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Note

Preparation of alginate gel beads containing chitosan salt and their function

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Abstract

Alginate gel bead containing chitosan salt was prepared and the function was investigated. When the bead was placed in bile acid solution it rapidly took bile acid into itself. The uptake amount of taurocholate was about 25 μmol per 0.2 g dried gel beads. This phenomenon was observed on the case of the beads incorporating colestyramine instead of chitosan. Therefore, it seems that the ion-exchange reaction accompanying the insoluble complex-formation between chitosan salt and bile acid occurs in calcium alginate gel matrix. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Chitosan salt; Alginate gel bead; Uptake of bile acid

1. Introduction

Natural polysaccharides such as alginic acid (Alg) and chitosan (CS) are food ingredients and have been used as additives for food and/or drug preparation because of the safety on per oral administration. These polymers are not absorbed from the gastrointestinal tract, thus they are recognized as a dietary fiber. They are also used as a vehicle for drug delivery system and have been studied for controlling drug release and biocompatibility (Takeuchi et al., 1996; Aspden et al., 1997). The polysaccharide has many anionic or cationic groups in the structure, therefore, it exhibits an unique physical property by the electrostatic interaction (Murata et al., 1993). For example, Alg forms cured gel beads in the presence of a divalent cation and it is able to fix a compound such as a drug or another polysaccharide within the gel matrix (Murata et al., 1996; Kikuchi et al., 1997). The CS was also utilized as a drug carrier for colonic targeting and/or as a

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microsphore for capsulation of enzymes (Dashevsky, 1998; Fernandez-Hervas et al., 1998; Lorenzo-Lamosa et al., 1998).

In this study, we prepared the calcium-induced alginate gel beads containing chitosan salt (Alg–CS) and investigated the predictable functions when Alg–CS would be administrated as a vehicle in gastrointestinal tract.

2. Material and methods

Alginate was purchased from Nacalai Tesque (Kyoto), and chitosan, CS (grade F, degree of deacetylation 75–85%) and chitin (fine powder grade) were obtained from Kimitsu Chemicals (Tokyo). Colestyramine was kindly provided by Bristol-Meyers Squibb (Tokyo). Taurocholate (T-CA), glycocholate (G-CA) were obtained from Nacalai Tesque. All other chemicals were of reagent grade.

2.1. Gel beads preparation

Alginate gel beads containing CS salt were prepared as follows. Sodium alginate was dissolved in distilled, demineralized water with agitation and CS was added to the solution. Two grams of alginate solution dispersed 5% (w/w) CS dropped into 20 ml of 0.2 M $CaCl_2$ dissolved in weak acid, e.g. 1% lactic acid and left to stand at 37°C for 3 h, after which the gel beads were transferred to 500 ml of distilled, demineralized water and left for 24 h. Then, the beads were taken out and dried at 35°C for 8 h, followed by vacuum in a desiccator in the presence of P_2O_5.

2.2. Uptake test of bile acid into Alg–CS

Fifteen millilitres of 2 mM bile acid solution was placed in a L-formed tube and maintained at 37°C. The dried Alg–CS (≈ 0.2 g) 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, Kyoto), a packed column (Nacalai Tesque, Cosmosil $5C_{18}$-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 v/v) at a flow rate of 1.0 ml/min and the detector wavelength was set at 254 nm. The uptake amount into Alg–Ca was calculated by the difference between the initial (30 μmol) and residual amount of bile acid in the solution. All tests were performed in triplicate.

3. Results and discussion

CS was held in alginate gel beads when a chelate structure, egg box junction was formed between alginate and calcium ions.

The diameter of hydro-beads was about 4.5 mm and it became about 2 mm in the dried state. Fig. 1 shows that the uptake of T-CA into Alg–CS. The gel bead containing CS salt gradually taken T-CA into itself from the solution and the uptake finished within 2 h in this condition. The uptake was also recognized on the other bile acid, such as GCA. For example, the amount was 18.9 ± 1.8 μmol after 1 h or 21.0 ± 1.4 μmol after 2 h, respectively. In all case, about 70–80% of each

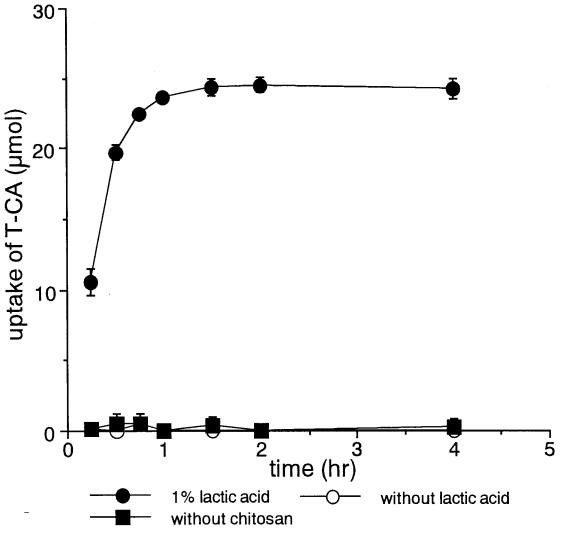

Fig. 1. Uptake of taurochorate (T-CA) into alginate gel beads containing chitosan.

