SUMMARY

Phosphate ester groups are known to link to some glucosyl residues in starch molecules We have prepared phosphoryl oligosaccharides (POs) from potato starch hydrolysates 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 maltohexaose to which at least two phosphoryl groups were attached POs had the ability to form a soluble complex with calcium and had an 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 Ca) as a food ingredient POs-Ca was an advantageous food ingredient as a soluble calcium source In relation to prevention of dental caries POs cannot be fermented by cariogenic microorganisms or mutans streptococci and they reduce the fall in plaque pH *in vitro* Moreover, POs-Ca effectively enhanced the remineralization of enamel lesions The aim of this study was to develop the application of POs-Ca for dental health to the enamel remineralization through the chewing of a sugar-free gum

Key words

Phosphoryl-Oligosaccharides Saliva Remineralization Enamel Chewing-gum

1 INTRODUCTION

The wide distribution of the ester phosphorus is observed in starches from various sources Potato starch is known to contain an esterified phosphoryl group amoung 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 [2] Furthermore approximately 60% to 70% of the phosphate groups were linked to C-6 of the glucosyl residues almost all the rest being linked to C-3 and a very small part possibly being linked to C-2 of the glucosyl residues[2] Our attention was focused on the utilization of the esterified phosphoryl group in potato starch and we succeeded in preparing new phosphoryl oligosaccharides (POs) from the starch hydrolysate [4] In this article we introduce our recent achievements in a new function of the oligosaccharides focusing on the application for soluble calcium and the effect of remineralization on enamel lesion

2 STRUCTURE AND CHARACTERIZATION OF POs

We developed a method of producing POs from potato starch using bacterial liquefying α-amylase (BLA) [EC 3 2 1 1] 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 the POs were analyzed by high-performance anion-exchange chromatography and pulsed amperometric detector system [4] The substance-linked phosphoryl group is detected by the system at different retention times according to the number and the positions of phosphate groups linked to each molecule The POs were fractionated into two fractions PO 1 and PO-2 [4 5] Fraction PO-1 was the major component of POs and was composed of maltotriose maltotetraose and maltopentaose to which one phosphoryl group is attached [5] Fraction PO 2 was predominantly composed of maltopentaose and maltohexaose to which at least two phosphoryl groups were attached (6) The average degree of polymerization of dephosphorylated PO 1 and PO-2 was evaluated to be 4 02 and 5 82 respectively [4] The detailed structure of the components of the 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 sites as those of porcine pancreatic α amylase (PPA) and human saliva α-amylase (HSA) as reported by Takeda *et al* [7] The phosphate group linked at C-6 of the glucosyl residue was detected as the content of the Glc-6-P after acid hydrolysis The spectrometric analysis by ^{13}C-NMR also distinguished the phosphate groups linked at C-3 and C-6 of the glucosyl residues respectively In conclusion, the PO-1 fraction was made up of oligosaccharides

phosphorylated at C-3 (3^3-phosphoryl maltotetraose and 3^4-phosphoryl maltopentaose) and oligosaccharides phosphorylated at C-6 (6^3-phosphoryl maltotriose, 6^2-phosphoryl maltotriose, 6^3-phosphoryl maltotetraose, and 6^4-phosphoryl maltopentaose) (Fig 1) [5] In the case of the PO-2 fraction, a little of these enzymes' treatment-resistant PO 2 remained It clearly indicated that two of the phosphoryl groups attached to C 6 and C-3 existed in PO 2 components [6] From these results the possible structures of PO-2 components were as shown in Fig 1

