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# Synthesis and inclusion property of α-cyclodextrin-linked alginate

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**Abstract**

The inclusion ability on alginate, a hydrogel forming polysaccharide, has been introduced by covalently linking α-cyclodextrin (α-CD). The secondary hydroxyl groups of sodium alginate were activated with cyanogen bromide and subsequently reacted with 6-amino-α-CD. The degree of substitution (DS) of the resulting α-CD linked alginate was 0.05–1.58 depending on reaction condition and the ratio of 6-amino-α-CD and alginate. Using *p*-nitrophenol as a model compound in UV–vis and circular dichroism spectroscopic examination, the modified polysaccharide showed an ability to form a host–guest complex. Furthermore, the α-CD linked alginate found to be provided the spherical beads by treating with a calcium chloride solution.


*Keywords:* Cyclodextrin; Alginate; Cyanogen bromide

## 1. Introduction

Cyclodextrin (CD) is a cyclic oligosaccharide made up of glucopyranose units bonded together via α(1→4) glycoside linkages. The most common CDs are α, β, and γ-CD, containing six, seven and eight units, respectively. Due to the cavities of CDs with internal diameters of 6–10 Å are relatively hydrophobic, various small apolar are able to be trapped to form inclusion complex with binding specificity [1]. Synthesis and application of CD containing polymers have been extensively studied in various fields such as the enhancement of enantioselectivity in capillary electrochromatography and treatment of some disorder by removal of cholesterol from the cell lines [2,3]. Furthermore, CD is also regarded as useful materials for the eradication environmental pollutants such as the endocrine disrupting chemicals, phenolic compounds, and aromatic compounds [4–6]. Various immobilized CD derivatives have been prepared as potential adsorbents for host–guest complex formation [7–10]. We have reported that CD-linked chitosan beads have a high selectivity in affinity chromatographic separation for *p*-nitrophenolates and bisphenol A [9,11]. These successful results prompted us to synthesize other CD-linked polysaccharides. Our interest focused on alginate, a linear polysaccharide containing a varying composition of β-(1→4) linked D-mannuronic acid and α-(1→4) linked L-guluronic acid residues. The alginate beads are known to be formed by cross-linking guluronic acid enriched segments with divalent ions such as $Ca^{2+}$ to form stable three-dimensional network, where $Ca^{2+}$ ions embedded like an egg-box model [12]. This polysaccharide gel beads has been widely used in many biotechnology applications including immobilization of bacteria or enzyme [13].

Here we would like to report the synthesis of α-CD linked alginate (CD–alginate) and its property to form inclusion complex with *p*-nitrophenol as a guest compound. The coupling reaction between α-CD and alginate was designed at the hydroxyl groups of alginate via CNBr method in order not to affect the carboxyl groups, which are necessary to form the calcium–alginate beads. The resulting CD–alginate was next examined the ability in the bead formation.

## 2. Experimental

### 2.1. Materials

Sodium alginate with medium viscosity (300–400 cP) was purchased from Wako Pure Chemicals (Japan). Its M/G

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Table 1
Preparation and physical properties of CD–alginate

| Ratio, CD:alginate | DS |
|---|---|
| 2:1 | 0.05 |
| 5:1 | 0.24 |
| 5:1[a] | 1.58 |

Coupling reactions were carried out in water for 2 days, washed by ultrafiltration and then freeze-dried to give a white polymer.
[a] Warmed up to 40 °C for further 1 h before washing by ultrafiltration.

(mannuronic acid/guluronic acid) ratio was 1.27, which determined by $^1$H NMR spectroscopic analysis as described below. All chemicals were reagent grade. Water used in this study was prepared by distillation and subsequent treatment with ion-exchanger.

