Studies on Mass Production and Application of Phosphoryl Oligosaccharides from Potato Starch*

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Abstract: Phosphate ester groups are known to link to some glucosyl residues in starch molecule. We focused on the utilization of esterified phosphoryl group in potato starch components. In starch processing, the actions of the amyloytic enzymes were hindered by the phosphoryl groups linked to the glucosyl residue, and phosphoryl oligosaccharides (POs) were obtained as indigestible components by the enzymes. The POs were composed of two fractions, PO-1 and PO-2. Fraction PO-1 was the main fraction, and it was composed of maltotriose, maltotetraose and maltopentaose to which one phosphoryl group was attached. Fraction PO-2 was predominantly composed of maltopentaose and maltotriose to which at least two phosphoryl groups were attached. The POs had the ability to form a soluble complex with calcium and had inhibitory effect on the formation of a calcium-phosphate precipitate. Based on the function of the POs, described above, we applied the POs of calcium (POs-Ca⁴) as a food ingredient. The POs-Ca was an advantageous food ingredient as a soluble calcium source. Relating to prevent of dental caries, POs can not be fermented by cariogenic microorganisms, and reduces the plaque pH fall in vitro. Moreover, POs-Ca was effectively enhanced remineralization of enamel lesion. The enhanced remineralization in the POs-Ca group was considered to be caused by increased soluble Ca contents in saliva. The chewing gum containing POs-Ca can produce the effective environment in saliva. That is, it can be said that POs-Ca is an ingredient which cannot be easily influenced of flavor and coloring matter, is the substance which performs re-calcification. The present results suggested that daily chewing of a sugar-free gum containing POs-Ca could be one effective approach to stimulate remineralization of enamel and thereby to prevent dental caries.

Key words: phosphoryl oligosaccharides, calcium, saliva, remineralization, chewing-gum

The wide distribution of the ester phosphorus is observed in starches from various sources. Potato starch is known to contain esterified phosphoryl group in its components.¹ ² Takeda and Hizukuri have reported that the phosphate groups were located mostly in the B-chain of amylopectin, whereas the phosphorylation of amylose was very little.³ Potato amylopectin contains 100–1000 ppm of the ester phosphorus.⁵ Furthermore, approximately 60% to 70% of the phosphate groups were linked to C-6 of the glucosyl residues, the almost all the rest being linked to C-3 and a very small part possibly being linked to C-2 of the glucosyl residues.⁵ Our attention was focused on the utilization of esterified phosphoryl group in potato starch, and succeed to prepare new phosphoryl oligosaccharides (POs) from the starch hydrolysate.⁶ In this article, we introduce our recent achievements in a new function of the oligosaccharides focusing on the application for soluble calcium and effect of remineralization on enamel lesion. In first, we tried to investigate a new functional food ingredient as soluble calcium. It is generally recognized that the absorbability of calcium is substantially dependent on its solubility in an aqueous solution,⁷ because calcium will be absorbed in its free or ionized form in the intestine.⁸ The adult human body contains about 1–1.2 kg of calcium, which amounts to about 1.5–2% of body weight. Of this, 99% is found in mineralized tissues, such as bones and teeth, where it is present as calcium carbonate, providing rigidity and structure.⁹ The remaining 1%, found in blood, extracellular fluid, muscle, and other tissues, play an important role in mediated vascular contraction and vasodilation, muscle contraction,

