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Research paper

Preparation of alginate gel beads containing chitosan nicotinic acid salt and the functions

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Abstract

Calcium-induced alginate gel beads containing chitosan salt (Alg-CS) was prepared using nicotinic acid (NA), a drug for hyperlipidemia, and investigated its two functions in gastrointestinal tract, (a) NA release from Alg-CS, (b) uptake of bile acids into Alg-CS. The amount of NA incorporated in Alg-CS increased according to increment of CS content. NA was rapidly released from Alg-CS in diluted HCl solution (pH 1.2) or physiological saline without disintegration of the beads. When Alg-CS was placed in bile acid solution it took bile acid into itself. About 80% of taurocholic acid dissolved in the medium was taken into Alg-CS. According to increment of bile acid concentration, the uptake amount increased and an approximately linear relationship existed among them.

Keywords: Alginate gel; Chitosan nicotinic acid; Bile acid

1. Introduction

Natural polysaccharide has been used in the food industry, in medicine and in drug preparation for release control [1,2]. It is also recognized as a dietary fiber, which regulates the intestinal functions. A cationic polysaccharide, chitosan (CS) has been studied on the utility for pharmaceutical preparations because of the biodegradable property or a lack of oral toxicity [3]. Recently, it was reported that CS has potency for interfering with the dietary fat absorption by surrounding oily particle which results from fat digestion [4]. On the other hand, alginic acid is an anionic polysaccharide, and the solution forms gel matrix in the presence of a divalent cation. It has been expected as a vehicle for controlling drug release because calcium-induced alginate gel bead (Alg-Ca) is able to fix a compound such as a drug or an other polysaccharide within the matrix [5,6].

We reported that CS weak acid salt can interact an anionic compound because it possesses the polycationic property [7], and Alg-Ca containing CS salt incorporated bile acids into itself by the electrostatic interaction [8]. It is known that biliary secretion is an important route for cholesterol excretion from the body [9], and the inhibition of enterohepatic circulation of bile acids stimulates the excretion. Actually, colestyramine is an anion-exchange resin which has been used for the decrease of the plasma cholesterol, thus, it is utilized for hyperlipidemia which is one of the most popular disease.

In this study, we prepared the calcium-induced alginate gel beads containing chitosan salt (Alg-CS) using nicotinic acid (NA), which is one of water soluble vitamins, a weak acid (pKa 4.85, Merck Index) or a drug for hyperlipidemia, and investigated the anticipate functions, (a) the NA release from Alg-CS and (b) the uptake of bile acids into Alg-CS, if Alg-CS would be administrated orally and then transferred in gastrointestinal tract.

2. Materials and methods

2.1. Materials

Sodium alginate was purchased from nacalai tesque (Kyoto), and a chitosan, CS(F) (degree of deacetylation 75-85%) was obtained from Kimitsu Chemical Industries (Tokyo) and two ones from Wako (CSW1, CSW2). Viscosity of each 1% chitosan-1% lactic acid solution was measured by Brookfield viscometer (Tokyo keiki Co., Tokyo) at 20°C and it was 9.4 cps (CS(F)), 3140 cps (CSW1) or 4090 cps (CSW2), respectively. Chitin was obtained from Kimitsu, curdlan and sodium dextran sulfate from Wako, and pullulan and xylan from Seikagaku Co. (Tokyo). Four cholic acids (sodium salt), namely taurocholate (T-CA), glycocholate (G-CA), taurodeoxycholate and

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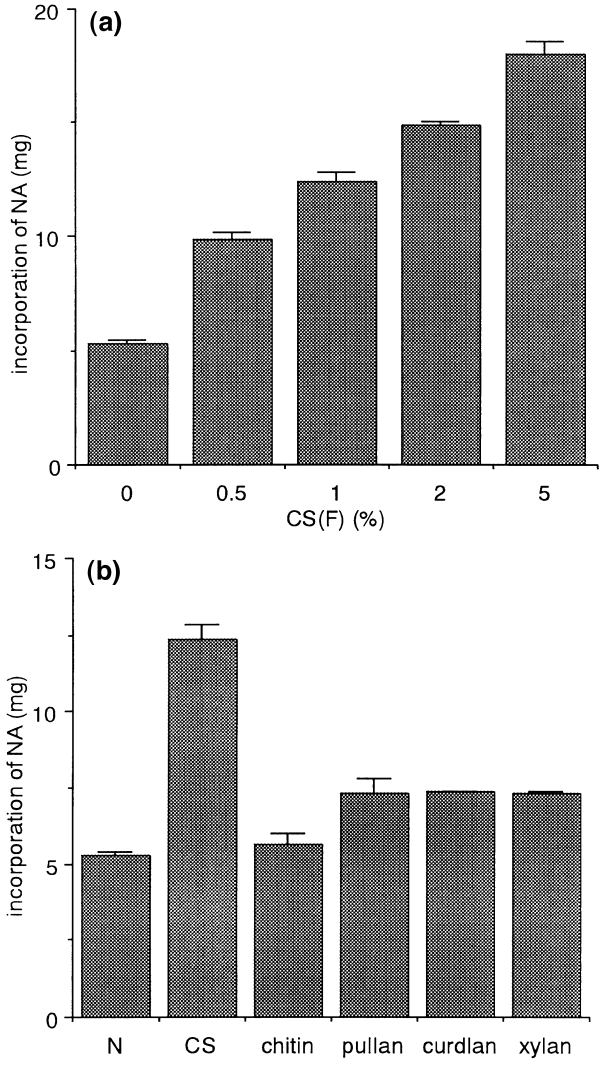

