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Note

Behavior of alginate gel beads containing chitosan salt prepared with water-soluble vitamins

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Abstract

Alginate gel beads were prepared which contained weak acid salts of chitosan (Alg-CS) and water-soluble vitamins (e.g. ascorbic acid (AS)) and the behavior of the beads, uptake of bile acids was investigated in vitro. The Alg-CS beads rapidly took up bile acid and this phenomenon was observed for both hydrogel beads and dried beads. About 120 μmol of taurocholic acid was taken up into Alg-CS (1 g) prepared with orotic acid. Dried Alg-CS is the granule which can be made easily, and keeps the ability of CS salt, and all elements can be taken as a food. Therefore, Alg-CS could serve as a useful dietary agent for the prevention of hyperlipidemia.

Keywords: Ascorbic acid; Alginate gel bead; Chitosan; Bile acid; Hyperlipidemia

1. Introduction

Water-soluble vitamins are responsible for many biochemical functions in the human body, and are mainly supplied by the diet. For example, pantothenic acid (PT, vitamin B_5) is a raw material in the production of coenzyme A and acyl carrier protein. Some vitamins are also utilized in food or food additives. It has been reported that some vitamins might have direct medicinal effects; e.g. ascorbic acid (AS) has been shown to decrease LDL cholesterol in the plasma of hyperlipidemia patients [1–3]. Natural polysaccharide, which has also been widely used in the food industry, serves as dietary fiber and is not absorbed in the gastrointestinal tract. It has been reported that some forms of natural polysaccharide, such as alginic acid and chitosan (CS), can suppress plasma cholesterol levels [4]. Recently, these two polysaccharides have been suggested as possible vehicles for controlling drug release. For example, alginate forms a calcium-induced gel matrix which is able to incorporate a drug or other polysaccharide [5,6]. CS forms gel beads under mild alkaline conditions, and its salt interacts with anionic compounds (due to the polycationic nature of the polysaccharide) [7]. We have previously reported the uptake of bile acids into alginate gel beads containing chitosan salts, which results from formation of a complex between CS and bile acid [8].

The purpose of this study was to prepare alginate gel beads containing a weak acid salt of CS and water-soluble vitamins. In this study, calcium-induced alginate gel matrix beads were formed which contained AS-Ca or PT-Ca. The behaviors of these gel beads, uptake of bile acids was investigated in vitro.

2. Materials and methods

2.1. Materials

L(+)-AS, L(+)-AS-Ca, PT-Ca, orotic acid, folic acid, citric acid were obtained from Wako Chemical Co. (Osaka). Alginate and taurocholate (T-CA) were purchased from Nacalai Tesque (Kyoto), and chitosan (CF(F); degree of deacetylation, 75–85%) was obtained from Kimitsu Chemical Industries (Tokyo). All other chemicals were of reagent grade.

2.2. Preparation of Alg-CS

Alg-CS beads containing AS were prepared as follows. One gram of sodium alginate was dissolved in 94 g of distilled, demineralized water with agitation, and 5 g of CS was added to the solution. Two grams of this solution dispersed CS was added to 10 ml of 0.02 M AS-Ca or PT-

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Ca, and left to stand at 37°C for 2 h. Next, 90 ml of AS solution (or 500 ml of saturated solution, in the cases of orotic acid or folic acid) was added to this solution, which was then left to stand at room temperature for 1–3 days, after which spherical hydrogel beads (approximately 3.5 mm in diameter) were obtained.

Dried Alg-CS beads (approximately 2.0 mm in diameter) were prepared as follows. Alg-CS (hydrogel) beads were incubated for 1 day and then taken out and dried at 35°C for 8 h on a dish, followed by vacuum treatment in a desiccator in the presence of P_2O_5.

2.3. Test of uptake of bile acid into Alg-CS

Fifteen milliliters of 2 mM bile acid solution was placed in an L-shaped tube and maintained at 37°C. The hydrogel beads (about 2 g) were added to the solution and shaken 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), at a flow rate of 1.0 ml/min, and the detector wavelength was set at 230 nm. The amount of bile acid taken up into the Alg-CS was calculated from the difference between the amount of each bile acid added and the residual amount at sampling time. An amount of dried Alg-CS beads corresponding to 2 g of hydrogel was also tested. All uptake tests were performed in triplicate.

