JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Multifunctionalities of Oleyl-Branched Oligosaccharide Phosphate from Potato Starch with a High Phosphate Content

Taichi Ikehara,[†] Reika Ogiwara,[†] Makoto Hattori,[†] Tadashi Yoshida,[†] Masatusgu Yamashita,[‡] and Koji Takahashi*[,†]

[†]Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-Cho, Fuchu City, Tokyo 183-8509, Japan

[‡]Quality Assurance Division, Taiyo Kagaku Company, Limited, 1-3 Takaramachi, Yokkaichi, Mie 510-0844, Japan

ABSTRACT: Branched oligosaccharide phosphate (BOS-P) and oleyl BOS-P (OA-BOS-P) were prepared from potato starch with a high phosphate content by limited amylolysis with α-amylase and exhaustive digestion with glucoamylase and oleylation of BOS-P through a lipase-catalyzed solid-phase synthesis. The multifunctional properties of OA-BOS-P were evaluated in terms of surface tension, emulsifying ability, Ca^{2+}-binding ability, and ability to control the gelatinization and retrogradation of potato starch. OA-BOS-P exhibited better emulsifying ability than BOS-P and Ca^{2+}-binding ability similar to that of BOS-P. OA-BOS-P elevated the gelatinization temperature and reduced viscosity more than BOS-P. OA-BOS-P also reduced retrogradation as indicated by the reduction in the setback viscosity, turbidity, development of the ordered structure and crystalline structure, and digestibility, whereas BOS-P elevated the setback and turbidness, despite reducing the development of the crystalline structure, except for development of the ordered structure, similar to that of the control. These results show that OA-BOS-P could be a useful material with novel emulsifying, Ca^{2+}-binding, and starchy food-controlling properties.

KEYWORDS: acylated branched oligosaccharide phosphate, branched oligosaccharide phosphate, emulsifying property, Ca^{2+}-binding property, gelatinization and retrogradation of starch

INTRODUCTION

Most foods contain oil and fat as important ingredients endowing them with body, as well as a calcium-absorption inhibitor, such as phosphate, phytate, or oxalate, and starch as a builder or modifier of those physical properties and structures. Considerable attention has thus been focused on controlling the stabilization of lipids, bioavailability of dietary calcium, and such physical properties as the gelatinization and retrogradation behavior of starch. In particular, controlling the physical properties of starch poses major difficulties, because starch only reveals its characteristic viscoelasiticity when heated in the presence of water and the swollen starch granules easily break down under a shearing stress that often results in a disliked sticky or pasty texture. Moreover, the resulting paste is deleterious to the physical properties of starchy foods during subsequent cooling, degrading their quality. These difficulties have encouraged the development of such emulsifiers as sucrose fatty acid ester (SE),[1] such Ca^{2+}-binding materials as alginic acid,[2] casein phosphopeptide,[3] phosphorylated oligosaccharide,[4,5] and citric acid,[6] and such starchy food-modifying materials as SE,[7] monoacylglycerol,[8] and related lipids.[9] However, because food processing often requires a food material with both specified and diverse functions, it is important to fully evaluate the characteristics of the food material and develop a new food material with multifunctionality.

We have previously reported that phosphorylated SE (SE-P), called an ionic glycolipid, could be prepared by dry-heating SE with metaphosphoric acid and that SE-P was a multifunctional material with better emulsifying ability than SE that was lost in the acidic region and presence of salt. SE-P also improved the physical properties of starch by reducing the viscosity and depressing retrogradation through forming a complex with the starch chain via the fatty acid moiety and endowing the Ca^{2+}-binding ability.[10] However, further functional improvements to SE-P are necessary, because SE-P showed relatively poor solubility at low temperature, an insufficient increase in the gelatinization temperature, and a reduction of viscosity. Improving the latter two shortcomings by stabilizing the starch could prevent the sticky or pasty texture from developing. It is likely that a larger saccharide moiety in an ionic glycolipid would improve the solubility because of its stronger hydration. Greater starch stability would also result from the reduced mobility of the starch chains endowed by stabilizing the water structure surrounding the starch chains through the interaction among starch, water, and the saccharide moiety.[11,12]

Phosphorylated oligosaccharides (POSs) have recently been developed from potato starch (PS) by thoroughly digesting with α-amylase, glucoamylase, and pulllanase as a mixture of mono- and diphosphoryl α-1,4-linked maltooligosaccharides, with an average degree of polymerization (DP) of 4.02.[4,5] The yield of POS was not reported, possibly because the low phosphate content of the original PS sample used resulted in a very low yield. If PS with a high phosphate content was digested without using a debranching enzyme, the high phosphate content and

Received: August 22, 2010
Accepted: December 17, 2010
Revised: November 24, 2010
Published: March 31, 2011

larger DP than those of POSs, together with the saccharide moiety of SE-P, could enable the yield of branched oligosaccharide phosphate (BOS-P) to be improved. However, BOS-P would probably show no emulsifying ability because of little content of the hydrophobic moiety. Fatty-acylating BOS-P by a mild procedure such as lipase-catalyzed solid-phase synthesis, which has endowed sericin with good emulsifying ability,[13] could thus enable a new anionic glycolipid with Ca^{2+}-binding ability and emulsifying ability to be developed. This acylated BOS-P would exhibit improved solubility and outstanding ability to control the thermal properties of starch, because it would have a more bulky hydrophilic saccharide moiety than SE-P. A saturated fatty acid can more easily form a complex with a starch chain than an unsaturated fatty acid.[14,15] However, an unsaturated fatty acid, such as oleic acid, is easier to handle than a saturated fatty acid, such as stearic acid, in SE-P because of its lower melting temperature than the latter.

Olelyl BOS-P (OA-BOS-P) was prepared in this study from PS with a high phosphate content by limited amylolysis with α-amylase and exhaustive digestion with glucoamylase and final oleylation of resulting BOS-P by lipase-catalyzed solid-phase synthesis. The multifunctionality of OA-BOS-P was demonstrated by its surface tension, emulsifying ability, Ca^{2+}-binding ability, and ability to increase the gelatinization temperature, reduce the viscosity, and reduce the retrogradation of PS.

