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Inclusion and Release of Hinokitiol Into/from MCT-β-CD Fixed on Japanese Washi Paper

TAKESHI FURUTA*, YOUKO KUSUYA, TZE-LOON NEOH, LARS REHMANN, SUN-HWA BEAK and HIDEFUMI YOSHII
Department of Biotechnology, Tottori University, 4-101 Koyamacho-minami, Tottori 680-8552, Japan

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Abstract

The potential for surface modification of paper by modified cyclodextrins was demonstrated by the model system of Japanese washi paper and monochlorotriazinyl-β-cyclodextrin (MCT-β-CD). MCT-β-CD was covalently bonded to the paper. The optimal bonded reaction conditions were found to be 10 min at 150 °C with a moisture content of less than 2.5 (g water/g dry paper). The bounded MCT-β-CD on the paper was able to include and release hinokitiol representing the group of antibacterial agents. The maximum molar inclusion ratio of hinokitiol in the immobilized MCT-β-CD was around 0.8. The release rates of hinokitiol, included in the fixed MCT-β-CD, were monitored at 50 °C for different relative humidities. The release rate was strongly influenced by the relative humidity. These results demonstrate that the antibacterial agent can be included and released from MCT-β-CD fixed on the paper. The antibacterial activity of the fixed hinokitiol against airborne microorganisms was demonstrated.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides and are natural byproducts of enzymatic starch degradation. The most interesting property of cyclodextrins lies in their hydrophobic cavity, which provides a hydrophobic microenvironment in aqueous solutions. Therefore, cyclodextrins are able to include hydrophobic components in their cavity, often resulting in an increase in the solubility of the included component.

Recently, some modified CDs were developed and used for inclusion of many chemical and pharmaceutical compounds, since they remarkably enhance the solubility. Monochlorotriazinyl-β-cyclodextrin (MCT-β-CD) is a commercially available reactive form of β-CD. It contains an average of two to three monochlorotriazinyl groups that are able to form covalent bonds with nucleophilic groups, such as –OH in cellulose [1–3]. The reaction mechanism is a nucleophilic substitution, and the reaction takes place in the presence of a catalyst, like sodium carbonate, mainly providing alkali conditions. Therefore, it offers the possibility to immobilize some hydrophobic flavor compounds on a cellulose surface such as paper, providing an interesting way in surface modification. MCT-β-CD could be successfully used to modify cotton [4]. The binding efficiency, however, was low and the binding reaction was not further characterized. Another way to bind cyclodextrin to cotton fibers, is the use of acrylamidomethylated β-cyclodextrin, as demonstrated by Lee *et al.* [5]. Because acrylamidomethylated β-cyclodextrin is not commercially available, it has to be synthesized. The cyclodextrin-bonded cloth could retain vanillin aroma after storing for seven days at 80 °C. Also antibacterial activity could be achieved by the inclusion of benzoic acid. Tanabe *et al.* could successfully immobilize cyclodextrin on a cellulose membrane and use it as a molecular detector [6].

Hinokitiol (or β-Thujapilicin) was chosen as a model component for the inclusion and release experiments. It can be extracted from wood [7], providing resistance to fungal decay and insect attacks [8]. In addition, hinokitiol also shows antibacterial activity.

In this study, a high amount of MCT-β-CD should be easily fixable on the surface of a Japanese washi paper to include antibacterial component (hinokitiol). The optimal fixing conditions were investigated in the aspect of the bonded amount of MCT-β-CD, as well as the inclusion of hinokitiol. The release characteristic of the included hinokitiol from MCT-β-CD was also studied at various relative humidities.

Experimental

Materials

MCT-β-CD with the degrees of substitution (DS) of 0.46 per anhydrous glucose unit was purchased from Wacker Chemicals East Asia Ltd. (Tokyo, Japan). If not

* Author for Correspondence. E-mail: takeshi@bio.tottori-u.ac.jp

further indicated, MCT-β-CD with DS of 0.46 was used. A washi paper was a kind gift from Taniguchi Washi Co., Ltd. (Tottori, Japan). Hinokitiol was gifted by Osaka Organic Chemical Co., Ltd. (Osaka, Japan). Sodium carbonate and chloroform were from Wako Pure Chemical Company (Osaka, Japan). Standard Method Agar was obtained from Kyokuto Kaihatsu Kogyo Co., Ltd. (Tokyo, Japan).

