Adsorption of Bile Acid by Chitosan-Orotic Acid Salt and Its Application as an Oral Preparation

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The orotic acid (OT) salt of chitosan (CS), CS-OT, and that of a CS derivative, CP, were prepared, and the adsorption of primary or secondary bile acid was investigated. Calcium-induced alginate gel beads (Alg-Ca) containing CS-OT were also prepared and autoclaved, and the possibility of these beads to act as a vehicle for oral administration to prevent hyperlipidemia was investigated. When taurocholate (TCA) and glycocholate (GCA) were present together in the medium, CS-OT adsorbed identical amounts of both bile acids. This trend was seen in all CPs, although the capacity to adsorb bile acid was affected by the number and/or structure of the amino groups in the CP. On the other hand, taurodeoxycholate, a secondary bile acid was preferentially adsorbed over TCA and GCA. Alg-Ca containing CS-OT took up bile acids in a similar manner as CS-OT irrespective of the water content of the gel matrix. As all elements can be taken as a food, Alg-Ca containing CS-OT could serve as a useful dietary agent for the prevention of hyperlipidemia, which is a lifestyle-related disease.

Key words chitosan; orotic acid; alginate gel bead; hyperlipidemia

Natural polysaccharides are dietary components and have been used as additives for controlling the viscosity of food. They are not only safe for oral intake but are also attractive because of their functions in the gastrointestinal tract.[1,2] One particular polysaccharide, chitosan (CS), has recently been studied for its utility in such preparations,[3—5] and it was reported that CS was able to reduce plasma cholesterol levels by interfering with absorption of dietary fat and/or bile acid (BA).[6—8]

BA is synthesized in the liver from cholesterol and is secreted in the gastrointestinal tract mainly as glycine or taurine conjugates, which are known as primary BAs. Secondary BAs, such as deoxycholate, are produced from primary BAs by intestinal microorganisms. Primary and secondary BAs are then absorbed in the ileum (enterohepatic circulation) and are reused as materials for freshly secreted BA.

Weak acid salts of CS interact with BA in the aqueous medium as a result of its polycationic properties.[9] Therefore, CS salts may be useful agents for inhibiting the enterohepatic circulation of BAs, thus decreasing plasma cholesterol levels, by acting as an anion-exchange resin, such as colestyramine (COL) or colestimide, in the intestinal tract after oral administration.[10] CS salts could also be used daily for the prevention of hyperlipidemia as they are easily prepared with acidic compounds, the food additive lactic acid, or water-soluble vitamins, all of which are mainly supplied by the diet.

We previously reported that the CS-orotic acid (OT) salt, CS-OT, was able to adsorb BAs, and that a remarkable decrease in serum cholesterol levels was observed when CS-OT was orally administered to rats.[11] OT is a water-soluble vitamin and the role of OT in lipid metabolism has been studied.[12] In the present study, we investigated the adsorption of BAs by CS-OT or OT salts of a CS derivative that was utilized as a carrier for enzyme immobilization *in vitro*.[13] And as CS-OT was fine powder hard to swallow, it was necessary to devise the pharmaceutical form of the salt for swallowing. Therefore, CS-OT was granulated with calcium-induced alginate gel beads (Alg-Ca), and the possibility of these beads to act as a vehicle to prevent hyperlipidemia by oral administration was investigated.

Experimental

Materials One type of CS (CS(F), degree of deacetylation; DA, 75—85%) was obtained from Kimitsu Chemical Industries (Tokyo), and four types of CS (7B: DA 70%, 8B: DA 80%, 9B: DA 90% and 10B: DA 100%) were obtained from Katokichi Co. Ltd. (Tokyo). Four types of the CS derivative, CP (chitopearl-BCW 2505, 2605, 3005 and 3505), were obtained from Fujibouseki Ltd. Co. (Tokyo) and the structures of these are shown in Fig. 1. The CPs were abbreviated as CP25, CP26, CP30 and CP35, respectively. COL (Questran) was kindly supplied by Bristol-Myers Squibb K.K. (Tokyo). OT was obtained from Wako Pure Chemical Ind., Ltd. (Osaka). Sodium alginate (polymerization: 450), sodium taurocholate (TCA), glycocholate (GCA), cholate (CA), and taurodeoxycholate (TDCA) were purchased from Nacalai Tesque (Kyoto). All other chemicals were of reagent grade.

