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Inhibiting or enhancing effect of sulfuric acid-treated wheat starch on antibody production induced by two types of adjuvant

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ABSTRACT

Glycogen has been reported to have immune-regulating activity. We examined in this study the immune-regulating activity of wheat starch of various molecular weights, because both starch and glycogen are made from glucose components linked by α-1,4 and α-1,6 glycoside bonds. Wheat starch was treated by sulfuric acid to prepare starch samples with differing molecular weight. The acid-treated starch inhibited cytokine production from murine splenocytes when the splenocytes were incubated with the antigen and a starch sample. The activity depended on the treatment time by sulfuric acid. Mice were then i.p. immunized with some antigens and the starch mixed with two types of adjuvant. The starch also inhibited the *in vivo* antibody production when administered with an alum adjuvant. In contrast, the starch enhanced the antibody response when administered with complete Fround adjuvant, indicating that the starch regulated immune responses depending on the molecular weight and surrounding circumstances.

1. Introduction

Allergic diseases of hay fever and asthma, as well as autoimmune diseases of colitis and rheumatoid arthritis are of immunological type induced by unbalanced or hyper immune responses. The increase of these immunological diseases has recently become one of the most severe health problems throughout the world, because the quality of life (QOL) of the patients is generally low. Many groups have tried to use the immune regulating function of food factors to improve QOL of these patients, for example with some kind of poly- or oligo-saccharides, tea catechins, and lactic acid bacteria.

Recent studies have shown that allergy is induced by Th2 type of immune response, while some autoimmune diseases, for example rheumatoid arthritis and multiple sclerosis, are induced by Th1-type responses (Finkelman & Urban, 2001). The Th17 type of response has also been considered to be involved in some inflammatory diseases (Kimura & Kishimoto, 2011). Food factors, such as lactic acid bacteria, have been found to inhibit allergy by suppressing the Th2 response (Enomoto et al., 2009; Yoshida, Hirano, Wada, Takahashi, & Hattori, 2004), while there are few foods known to inhibit Th1 or Th17. Such activity of food factors could also be useful to improve QOL of the patients with those diseases. However, such Th1, Th2 and Th17 immune responses are also necessary to protect us from infections. The complete inhibition of these immune response by some medicines is therefore not always the best way to treat allergy or autoimmune diseases. A method to specifically inhibit an unbalanced or needlessly strong response is necessary. Foods do not generally make a major or rapid difference to our health, but a daily intake of functional foods might gradually improve our health without any serious side effects. Controlling the intensity and balance of such immune responses as Th1, Th2 and Th17 by some food factors can be expected to maintain a healthy life.

Amylopectin, a type of starch, consists of polymerized glucose units linked by α-1,4 and α-1,6 glycoside bonds and are widely contained in grain. Starch in foods is not only necessary as a source of energy, but also plays an important role in processing food products. In contrast, glycogen, which also consists of polymerized glucose units, provides a stock of glucose in animals, and the structure and character of glycogen are known to be different from those of starch. Glycogen has more branches, which are formed by α-1,6 glycoside bonds, and higher water solubility than starch. Recent studies have revealed glycogen to possess several immune-regulating functions (Ryoyama, Kidachi, Yamaguchi, Kajiura, & Takata, 2004; Takaya et al., 1998). The effect of glycogen obtained from shellfish, for example scallops and oysters, on the anti-tumour response was initially studied, and it had been thought that the activity of glycogen strongly depended on its structure (Takaya et al., 1998). However, Kakutani et al. (2007) have demonstrated that some enzymatically synthesized types of glycogen also had strong activity, like the glycogen obtained from shellfish, and that this activity strictly depended on the molecular weight and not the

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structure. This finding implies that starch may have similar activity if its molecular weight can be appropriately adjusted.

The immune regulating function of starch has hardly been reported, because the molecular weight of amylopectin in foods is in the range of 3.1–5.2 $\times$ 10^8 (Yoo & Jane, 2002). According to the report by Kakutani et al. (2007), glycogen with a molecular weight of about 5 $\times$ 10^6 demonstrated the strongest activity, indicating that some type of treatment would be required for starch to provide such activity. Treating starch with sulfuric acid is widely used by the food industry to imbue starch with appropriate properties such as viscosity and water solubility. Although there is no reported study examining the immune regulating function of sulfuric acid-treated starch, this or other methods to reduce the molecular weight of starch would be useful to generate special properties for food processing and also to develop novel food materials with immune regulating functions; it is therefore necessary to confirm whether or not sulfuric acid-treated starch can regulate immune responses.

Any examination of the immune-regulating activity of acid-treated starch requires samples of starch with varying molecular weight. We therefore prepared three types of starch by treating with sulfuric acid for different periods of time. The differing effects of these different types of starch on the T cell response, especially on the cytokine production, were first examined. The effects of these starch samples on the antibody production were then investigated *in vivo*. Our findings will help to provide novel functions of starch for immune regulation, and expand its application to various food products.

2. Materials and methods

2.1. Mice

Six-week-old BALB/c mice were purchased from Clea Japan (Tokyo, Japan). Ovalbumin (OVA)-specific T cell receptor transgenic mice (TCR-Tg mice; DO11.10) were used at 6–14 weeks old, the T cells of DO11.10 mice recognizing $OVA_{323-339}$ restricted to $I\text{–}A^d$. All the mice were maintained and used in accordance with the guidelines for the care and use of experimental animals of Tokyo University of Agriculture and Technology.