Preparation of washi paper bonded with MCT-β-CD

The subject of these experiments is to covalently bond MCT-β-cyclodextrin to the surface of the washi paper. MCT-β-CD is a reactive cyclodextrin capable of forming covalent bonds with nucleophilic groups. The following approach to bond MCT-β-CD to the paper was developed in this study, based on the method devised by Rehmann *et al*. [9].

In a 50 mL beaker, MTC-β-CD was dissolved in 25 mL H_2O at room temperature to prepare 0.14–0.35 g/mL solution. After the addition of 0.8 g Na_2CO_3, it was stirred at 40 °C for 2 min. The mixture was poured in a stainless steel square vessel ($100 \times 150 \times 20^{H}$) containing 10 g of washi paper, followed by shaking at 30 °C for 20 min (Titec BR-13UM shaker, Titec, Tokyo, Japan). Thereafter, the wetted paper was gently wiped on the surface and dried under vacuum at 50 °C in a vacuum oven (Advantec VR-320 Vacuum Drying Oven, Toyo Seisakusho Co., Ltd., Tokyo, Japan), in order to control the moisture content of the paper.

The sample paper was heated at 100–150 °C in a furnace (Isuzu ETR-23K, Tokyo, Japan) for 2–10 min for a thermal fixing reaction. The resulted paper was then washed under running water for 10 min and dried in vacuum for 24 h at 70 °C. The quantity of MCT-β-CD bonded to the paper was estimated by the weight difference of the sample of paper before and after the fixing process described above. MCT-β-CD bonded washi paper will be designated hereafter as MCT-β-CD-paper.

Molecular inclusion of hinokitiol in MCT-β-CD-paper

The conditions for the inclusion reaction were as follows: An MTC-β-CD bonded washi paper was immersed in a stainless steel vessel containing 20 mg/mL hinokitiol solution. The vessel was covered by a plastic plate and shaken at 180 rpm in the Titec BR-13UM shaker at 50 °C. After 3 h of shaking, the samples were washed in water, followed by vacuum drying at 50 °C for 1h and 90 °C for 24 h.

Release of included hinokitiol from MCT-β-CD-paper

Hinokitiol included in MCT-β-CD-Washi paper was chopped into 2×2 mm square chips. About 0.1 g of the paper chips was spread into a 15 mL ($20^{\phi} \times 48$ mm) glass bottle, and stored in a constant temperature air bath at 50 °C. A humid-controlled air (7–90% of relative humidity) was flowed through the bottle. The general set-up of the equipment was shown elsewhere [10]. At a prescribed time interval, the glass bottle containing the sample paper was removed, sealed and stored at 4 °C until the included component was extracted and measured via gas chromatography.

Extraction of hinokitiol from MCT-β-CD- paper

The hinokitiol concentration was measured with gas chromatography after chloroform extraction according to the previous report [11]. An approximately 0.1 g paper chip was weighed in a capped glass tube, and 4 mL of water and 1 mL of chloroform was added in that order. The solution was heated in a water bath for 25 min at 90 °C to extract hinokitiol into the chloroform. During heating the tube was periodically shaken with a vortex mixer. After the extraction the samples were centrifuged in a Kubota 2010 (Kubota Inc., Kyoto, Japan) centrifuge for 20 min at 3000 rpm to separate water and chloroform phases.