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Abbreviations: POs, phosphoryl oligosaccharides; POs-Ca, phosphoryl oligosaccharides of calcium; Ca, calcium; P, phosphate; BSA, bacterial saccharifying α-amylase; GA, glucoamylase; BLA, bacterial liquefying α-amylase; PPA, porcine pancreatic α-amylase; HSA, human salivary α-amylase; TAA, Taka-amylase A; CTGase, cyclomaltodextrin glucanotransferase; NPL, neopullulanase; Glc-6-P, d-glucose-6-phosphate; 6'-phosphoryl maltose, O-6-phosphoryl-α-d-glucopyranosyl-(1→4)-O-α-d-glucopyranose; 6'-phosphoryl maltotriose, O-6-phosphoryl-α-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-d-glucopyranose; 6'-phosphoryl maltotetraose, O-6-phosphoryl-α-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-d-glucopyranose; 6'-phosphoryl maltopentaose, O-6-phosphoryl-α-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-d-glucopyranose; 6'-phosphoryl maltotriose, O-α-d-glucopyranosyl-(1→4)-O-β-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-d-glucopyranose; 6'-phosphoryl maltotetraose, O-α-d-glucopyranosyl-(1→4)-O-β-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-d-glucopyranose; 3'-phosphoryl maltotetraose, O-α-d-glucopyranosyl-(1→4)-O-β-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-d-glucopyranose; 3'-phosphoryl maltotetraose, O-α-d-glucopyranosyl-(1→4)-O-β-d-glucopyranosyl-(1→4)-O-3-phosphoryl-α-d-glucopyranosyl-(1→4)-O-β-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-O-α-d-glucopyranosyl-(1→4)-d-glucopyranose.
nerve transmission and glandular secretion. Calcium is required for natural growth and development of the skeleton. Calcium requirements vary throughout an individual’s life, with greater needs during the periods of rapid growth in childhood and adolescence, during pregnancy and lactation, and in later life.

In second, we examined the effects of POs on remineralization of artificial early caries lesions in bovine enamel in vitro. The effects of daily application of a sugar-free chewing gum containing POs-Ca on remineralization were investigated on enamel in situ. Dental caries is a dynamic biological process characterized by sequences of de- and remineralization and, therefore, caries prevention can be achieved when the positive mineral balance surpasses the rate of demineralization over a prolonged time. In these biological reactions, remineralization in caries lesions is stimulated in principal by 3 environmental factors, i.e. supersaturation of calcium and phosphate ions, maintenance of neutral zone pH and presence of low fluoride ions. Of the 3 factors, stabilization of supersaturated mineral ions in outer and/or inner liquid phase is the most fundamental. In these studies, we introduce our recent achievements in a new function of the oligosaccharides focusing on the application for soluble calcium and effect of remineralization on enamel lesion.

1. Preparation and structure of phosphoryl oligosaccharides.

We developed a production method of POs from potato starch, using bacterial liquefying α-amylase (BLA) [EC 3.2.1.41], glucoamylase (GA) [EC 3.2.1.3] and pullulanase [EC 3.2.1.41]. The actions of the amylolytic enzymes were hindered by the phosphoryl groups linked to the glucosyl residues, and POs were obtained as indigestible components by the enzymes. The components of POs were analyzed by high-performance anion-exchange chromatography (HPAEC) and pulsed amperometric detector system. The substance linked phosphoryl group is determined by the system at different retention times according to the number and the positions of phosphate groups linked to each molecule (Fig. 1). The POs were fractionated into two fractions; PO-1 and PO-2, and analyzed components by alkaline phosphatase treatment. The fraction PO-1 was the major component of POs and composed of maltotriose, maltotetrose and maltohexaose to which at least two phosphoryl groups were attached.

The fraction PO-2 was predominantly composed of maltoptaoose and maltohexaose to which at least two phosphoryl groups were attached. The average degree of polymerization of dephosphorylated PO-1 and PO-2 was evaluated to be 4.02 and 5.82, respectively. The detail structure of the components of PO-1 fraction was analyzed by using the different hydrolytic properties of bacterial saccharifying α-amylase (BSA) and GA on the phosphoryl oligosaccharides.