### 2.2. Instruments

Photographs of scanning electron microscopy (SEM) were taken on a Hitachi S-2400 instrument operating at 10 kV, after the sample was subjected to sputtering with gold. Infrared spectra were recorded using a Horiba FT-210 spectrophotometer with a potassium bromide pellet. $^1$H NMR spectra were recorded with a Bruker ASX-300 spectrometer for 2.5 mg-samples in deuterium oxide (0.5 ml) at 300.13 MHz, kept the temperature of the sample at 90 °C during measurement. The peak of HDO was calibrated at $\delta$ 4.05 ppm. UV–vis and circular dichroism spectra were recorded with a Hitachi U-3200 spectrometer and a JASCO J-720 spectropolarimeter, respectively.

### 2.3. Preparation of 6-amino-α-cyclodextrin

According to reported procedures [14,15], dried α-CD (10 g) and *p*-toluenesulfonyl chloride (5 g) was dissolved in pyridine (750 ml) at 4 °C for 4 h, separated with Diaion HP-20 to give mono-tosyl-α-CD (4.7 g). The product was dissolved in water (50 ml) at 80 °C and then sodium azide (3 g) was added. The suspended mixture was stirred at the same temperature for 5 h, then cooled to room temperature and precipitated with acetone to give white powder of 6-azido-α-CD (3 g). The resulting product (3 g) was dissolved in water (20 ml) and hydrogenated in the presence of 10% palladium carbon (100 mg) for 2 days under atmospheric pressure. The filtered reaction mixture was applied to CM-Sephadex C-25 column ($NH_4^+$ form), then eluted with 1 M $NH_3$ to obtain 6-amino-α-CD (1.8 g).

### 2.4. Preparation of CD–alginate

Sodium alginate (0.1 g) was dissolved in water (100 ml) and then reacted with CNBr (60 mg). The pH of reaction mixture was controlled to between 10.0 and 11.0 by dropping aqueous NaOH solution (6 M) for 1 h. The CN-treated alginate was subjected to ultrafiltration through a membrane with molecular weight cut-off 10,000. The product was washed with water for 2 days. Subsequently, 6-amino-α-CD was added to the resulting solution, allowed to stir for 2 days and then washed by ultrafiltration for 2 days. The ratio of CN–alginate and 6-amino-α-CD and the DS elucidated from $^1$H NMR summarized in Table 1. CD–alginate (DS 0.24): $^1$H NMR ($D_2O$) $\delta$ 5.2–5.0 (m, 1H, H-1 of alginate and α-CD), 4.73 (s, 0.36 H, H-1 of mannuronic acid and H-5 of alginate), 4.45 (s, 0.17 H, H-5 of guluronic acid), 3.7–3.4 (m, ca. 2H, H-2 and H-4 of α-CD); CD–alginate (DS 1.58): $^1$H NMR ($D_2O$) $\delta$ 5.2–5.0 (m, 1H, H-1 of alginate and α-CD), 4.73 (s, 0.10H, H-1 of mannuronic acid and H-5 of alginate), 3.7–3.4 (m, H-2 and H-4 of α-CD), 2.8–2.5 (m, 0.16H, H-6 of α-CD with amino group) (peak area shown above are relative values and the spectra are shown in Fig. 2).

### 2.5. Inclusion property

Inclusion ability was estimated by recording the UV–vis spectra and circular dichroism spectra of CD–alginate containing *p*-nitrophenol ($3.59\times10^{-5}$ M, aqueous NaOH, pH 11) as a guest compound.

### 2.6. Bead formation

Bead formation was performed by adding dropwise of the CD–alginate solution (2% w/v) into a stirring $CaCl_2$ solution (2% w/v). The resulting white beads were allowed to stand for 24 h in the $CaCl_2$ solution and then washed with water several times.