The POs can form solubilized complexes with Ca and iron [4 8] The inhibitory effect of POs on the formation of Ca-P precipitate was dependent upon the covalently bound phosphoryl groups in the molecule [4 8 10] POs-bound calcium was thought an advantageous food ingredient as a soluble calcium source[11] In addition, POs can not be metabolized by cariogenic bacteria as mutans streptococci, as is true for xylitol and the preventive effect was shown on reducing the fall in plaque pH despite the buffering power sucrose-dependent fermentation[12] Sugar alcohols such as xylitol are widely used as sweeteners in chewing-gum to prevent dental caries and to promote remineralization [13 14] The POs can also reduce the amount of artificial plaque and demineralization on enamel even in the presence of sucrose [15] Furthermore the effects of POs on remineralization of caries-like lesions in enamel were examined *in vitro* [16] The results showed the possibilities that POs may have a synergistic effect with fluoride on the rate of remineralization[16] Based on the previously revealed features of POs, we examined the effect of remineralization on enamel by a chewing gum containing a calcium salt of POs (POs-Ca)

3 APPLICATION OF POs-Ca FOR ORAL HEALTH

The effects of daily application of a sugar-free chewing gum containing 2 5 wt% POs-Ca on remineralization of enamel were examined POs-Ca was prepared as 5 wt% calcium in the molecule The gum was concluded to be a non-cariogenic product since it was proven by intraoral plaque pH-telemetry tests in four human volunteers not to depress the pH of interdental plaque below 5 7 by bacterial fermentation either during consumption or during a period of 30 minutes following consumption by the general method of the Association for Toothfriendly Sweets [17] First the effect of the gum containing POs-Ca on remineralization of caries-like lesions in enamel was examined by using a human saliva immersing (HSI) test [18] The HSI-test would be a useful system for detection and evaluation of the remineralization effect using human saliva since it is easy to control condition for the test and light demands are made on volunteers, making it preferable to an intraoral study The results suggested that the HSI-test and intraoral study have relevance to the effect on enamel remineralization of POs-Ca [18 20] It is thought that human saliva plays some important role in oral health [21] In particular stimulated saliva for chewing would have some influence on remineralization

1) SALIVARY ASSESSMENT

We produced two types of sugar free chewing gum (tablet type) for the experiments One contained 2 5% POs-Ca (POs Ca (+) gum) and 46% xylitol and the other contained 48 5% xylitol without POs (POs-Ca (-) gum) The average weight of each chewing gum tablet was about 1 5g All saliva stimulated while chewing 2 tablets of POs-Ca (+) gum or POs Ca ( ) gum was collected from 12 healthy adult volunteers (6 males and 6 females mean age = 29 9 y old) Each volunteer chewed 2 pieces of gum 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 Immediately after the salivary treatments the enamel slabs were rinsed with deionized water This procedure was repeated 4 times a day for 4 days During the study period no fluoride agent was used and great care was taken not to dry the enamel disk samples The human saliva was used in the HSI-test within 1h after sampling Salivary volume and mineral contents were compiled in Table 1 The volume and pH of saliva from each volunteer were measured immediately after sampling Subsequently an aliquot of the saliva was centrifuged (10 000 g) for 5 min and the supernatant was prepared 0 1N HCl solution with addition of 1N HCl solution After re-centrifugation the supernatant was filtrated with an ultra filter (0 45 μm) The filtered saliva was assayed for the concentrations of inorganic phosphate (P) and calcium (Ca) The concentrations of P and Ca were measured by the methods using molybdenum reagent (22) and by *o*-cresolphthalein complexion (OCPC) method using the calcium-C-test (Wako Pure Chemical Japan) respectively The salivary volume of Fs and Ls secreted by chewing of POs Ca (+) gum and that by POs-Ca (-) gum were nearly equal values and no significant difference was observed The concentration of Ca in Fs in POs-Ca (+) gum was much higher than that of POs Ca (-) gum (p<0 0001) However the Ca concentration of Ls in POs Ca (+) gum was similar to that of Ls and Fs from POs Ca ( ) gum The difference in chewing gum did not largely influence the concentration of P in saliva samples Neither gum included any ingredients containing P Human saliva includes abundant P compared with Ca The time course of soluble Ca and P during 20 min was measured in chewing each gum (Fig 2 a b) In the experiment human whole stimulated saliva was collected from 17 healthy adult volunteers (9 males and 8 females mean age = 29 0 y old) including the former 12 volunteers during chewing 2 tablets of POs-Ca (+) gum or POs-Ca (-) gum The results indicated that most Ca in POs Ca (+) gum was extracted into saliva within the first 10 min However the concentration of P is almost fixed in saliva and increases in proportion to the amount of saliva The Ca/P ratio values in Fs from POs-Ca (+) gum (1 12±0 31) were significantly higher than the values in the other (p<0 0001) Especially at the beginning the Ca/P ratio ranged to 1 67 which is the value of hydroxyapatite in enamel (Fig 2 c) The pH of saliva was measured at 1 3 6 10 and