The limiting cleavage points of BSA on PO-1 components were the same site as those of porcine pancreatic α-amylase (PAA) and human saliva α-amylase (HSA), as reported by Takeda et al. In addition, the limiting cleavage points of neopullulanase and some α-amylases to PO-1 fraction were investigated. The phosphoryl oligosaccharides of fraction PO-1 were good substrates to distinguish the enzymes of α-amylase family based on their specificities. The reaction specificity of neopullulanase was compared with those of other α-amylase family enzyme; BSA, BSA, PAA, HSA and Taka-amylase A (TAA) by using the PO-1 components as the substrate. The neopullulanase can hydrolyze pullulan and also show unique macromolecule recognition among the α-amylase family enzyme; the neopullulanase exhibits distinct specificity toward amylose and amylopectin. The specificity of neopullulanase on PO-1 components was quite different from those of α-amylases. Neopullulanase can hydrolyze the glucosyl linkage at the reducing site up to one glucosyl residue far from the glucosyl residue phosphorylated at C-6 (and up to two glucosyl residues far from the glucosyl residue phosphorylated at C-3, respectively (Table 1).

The phosphate group linked at C-6 of the glucosyl resi-
due was detected as the content of the Glc-6-P after acid hydrolysis. The spectrometric analysis by 13C-NMR also distinguished the phosphate groups linked at C-3 and C-6 of the glucosyl residues, respectively. In conclusion, the PO1 fraction was made up of oligosaccharides phosphorylated at C-3 (3'-phosphoryl maltotetraose and 3'-phosphoryl maltopentaose) and oligosaccharides phosphorylated at C-6 (6'-phosphoryl maltotriose, 6'-phosphoryl maltotriose, 6'-phosphoryl maltotetraose and 6'-phosphoryl maltopentaose) (Fig. 2).15) In case of PO2 fraction, these enzymes treatment-resistant PO2 slightly remained. It clearly indicated that two of the phosphoryl groups attached to C-6 and C-3 existed in PO2 components.16) From these results, the possible structures of PO2 components were as shown in Fig. 3. The PO2 fraction, which contains at least two phosphoryl groups attached to the molecule, might have been newly found in potato starch. By the coupling reaction of cyclomaltodextrin glucoamylase (CGTase), we found that the fraction of PO1 decreased and the fraction with at least two phosphoryl groups like a PO2 increased.16)

The POs can form solubilize complexes with calcium and iron.4,23) Both fractions PO1 and PO2 had the ability to form a soluble complex with calcium, and fraction PO2 had stronger inhibitory effect on the formation of a calcium-phosphate precipitate and made more stable and soluble complex with the calcium ion.49 In addition, it was found that the fraction PO1 was a suitable compound in producing the conjugate with ovalubmin (OVA) through the Maillard reaction, and the conjugate could exhibit the inhibitory effect on the formation of a calcium-phosphate precipitate.24) We can easily vary the number of phosphate groups incorporated into each molecule by using this method for POs. The OVA conjugate had plural number of phosphate groups in one molecule. By the coupling reaction of CGTase to POs, the inhibitory effect on the formation of a calcium-phosphate precipitate was increased.16) These results would also support the hypothesis; the inhibitory effect on the formation of a calcium-phosphate precipitate depends upon its amount of carrying phosphate groups in the molecule. On the other hand, an iron is also hardly dissolved in the intestinal canal at the physiological pH level so that its absorption is extremely low. The PO1 fraction solubilized equivalent moles of iron ion, and the PO2 fraction solubilized more than 10-fold equivalent moles of iron ion (Fig. 4).23) It indicated that the iron solubilizing ability also depends upon the number of covalently bound phosphoryl group per molecule. POs bound calcium was thought an advantageous food ingredient as a soluble calcium source.25,26) In addition, POs can not be metabolized by cariogenic bacteria as mutans streptococci as same as xylitol and the preventive effect was shown on reducing the fall in plaque pH even though sucrose-dependent fermentation for acting buffering power.27)
Sugar alcohols such as xylitol are widely used as a sweetener in chewing-gum to prevent dental caries and to promote remineralization.\textsuperscript{3,20} The POs can also reduce a content of artificial plaque and demineralization on enamel, even if sucrose existed.\textsuperscript{20}

Furthermore, the effects of POs were examined on remineralization of caries-like lesions in enamel in vitro.\textsuperscript{31} In results, there were possibilities that POs may have synergistic effect with fluoride in rate of remineralization. Based on the previously revealed features of POs, we examined the effect of remineralization on enamel by a chewing gum containing calcium salt of POs (POs-Ca).