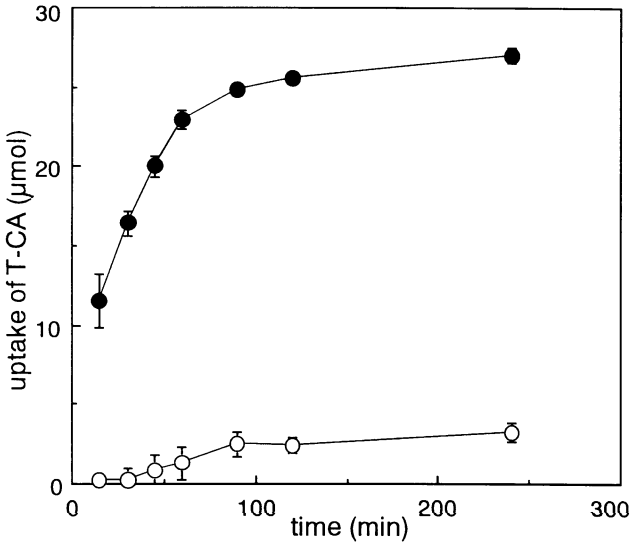

Fig. 2. Uptake of T-CA into Alg-CS treated with orotic acid (OA) or folic acid (FA).

3. Results and discussion

3.1. Uptake of bile acid into Alg-CS

Alg-CS prepared with AS-Ca and AS rapidly adsorbed the bile acid via an ion-exchange reaction. Fig. 1 shows the effect of preparation time on the uptake of T-CA into Alg-CS (hydrogel). After 1–1.5 h, about 20 μmol of T-CA was taken up into 2 g of hydrogel beads containing CS (0.1 g). There were no marked differences, in the amounts of bile acid taken up into Alg-CS, between the different preparation times. Thus, it appears that formation of the CS-AS salt (which is responsible for the uptake of bile acid) is complete after the 1st day of preparation. In the case of Alg-CS

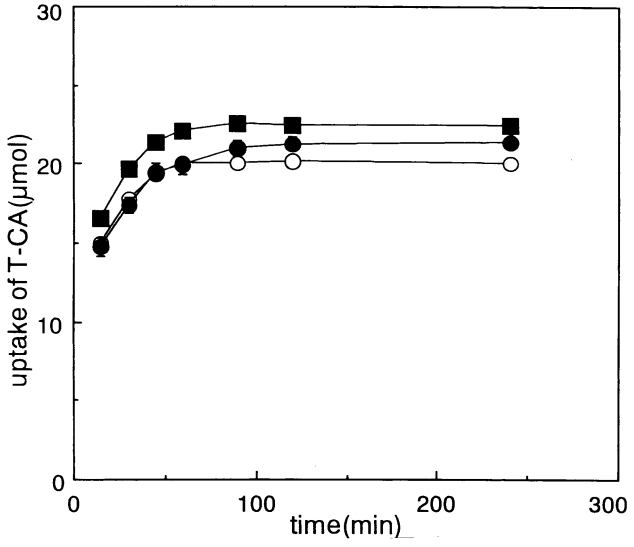

Fig. 1. Effect of incubation time on the uptake of taurocholate (T-CA) into Alg-CS treated with 0.2% AS.