20 min during saliva sampling from 17 volunteers 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 After human salivary treatments planoparallel sections of about 500μm thickness were cut from the enamel samples using a water cooled diamond coated saw (Isomet Buhler USA) These sections were ground planoparallely on a wet 800 grit abrasive paper to a thickness of about 200 μm The sections were fitted on a high resolution positive film (Fuji, Japan) together with an aluminum step wedge and microradiographed (PW-1830 Philips The Netherlands) by Cu K α x ray generated at 25 kV and 25 mA for 24 sec The films were developed fixed and rinsed under standardized conditions The degree of remineralization was evaluated on digitized microradiographic images by combined means of computer-assisted videodensitometry (CAV) and a mineral distribution analysis program (MDA) developed by Inaba *et al* [23 24] Finally the mineral distribution parameters namely the lesion depth (ld μm) and mineral loss value (ΔZ vol% μm) were measured The data values were analyzed statistically by the repeated measure ANOVA followed by the Tukey Kramer test for multiple comparison The ld and ΔZ values are shown in Fig 3 by individuals in types of gum In all the individuals participating significantly lower ld and ΔZ values were observed in the case of POs-Ca (+) gum indicating enhanced enamel remineralization No remarkable mineral recovery was observed in the POs-Ca ( ) gum group The ld (means±SD = 81±15 μm) and ΔZ (2 825±593 vol% μm) in the POs-Ca (+) gum group were significantly lower (p<0 001) compared with those after initial demineralization and in POs Ca (-) gum group The saliva secreted by chewing POs-Ca (+) gum had higher remineralization enhancement activity than that from POs-Ca (-) gum No difference was observed in volume time-course change in pH level or contents of soluble P of saliva between chewing gum types or among volunteers There were adequate amounts of P compared with Ca in saliva collected by chewing of the POs-Ca ( ) gum In the case of POs Ca (+) gum the Ca content was higher than that from chewing the POs-Ca ( ) gum The initial Ca/P ratio value in POs-Ca (+) gum induced saliva was higher than that in POs Ca ( ) gum induced saliva The Ca/P ratio was 0 3 or less for POs Ca (-) gum These results suggested that remineralization enhanced by chewing the POs Ca (+) gum was due to the increased soluble calcium in saliva that resulted in a higher Ca/P ratio value corresponding with the value (1 67) of hydroxyapatite