2. Bioavailability of POs-Ca in rats.

1) In vivo digestibility of POs-Ca.

POs-Ca, glucose or high maltotetraose syrup (TETRUP-H, Hayashibara Biochemical Laboratories Inc., Okayama, Japan) was administered orally by direct stomach intubation for one group.\textsuperscript{22} Before and after administration, blood samples were collected from the tail vein at each time point.

Although the glucose concentration of POs-Ca group was significantly lower than those of other groups at 0.25 h after administration, the overall rates of plasma glucose response in the three groups were almost similar. The maximum concentration of the POs-Ca group was lower than those of the glucose group and the TETRUP-H group, although the same amount of saccharides was administered (Fig. 5). Plasma calcium concentration of the POs-Ca group showed slight but significant increase during 0.25–0.5 h after administration concurrent with the increase of plasma glucose (Fig. 6a). On the other hand, the transient decrease during the rise of plasma glucose and the following recovery of plasma phosphate concentration was observed in all groups (Fig. 6b). We thought that the phosphate groups of POs-Ca did not influence the absorption and the dephosphorylation of POs-Ca by alkaline phosphatase localizing at the brush border membrane in the small intestine would not be rate limiting in POs-Ca absorption. As to the digestibility of POs-Ca by HSA, it could hydrolyze a part of the PO-1 fraction.\textsuperscript{20} However, it took 24 h to hydrolyze POs. Consequently, we thought that POs-Ca would not be substantially hydrolyzed by HSA when humans ingest POs-Ca in foods or drinks. As to the changes of calcium and phosphate concentration in plasma when administered POs-Ca, the slight but significant increase of plasma calcium during 0.25–0.5 h after oral administration of POs-Ca was in accordance with the increase of plasma glucose. Thus, we thought that the calcium increased was brought from POs-Ca. From these results, it was suggested that the POs-Ca orally administered would be hydrolyzed into glucose, calcium and phosphate and then absorbed completely in the small intestine, therefore not causing acute diarrhea.

2) Calcium absorbability of POs-Ca in comparison with various calcium compounds.

POs-Ca was prepared as 5 wt% calcium in the molecule and can be expected to be a useful calcium supplement because of its high solubility in water. We investigated the absorbability of calcium from POs-Ca using in situ rat ligated jejunum loop system.\textsuperscript{26} A comparison of the calcium absorption rates in the ligated jejunum loop between POs-Ca and the soluble calcium compounds (CaCl\textsubscript{2} and Ca-lactate) is shown in Fig. 7a. The calcium absorption rates of the three groups were all approximately 60%, so the intestinal calcium absorption rate of the POs-Ca group was almost comparable with that of the soluble calcium groups. However, as shown in Fig. 7b, the calcium absorption rate of POs-Ca was significantly higher (p<0.05) than that of the insoluble calcium compounds (CaCO\textsubscript{3} and CaHPO\textsubscript{4}).

We investigated whether the total calcium absorption would be increased at the ligated loop when mixed POs-Ca and a whey mineral complex (WMC), which is widely
but calcium chloride is a relatively inferior calcium source compared to calcium citrate and calcium carbonate by normal subjects, and by patients with achlorhydria.

The calcium ratio of WMC to POs (+) was substituted for POs (−). Each value is the means±SD for 8 rats. The total Ca concentration of each test solution was adjusted to 1.2 mg/mL by varying the calcium ratio of WMC to POs-Ca. Significantly different (p< 0.05) from the WMC and 1:1 groups.

used as a calcium source in various foods or drinks, were substituted for POs-Ca in the test solution. When part of the WMC was substituted for POs-Ca in the test solution, the total calcium absorption rate in the ligated jejunum loop increased with increasing of calcium from POs-Ca (Fig. 8). The calcium absorption rate of the group with a calcium ratio of 1:1 in the test solution was significantly higher (p<0.05) than that of the WMC group.