## 3. Results and discussion

### 3.1. Preparation of α-CD linked alginate

Since the carboxylic groups of alginate chain are necessary for the calcium-ion mediated beads formation, we planned that the coupling reaction between CD and alginate was performed at the secondary hydroxyl groups. CNBr method has been widely used for covalent fixation of proteins or enzymes to insoluble polysaccharides like Sephadex, Sepharose, and cellulose [16]. The polysaccharides were treated with CNBr to activate the hydroxyl groups, followed by coupling with various ligands containing amino groups. Therefore, it seemed to be suitable for our purpose. According to the reported mechanism proposed in the modification of Sephadex or glucose resembling model compounds, the reaction of CNBr yields cyanate esters, which were regarded as highly unstable and transitory intermediates, which are more likely for the activation reaction in an alkaline condition [17]. Our synthetic plan of CD–alginate is shown in Fig. 1, which involves two steps, i.e. activation of the hydroxyl groups in alginate and subsequent coupling with 6-amino-α-CD. Thus, a solution of alginate and CNBr in water was treated with NaOH to

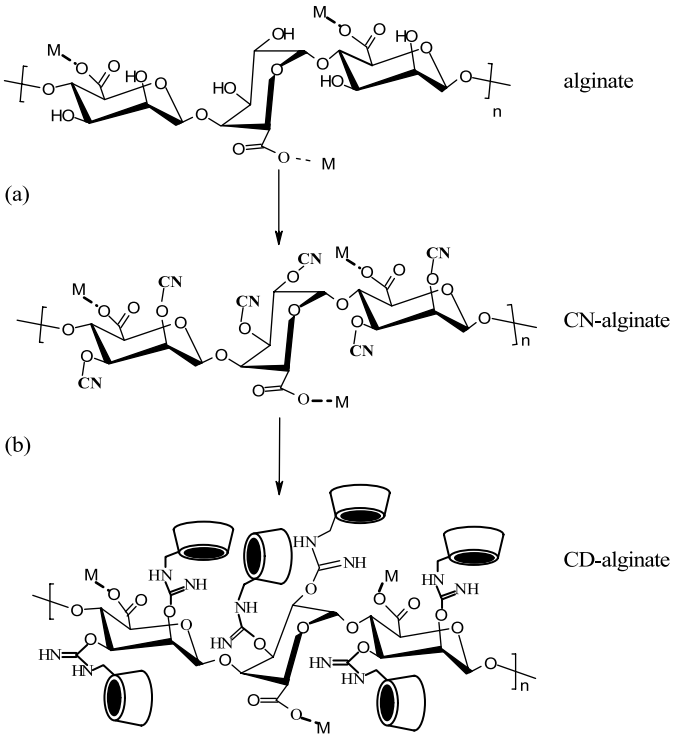


Fig. 1. Synthesis of CD–alginate. M means metal ions such as $Na^+$ and $Ca^{2+}$. Reagent and conditions: (a) CNBr in water, controlled pH 10.0–11.0, room temperature, 1 h; (b) 6-$NH_2$-α-CD in water, 2 days.

activate, and the pH of the reaction mixture was maintained between 10.0 and 11.0 by addition of aqueous NaOH for 1 h. After purification, the resulting CD-activated alginate was couple with 6-amino-α-CD, which was prepared from α-CD by established methods firstly activated α-CD to the involving mono-6-O-tosylation, azido-substitution, and hydrogenation [14,15]. Coupling between CN–alginate and 6-amino-α-CD was performed in aqueous solution at room temperature. Similarly to Sephadex and glucose activation, our product contained the isourea linkage between alginate and α-CD. The degrees of introducing CD residue into alginate were depending on the ratio of α-CD and alginate as well as the reaction temperature, as shown in Table 1. Maximum DS in our experiments was reached 1.58 when the coupling reaction was conducted at 40 °C using 5 mol equiv of 6-amino-α-CD.