MATERIALS AND METHODS

Materials. PS (Konafubuki var.) with a high phosphate content (76.4 mg %)[16] and normal large granule PS were supplied by Hokuren Research Institute (Sapporo, Japan) and used after being repeatedly washed with distilled water at 4 °C and air-dried (16.3 and 14.1% moisture contents, respectively). α-Amylase (*Bacillus subtilis*, 20 units/mg) and glucoamylase (*Rhizopus* sp., 20 units/mg) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Oriental Yeast (Tokyo, Japan), respectively. An immobilized lipase (Novozym 435; *Candida antartica*, 10 000 PLU/g) was supplied by Novozyme Japan (Tokyo, Japan), and acid phosphatase (from potato, 2 units/mg) was purchased from Roche Diagnostics (Basel, Switzerland). All other reagents used were commercially available.

Preparation of BOS-P. A PS (Konafubuki var.) suspension (8 g/800 mL) in a 0.02 M acetate buffer (pH 6.0) containing 6 mM NaCl and 2 mM $CaCl_2$ was heated at 100 °C for 10 min while stirring to give a paste and then immediately cooled to 70 °C. The paste was hydrolyzed at 70 °C for 2 min by adding 0.4 mL of an α-amylase solution (60 units/mL) and then heated at 100 °C for 15 min to stop further hydrolysis. After the hydrolysate was cooled to 45 °C, it was adjusted to pH 5.0 with 0.02 M acetic acid and further hydrolyzed at 45 °C for 1 h by adding 8 mL of a glucoamylase solution (600 units/mL). The resulting hydrolysate was heated at 100 °C for 10 min to stop the amylolysis, then centrifuged at 17000*g* for 15 min, and filtered with no. 2 filter paper (Advantec, Tokyo, Japan) to obtain the reaction product. The reaction product adjusted to pH 7.0 was applied to a DEAE-Sepharose Fast Flow column (2.8 cm inner diameter × 7.5 cm) that had been equilibrated with a 0.02 M borate buffer (pH 7.0) and eluted stepwise with 800 mL of the same buffer and then 800 mL of the same buffer containing 1.0 M NaCl. The NaCl−eluate was desalted by an electrodialyzer (Astom S3 Microacylazer, Tokyo, Japan) to give 0.15 mS/cm as previously described.[17] The resulting desalinate was concentrated in a rotary evaporator (Shibata, Tokyo, Japan) to 300 mL and then lyophilized to recover BOS-P.

Preparation of OA-BOS-P. BOS-P was oleylated according to the method previously described.[13] In brief, 100 mg of BOS-P in a screw-capped Erlenmeyer flask was pulverized in 20 mL of dehydrated *n*-hexane by a Polytron PTA-7 homogenizer (Kinematica, Switzerland) at 24 000 rpm for 1 min. Molecular sieves (0.2 g) activated at 400 °C for 4 h were added, and the mixture was deaerated under reduced pressure for 1 min in an ice bath. Oleic acid (1.0 mL) and 1 g of Novozym 435 were then added, and the headspace gas was replaced with nitrogen gas. The reaction mixture was incubated at 60 °C for 24 h while shaking at 180 cycle/min. The reaction product was passed through a stainless-steel mesh to remove the molecular sieves and then passed through a membrane filter (0.2 μm pore size; Advantec, Tokyo, Japan) to recover the residue. This residue was air-dried and then dispersed in chloroform/*n*-hexane (5:1, v/v). The dispersion was centrifuged at 1700*g* for 5 min to separate the added Novozym 435 rising to the surface of the solvent. The recovered Novozym 435 was washed twice with *n*-hexane and then with ethanol. The reaction product adhering to the Novozym 435 was separated by filtering at reduced pressure while adding a small amount of chilled water. The filtrate was lyophilized to recover OA-BOA-P.

Measurement of the Unit Chain-Length Distribution of BOS-P. BOS-P was dephosphorylated with acid phosphatase. BOS-P (10 mg) dissolved in 2 mL of a 0.2 M acetate buffer (pH 4.8) was treated at 27 °C for 6 h by adding 2 mL of an acid phosphatase solution (1 mg/mL) and then heated at 100 °C for 15 min to stop the reaction, prior to centrifuging at 1700*g* for 5 min. The resulting supernatant was adjusted to pH 3.5 with 0.2 M acetic acid and then completely debranched with isoamylase (26 IU) and pullulanase (170 IU) according to the previously reported method.[18] The debranched sample solution was thoroughly dialyzed against distilled water using a Spectra/Por dialyzing membrane (100 molecular weight cutoff; Medical Industry, Breda, The Netherlands) before lyophilizing to recover the debranched sample. The debranched sample was dissolved in 75% dimethyl sulfoxide (4.6 mg/0.8 mL). The sample solution was applied to a Dionex high-performance anion-exchange chromatograph coupled with a Dionex Carbopac PA guard column (3.0 mm inner diameter × 25 mm), a Carbopac PA1 separation column (4.0 mm inner diameter × 250 mm), and a pulsed amperometric detector (HPAEC-PAD; Dionex Japan, Tokyo, Japan) and eluted by a linear gradient from 0.1 M NaOH to 0.1 M NaOH containing 0.6 M sodium acetate at a flow rate of 1.0 mL/min.

Differential Scanning Calorimetry (DSC). A BOS-P or OA-BOA-P solution (10 μL) containing 5 and 10% (w/w) of each based on the dry starch weight, which had been adjusted to pH 7.0 with 0.1 M NaOH, was added to 5 mg of PS in an airtight anodized aluminum capsule. DSC was conducted to determine the gelatinization temperature (GT) and enthalpy change of gelatinization in the range of 5−100 °C at a heating rate of 2 K/min using a SSC-5020 DSC-6100 instrument (SII NanoTechnologies, Chiba, Japan) as previously described.[19] Distilled water (15 μL) was used as a reference, and five measurements were performed. DSC for the starch samples preserved at 4 °C for 7 days was also carried out to evaluate the re-gelatinization enthalpy as an index for relatively long-term retrogradation.