2) INTRAORAL EVALUATION

Based on the former results we investigated the effects of the POs-Ca (+) gum on the 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 and participated in a double-blind intraoral study In first, 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 [18] The three groups were (i) the POs-Ca (-) gum group, (ii) the POs-Ca (+) gum group or (iii) sugar gum containing 62 wt% sucrose (the sucrose gum group) The chewing time was always 20 min and the palatal plate was preserved in the oral cavity for an additional 20 min Except for the time of chewing the gum and the subsequent 20 min, the appliances were stored in a plastic container with 100% humidity The results were that the remineralization rates (ld reduction percentage with respect to the mean ld value after initial demineralization) in the POs-Ca (+) group were about 67%, 54% and 76% at the 1st, 2nd and 4th week, respectively The remineralization rates in the POs-Ca (-) group ranged from 12 to 23% being much lower than that in the POs-Ca (+) group The sucrose group showed a negative value by the 4th week, indicating progression of demineralization The remineralization rate of the POs-Ca (+) group was higher than that of the POs-Ca (-) group at the 1st, 2nd and 4th week [18] The present results were well consistent with the results of the *in vitro* evaluations with the HSI-test Furthermore, the promoting of enamel remineralization of the POs-Ca (+) group was reconfirmed *in situ* by a two-week double-blind and cross-over design intraoral study [25] The ld of the POs-Ca (+) group was significantly reduced by 51% compared with that in the sucrose group and by 44% compared with that in the POs-Ca (-) group (Fig 4a) The ΔZ in the POs-Ca (+) group was also significantly lower by 30% compared with that in the sucrose group and by 25% compared with that in the POs-Ca (-) group (Fig 4b) The effects of the gum on remineralization of dentin were also confirmed in a double-blind cross-over design intraoral study [26]

4 CONCLUSION AND PERSPECTIVE

The promoted remineralization of enamel and dentin lesions by the POs-Ca (+) group can be explained as follows The pH of the saliva during the chewing of the gums is estimated to increase from about 7 to 7 5 Since, in general, this relatively higher pH is not suitable for the solubilizing of Ca and phosphate it is considered that POs-Ca in the saliva would aid to maintain the 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 and dentin 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) In a former study, some ratios of Ca/P was investigated *in vitro* [27] The Ca/P ratio of 1 67 showed significant reduction in ld and ΔZ of enamel lesion compared with other ratios It was also suggested the elevation of the Ca/P ratio in saliva enhanced the remineralization The gum containing POs-Ca would be able to compensate the 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 a nutritional approach It is generally known that a gastrointestinal disorder would occur when we take a food containing an

excess amount of sugar alcohol at one time We have already shown that the consumption of an excess amount of POs-Ca does not cause a gastrointestinal disorder [28] [29] We also confirmed that the orally administered POs-Ca was hydrolyzed and then absorbed completely in the small intestine in rats [30] In conclusion, daily use of a sugar-free chewing gum containing POs-Ca can effectively enhance the remineralization both in enamel and dentin lesions POs-Ca enhanced enamel remineralization by increasing the solubility of Ca in the oral environment and could be a beneficial material for oral health

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馬鈴薯澱粉由来リン酸化オリコ糖カルシウムのオーラルヘルスへの応用

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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 つまりリン酸化オリコ糖カルシウム（POs-Ca）を調製してきた。このリン酸化オリコ糖は2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画分はリン酸化オリコ糖の主な成分であって マルトトライオース マルトテトラオース およびマルトヘ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画分は主にマルトヘ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有していた リン酸化オリコ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化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 リン酸化オリコ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産生する酸によるプラーク内の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 ここては POｓ-Caを関与成分としたノ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。

REFERENCES

1 C Takeda Y Takeda and S Hizukuri Structure of the amylopectin fraction of amylomaize *Carbohydr Res* **246** 273-281 (1993)

2 S Hizukuri Carbohydrates in Food A -C Eliasson ed Marcel Dekker Inc , New York pp 375 379 (1996)

3 Y Takeda S Hizukuri Location of phosphate groups in potato amylopectin *Carbohydr Res* **102,** 321 327 (1982)

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

5 H Kamasaka K To-o K Kusaka T Kuriki T Kometani H Hayashi S Okada The structures of phosphoryl oligosaccharides prepared from potato starch *Biosci Biotech Biochem* **61** 238 244 (1997)

6 H Kamasaka K To o K Kusaka T Kuriki T Kometani S Okada A way of enhancing the inhibitory effect of phosphoryl oligosaccharides on the formation of calcium phosphate precipitate by using the coupling reaction of cyclomaltodextrin glucanotransferase *J Appl Glycosci* **44** 285-293 (1997)

