S. Recent advances in knowledge of starch structure. Starch/Stärke 1991, 43, 375−384.

(13) Bertot, E. Lintnerisation of two amylose-free starches of A- and B-crystalline types, respectively. Starch/Stärke 2004, 56, 167−180.

(14) Imberty, A.; Perez, S. A revisit to the three-dimensional structure of B-type starch. Biopolymers 1988, 27, 1205−1221.

(15) Baldwin, P. M. Starch granule-associated proteins and polypeptides: a review. Starch/Stärke 2001, 53, 475−503.

(16) Azudin, M. N.; Morrison, W. R. Non-starch lipids and starch determination in genetically modified starches. Starch/Stärke 2019, 74, 299−313.

(17) Yu, L.; Dean, K.; Li, L. Polymer blends and composites from renewable resources. Prog. Polym. Sci. 2006, 31, 576−602.

(18) Mayowa-A, A.; Olatunde-C, O. Recent Development on the Application of Carbohydrate Polymers. J. Appl. Chem. 2018, 11, 68−80.

(19) Sangsuwan, R.; Sangher, S.; Aree, T.; Mahidol, C.; Ruchirawat, S.; Kittakoop, P. An organocatalyst from renewable materials for the synthesis of coumarins and chromenes: three-component reaction and multigram scale synthesis. RSC Adv. 2014, 4, 13708−13718.

(20) López-Iglesias, C.; Barros, J.; Ardao, I.; Monteiro, F. J.; Alvarez-Lorenzo, C.; Gómez-Amoza, J. L.; García-González, C. A. Vancomycin-loaded chitosan aerogel particles for chronic wound applications. Carbohydr. Polym. 2019, 204, 223−231.

(21) Zakeri-Milani, P.; Lovyymi, B. D.; Jelvehgari, M.; Valizadeh, H. The characteristics and improved intestinal permeability of vancomycin PLGA-nanoparticles as colloidal drug delivery system. Colloids Surf. B 2013, 103, 174−181.

(22) Xu, J.-J.; Xu, B.; Shou, D.; Xia, X.; Hu, Y. Preparation and Evaluation of Vancomycin-loaded N-trimethyl Chitosan Nanoparticles. Polymers 2015, 7, 1850−1870.

(23) Rosniyana, A.; Hashifah, M. A.; Shariffah Norin, S. A. Effect of heat treatment (accelerated ageing) on the physicochemical and cooking properties of rice at different moisture contents. J. Trop. Agric. Food Sci. 2004, 32, 155−162.

(24) Zhi-Peng, Y.; Na, L.; Li, Y.; Zhi-Qiang, J.; Zong-Quan, W. One-Pot Synthesis, Stimuli Responsiveness, and White-Light Emissions of Sequence-Defined ABC Triblock Copolymers Containing Polytioaphene, Polyyallene, and Poly(phenyl isocyanide) Blocks. Macromolecules 2017, 50, 3204−3214.

(25) Wang, Q.; Ben-Fa, C.; Jia-Hong, C.; Na, L.; Zong-Quan, W. Facile Synthesis of Optically Active and Thermoresponsive Star Block Copolymers Carrying Helical Polysicyanide Arms and Their Thermo-Triggered Chiral Resolution Ability. ACS Macro Lett. 2018, 7, 127−131.

(26) Song-Qing, Z.; Guiju, H.; Xun-Hui, X.; Shu-Ming, K.; Na, L.; Zong-Quan, W. Synthesis of Redox-Responsive Core-Linked Micelles Carrying Optically Active Helical Poly(phenyl isocyanide) Arms and Their Applications in Drug Delivery. ACS Macro Lett. 2018, 7, 1073−1079.