Preparation of CS-OT CS-OT was prepared as reported previously.[11] CS-OT powder (75—200 μm) was obtained by sieving. In this paper, the salt prepared with CS(F) and OT is abbreviated as CS(F)-OT.

Preparation of CP OT Salt (CP-OT) Each CP was washed with distilled demineralized water and ethanol and was dried at room temperature. Dried CPs were treated with OT solution as follows: CP (0.5 g) was added to 500 ml of 0.072% OT solution and stirred for 1 d at room temperature. The suspension was centrifuged at 3000 rpm for 5 min. The precipitate was collected and washed twice with 50 ml of distilled demineralized water and ethanol, dried on a dish, and was desiccated under a vacuum in the presence of P_2O_5.

Preparation of Alg-Ca Containing CS-OT Two grams of 1(w/w)% sodium alginate solution dispersed 5% CS was added to 10 ml of 0.1 M $CaCl_2$, and was left to stand at room temperature for 1 h. Next, the resulting spherical hydrogel beads were transferred to 50 ml of 0.072% OT solution, which was then autoclaved at 121 °C for 15 min. After cooling at room temperature, Alg-Ca (hydrogel bead) was obtained. The dried gel beads were prepared as follows: hydrogel beads were collected and dried at 35 °C for 8 h on a dish, and were desiccated under a vacuum in the presence of P_2O_5.

Adsorption of BA by CS-OT or Alg-Ca Containing CS-OT Fifteen milliliters of a solution containing bile acid was placed into an L-shaped tube and was maintained at 37 °C. Between 10 and 20 mg of CLS, CS-OT or CP-OT and/or Alg-Ca containing CS(F)-OT was added to the solution and was shaken at 67 times per min. A 0.2-ml aliquot of each solution was removed periodically and filtered with a membrane filter (0.45 μm) for HPLC analysis.[9] The amount of BA adsorbed by the CS sample was calculated from the difference between the initial amount of BA and the residual amount at each sampling time. All uptake tests were performed in triplicate.

Results and Discussion

TCA, which is a primary BA, is adsorbed by anion-exchange resins such as an anti-hyperlipidemia medicine COL.

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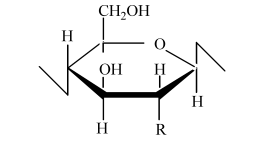

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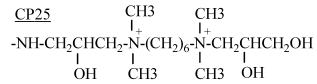

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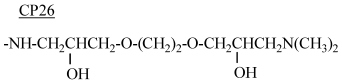

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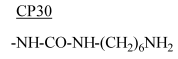

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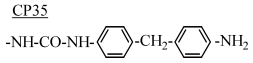

Fig. 1. Structures of CPs

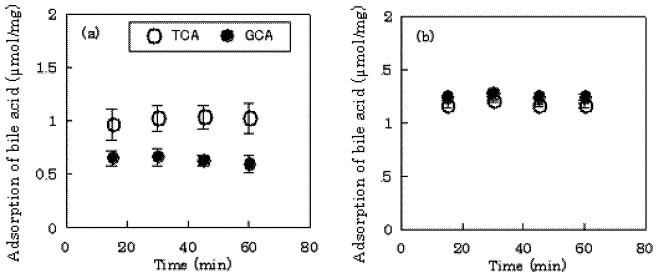

Fig. 2. Adsorption of BAs by COL (a) and CS(F)-OT (b)

Initial BA concentration; 2 mM TCA, 2 mM GCA. Results are expressed as means and the bars indicate S.D. ($n=3$).