Viscosity Measurement. After 25 mL of the BOS-P and OA-BOA-P solution containing 0.5 and 1.0% (w/w) of each was added based on the dry starch weight, which had been adjusted to pH 7.0 with 0.1 M NaOH, to 2.0 g of PS in an aluminum container, a RVA Super 3 Rapid Visco Analyzer (Newport Scientific, New South Wales, Australia) was used to investigate the pasting properties. Each sample was held at 50 °C for 1 min and then heated to 95 °C at a heating rate of 5.6 K/min. After the sample was held at 95 °C for 2 min, it was cooled to 50 °C at a cooling rate of 5.6 K/min. Triplicate measurements for PS had previously shown a high degree of reproducibility, with the coefficient of variation of the peak viscosity being evaluated as only 0.19% [159.2 ± 0.3 relative value units (RVUs); mean ± standard deviation (SD)].[10] Each measurement was thus subsequently taken only once. After the RVA measurement, the paste was preserved at 4 °C for 1 week and then dehydrated with ethanol to obtain a powdered sample for analyzing the degree of gelatinization. The powdered sample containing 0.2, 0.4, 5, and 10% (w/w) BOS-P and OA-BOS-P were also prepared to examine the extent of the recovered crystalline and ordered structure.

Measurement of the Absorbance of the Retrograded Paste. The paste after RVA had been measured was held between two slide glasses separated by the thickness of one slide glass without any entrapped air bubbles and then left at 4 °C for 24 h according to the previously described method.[20] After warming to room temperature, the absorbance at 500 nm of the paste was measured to evaluate the turbidness because of macroscopic aggregation of the PS chains during short-term retrogradation.

X-ray Diffractometry. X-ray diffractometry of the retrograded and powdered starch samples after RVA was carried out by a Rint 2100 Ultima$^+$/PC X-ray diffractometer (Rigaku Co., Tokyo, Japan) with a copper target at 50 kV and 40 mA producing Cu Kα of 1.54 Å as an index for relatively long-term retrogradation according to the method previously described.[10]

Measurement of the Degree of Gelatinization. The degree of gelatinization of the retrograded starch sample after RVA was measured by the β-amylase−pullulanase (BAP) method[21] as an index for relatively long-term retrogradation. The starch sample (80 mg) was dispersed in 8 mL of distilled water with a Polytron PTA-7 homogenizer (Kinematica, Switzerland) at 24 000 rpm for 30 s, and 2 mL of the dispersion was diluted to 25 mL with a 0.8 M acetate buffer (pH 6.0) for the examination solution. Another 2 mL of the dispersion was completely gelatinized with 0.2 mL of 10 M NaOH, heated at 50 °C for 5 min, and then neutralized with 1 mL of 2 M acetic acid, prior to diluting to 25 mL with the same buffer. The enzyme solution (1 mL) was added to 4 mL of the examination or gelatinized solution and reacted at 40 °C for 30 min. After heating in boiling water for 5 min, the reaction product was diluted 5-fold and the reducing sugar and total sugar were determined by the Somogyi[22]−Nelson[23] method and the phenol−sulfuric acid method,[24] respectively. A blank was prepared by adding 1 mL of the enzyme solution that had previously been heated in boiling water for 10 min. Pullulanase (170 mg, 2 units/mg; Hayashibara Biochemicals Laboratories, Tokyo, Japan) and 9.59 mg of β-amylase (8.34 units/mg; Nagase Biochemicals, Tokyo, Japan) were dissolved in the 0.8 M acetate buffer (pH 6.0), diluted to 100 mL, and filtered for use as the enzyme solution. The degree of gelatinization was calculated by the following equation:

$$\text{degree of gelatinization (\%)} = \{(A-a)/B\}/\{(A'-a)/B'\}$$

where A, A', and a are the reducing sugar contents of the examination sample, the gelatinized sample, and the blank, respectively, and B and B' are the total sugar contents of the examination sample and gelatinized sample, respectively.

Measurement of the Surface and Interfacial Tension of the OA-BOS-P Solution. The surface tension of the 1% OA-BOS-P solution and interfacial tension of a corn oil (J-Oil Mills, Tokyo, Japan)−1% OA-BOS-P solution were measured at 25 °C with a CBVP-A3 automatic surface tensiometer (Kyowa Interface Science, Niiza, Japan) by the Wilhelmy method.[25]

Measurement of the Emulsifying Property. OA-BOS-P and BOS-P were dissolved in a 0.02 M citrate−0.01 M phosphate buffer (pH 4 or 7) or in the same buffer containing 1.0% NaCl to give a concentration of 1.0 mg/mL. The sample solution (2 mL) and 0.5 mL of corn oil in a test tube (18 mm inner diameter × 85 mm) were homogenized by a Polytron PTA-7 homogenizer (Kinematica, Switzerland) at 24 000 rpm for 1 min. The emulsion was diluted 50-fold with a 0.1% SDS solution at 0, 10, 30, 60, and 120 min after emulsification, and the absorbance at 500 nm was measured according to the method previously described.[26] The emulsifying activity index (EAI) was calculated by the following equations:[27]

$$\text{EAI} = 2T/\phi C \quad \text{and} \quad T = 2.3A/L$$

where A is the absorbance at 500 nm just after emulsification, L is the light path (10^{-2} m), C is the concentration of a sample (10^3 g/m^3), and ϕ is the oil phase volume (0.2). The absorbance at 60 min after emulsification approaching the equilibrium level was selected for the emulsion stability index (ESI).