There might be some hypoaosorption of calcium in the elderly because of the known increase in the prevalence of achlorhydria with age. The solubility and absorbability of calcium chloride were the same as those of POs-Ca, but calcium chloride is a relatively inferior calcium source because it irritates the gastrointestinal membrane. Recker has found no difference in calcium absorption between calcium citrate and calcium carbonate by normal subjects, but a large difference in patients with achlorhydria. Thus, we considered that the high solubility of POs-Ca throughout the acidic, neutral and alkaline pH ranges would be advantageous for calcium absorption by the elderly and/or by patients with achlorhydria.

Partial substitution by POs-Ca for the whey mineral complex (WMC) in the test solution elevated the total calcium absorption. It might be thought that the increase in the ratio of soluble calcium in the test solution resulted in the elevation of total calcium absorption. It is thus suggested that the increase in the ratio of soluble calcium in foods and in the intestinal tract would enhance the total calcium absorption. WMC is added as a calcium source to many kinds of processed food, especially to dairy products such as milk beverages and ice cream, due to its favorable flavor and image of being manufactured from milk, although its solubility is relatively low. Thus, we thought that the addition of POs-Ca to foods and drinks containing WMC or other insoluble calcium sources for the purpose of calcium fortification would be helpful to elevate the total absorption of calcium from them. As a calcium supplement for various foods, especially for beverages, POs-Ca would be a favorable soluble calcium material with relatively high absorbability from the intestinal tract.

3. Application of POs-Ca for oral health.

Most studies to measure mineral contents in sub-surface of enamel sections used transversal microradiography (TMR). The TMR analysis can measure the mineral uptake or loss directly from microradiography of thin sections of enamel and is recognized as a primary standard method for the analysis of de- and re-mineralization. The mineral distributions were quantified by TMR analysis. The lesion depth and mineral loss value were measured from microradiographies. The effects of POs-Ca on remineralization of caries-like lesions in enamel in vitro were investigated.

Bovine enamel slabs were demineralized to produce artificial early caries lesion, and subsequently remineralized in a mineral solution containing POs-Ca. The mineral contents treated by the mineral solution containing POs-Ca were caused mineral recovery compared with demineralized enamel samples. In addition, the effect of the gum containing POs-Ca on remineralization of caries-like lesions in enamel was examined by using human saliva immersing (HSI) test. It was examined the effects of daily application of a sugar-free chewing gum containing 2.5 wt % POs-Ca on remineralization of enamel. The gum was concluded non-cariogenic product since they were proven by intraoral plaque pH-telemetry tests in four human volunteers to not depress the pH of interdental plaque below 5.7 by bacterial fermentation, neither during consumption nor during a period of 30 min following consumption by the general method of Association for Toothfriendly Sweets. The HSI-test would be a useful system for detection and evaluation of remineralization effect using human saliva, since the test is easy to control condition and light works for volunteers prefer to intraoral study. These results suggested that the HSI-test and intraoral study have relevance to the effect on enamel remineralization of POs-Ca each other. It is thought that human saliva plays some important role in oral health. Especially, stimulated saliva during chewing would influence some effect to remineralization.

1) Salivally assessment.

We applied two types of sugar-free chewing gum. One contained 2.5% POs-Ca gum (POs-Ca (+)) and the other without POs gum (POs-Ca (−)). Human whole stimulated saliva was collected from 12 healthy adult volunteers during chewing 2 tablets of POs-Ca (+) or POs-Ca (−). Each volunteer chewed 2 pieces of gum tablets (average weight: 1.5g) for 20 min and the whole saliva was collected for the first 10 min (Fs) and last 10 min (Ls) separately. Demineralized bovine enamel slabs were immersed in the Fs for 10 min and subsequently in the Ls for 10 min at 37°C, continuously. Immediately after the salivary treatments, the enamel slabs were rinsed with deionized water. This procedure was repeated 4 times a day for 4 days. Salivary volume and the concentrations of inorganic phosphate ion (P) and calcium ion (Ca) were measured.