### 3.2. $^1H$ NMR analysis

The typical $^1H$ NMR spectra of alginate and CD–alginate are shown in Fig. 2. Three characteristic signals corresponding to the substitution of alginate were observed at 5.05, 4.73, and 4.42 ppm, which were assigned to anomeric proton and H-5 proton of alginate. From these peaks, monomer composition and the fractions of nearest neighbours along the copolymer chain of alginate could be calculated. The signal at δ 5.05 ppm was assigned to G-1 (H-1 proton of guluronic residue), 4.73-position was assigned to M-1 and GM-5 (H-1 proton of mannuronic acid residues and H-5 proton of mannuronic acid residue in a having neighbouring G residue, respectively), and that of δ 4.42-position was assigned to GG-5 (H-5 proton of guluronic acid residue having neighbouring G residue) [18]. As shown in Fig. 2, these peaks of alginate used as the starting material were presented as peak A (G-1), B (M-1 and GM-5), and C (GG-5), respectively. The integrated peak area ($I$) were quantitatively elucidated the mole fraction by: $F_G = I_A/(I_B + I_C)$

Contrastly, $^1H$ NMR spectrum of CD–alginate obviously exhibited an increased peak area of $A'$ peak, which was corresponding to anomeric protons of both alginate and α-CD. The substitution ratio was estimated by: $F_{G'} = I_{A'}/(I_{B'} + I_{C'})$. The α-CD substituted in alginate (DS) was evaluated by the following relationship: $DS = (F_{G'} - F_G)/6$. The results of calculation are shown in Table 1. DS may be reached 2.00 if the reaction is completely coupled at hydroxyl groups of alginate polymer. The nomenclature used for the modified alginate was defined as CD–alginate ($n$) where $n$ is the substitution degree.

### 3.3. FT-IR analysis

The FT-IR spectra of CD–alginate (DS 0.24 and 1.58) were compared with the spectrum of sodium alginate (Fig. 3). It is obvious that sodium alginate shows a broad band at 3453 $cm^{-1}$ for –OH stretching, two bands at 1653

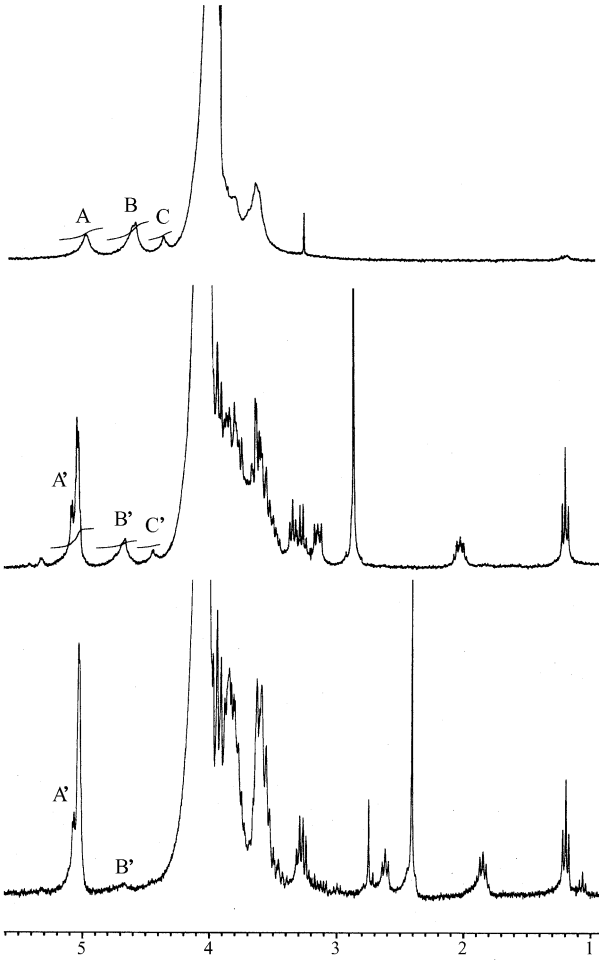


Fig. 2. Typical $^{1}$H NMR spectra of alginate (up), CD–alginate (DS 0.24) (middle), and CD–alginate (DS 1.58) polymer (down).