Conductometric Titration. Conductometric titration was carried out to measure the calcium ions bound with OA-BOS-P and BOS-P as described previously.[10] $CaCl_2$ (1 or 2 mM) was added to 16 mL of an OA-BOS-P (0.31 mg/mL) or a BOS-P (0.45 mg/mL) solution in small increments (50 μL at a time), and the conductivity was measured with an ES-12 conductometer (Horiba, Kyoto, Japan) at room temperature. The end point for conductometric titration was determined as the intersection of the linear regression curves obtained from the initial titration and the ultimate titration.

Analytical Methods. The moisture content was determined by heating a sample at 110 °C until a constant weight had been obtained. The total sugar and reducing sugar were determined by the phenol−sulfuric acid method[24] and the Somogyi[22]−Nelson[23] method, respectively. The average degree of polymerization of BOS-P was determined by the ratio of the total sugar content to reducing sugar content of the sample solution according to the previously described method[18] after dephosphorylation with acid phosphatase. BOS-P (18 mg) dissolved in 8 mL of a 0.05 M acetate buffer (pH 4.8) was treated at 27 °C for 6 h with adding 3.2 mL of acid phosphatase in the same buffer solution (1 mg/mL) and then heated at 100 °C for 5 min to stop the reaction, prior to centrifuging at 1700g for 5 min to obtain the supernatant for measuring sugars after heating at 100 °C for 5 min. Each sugar content was corrected for the amount of sugar in the acid phosphatase preparation. The oleic acid content of OA-BOS-P was determined by gas−liquid chromatography (GLC) with a GC 4CM apparatus (Shimadzu, Kyoto, Japan) and a DEGS Chromosorb WAW column (GL Science, Tokyo, Japan) after fatty acid methyl esters had been prepared from OA-BOS-P by methanolysis according to the method previously described.[28] The phosphoric acid contents of BOS-P and OA-BOS-P were determined by wet ashing with sulfuric acid and perchloric acid, followed by use of the Phospher-C test kit (Wako Pure Chemical Industries, Osaka, Japan) as described previously.[10]

Statistical Analysis. The Turkey−Kramer test was used to compare mean values at the 5% significant level.

RESULTS AND DISCUSSION

Chemical Features of OA-BOS-P. The yields of BOS-P and OA-BOS-P were about 1 and 20% based on the used starch weight and BOS-P, respectively. DP of BOS-P was evaluated to be 22.5 after dephosphorylation with acid phosphatase. The unit chain-length distribution of dephosphorylated and debranched BOS-P, which had been separately prepared, showed a maltooligosaccharide composition of G2/G3/G4/G5/G6/G7 = 3.5:5.2:4.4:1.5:2.8:1 (molar ratio) without higher chains. These results show BOS-P to have a branched oligosaccharide population containing different chain lengths. The phosphate content of BOS-P was 0.61% based on the sample weight, and the oleic acid and phosphate contents of OA-BOS-P were evaluated to be 0.77 and 0.78% based on the sample weight, respectively.

Interfacial Ability. The interfacial properties of OA-BOS-P were investigated in terms of the surface tension in a water−air system, interfacial tension in a corn oil−water system, and emulsifying property. A solution of BOS-P had a lower surface tension than that (71.99 mN/m) of distilled water at 25 °C,[29] and there was a little decrease in the interfacial tension during 200 s (Figure 1). This indicates that BOS-P had a little degree of surface activity. OA-BOS-P exhibited markedly lower surface tension and interfacial tension than those of BOS-P and soybean lecithin (43.1 and 13.6 mN/m, respectively), indicating better

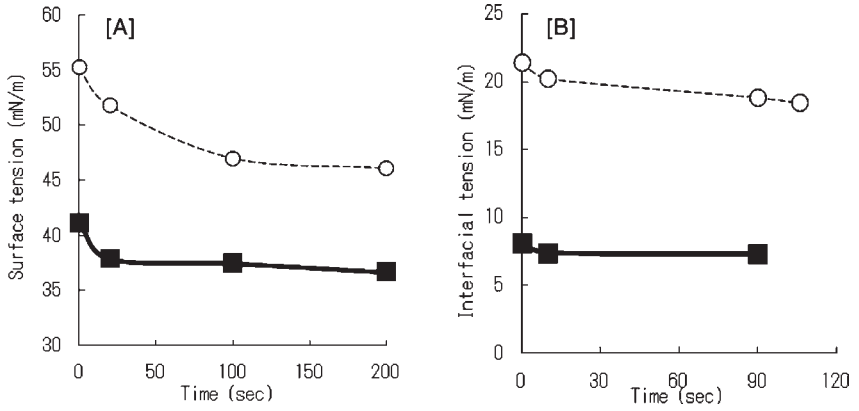

Figure 1. Comparative effects of OA-BOS-P (■) and BOS-P (○) on the (A) surface tension in a water−air system and (B) interfacial tension in a water−corn oil system. The interfacial properties were measured at 25 °C at a 1% concentration.

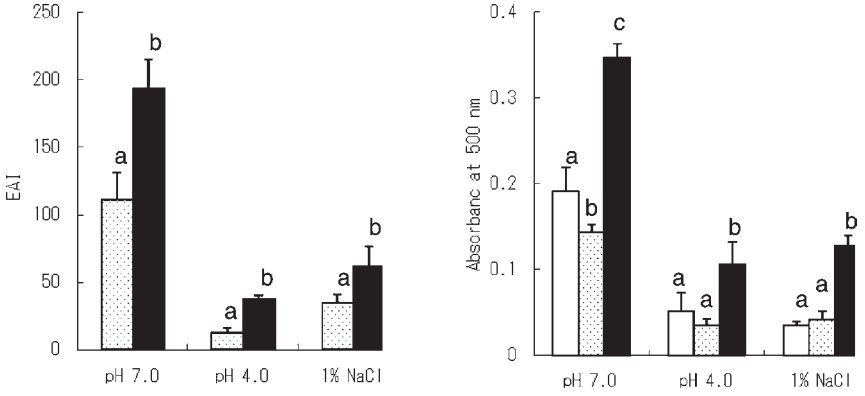

Figure 2. Comparative EAI and ESI of the O/W emulsions prepared with OA-BOS-P and BOS-P. EAI and ESI were derived from the respective absorbance just after emulsification and after 60 min at 500 nm of an O/W emulsion prepared with OA-BOS-P and BOS-P (1% concentration each) at pH values of 4.0 and 7.0 and in the presence of 1.0% NaCl at pH 7.0. Each value is the mean ± standard error (SE) (n = 3). Different letters show significant difference at $p < 0.05$. Black bars, OA-BOS-P; dotted bars, BOS-P; and white bars, control.

interfacial activity than BOS-P and lecithin, probably because of the improved hydrophile−lipophile balance by the acylation of oleic acid.