7 Y Takeda S Hizukuri Y Ozono and M Suetake Actions of porcine pancreatic and Bacillus subtilis α-amylases and Aspergillus niger glucoamylase on phosphorylated (1 4) α -D-glucan *Biochim Biophys Acta* **749** 302 311 (1983)

8 H Kamasaka K To-o K Kusaka T Kuriki T Kometani S Okada Effect of phosphoryl oligosaccharides on iron solubility under neutral conditions *Biosci Biotech Biochem* **61** 1209 1210 (1997)

9 H Kamasaka K Kusaka K To o T Kuriki T Kometani S Okada Inhibitory effect on formation of calcium phosphate precipitate by conjugates of ovalbmin and phosphoryl oligosaccharides *J Appl Glycosci* **44** 295 302 (1997)

10 H Kamasaka K To-o M Uchida K Kusaka T Kuriki T Kometani H Hayashi S Okada T Ichikawa Studies of phosphoryl oligosaccharides prepared from potato starch *J Appl Glycosci* **44** 253 261(1997)

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

12 H Kamasaka, S Imai T Nishimura T Kuriki T Nishizawa Effect of phosphoryl oligosaccharides from potato starch on acid fermentation by *mutans streptococci J Dent Hlth* **52** 66-71(2002)

13 K Wennerholm J Arends D Birkhed J Ruben C G Emilson and A G Dijkman Effect of xylitol and sorbitol in chewing-gums on mutans streptococci plaque pH and mineral loss of enamel *Caries Res* **28** 48-54 (1994)

14 K K Makinen and E Soderling Solubility of calcium salts enamel and hydroxyapatite in aqueous solutions of simple carbohydrates *Calcif Tissue Int* **36** 64 71 (1984)

15 S Imai H Kamasaka, D Inaba T Nisizawa and N Hanada Inhibitory Effect of Phosphoryl Oligosaccharides against Enamel Demineralization by Mutans Streptococci *J Dent Res* **81** A351 (2002)

16 D Inaba K Minami, H Kamasaka, M Yonemitsu Effect of phosphoryl oligosaccharides (POs) on remineralization of enamel lesion in vitro *Dent Soc Iwate Med Univ*, **27**, 197-202 (2002)

17 S Takahashi-Abbe, K Abbe, N Takahashi, Y Tamazawa, T Yamada Inhibitory effect of sorbitol on sugar metabolism of Streptococcus mutans in vitro and on acid production in dental plaque in vivo *Oral Microbiol Immunol*, **16**, 94-99 (2001)

18 H Kamasaka D Inaba K Minami T Nishimura T Kuriki S Imai M Yonemitsu Remineralization of enamel by phosphoryl oligosaccharides (POs) supplied by a chewing gum Part I Salivary assessment in vitro *J Dent Hlth* **52** 105 111 (2002)

19 D Inaba H Kamasaka K Minami T Nishimura T Kuriki S Imai M Yonemitsu Remineralization of enamel by phosphoryl-oligosaccharides (POs) supplied by a chewing gum Part II Intraoral evaluation *J Dent Hlth* **52** 112-118 (2002)

20 H Kamasaka D Inaba K Minami T Nishimura T Kuriki M Yonemitsu Production and application of phosphoryl oligosaccharides prepared from potato starch *Trends in Glycosci Glycotech* **15** 75 89 (2002)

21 F J Dowd Saliva and dental caries Dent Clin North Am 43 579-597 (1999)

22 Ui M Itaya K A new micromethod for the colorimetric determination of inorganic phosphate *Clin Chim Acta* **14** 361 366 (1966)

23 D Inaba O Takagi J Arends A computer-assisted videodensitometric method to visualize mineral distributions in in vitro and in vivo formed root caries lesions *Eur J Oral Sci* **105** 74-84 (1997)