2.2. Antigens and antibodies

Crude bovine β-lactoglobulin (β-LG; genotype AA) was prepared from fresh milk of a Holstein cow according to the method of Armstrong, McKenzie, and Sawyer (1967). This crude β-LG was purified in a DEAE–Sepharose Fast Flow column (3.0 ID $\times$ 40 cm; Amersham Pharmacia Biotech, Buckinghamshire, UK) by the method described previously (Hattori, Nagasawa, Ametani, Kaminogawa, & Takahashi, 1994), the degree of purity being confirmed by polyacrylamide gel electrophoresis (PAGE) performed by the method of Davis (Davis, 1964). OVA was purchased from Seikagaku Kogyo (Tokyo, Japan). Concanavalin A (Con A; Sigma, Tokyo, Japan) was used for antigen-independent T cell activation.

Purified rat anti-mouse IFN-γ mAb (R4-6A2, BD Pharmingen, San Diego, CA), purified rat anti-mouse IL-4 mAb (11B11, BD Pharmingen), biotinylated rat anti-mouse IFN-γ mAb (XMG1.2, BD Pharmingen), biotinylated rat anti-mouse IL-4 mAb (BVD6-24G2, BD Pharmingen), biotinylated rat anti-mouse IgG1 Ab (Zymed, San Francisco, CA), biotinylated rat anti-mouse IgG2a Ab (Zymed) and biotinylated rat anti-mouse IgE mAb (R35-118, BD Pharmingen) were used for ELISA.

2.3. Preparation of sulfuric acid-treated starch

Wheat starch (Ukiko, Manto, Tokyo, Japan) was suspended in an 18% sulfuric acid solution and kept at 38 °C for 0, 4, 8 and 12 days. Each sample was collected by a centrifugation (800*g*), the supernatant being used for analyzing the soluble sugar amount to evaluate the progress of the acid treatment. After removing the supernatant, the precipitate was washed by ice-cooled water. We used ice-cooled water to avoid the gelatinization of the starch caused by heat which occurred by mixing the water and the residual acid. The gelatinization results in loss of yield after re-centrifugation. This procedure was conducted at least five times until the supernatant showed a neutral pH value. A part of each of these samples was subdivided and dried at 110 °C to estimate the weight of the sample obtained. The starch samples were used for the experiments after suspending in PBS and autoclaving. The progress of the sulfuric acid treatment was evaluated by measuring the amount of soluble saccharide in the supernatant by the phenol–sulfuric acid method. The amount of insoluble saccharide was calculated by subtracting the soluble saccharide amount from the total starch amount. The difference in the amount of insoluble saccharide between the starch treated for 0 days and each other treated sample is shown in Table 1 as dissolved saccharides. The percentage of the amount of dissolved saccharide in each treated sample compared with the amount of insoluble saccharide in the starch treated for 0 days is also shown in Table 1 as the acid hydrolysis rate. Untreated starch was used as a control in the Figs. 1–3, while 0 day-treated starch was used in Figs. 4 and 5. We confirmed those two starch samples had similar activity by performing the same experiment as that shown in Fig. 1 (data not shown).

2.4. Cell culture

The effects of the starch on a splenocyte culture were evaluated by incubating spleen cells (2 $\times$ 10^6 cells/ml) of DO11.10 with OVA (0 and 10 μM) and each starch (0.1 and 1 mg/ml) in an RPMI1640 medium (Nissui Pharmaceutical, Tokyo, Japan) containing 10% heat-inactivated fetal calf serum (JRH Bioscience, Lenexa, KS). Each supernatant was collected after 2 days of incubation for use in subsequent cytokine and cytotoxicity assays.

The results shown in Fig. 3 were obtained from spleen cells of DO11.10 (2.5 $\times$ 10^6 cells/ml) and CD90-positive spleen cells (8 $\times$ 10^5 cells/ml) that had been incubated with ConA (2 μg/ml) and each starch (1 mg/ml). The CD90-positive cells (T cells) in this experiment were isolated from spleen cells by magnetic separation, using CD90 MACS beads and a MACS separation column (Miltenyi Biotec, Bergish Gladbach, Germany).

2.5. Immunization and preparation of the sera

BALB/c mice (5 mice/group) were i.p. immunized with β-LG. The immunization was carried out after, respectively, dissolving β-LG and each starch in PBS at 2 mg/ml and 20 mg/ml. This solution was mixed with an equal volume of an alum adjuvant (20 mg/ml), and 100 μl was injected into each mouse. In the other experiment, BALB/c mice (5 mice/group) were i.p. immunized with OVA. In this case, OVA and each starch were, respectively, dissolved in PBS at 2 and 20 mg/ml. This solution was then emulsified with an equal volume of complete Froud adjuvant (CFA; Difco Laboratories, Detroit, MI), and 100 μl was injected into each

Table 1
Progress of the acid treatment.

Treatment	Dissolved saccharides (mg/ml)	Acid hydrolysis rate (%)
0-day	–	–
4-day	2.9	24.8
8-day	3.6	30.8
12-day	8.6	73.5