The emulsifying properties of BOS-P and OA-BOS-P were evaluated by the absorbance at 500 nm of oil-in-water (O/W) emulsions prepared at pH values of 7.0 and 4.0 and in the presence of 1% NaCl at pH 7.0 in terms of the EAI and ESI derived from the absorbance just after and 60 min after emulsification, respectively. BOS-P showed absorbance just after emulsification (data not shown) and an ESI value similar to that of the control, except for the lower value at pH 7.0 (Figure 2), although BOS-P had some surface activity, thus indicating little emulsifying property. On the other hand, OA-BOS-P exhibited significantly higher EAI and ESI values than those of BOS-P and the control under every emulsifying condition, with especially high values in the neutral pH range (Figure 2). It is thus concluded that OA-BOS-P had good emulsifying ability similar to that of SE-P because of the interfacial tension improved by oleylation.

Calcium Ion-Binding Ability. The interaction of OA-BOS-P with calcium was evaluated by conductometric titration with a $CaCl_2$ solution, and the end point of titration was determined as the intersection between the two regression lines with high determination coefficients.[10] The bound Ca^{2+} was calculated to be 5.8 mg/g of sample. Because the phosphate content of OA-BOS-P was 0.78%, the molar binding ratio of Ca^{2+} to the phosphate moiety was evaluated to be 1.7:1. It is thus concluded that OA-BOS-P exhibited Ca^{2+}-binding ability.

Control of the Gelatinization Behavior. The gelatinization behavior of PS containing BOS-P and OA-BOS-P was investigated by DSC and RVA. DSC data showed that BOS-P significantly elevated the gelatinization temperature (T_o) according to the level of addition (Figure 3). Because the phosphate group[30] and saccharide moiety[31] elevate T_o depending upon their concentrations, the effect of BOS-P on the increase in T_o is probably caused by the complex effects of the phosphate and saccharide moieties. BOS-P significantly reduced the enthalpy independent of the added amount. OA-BOS-P exhibited a greater effect than BOS-P on increasing T_o and decreasing the enthalpy (Figure 3). To evaluate the strong effect of OA-BOS-P, the retained amount of sucrose monostearate (SE) (S-1670; Mitsubishi Chemical Co., Tokyo, Japan) by PS was determined by substituting OA-BOS-P. PS (2 g) was dispersed in 25 mL of a SE solution containing 0.2% SE based on PS weight, and the dispersion was heated at 50 and 75 °C for 30 min while shaking. After centrifugation at 29000g for 30 min, retained SE was evaluated by measuring the fatty acid concentration of the resulting supernatant according to the previous method.[28] As a result, 25 and 88% of added SE were retained with PS at 50 and 75 °C, respectively (corresponding to 1.3 and 4.8 mol of SE/10 000 glucose residues of PS), probably through a starch−fatty acid

dx.doi.org/10.1021/jf1032558 |J. Agric. Food Chem. 2011, 59, 4962–4968

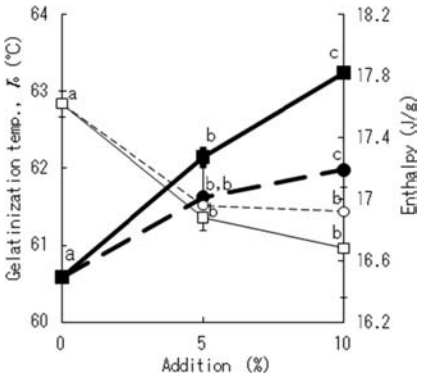

Figure 3. Comparative effects of OA-BOS-P and BOS-P on the thermal characteristics of PS evaluated by DSC. ■ and ●, gelatinization temperature (onset temperature, T_o); □ and ○, enthalpy change. ■ and □, OA-BOS-P; ● and ○, BOS-P. Each value is the mean ± SE (n = 5). Different letters show significant difference at $p < 0.05$. Any omitted error bars were too close to show.

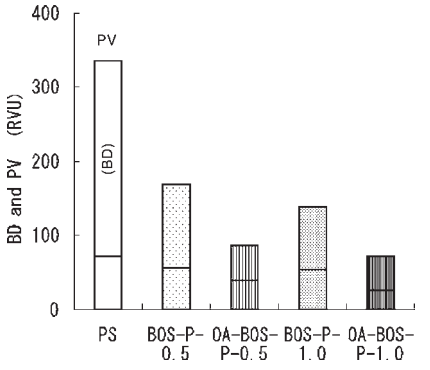

Figure 4. Comparative effects of OA-BOS-P and BOS-P on the PV and BD of PS evaluated by RVA. The viscosity of PS containing 0.5 and 1.0% OA-BOS-P or BOS-P was measured by RVA.

complex.[10,14,15] This suggests that OA-BOS-P having the fatty acid moiety would be similarly retained and that retained OA-BOS-P could inhibit the hydration of PS, probably resulting in the increase in T_o. Although T_o was only increased over the control level by 0.5 °C from adding SE-P at 5%,[10] the T_o increase from adding OA-BOS-P was 1.7 °C higher than that with SE-P, indicating the markedly enhanced effect of OA-BOS-P.