24 D Inaba R Tanaka, O Takagi M Yonemitsu J Arends Computerized measurements of microradiographic mineral parameters of de- and remineralized dental hard tissues *J Dent Hlth* **47** 67-74 (1997)

25 D Inaba K Minami H Kamasaka, T Kuriki S Imai, M Yonemitsu Intraoral effects of phosphoryl-oligosaccharides calcium on remineralization on enamel lesion *J Dent Hlth*, **53** 8-12 (2003)

26 D Inaba, K Minami, H Kamasaka, M Yonemitsu Remineralization of enamel and dentin by a chewing gum containing phosphoryl oligosaccharides calcium (POs-Ca) in situ *Dent Soc Iwate Med Univ*, **27**, 203-209 (2002)

27 T Moriya In vitro Remineralization of bovine enamel with various Ca/P ratios *J Dent Hlth*, **49**, 40-54 (1999)

28 H Takii, H Kamasaka, T Nishimura, K To-o K Sugimoto, T Kuriki The influence of sugar alcohol chewing gum containing phosphoryl-oligosaccharides on gastrointestinal condition in humans *J Nutr Food* **5** 61 68 (2002)

29 H Takii, M Yoshiyama, M Yanase, H Kamasaka T Nishimura, T Kuriki The relationship of phosphoryl-oligosaccharides or sugarless chewing gum containing phosphoryl-oligosaccharides ingestion and gastrointestinal condition in humans *J Appl Glycosci* **50**, 51-54 (2003)

30 K To-o, H Kamasaka, T Nishimura, T Kuriki, S Saeki Y Nakabou Bioavailability of calcium-bound phosphoryl oligosaccharides in rats *J Appl Glycosci* **49** 159-165 (2002)

Fig 1 Structure of Phosphoryl Oligosaccharides
Symbols P 3 and P-6 phosphoryl groups linked at C 3 and C-6 of glucosyl residues

○ glucosyl residue ○, glucosyl residue which possibly exists ○ reducing end

Fig 2 Time Course of Salivary Soluble Phosphate (a), Calcium (b) and Ca/P ratio during Chewing Gum with (■) or without (◇) POs-Ca
Human whole saliva was collected from 17 healthy adult volunteers (9 males and 8 females mean age = 29 0 y old) by chewing 2 tablets of POs-Ca (+) gum or POs-Ca (-) gum Each volunteer chewed the tablets for 20 min and the whole saliva was collected Vertical bars, SD

Fig 3 Enhanced Remineralization by the POs-Ca Gum
Numbers of volunteers were 12 persons (6 males and 6 females, mean age = 29 9 y old)
Vertical bar, S D , *p<0 001

Fig 4 Comparison of the lesion depth (a) and mineral loss values (b) after *in situ* experiments
Numbers of volunteers were 12 persons (6 males and 6 females, mean age, 21 y old) Vertical bar, S D , *p<0 05 **p<0 01