The volume and pH of saliva from each volunteer were measured immediately after sampling. The salivary volume of Fs and Ls secreted by chewing of POs-Ca (+) and that by POs-Ca (−) showed no significant difference. The concentration of Ca in Fs in POs-Ca (+) was greatly higher than that of POs-Ca (−). The difference of chewing
gum did not largely influence the concentration of P in saliva samples. Human saliva includes abundant content of P, comparing with Ca. Time course of soluble Ca and P during 20 min was measured in chewing each gum (Figs. 9a, b). The results indicated that most of Ca in POs-Ca (+) was extracted into saliva within first 10 min. However, the concentration of P is almost fixed in saliva and increases in proportion to the amount of saliva. Especially of the beginning, the Ca/P ratio was ranging to 1.67 that was the value of hydroxyapatite in enamel (Fig. 9c). The pH of saliva was measured at 1, 3, 6, 10 and 20 min during chewing. The pH was about 7.0 at the beginning, rose to about 7.5 in the first 6 min, and thereafter remained around 7.5 during the chewing period in both cases (Fig. 9d). No significant difference was observed between the salivary volumes secreted by chewing of POs-Ca (+) and that by POs-Ca (−) (Fig. 9e). After human salivary treatments, thin planoparallel sections were cut from the enamel samples. Finally, the mineral distribution parameters, namely the lesion depth (ld, µm) and mineral loss value (ML, vol%·µm) were measured. The ld and ML values were shown in Fig. 10 by individuals in types of gum. In all the individuals participated, significantly lower ld and ML values were observed in case of POs-Ca (+) indicating enhanced enamel remineralization. No remarkable mineral recovery was observed in case of POs-Ca (−) group. The saliva secreted by chewing POs-Ca (+) had higher remineralization-enhancement activity than that from POs-Ca (−). No difference was observed in volume, time-course change in pH level and contents of soluble P of saliva between chewing gum types and among volunteers. The contents of P existed adequately comparing with Ca in saliva collected by chewing of the POs-Ca (−). In case of POs-Ca (+), a content of Ca was higher than that from chewing the POs-Ca (−). Initial Ca/P ratio value in POs-Ca (+) gum induced saliva was higher than that in POs-Ca (−) induced saliva. The Ca/P ratio was 0.3 or less for POs-Ca (−). These results suggested that enhanced remineralization by chewing of the POs-Ca (+) were due to the increased soluble calcium in saliva that resulted in higher Ca/P ratio value corresponding with the value (1.67) of hydroxyapatite.

2) Intraoral study.

Based on the former results, we investigated the effects of the POs-Ca (+) gum on remineralization of enamel in situ. Twelve healthy adult volunteers (6 males and 6 females; mean age, 21 years old) were randomly divided into 3 groups (n=4 per group) and participated in a double-blind intraoral study. Each volunteer wore a palatal appliance containing 3 demineralized enamel disks, and chewed one of the following experimental gums 4 times a day (after meals and before bed time) for up to 4 weeks. The three groups were (1) POs-Ca (−) group, (2) POs-Ca (+) group or (3) sugar gum containing 62 wt % sucrose (sucrose gum group). The chewing time was always 20 min and the palatal plate was preserved in the oral cavity for additional 20 min. All the gums were supplied as a tablet form and the volunteers had 2 pieces of the gum each time. The results were that the remineralization rates (ld reduction %age with respect to the mean ld value after initial demineralization) in the POs-Ca (+) group were about 67, 54 and 76% at week 1, 2 and 4, respectively. The remineralization rates in the POs-Ca (−) group ranged from 12 to 23% being much lower than these in the POs-Ca (+) group. The sucrose group showed positive remineralization rates by week 2, but finally reached to a negative value by week 4 indicating progression of demineralization. The study of the promoting on enamel remineralization of a POs-Ca (+) group was reconfirmed in situ during 2 weeks with double-blind and cross-over design intraoral study. These results also suggested that the HSI-test and intraoral study have high relevance to the effect on enamel remineralization of POs-Ca each other.