The addition of BOS-P and OA-BOS-P resulted in a marked decrease in the peak viscosity (PV) and breakdown (BD) of PS (Figure 4) without any change in the pasting temperature according to the level of addition. In particular, OA-BOS-P more strongly inhibited swelling of the starch granules, increasing the PV and resulting in BD of the swollen granules, probably because of hydrophobilization with the oleic acid moiety in retained OA-BOS-P. OA-BOS-P elevated T_o by 1.7 °C and reduced PV to 26% of the control value at 5 and 0.5% addition, respectively. However, the corresponding values for SE-P were only 0.2 °C and 71%, respectively.[10] These suggest a more contributive effect on inhibiting the development of a sticky and pasty texture than that of SE-P.

Control of the Retrogradation Behavior. The effect of BOS-P and OA-BOS-P on the initial and short-term retrogradation behavior was evaluated by the setback (SB) during cooling to 50 °C with the RVA measurement and the increased absorbance at 500 nm of the paste retrograded at 4 °C for 24 h after

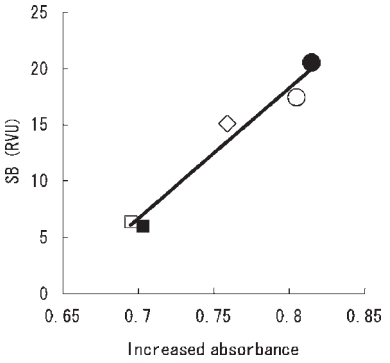

Figure 5. Relationship between the SB value of RVA and increased absorbance at 500 nm of PS paste containing OA-BOS-P and BOS-P during retrogradation at 4 °C for 24 h. OA-BOS-P: □, 0.5% addition; ■, 1.0% addition. BOS-P: ○, 0.5% addition; ●, 1.0% addition. ◇, Control.

RVA, respectively. BOS-P elevated SB to some extent depending upon the amount of addition (Figure 5). It has been reported that phosphate with an anionic charge could stabilize the structure of starch as shown by the markedly increased gelatinization temperature in the presence of phosphate[30] and that a charged amino acid, such as lysine and glutamic acid, elevated SB[32] and had a high binding ability to starch chains, probably through electrostatic interaction between the starch chains and charged amino acids.[33] The phosphate moiety of BOS-P would thus presumably have promoted entanglement of the dispersed starch chains through this electrostatic interaction. However, OA-BOS-P reduced SB with a 0.5% addition, probably because of a reduction in the leached amylose and excessive inhibition of the entanglement, resulting from forming a starch−OA-BOS-P complex through the oleyl moiety, as compared to promoting the entanglement through the phosphate moiety; a separate experiment had shown that PS containing OA-BOS-P showed a small reversal endothermic peak in a region from about 108 to 111 °C (data not shown), corresponding to the melting endothermic peak of a starch−fatty acid complex.[10,15] However, there was little further decrease with 1.0% addition. The increase in the absorbance of the starch paste containing BOS-P was higher than that of the control, whereas OA-BOS-P reduced the increased absorbance (Figure 5). There was a relationship between SB (Y) and the increased absorbance (X) that could be expressed by the linear regression equation with a high coefficient of determination, $Y = 116X - 77.4$ ($R^2 = 0.965$). This suggests that entanglement of the starch chains presumed from the SB value corresponded well to aggregation of the starch chains presumed from the increased absorbance during placing the paste at 4 °C for 24 h. It is thus concluded that OA-BOS-P could inhibit the initial and short-term retrogradation in terms of inhibited entanglement and aggregation of the starch chains, whereas BOS-P accelerated them.

The effect of BOS-P and OA-BOS-P on the relatively long-term retrogradation in terms of development of the ordered structure and enzymatic digestibility was examined by DSC and digestion with amylases (the BAP method) of the sample preserved at 4 °C for 7 days after the first DSC and RVA measurements. DSC showed that the BOS-P-added samples tended to have lower phase transition enthalpy (ΔH_2), although there was no significant difference (Figure 6). On the other hand, OA-BOS-P significantly reduced ΔH_2 to about 73% of the control value. The OA-BOS-P-containing samples from the retrograded pastes after RVA also showed significantly low ΔH_2 values (9.2 ± 0.9 J/g for 5% addition and 8.7 ± 1.0 J/g for 10% addition compared to 11.6 ± 1.2 J/g for the control,

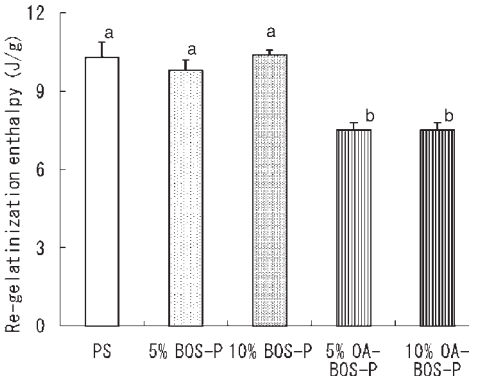

Figure 6. Comparative effects of OA-BOS-P and BOS-P on the regelatinization enthalpy of samples preserved at 4 °C for 7 days after the first DSC measurement. Each value is the mean ± SE (n = 5). Different letters show significant difference at $p < 0.05$.

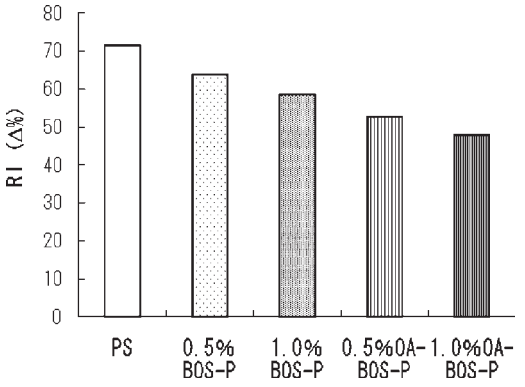

Figure 7. Comparative effects of OA-BOS-P and BOS-P on the RI. The gelatinized samples and their retrograded samples were prepared by dehydrating with ethanol the paste after RVA measurements and the preserved paste at 4 °C for 7 days, respectively. RI is defined by the difference between the degree (%) of gelatinization of the gelatinized samples and that of each retrograded sample evaluated by the BAP method.

n = 5), despite no significant difference (9.8 ± 0.4 J/g for 5% addition and 10.0 ± 0.8 J/g for 10% addition, n = 5) for the BOS-P-containing sample. These results mean that OA-BOS-P could inhibit the development of the ordered structure during relatively long-term retrogradation. This inhibition was probably caused by forming a complex with a starch chain via the olelyl moiety.