Fig. 1. (a) Effect of CS(F) concentration on the incorporation of nicotinic acid (NA) in Alg-Ca. (b) Effect of 1% polysaccharides addition on the incorporation of NA in Alg-Ca. (N: no additive).

glycochenodeoxycholate were obtained from nacalai tesque. All other chemicals were of reagent grade.

2.2. Preparation of Alg-CS

Alg-CS were prepared as follows. Sodium alginate (1% W/W) was dissolved in distilled, demineralized water with agitation and 0-0.5 g of CS was dispersed in the solution (10 g). Two grams of this solution was dropped into 20 ml of 0.2 M CaCl2 containing 1% nicotinic acid and left to stand at 37°C for 3 h. These hydrogel beads were washed twice with 50 ml distilled water and dried at 35°C for 8 h on a dish, followed by vacuum in a desiccator in the presence of P_2O_5.

2.3. Dissolution test of NA from Alg-CS

The release of NA from Alg-CS in 500 ml dissolution medium was on a JP XIII dissolution test apparatus using paddle method (150 rev./min, 37 ± 0.5°C). The dissolution media included physiological saline and JP XIII disintegration test solution (1st. fluid). A 4 ml aliquot of test solution was removed periodically and 4 ml of new medium (37°C) was added to maintain a constant volume. The absorbance of each solution removed was determined at 261 nm (saline) or 262 nm (1st. fluid) using spectrometer UV-1200 (Shimazu). All dissolution tests were performed in triplicate.

2.4. Uptake test of bile acid into Alg-CS

15 ml of bile acid solution (1–4 mM) was placed in an L-formed tube and maintained at 37°C. The dried Alg-CS corresponding to 2 g of hydrogel was added to the solution and shaken at 67 times per min. A 0.2 ml aliquot of each solution was removed periodically for HPLC analysis, as follows. The system comprised an LC-6A pump (Shimazu,