The HSI-test is a useful system for standard evaluation of remineralization effect.

The promoted remineralization of enamel and dentin lesions by POs-Ca (+) group can be explained as follows.
The pH of the saliva during chewing the gums is estimated to increases from about 7 to 7.5. Since, in general, this relatively higher pH is not suitable for Ca and phosphate to be solubilized, it is considered that POs-Ca in the saliva would aid to maintain solubility of mineral ions even at pH 7–7.5 and, thereby, ionized Ca and P had potential to redeposit onto the residual hydroxyapatite crystals in enamel lesions. Thus, under the presence of POs, soluble Ca in saliva increases efficiently and, thereby, the salivary Ca/P ratio can increase nearly up to the rate of hydroxyapatite (1.67). The gum containing POs-Ca could be able to compensate the ability of remineralization effect of saliva itself. The results suggested that POs may be a novel and unique substance to enhance enamel remineralization, and could be utilized for caries prevention by nutritional approach. It is generally known that a gastrointestinal disorder would happen when we take a food containing excess amount of sugar alcohol at a time. We have already shown that the consumption of excess amount of POs-Ca does not cause a gastrointestinal disorder. We also confirmed that the orally administered POs-Ca was hydrolyzed and then absorbed completely in the small intestine in rats.

Daily use of a sugar-free chewing gum containing POs-Ca can effectively enhance the remineralization in enamel lesion. POs-Ca enhanced enamel remineralization by increasing the solubility of Ca in oral environment and could be a beneficial material for oral health. It has been reported that oligosaccharides improve intestinal bacterial flora, prevent carcinogenesis, and decrease the calorie effects of foods. We developed a new acid oligosaccharides; inhibition on the formation of a Ca-P precipitate, which lead to an advantageous food ingredient as a soluble calcium, and promoting the remineralization of enamel lesion.

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澱粉には、その構成糖にリン酸基がエステル結合して
いる糖を含むことが知られている。著者らは馬鈴薯澱粉
の加水分解物より、リン酸基がエステル結合している糖、
つまり、リン酸オリゴ糖カルシウム (POs-Ca) を調製し
てきた。このリン酸オリゴ糖は二つの画分 PO-1 画分およ
び PO-2 画分から構成されていた。PO-1 画分はリン酸オ
リゴ糖の主な成分であって、マルトトライオース、マルト
トタロース、およびマルトペンタオースから構成さ
れており、分子内に 1 個のリン酸基を有していた。PO-2
画分は主にマルトペンタオースおよびマルトヘキサオー
スから構成されており、少なくとも 2 個のリン酸基を分
子内に含有していた。リン酸オリゴ糖はカルシウムと水溶
性の複合体を形成し、カルシウム-リン酸の沈殿形成を阻
害する効果を有していた。以上の結果をもとにリン酸オ
リゴ糖のカルシウム塩 (POs-Ca) を食品素材として開発し
てきた。POs-Ca は、水溶性カルシウム供給のための食品
素材として優れていた。また、う蝕予防の観点から、リ
ン酸オリゴ糖はう蝕原因細菌であるミュータンス連鎖球
菌の栄養源にならず、本菌の生産する酸によるプラック
内の pH の低下も抑制する作用を有していることを明らか
にした。さらに、POs-Ca は初期う蝕を誘発したエナメル
質の再石灰化を効果的に促進する作用も有していること
がわかった。ここでは、POs-Ca を関与成分としたシュ
ガーレスガムの初期う蝕の再石灰化効果を明らかにし、
POs-Ca の口腔保健への応用開発について紹介する。