Analysis of included fraction of hinokitiol by gas chromatographic measurement

The concentration of hinokitiol in the chloroform was determined by FID gas chromatography (GC-14A, Shimadzu, Kyoto, Japan). The sample was separated on a PEG 20M column (Shimadzu) at 180 °C with nitrogen as carrier gas. The injection and detector temperature were 190 °C and 230 °C, respectively. The gas pressure of the air and hydrogen was 0.5 bar, and the nitrogen pressure was 0.1 bar. External standard method was used for analysis. As a standard solution, 10 mg hinokitiol was dissolved in 4 mL water and mixed with 1 mL chloroform. The chloroform/water ratio in the standard was chosen to be the same as for the extraction, considering the distribution coefficient of hinokitiol in the two phases.

Antibacterial Activity of the Included Hinokitiol

To investigate the antibacterial abilities of the included hinokitiol, *ca*. 2×3 cm of the MCT-β-CD-paper with included hinokitiol was exposed to normal air in the laboratory for 48 h. As a control, MCT-β-CD bonded paper without hinokitiol was used. The paper was then pressed on to a standard growth agar medium to transfer possible accumulated microorganisms to the growth medium. After 5 min, the paper was removed and the Petri dishes were incubated at 37 °C for 48 h. The number of colonies represented the number of vital airborne microorganisms, which accumulated in the paper.

Results and discussion

Factors influencing the amount of MCT-β-CD bonded to washi paper

MCT-β-CD is a cyclodextrin reactive to nucleophilic groups, such as –OH in paper. The quantity of MCT-

β-CD on the paper was determined by gravimetric measurements. The amount of MCT-β-CD bonded on the paper was dependent on the concentration of aqueous MCT-β-CD solution, the moisture content of the paper before heat treatment, and the heating temperature.

Figure 1 illustrates the influence of the moisture content in the paper before the heat treatment at 150 °C for 10 min. At a higher moisture content above 2.6 (g water/g dry paper), the amount of bonded MCT-β-CD decreased markedly, but at lower moisture content below 2.5, the amount of bonded MCT-β-CD was nearly constant. Therefore, before heat treatment, we reduced the moisture content of the paper by the vaccume dryer at 50 °C for 10 min. The resulting moisture content was $1.0 \pm 10\%$.

The effect of temperature of the heat treatment is shown in Figure 2. The paper was dipped in the MCT-β-CD aqueous solution of 0.28 g/L concentration, followed by drying to the moisture content of 10%. The bonded amount of MCT-β-CD was nearly proportional to the heating temperature between 90 and 160 °C. However, the color change of the paper from white to brown took place at 160 °C. The length of time of the heat treatment was also investigated. Ten minutes was enough to obtain a constant bonded amount of MCT-β-CD.

The concentration of MCT-β-CD in aqueous solution influenced also the bonded amount on the paper, as shown in Figure 3. An almost linear increase against the concentration was found. From these results, the most appropriate heat treatment condition was considered as; the heat treatment temperature of 150 °C and the treating time of 10 min.

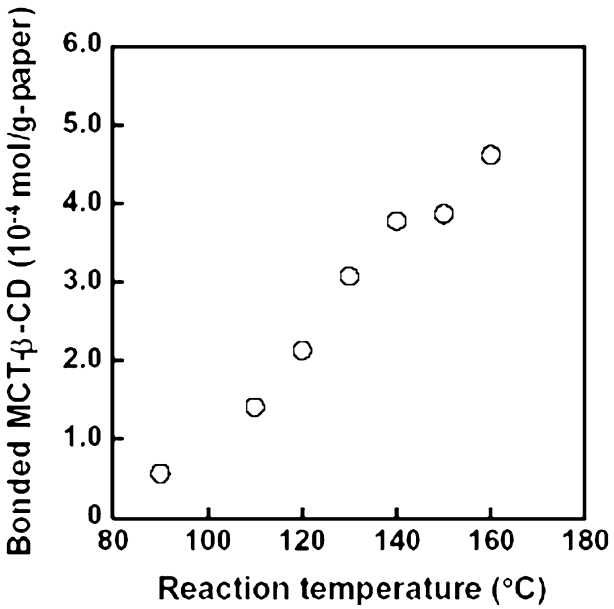

Figure 2. Influence of reaction temperature on the content of MCT-β-CD in the paper. The initial MCT-β-CD concentration of the dipping solution is 0.28 g/mL.