The retrogradation index (RI, Δ%) of enzymatic digestibility is defined by the difference between the degree (%) of gelatinization of a gelatinized sample and that of each retrograded sample, as evaluated by the BAP method in this study. BOS-P reduced RI depending upon the level of addition (Figure 7). This suggests the contribution of BOS-P to inhibit the development of the structure resistant to the enzymatic digestion, being different from the result that BOS-P reconstituted the ordered structure to a degree similar to the control, as evaluated by DSC. OA-BOS-P reduced RI more than BOS-P, depending upon the level of addition and corresponding to the results by DSC. This suggests that OA-BOS-P could contribute to inhibiting the development of the ordered structure and resistant structures to amylase digestion.

The crystalline structure developed during retrogradation at 4 °C for 7 days after RVA in a separate experiment was also

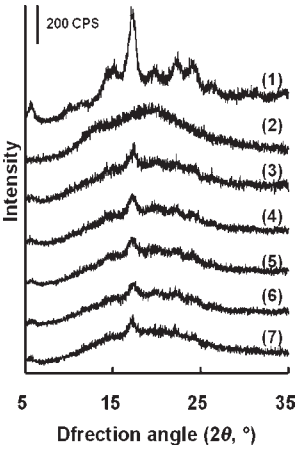

Figure 8. X-ray diffraction patterns of the retrograded samples. The paste containing OA-BOS-P or BOS-P after RVA was preserved at 4 °C for 7 days and then recovered as a retrograded powdered sample by dehydrating with ethanol and air drying. (1) Native PS, (2) gelatinized PS, (3) retrograded PS, (4) retrograded PS containing 0.2% BOS-P, (5) retrograded PS containing 0.4% BOS-P, (6) retrograded PS containing 0.2% OA-BOS-P, and (7) retrograded PS containing 0.2% OA-BOS-P.

examined by X-ray diffractometry. Because the X-ray diffraction pattern showed only a small peak at 2θ = 17° because of the crystalline structure developed during retrogradation (Figure 8), the relative crystalline content is defined as the relative ratio of each developed crystalline content to that of the PS paste, which was estimated as the ratio of the diffraction intensity at 2θ = 17° for each sample to that for the native PS according to the method described previously.[10] Consequently, 0.2 and 0.4% additions of BOS-P and OA-BOS-P reduced the content to 94.4 and 87.7% for BOS-P and 93.5 and 87.1% for OA-BOS-P, respectively . The samples containing OA-BOS-P indicated a somewhat lower relative crystalline content than those of BOS-P, but there was no clear difference, probably because of too low of an addition. It is thus suggested that OA-BOS-P could inhibit relatively long-term retrogradation in terms of inhibited development of the ordered and crystalline structure and reduced digestibility. These results imply that OA-BOS-P would contribute to controlling the gelatinization and retrogradation behavior of PS.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +81-42-367-5712. Fax: +81-42-360-8830. E-mail: k-taka@cc.tuat.ac.jp.

Funding Sources

This study was partly supported by the Japan Food Chemical Research Foundation.

ACKNOWLEDGMENT

We are grateful to Associate Professor T. Kondo of Kyoritsu Women's University for measuring RVA and T. Itaya of Hokuren Research Institute for providing PS samples with a high phosphate content and large granules.

ABBREVIATIONS USED

BOS-P, branched oligosaccharide phosphate; OA-BOS-P, oleyl branched oligosaccharide phosphate; SE, sucrose fatty acid ester;

SE-P, phosphorylated sucrose fatty acid ester; POS, phosphorylated oligosaccharide; PS, potato starch; DP, degree of polymerization; DSC, differential scanning calorimetry; RVA, rapid viscoanalyzer; EAI, emulsifying activity index; ESI, emulsion stability index; PV, peak viscosity; BD, breakdown; SB, setback.

REFERENCES

(1) Watanabe, T. Sucrose fatty acid esters—Past, present and future. *Foods Food Ingredients J. Jpn.* **1999**, *180*, 18–25 (in Japanese).

(2) Yamamoto, K.; Kumagai, H.; Sakiyama, T.; Song, M.; Yano, T. Inhibitory activity of alginates against the formation of calcium phosphate. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 90–93.

(3) Lee, Y.-S.; Noguchi, T.; Naito, H. Phosphopeptides and soluble calcium in the small intestine of rats given a casein diet. *Br. J. Nutr.* **1980**, *43*, 457–467.

(4) Kamasaka, H.; Uchida, M.; Kusaka, K.; Yoshikawa, K.; Yamamoto, K.; Okada, S.; Ichikawa, T. Inhibitory effect of phosphorylated oligosaccharides prepared from potato starch on the formation of calcium phosphate. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1412–1416.

(5) To-o, K.; Kamasaka, H.; Nishimura, T.; Kuriki, T.; Saeki, S.; Nakabou, Y. Absorbability of calcium-bound phosphoryl oligosaccharides in comparison with that from various calcium compounds in the rat ligated jejunum loop. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 1713–1718.

(6) Williams, G.; Sallis, J. D. Structure−activity relationship of inhibitors of hydoxyapatite formation. *Biochem. J.* **1979**, *184*, 181–184.

(7) Nakazawa, F.; Takahashi, J.; Takada, M. Interaction between potato starch and sucrose−lipid monoesters studied by differential scanning calorimetry. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 248–252.

(8) Krog, N.; Nybo-Jensen, B. Interaction of monoglycerides in different physical states with amylose and their anti-firming effects in breads. *J. Food Technol.* **1970**, *5*, 77–87.