When 10 mg of COL was soaked in 15 ml of 4 mM TCA, the BA was immediately adsorbed and the amount of adsorbed TCA was 1.9 ± 0.1 μmol/mg after 30 min. In the case of GCA, it was 2.1 ± 0.4 μmol/mg. CS(F)-OT also adsorbed these BAs and the amounts of TCA and GCA were 2.4 ± 0.3 μmol/mg and 2.4 ± 0.4 μmol/mg, respectively. These values may indicate the maximum degree that CS(F)-OT was able to adsorb these BAs, as the amount of the BAs was 2.2 ± 0.1 μmol/mg after 30 min in 8 mM TCA (initial concentration).

When TCA and GCA (2 mM each) were both present in the medium, COL adsorbed TCA preferentially and the amount of TCA adsorbed by the resin was 1.5 times that of GCA, as shown in Fig. 2a. On the other hand, CS(F)-OT equally adsorbed both BAs (1.2 μmol/mg) under the same conditions (Fig. 2b). For COL, in the presence of 2 mM TCA and 2 mM CA, the amounts of adsorbed TCA and CA after 30 min were 1.1 ± 0.1 μmol/mg and 0.8 ± 0.1 μmol/mg, while those for CS(F)-OT were 1.0 ± 0.1 μmol/mg and 1.1 ± 0.1 μmol/mg, respectively.

CP is a polycationic resin, and is a derivative of CS, as shown in Fig. 1. TCA and GCA were adsorbed by CP-OT in a similar manner as CS(F)-OT. The amount of BAs adsorbed was affected by the number and/or the structure of the amino groups in CP. CP25 adsorbed similar amount of BAs as CS(F)-OT, as shown in Fig. 3. The capacity for adsorption lowered according to decreases in the number of amino groups, and in the case of CP35, the amount of adsorbed

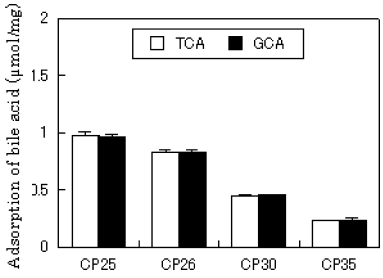

Fig. 3. Adsorption of BAs by CP-OT

Initial BA concentration; 2 mM TCA, 2 mM GCA. Results are expressed as means and the bars indicate S.D. ($n=3$).

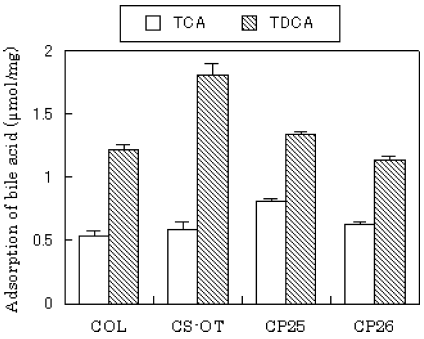

Fig. 4. Adsorption of BAs

Initial BA concentration; 2 mM TCA, 2 mM TDCA. Results are expressed as means and the bars indicate S.D. ($n=3$).

Table 1. Effects of DA on Adsorption of BAs by CS-OT (after 30 min)

DA (%)	TCA (μmol/mg)	TDCA (μmol/mg)
70	0.53 ± 0.03	1.53 ± 0.11
80	0.72 ± 0.02	1.85 ± 0.06
90	0.80 ± 0.06	1.75 ± 0.04
100	0.70 ± 0.03	1.81 ± 0.02

Initial BA concentration; 2 mM TCA, 2 mM TDCA. Results are expressed as means and the bars indicate S.D. ($n=3$).

BAs was half that of CP30. Adsorption of BAs by the resin may be dependent on weakly basic aromatic amines. Irrespective, TCA and GCA were almost equally adsorbed by CP-OT when both of these BAs were present, although the structures of the amino group contained in these resins were different. Therefore, the characteristics observed for both CS(F)-OT and CP-OT appear to be attributable to their polymeric structures, *i.e.*, the fact that they are polysaccharides. CP25 alone adsorbed small amounts of the BAs as it contained a quaternary ammonium salt; however, the other CPs had little capacity to adsorb BAs when they were not treated with OT (data not shown).