Inclusion of hinokitiol in MCT-β-CD bonded on washi paper

The inclusion of hinokitiol was conducted into MCT-β-CD covalently bonded to the paper. Figure 4 shows the inclusion abilities of MCT-β-CD-papers with different MCT-β-CD contents in the paper, which were prepared at different heat treatment temperatures in Figure 1. The molar inclusion ratio of hinokitiol to MCT-β-CD (inclusion fraction) for samples with the MCT-β-CD

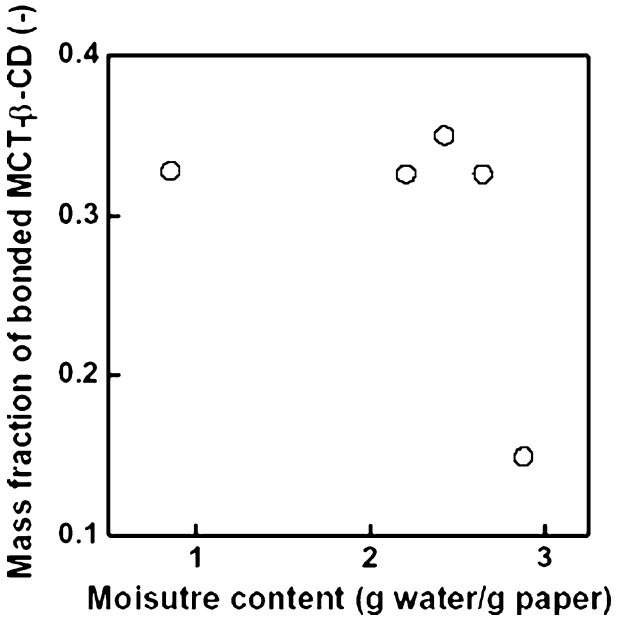

Figure 1. Influence of the initial moisture content of paper on the fixing efficiency. Initial concentration of MCT-β-CD is 30%, and the reaction temperature is 150 °C.

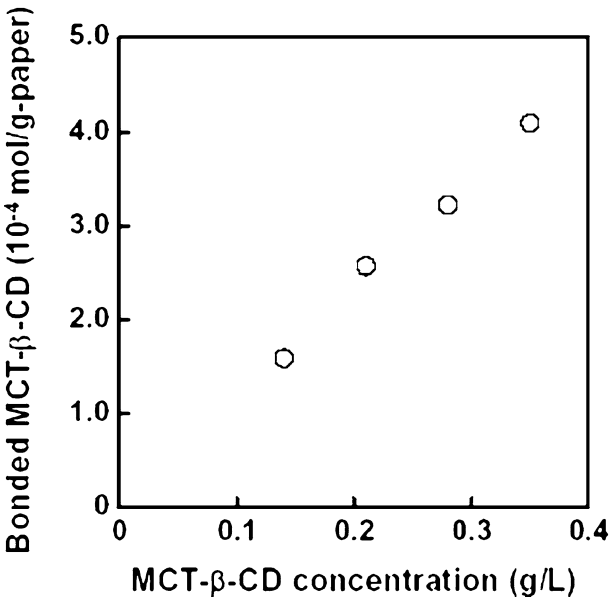

Figure 3. Effect of the concentration of MCT-β-CD in the dipping solution. The fixing conditions are 150 °C of the reaction temperature and the length of the reaction time is 10 min.