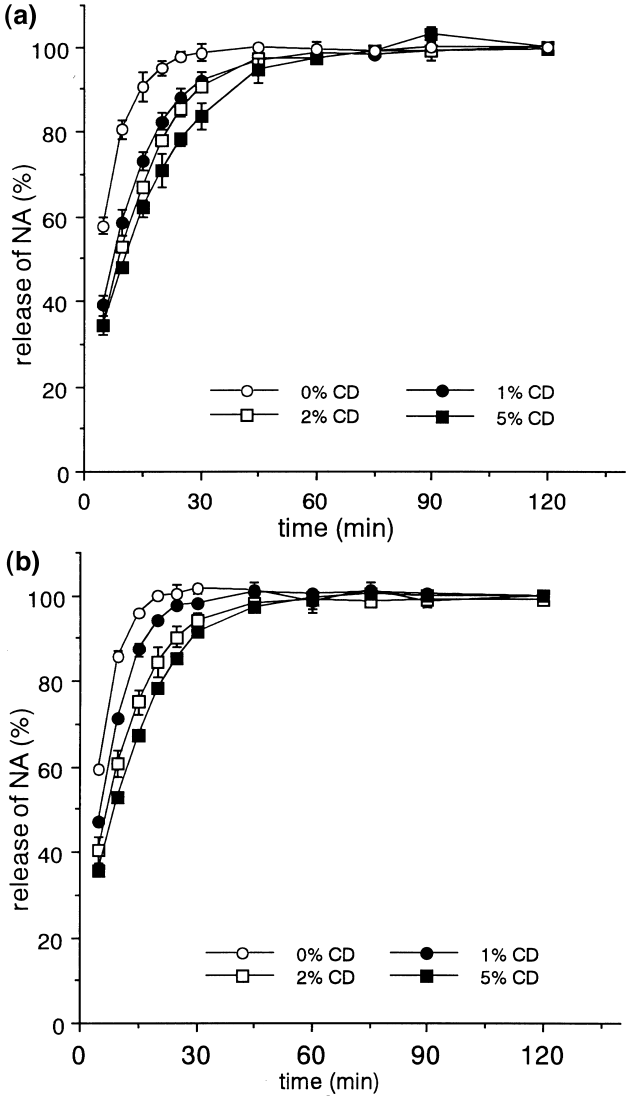

Fig. 2. (a) Release profiles of NA from Alg-Ca containing 5% CS(F) and curdlan (CD) in physiological saline. (b) Release profiles of NA from Alg-Ca containing 5% CS(F) and CD in 1st. fluid.

Kyoto), a packed column (nacalai tesque, cosmosil 5C18-MS 150 × 4.6 mm), and a SPD-6A UV detector (Shimazu). HPLC was conducted at ambient temperature using an eluent comprising methanol, 30 mM phosphate buffer (pH 3.4) and acetonitrile (6:3:1) at a flow rate of 1.0 ml/min and the detector wavelength was set at 254 nm. The uptake amount of bile acid into Alg-CS was calculated by the difference between the initial and residual amount of bile acid in the solution. All tests were performed in triplicate.

3. Results and discussion

3.1. Loading of NA in Alg-CS

The amount of NA incorporated in Alg-CS increased according to increment of CS content, and this phenomenon was not observed with Alg-Ca containing chitin, pullulan, curdlan or xylan (Fig. 1a,b). And the change resulting from the addition of CS did not occur when nicotinamide instead of NA was incorporated in Alg-Ca (data not shown). This means that CS-NA salt formed inalginate gel matrix when the gel bead was prepared by dropping alginate solution dispersing CS into $CaCl_2$ which contained NA, and that CS-NA stayed in Alg-Ca.

3.2. Release of NA from Alg-CS

Fig. 2a,b shows the release profiles of NA from Alg-CS. NA was rapidly released from Alg-CS in 1st fluid or physiological saline and disintegration of the beads was not observed throughout the dissolution test. The release profile of water-soluble compound from Alg-Ca changed by addition of any additives [10], and actually, the apparent release rate gradually decreased according to the increment of curdlan amount incorporated in Alg-CS as shown in Fig. 2. Same effect was recognized when dextran was added to

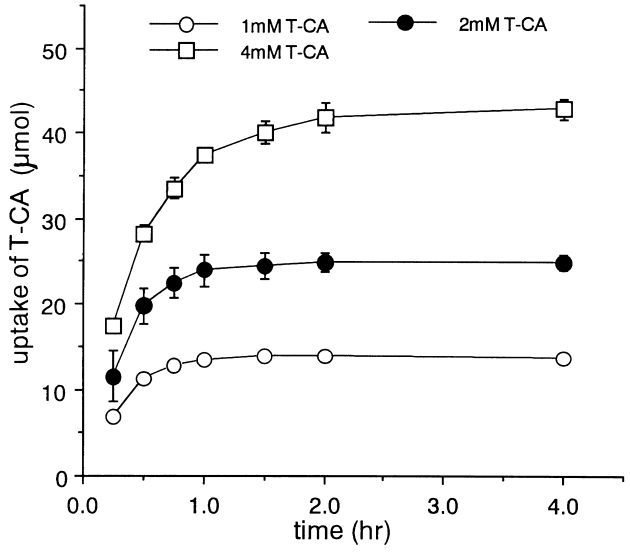

Fig. 3. Uptake of taurocholate (T-CA) into Alg-Ca containing 5% CS(F).

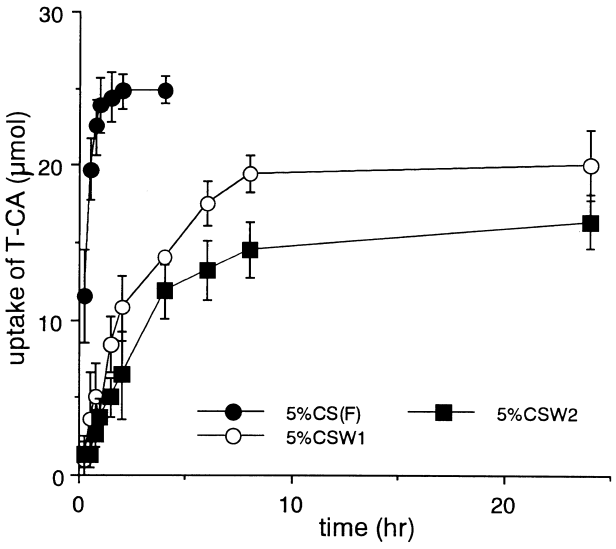

Fig. 4. Uptake of T-CA into Alg-Ca containing various chitosan (5%) (2 mM T-CA).