COL also adsorbed secondary BAs, and the resin preferentially adsorbed TDCA when TCA (2 mM) and TDCA (2 mM) were both present in the medium, as shown in Fig. 4. The amount of TDCA adsorbed by COL was about twice that of TCA. Furthermore, a similar trend was observed for the adsorption of BAs by CS(F)-OT and CP-OT. The other CS-OT prepared with CS that the DA was 70—100%, preferentially adsorbed TDCA (Table 1). In all cases, the total amount of the two BAs adsorbed was 2—2.5 μmol/mg.

The Alg-Ca (hydrogel bead) prepared in this study theoret-

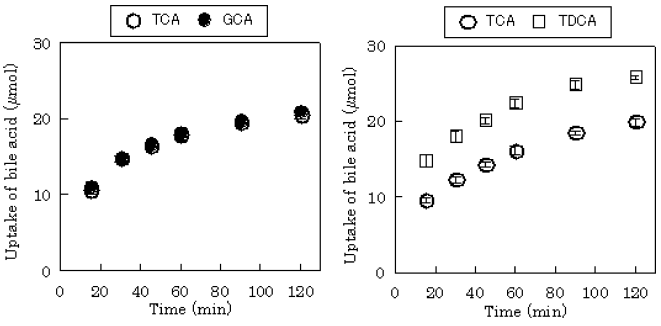

Fig. 5. Uptake of BAs into Alg-Ca (Hydrogel Bead) Containing CS(F)-CT

Initial BA concentration; 2 mM TCA, 2 mM GCA, 2 mM TDCA. Results are expressed as means and the bars indicate S.D. (n=3).

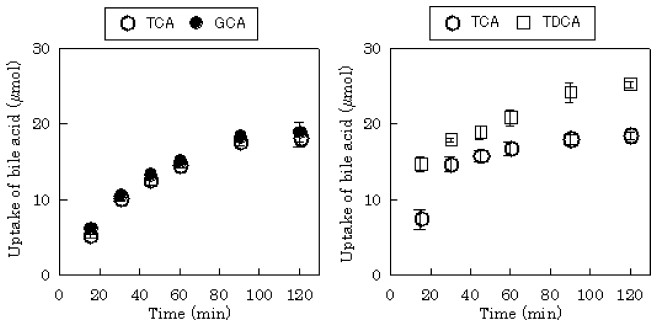

Fig. 6. Uptake of BAs into Alg-Ca (Dried Gel Bead) Containing CS(F)-CT

Initial BA concentration; 2 mM TCA, 2 mM GCA, 2 mM TDCA. Results are expressed as means and the bars indicate S.D. (n=3).

ically contains about 100 mg of CS, and the CS was converted to the OT salt in the gel matrix by autoclaving in OT solution. Alg-Ca gradually took up BAs from solution containing two types of BA, as shown in Fig. 5. When TCA and GCA were both present in the medium, the same amounts (about 70% of the amount originally dissolved in the medium) of these BAs were adsorbed by Alg-Ca. In the case of dried Alg-Ca, a similar BA uptake profile was observed, although the initial uptake rate was smaller than that of the hydrogel beads (Fig. 6). Furthermore, the preference in adsorption of BAs was similar to that observed for CS(F)-OT powder in the solution containing both TCA and TDCA. These results suggest that the adsorption of BAs was able to progress in the polymer gel matrix at any given water content. In addition, Alg-Ca containing CS-OT can be daily used as a vehicle for oral administration because the uptake of BAs took place in beads sterilized by autoclaving.

It has been reported that 12—36 mg of BAs are secreted per day in humans and that the principal primary BA is GCA.[14] CS-OT salts immediately adsorb BAs and this adsorption occurs in polymer matrixes, such as Alg-Ca, which are suitable for oral administration.[15] Because hyperlipidemia is a lifestyle-related condition, preventive measures applied to latent patients are required in order to overcome the disease.[16,17] Therefore, Alg-Ca containing CS-OT may be a candidate preparation for prevention of hyperlipidemia.

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