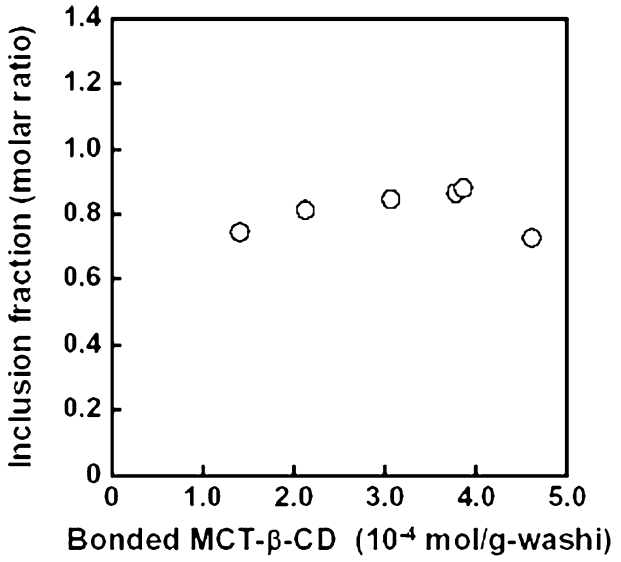

Figure 4. The inclusion abilities for hinokitiol of MCT-β-CD binding on the paper. The concentration of MCT-β-CD in the dipping solution is 0.28 mg/mL, the reaction temperatures are 110–160 °C. The sample paper used are the same as prepared in Figure 2.

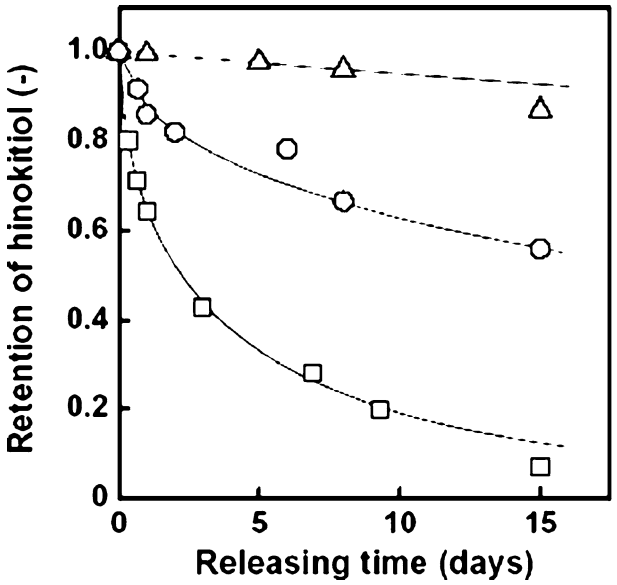

Figure 5. Release of hinokitiol from MCT-β-CD-paper at 50 °C at various relative humidities. Δ RH = 50%, ○ RH = 75%, □ RH = 98%. The solid lines represent the Avrami simulations.

content between 1.5×10^{-4} and 3.9×10^{-4} (mol/g-washi) were nearly constant of ≈0.8. These samples were prepared at the heat treatment temperature between 110 and 150 °C. However the sample prepared at a higher temperature of 160 °C (MCT-β-CD content is 4.6×10^{-4} (mol/g-paper)) shows a significant lower molar inclusion ability than the ones prepared at lower temperatures. A possible reason would be a heat degradation of the cyclodextrin, which inactivates its inclusion ability. To avoid this, 150 °C was chosen as the favored reaction temperature due to low heat degradation of the MCT-β-CD and a high binding and inclusion efficiency.

Release characteristic of hinokitiol

The retention time curve of hinokitiol during release at 50 °C for different air humidities can be seen in Figure 5. The initial hinokitiol inclusion ratio was 0.8, the maximum achieved inclusion ratio. Over the time period of 15 days, the hinokitiol retention at relative humidity (RH) of 90% decreased to less than 0.1, and to 0.6 at 75% RH, whereas it only decreased to 0.9 at RH of 50%. According to Rehmann *et al.* [9], the retention time curve could be described by the following Avrami equation.

$$R = \exp[-(k \cdot t)^n] \quad (1)$$

Where R is the flavor retention (the amount of remaining included flavor divided by the amount of initially included flavor), k, the release rate constant and n, a parameter represents the release mechanism. The Avrami equation was originally developed to describe the crystallization of polymers, and could be transferred to describe the flavor release from spray dried flavor emulsions [12], where the release mechanism is different. The estimated parameters are shown in Table 1. For 75 and 90% RH, the value for n is identical (0.54), indicating that the release is diffusion controlled process. For 50%, it is higher but still in the same range, and indicates the same release mechanism. In general, n should more likely be seen as just a fitting parameter.