Table 1
Uptake amount of T-CA into alginate gel beads containing colestyramine or chitin

	Uptake amount (μmol)	
	1 h	2 h
Colestyramine	23.6 ± 0.94	28.0 ± 0.71
Chitin	ND	ND

ND, not detected.

bile acid dissolved in the solution was taken into Alg–CS. On the other hand, this phenomenon was not observed in the case of the bead without CS salt or it dispersed CS; that is, lactic acid was not added when the bead was prepared. These data mean that the uptake of bile acid into Alg–CS is attributable to the electrostatic interaction between an amine group of chitosan and a carboxylic group of bile acid. Actually, the fibrous precipitate developed when the bile acid solution, such as T-CA (2 mM), was added to the 1% (w/w) CS solution dissolved with lactic acid.

Table 1 shows the amounts of T-CA taken into the alginate gel beads incorporating chitin or colestyramine, an ion-exchange resin which has been clinically used in therapy for hypercholesteremia. Uptake of T-CA into alginate gel beads containing chitin was not recognized even after 4 h. The beads incorporating colestyramine, which contain quaternary ammonium salts in the structure, rapidly took T-CA into itself and the amount after 2 h went up to 90% of bile acid dissolved in the medium. These results show that a polymer containing amino group is needed in order to take up bile acid. Moreover, similar results was obtained when polyallylamine (Nittobo, Tokyo) lactic acid salt was incorporated into the beads instead of colestyramine (data not shown).

It can, therefore, be presumed that ion-exchange reaction accompanying the insoluble complex formation between CS and bile acid occurs in calcium alginate gel matrix as follows;

$$\text{CS–lactic} + \text{Na–bile acid} \rightarrow \text{CS–bile acid} + \text{Na–lactate}$$

Fig. 2 shows the amount of T-CA taken into

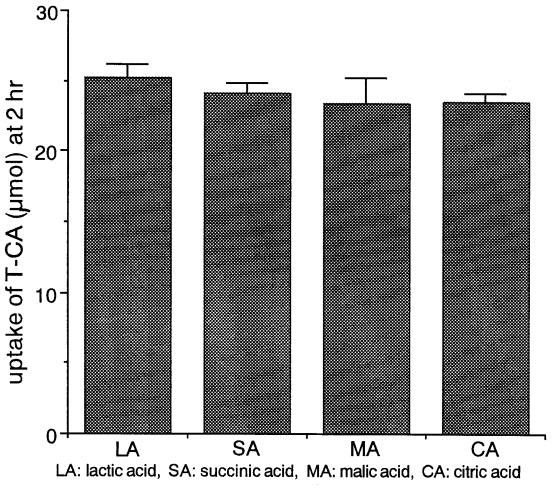

Fig. 2. The change of uptake amount of T-CA into Alg-CS prepared with each weak acid.

Alg–CS prepared with lactic acid, succinic acid, malic acid or citric acid. About 80% of the bile acid dissolved in the solution was taken into gel beads containing mono-, di-, or tricarboxylic acid salt of CS. No remarkable change of the amount was found in all case. Furthermore, Alg–CS prepared with an acidic amino acid, such as aspartic acid or glutamic acid, also took T-CA as shown in Fig. 3.

Alg–CS consists of two natural polysaccharides, calcium ion and a weak acid, such as lactic acid,

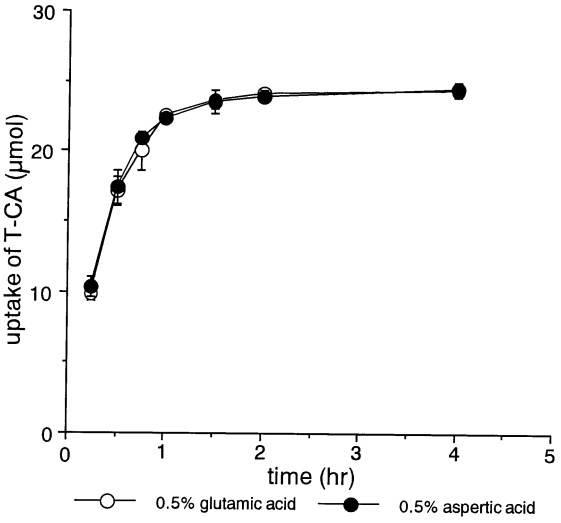

Fig. 3. Uptake of T-CA into Alg-CS prepared with acidic amino acid.

and all of these components has been utilized for food additives. After Alg–CS is administered orally it is going to bind bile acids in the small intestine, such as colestyramine, which is an anion exchange resin used for the decrease of plasma cholesterol (Shepherd et al., 1980). Thus, it seems that hyperlipidemia, which is a very common disease, is preventable by the continuous administration of Alg–CS. Currently, we are preparing Alg–CS with nicotinic acid, a drug for hyperlipidemia, and investigating its function.

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