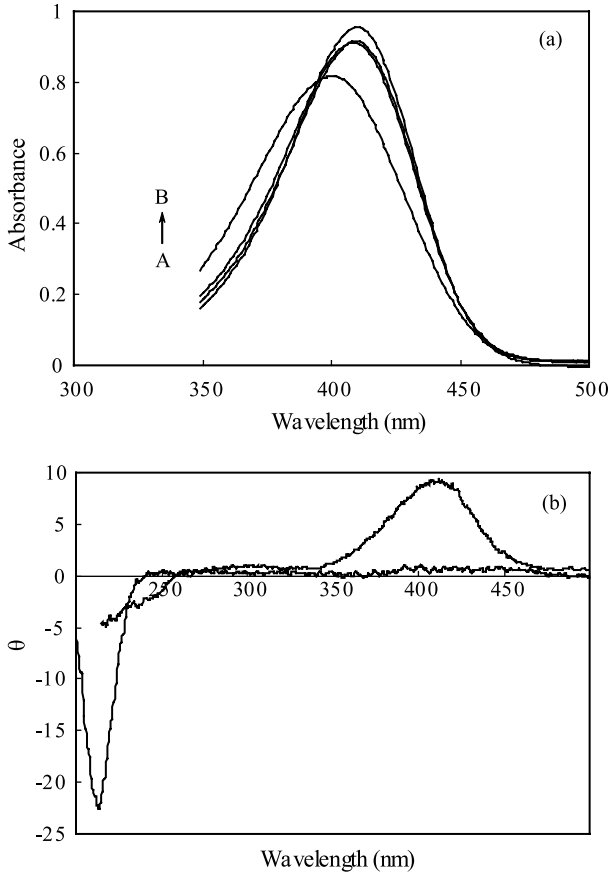


Fig. 4. Spectroscopic examination of the inclusion ability of CD–alginate and *p*-nitrophenol ($3.59\times10^{-5}$ M): Solvent: Aqueous NaOH, pH 11.0. (a) UV–vis spectra of *p*-nitrophenol in the presence of CD–alginate (α-CD concentration 0, 0.20, 0.55 and 0.88 mM, read from A to B.); (b) circular dichroism spectra of *p*-nitrophenol in the presence of CD–alginate (DS 1.58) and parent alginate.

and 1408 $cm^{-1}$ for the $-COO^{-}$ stretching and one sharp peak at 1054 $cm^{-1}$, which is for the C–O stretching. The CD–alginate spectra showed the strong signal of C–O stretching vibration band at 1158 and 1030 $cm^{-1}$, denoting the presence of the α-CD. Additional bands can be observed, at 3300 and 1550 $cm^{-1}$, identified in the literature as N–H stretching vibration [19]. A small peak at 1400 $cm^{-1}$ might be assignable to C–N stretching also verified the formation of isourea linkage [20]. The results of $^{1}$H NMR and FT-IR indicated that the CD–alginate was obviously synthesized by the CNBr method.

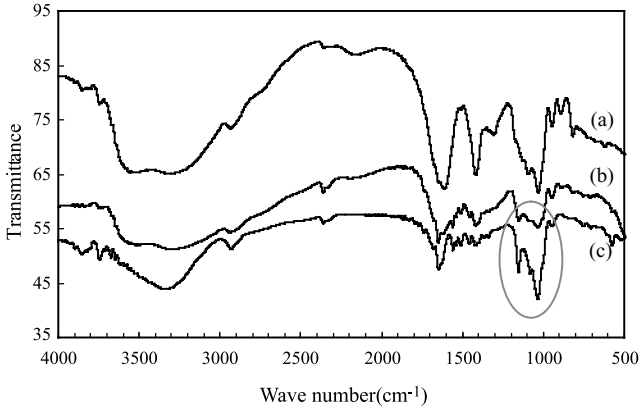


Fig. 3. IR spectra of cross-linked and uncross-linked alginate; (a) alginate; (b) CD–alginate (DS 0.24); and (c) CD–alginate (DS 1.58).