As expected from previous works [13, 14], the relative humidity has a strong influence on the release rate. Yoshii *et al.* have reported that during the inclusion of *d*-limonene in β-CD, a minimum amount of water molecules is required [11]. The inclusion takes place only if the flavor can substitute an included water molecule in the cavity of cyclodextrin. The release, however, follows the same mechanism; water substitutes the included compound. As a consequence, the release at 50% RH is very slow, due to the lower water concentration in the gas phase. Compared to the release of *d*-limonene in the cellulose bonded MCT-β-CD, a lower release rate of hinokitiol was obtained. This is desired in its later application as an antibacterial surface modifier. In realistic application, hinokitiol can keep the surface of the paper or wallpaper partly sterile.

Antibacterial activity of the included hinokitiol

It should be demonstrated, that surfaces modified with MCT-β-CD including hinokitiol, can inhibit contami-

Table 1. Parameters of the Avrami equation describing the release of *hinokitiol* from MCT-β-CD-paper for various relative humidities at 50 °C

Relative humidity (%)	k (day^{-1})	n (–)
50	0.0051	1.0
75	0.0244	0.54
90	0.33	0.54

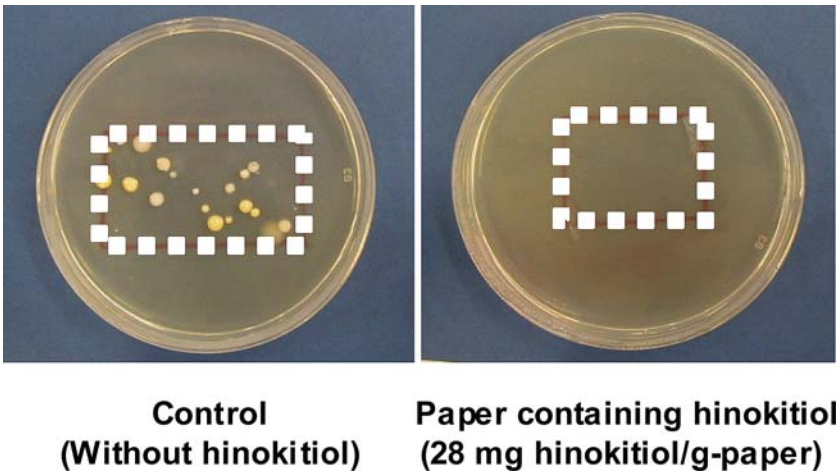

Figure 6. Antibacterial activity of hinokitiol included in MCT-β-CD-paper towards airborne microorganisms.

nation by airborne bacteria. Therefore, hinokitiol containing MCT-β-CD-paper, was exposed to laboratory air, followed by a transfer of the accumulated vital microorganism to standard growth agar. Figure 6 shows the photos of colonies found on the growth medium incubated at 30 °C. The square dotted lines show the region where the hinokitiol containing MCT-β-CD-paper or the control paper were pressed on to the standard growth agar medium. There was no bacteria growth observed in the medium pressed with the MCT-β-CD-paper containing hinokitiol, demonstrating the antibacterial activity of hinokitiol towards airborne bacteria populations. This implies that the paper prepared in this study was very useful as antibacterial material.

Conclusion

MCT-β-CD could be successfully bonded to the Japanese washi paper. At optimal conditions, 4.0×10^{-4} mol of MCT-β-CD could be bound to one g of the paper. The inclusion and release of antibacterial agents could be shown by the example of hinokitiol. The produced MCT-β-CD-paper has the potential to include various other substances. The observed lower release rate of hinokitiol is favorable in its application as an antibacterial surface modifier. The potential of the MCT-β-CD-paper included hinokitiol to provide sterile surfaces was demonstrated. The conducted experiments clearly show that the reduced number of vital microorganisms was caused by the included hinokitiol. However, this needs further investigation, regarding the minimum amount of required hinokitiol to inhibit microbial growth.

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