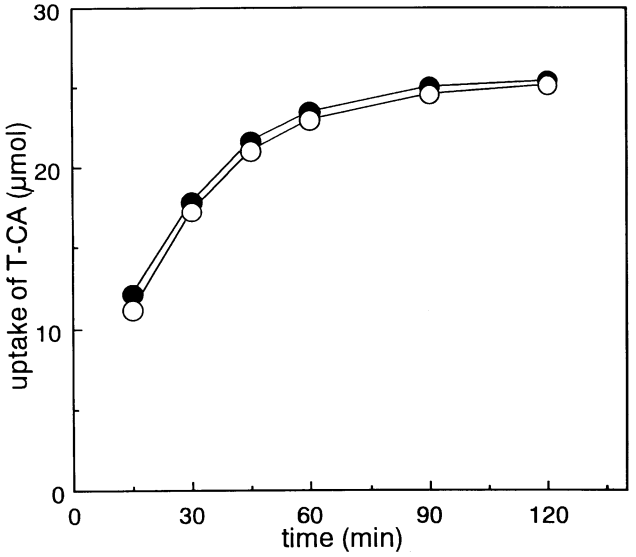

Fig. 3. Uptake of T-CA into dried Alg-CS prepared with 0.02 M calcium ascorbate (AS-Ca) or calcium pantothenate (PT-Ca).

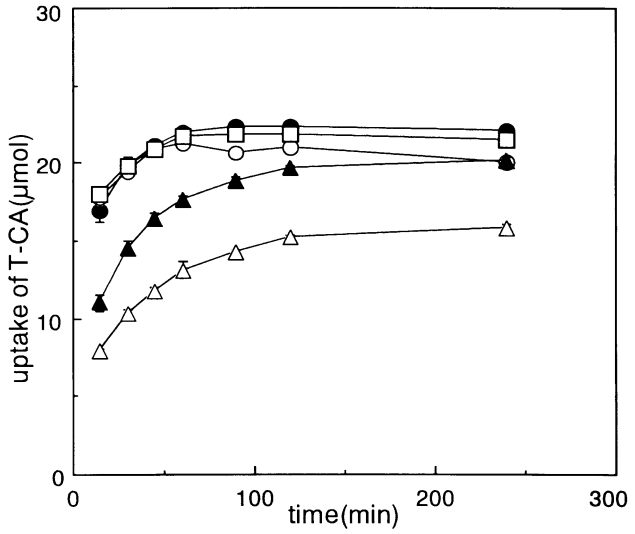

Fig. 4. Uptake of T-CA into Alg-CS treated with AS and citric acid (CR)

prepared with orotic acid, approximately 25 μmol of T-CA had been taken up per sample of Alg-CS at 2 h (Fig. 2). However, Alg-CS prepared with folic acid took up extremely little T-CA. During preparation with folic acid, CS salt (which participates in an ion-exchange reaction with T-CA) might not form adequately because the water-solubility of this vitamin is low.

Dried Alg-CS beads also swelled gradually in T-CA solution and took up bile acid as shown in Fig. 3. After 2 h, they had taken up 85% of the bile acid initially dissolved in the solution. A similar bile acid uptake profile was obtained for dried Alg-CS prepared with PT-Ca as a gelation agent (Fig. 3). Thus, the dried alginate gel matrix prepared with CS-AS salt was capable of containing not only AS but also PT.

AS is frequently used as a food additive, and may be added along with acids which can form CS salts. Fig. 4 shows T-CA uptake rates for Alg-CS prepared with a mixture of AS and citric acid. About 50% of T-CA dissolved in the medium was taken up by Alg-CS prepared with 0.1% AS and 0.1% citric acid. Both the uptake rate and the total amount of bile acid taken up increased in direct proportion to the combined total amount of ascorbic and citric acid.

In this study, we demonstrated that Alg-CS could potentially be used for the uptake of bile acids. Alg-CS is suitable for daily intake, because all of its components are either food materials or food additives, all of which have a long history of safe use. Orally administered Alg-CS can release water-soluble vitamin such as PT-Ca or AS, and it can then inhibit enterohepatic circulation of bile acids, by taking up those that have been secreted into the intestinal tract [9,10]. Alg-CS could be a useful agent in the prevention of hyperlipidemia, which is a lifestyle-related disease that requires dietary therapy. Now, we administer orally CS salt to rats and investigate the influence on the cholesterol levels in plasma because the environment of the gastrointestinal tract will have a great influence upon the uptake of bile acid by Alg-CS.

Acknowledgements

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