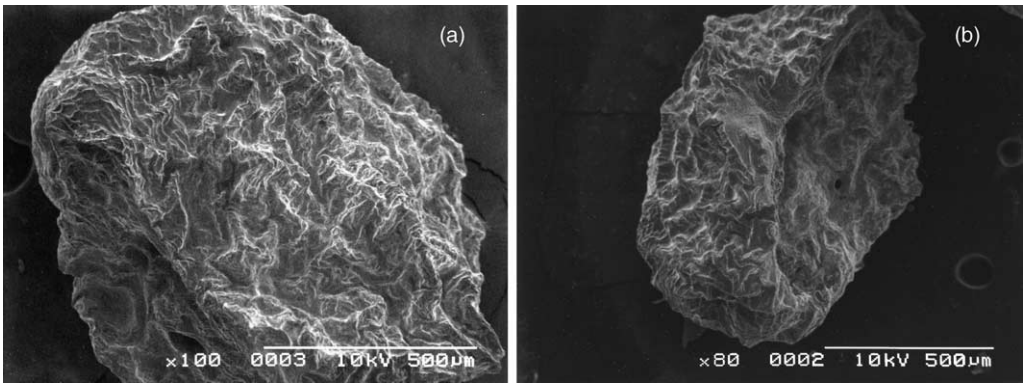


Fig. 5. SEM photographs of α-CD-linked alginate bead prepared from CD–alginate (DS 0.24) in surface (a) and cross-section view (b).

### 3.4. Inclusion ability

The inclusion ability of the CD–alginate was examined in terms of UV–vis spectroscopic titration using *p*-nitrophenol as a guest compound. Fig. 4(a) showed UV–vis spectra of *p*-nitrophenol, which contained CD–alginate with the CD concentration of 0, 0.20, 0.55 and 0.88 mM. Similar to the parent α-CD, a bathochromic shift was observed in the UV–vis spectrum with increasing CD concentration of the CD–alginate molecule, and the isosbestic points were observed at 395 and 462 nm. Using the Benesi–Hildebrand equation [21], calculated on the basis of the α-CD residues coupled in alginate showed that the equilibrium constant of the host–guest complex with *p*-nitrophenol was $1.4\times10^{4}\,M^{-1}$ while the parent α-CD was reported to $4.5\times10^{3}\,M^{-1}$ [7]. Furthermore, the inclusion ability of CD-linked alginate (DS 1.58) towards *p*-nitrophenol was also revealed by the large induced circular dichroism effect observed at 410 nm (Fig. 4(b)) and also observed the circular dichroism spectra of alginate that characterized by a peak at ~200 nm and a trough at ~212 nm.

### 3.5. Bead formation

The CD–alginate, which accomplished in the introducing of inclusion property to normal alginate polymer was examined the ability in bead formation. Stable spherical beads were obtained by dropping an aqueous solution of CD–alginate (DS 0.24) into and aqueous $CaCl_2$ solution (2% w/v). As shown in Fig. 5 the CD–alginate performed highly cross-linked materials with increased surface area. Inside of the beads also had highly cross-linked structure. This characteristic was quite different when compared to conventional alginate bead performed in earlier publications [22,23], which the surface was a mostly smooth and displayed a less folded structure. We speculated our CD–alginate beads would provide a suitable structure for bacterial cell encapsulation in a future purpose, in addition to the inclusion ability for guest compound.

## 4. Conclusion

The approach for coupling CD to alginate through the CNBr method showed the good results of guest-responsive capability on the alginate. Stable and spherical beads were also obtained simply by dropping an aqueous solution of modified CD–alginate into a calcium chloride solution. This novel synthesis may provide a useful in combination between inclusion ability and gel-bead formation, which are in our great interest in the field of environmental remediation as for bacterial encapsulation. Further studies along this line are now in progress.

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