(9) Yamashita, M.; Adachi, H.; Nakamura, T.; Tokuriki, N.; Taniguchi, H.; Hisamatsu, M. Effect of soy lysophosopholipid on rheological properties of wheat starch gel. *J. Appl. Glycosci.* **2001**, *48*, 271–278.

(10) Yamagishi, Y.; Hattori, M.; Yoshida, Y.; Takahashi, K. Improvement of the functional properties of sucrose stearate by phosphorylation. *J. Agric. Food Chem.* **2004**, *52*, 8039–8045.

(11) Katsuta, K.; Miura, M.; Nishimura, A. Kinetic treatment for rheological properties and effects of saccharides on retrogradation of rice starch gels. *Food Hydrocolloids* **1992**, *6*, 187–198.

(12) Katsuta, K.; Nishimura, A.; Miura, M. Effect of saccharides on stabilities of rice gels. 1. Mono- and disaccharides. *Food Hydrocolloids* **1992**, *6*, 387–398.

(13) Ogino, M.; Tanaka, R.; Hattori, M.; Yoshida, T.; Yokote, Y.; Takahashi, K. Interfacial behavior of fatty-acylated sericin prepared by lipase-catalyzed solid-phase synthesis. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 66–75.

(14) Tang, M. C.; Copeland, L. Analysis of complexes between lipids and wheat starch. *Carbohydr. Polym.* **2007**, *67*, 80–85.

(15) Zhou, Z.; Robards, K.; Helliwell, S.; Blanchard, C. Effect of the addition of fatty acids on rice starch properties. *Food Res. Int.* **2007**, *40*, 209–214.

(16) Karim, A. A.; Toon, L. C.; Lee, V. P. L.; Ong, W. Y.; Fazilah, A.; Noda, T. Effects of phosphorous contents on the gelatinization and retrogradation of potato starch. *J. Food. Sci.* **2007**, *72*, C132–C137.

(17) Sakauchi, S.; Hattori, M.; Yoshida, T.; Yagishita, T.; Ito, K.; Akemitsu, S.; Takahashi, K. Thermal behavior of potato starch and water-vaporization behavior of its paste controlled with amino acid and peptide-rich food materials. *J. Food Sci.* **2010**, *75* (2), C177–C183.

(18) Takahashi, K.; Kondo, H.; Kuroiwa, H.; Yokote, Y.; Hattori, M. Reversible thermal transition of soluble branched chains from slightly acid-treated potato starch. *Biosci., Biotechnol., Biochem.* **2000**, *64*, 1365–1372.

(19) Takahashi, K.; Shirai, K.; Wada, K. Melting behavior of gels prepared from isolated subunits of collagen. *J. Food Sci.* **1988**, *53*, 1920–1921.

(20) Katayose, S.; Kegai, K.; Nishimura, M.; Daud, N. A. bt.; Hattori, M.; Yoshida, T.; Ishii, Y.; Takahashi, K. Starch-ε-poly(L-lysine)-fatty acylated saccharide and ε-poly(L-lysine)-fatty acylated saccharide conjugates exhibit emulsifying ability, antibacterial activity, and controlling ability of thermal behavior of potato starch. *J. Appl. Glycosci.* **2007**, *54*, 173–180.

(21) Kainuma, K.; Matsunaga, A.; Itagawa, M.; Kobayashi, S. New enzyme system−β-amylase−pullulanase−to determine the degree of gelatinization and retrogradation of starch and starch products. *J. Jpn. Soc. Starch Sci.* **1981**, *28*, 235–240 (in Japanese).

(22) Somogyi, M. Notes on sugar determination. *J. Biol. Chem.* **1952**, *195*, 19–23.

(23) Nelson, N. A photometric adaptation of Somogyi method for the determination of glucose. *J. Biol. Chem.* **1944**, *153*, 357–380.

(24) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.

(25) Wilhelmy, L. Ueber die abhängigkeit der capillartäts-consten des alcohols von substanz und des benetzten fasten körpers. *Ann. Phys.* **1863**, *12*, 177–217.

(26) Takahashi, K.; Hirano, Y.; Araki, S.; Hattori, M. Emulsifying ability of porphyran prepared from dried nori, *Porphyra yezonsis*, a red alga. *J. Agric. Food Chem.* **2000**, *48*, 2721–2725.

(27) Perce, K. N.; Kinsella, J. E. Emulsifying properties of protein: Evaluation of a turbidmetric technique. *J. Agric. Food Chem.* **1978**, *26*, 716–724.

(28) Takahashi, K.; Lou, X.-F.; Ishii, Y.; Hattori, M. Lysozyme−glucose stearic acid monoester conjugate formed through the Maillard reaction as an antibacterial emulsifier. *J. Agric. Food Chem.* **2000**, *48*, 2044–2049.

(29) Vargaftik, N. B.; Volkov, B. N.; Voljak, L. D. International tables of the surface tension of water. *J. Phys. Chem. Ref. Data* **1983**, *12*, 817.

(30) Takahashi, K.; Shirai, K.; Wada, K.; Kawamura, A. Effects of salts and saccharides on the gelatinization of starch. *J. Jap. Soc. Starch Sci.* **1980**, *27*, 22–27 (in Japanese).

(31) Beleia, A.; Miller, R. A.; Hoseny, R. C. Starch gelatinization in sugar solutions. *Starch/Staerke* **1996**, *48*, 259–262.

(32) Ito, A.; Hattori, M.; Yoshida, T.; Takahashi, K. Reversible regulation of gelatinization of potato starch with poly(L-lysine) and amino acids. *Starch/Staerke* **2004**, *56*, 570–575.

(33) Ito, A.; Hattori, M.; Yoshida, T.; Watanabe, A.; Sato, R.; Takahashi, K. Regulatory effect of amino acids on the pasting behavior of potato starch is attributable to its binding to the starch chain. *J. Agric. Food Chem.* **2006**, *54*, 10191–10196.