Alg-CS. Thus, NA release from Alg-Ca is controlled by the modifying the gel matrix structure, however, will be rapid in gastric cavity.

3.3. Uptake of bile acid into Alg-CS

We have reported that alginate gel bead containing CS lactic acid salt adsorbed bile acids by ion-exchange reaction such as colestyramine, an anion exchange resin [8]. Fig. 3 shows the uptake of T-CA into Alg-CS which was prepared using NA instead of lactic acid. Dried Alg-CS gradually took T-CA into itself from the solution and the uptake finished after 1.5–2.0 h. It is known that the amount of bile acids secreted change by the condition of human intestinal tract [9]. According to increment of T-CA concentration, the uptake amount increased and an approximately linear relationship existed among them. Thus, that Alg-CS will take bile acids into itself in response to intestinal condition. However, this phenomenon did not occur for Alg-Ca dispersed CS in the place of CS nicotinic acid salt. Though the uptake of T-CA was observed in the case of Alg-Ca loaded CSW1 or CSW2 the rate was significantly slower than that of Alg-CS (Fig. 4). It means that the complex formation between CS and bile acid inside of gel bead was affected by CS property, e.g., molecular weight. The uptake was also recognized on the other bile acid such as

Table 1
Uptake of bile acids into Alg-Ca (after 1.5 h)

Bile acid	Amount (μmol)
Taurocholate	24.4 ± 1.6
Glycocholate	26.0 ± 1.0
Glycochenodeoxyholate	23.6 ± 0.4
Taurodeoxyholate	25.2 ± 2.1

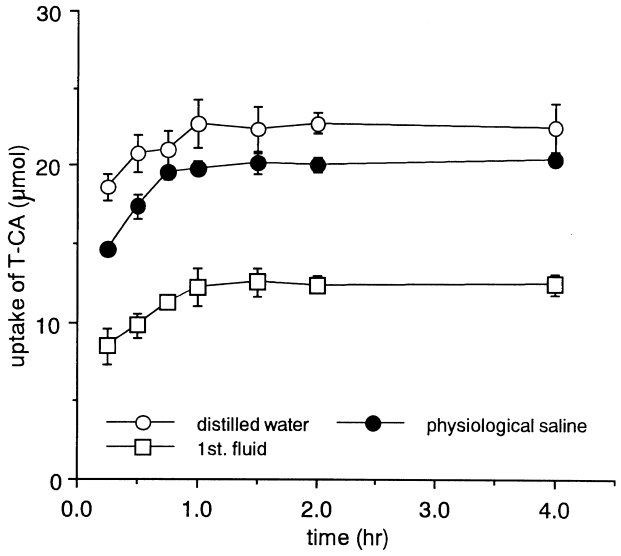

Fig. 5. Uptake of T-CA into Alg-Ca containing 5% CS(F) after dissolution test of NA.

glycocholate, taurodeoxycholate or glycochenodeoxycholate as shown in Table 1. In all case, about 80% of each bile acid dissolved in the solution was taken into Alg-CS.

3.4. Uptake of bile acid into Alg-CS after NA release

If Alg-CS is administered orally, it may release NA in stomach, then transfers into intestinal tract in which bile acids are secreted. Fig. 5 shows the uptake of T-CA into Alg-CS which was carried out the dissolution test for 2 h in several solutions. After the dissolution test in physiological saline or distilled water, Alg-CS was transferred in T-CA solution, and it took the bile acid up itself and the amount was about 20–23 μmol/sample. However, the amount decreased significantly after the test with 1st fluid, which mimics the gastric juice. In this experiment, Alg-Ca containing CS-HCl salt was not tested because changing sodium alginate to alginic acid and formation of calcium-induced gel matrix would take place in dilute hydrochloric acid solution at the same time. Therefore, it is not clear whether a lowering of uptake efficiency is caused by formation of CS-HCl salt in Alg-CS or by the change of gel matrix structure.

Conventionally, the preparation for oral administration has played the role as a vehicle which contains a drug and releases it in the gastrointestinal tract. In this paper, we tried to give two-functions to the vehicle, Alg-CS as follows; (a) the NA release and (b) the uptake of bile acids. Thus, if these work well, Alg-CS would be a excellent preparation for hyperlipidemia. Now, we try to prevent the lowering of uptake efficacy of bile acids after treating with 1st fluid.

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