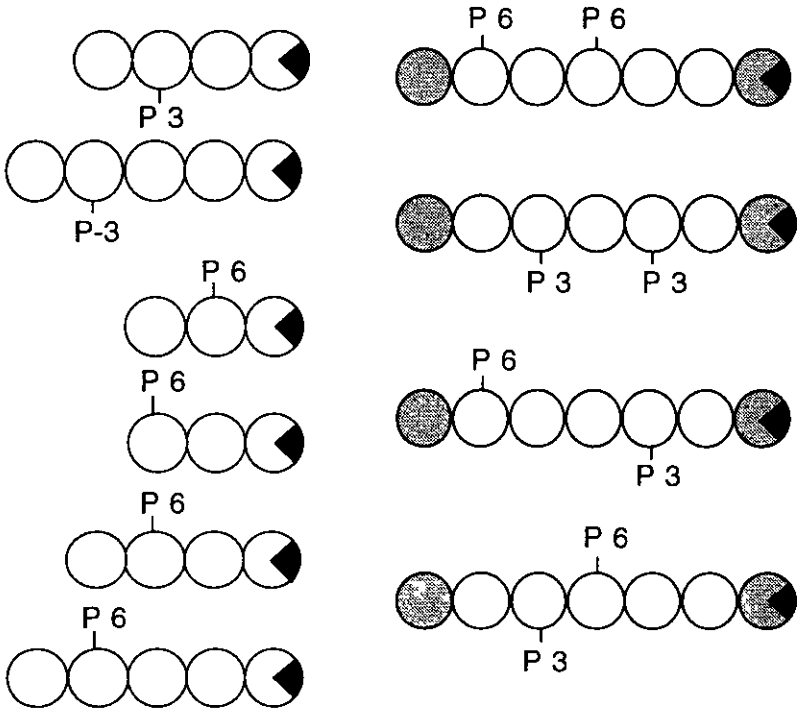

Fig 1

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Table 1 Analysis of Volumes and Mineral Contents in Saliva

	POs-Ca	Fs		Ls		P[b]
		Means±SD	P[a]	Means±SD	P[a]	
Salivary volume (ml)	+	20 34±4 13	ns	9 35±3 24	ns	**
	-	20 74±4 43		9 65±3 35		**
Ca (mM)	+	6 29±2 44	**	1 72±0 27	*	**
	-	1 69±0 41		1 39±0 37		ns
P (mM)	+	5 62±1 41	ns	6 22±1 31	ns	ns
	-	6 15±1 35		6 49±1 15		ns
Ca/P	+	1 12±0 31	**	0 27±0 05	*	**
	-	0 28±0 08		0 22±0 05		ns

Fs Collected whole saliva for the first 10 min
Ls Collected whole saliva for the last 10 min
a *p* for POs-Ca (+) vs POs-Ca (-), *b* *p* for FS vs LS
ns not significant, ** $p<0.0001$, * $p<0.05$

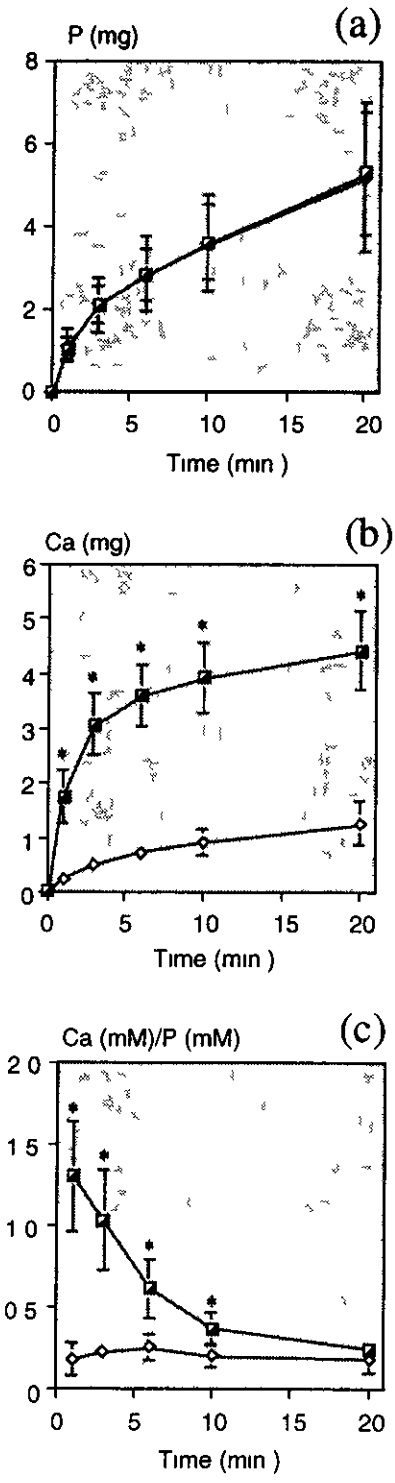

Fig 2

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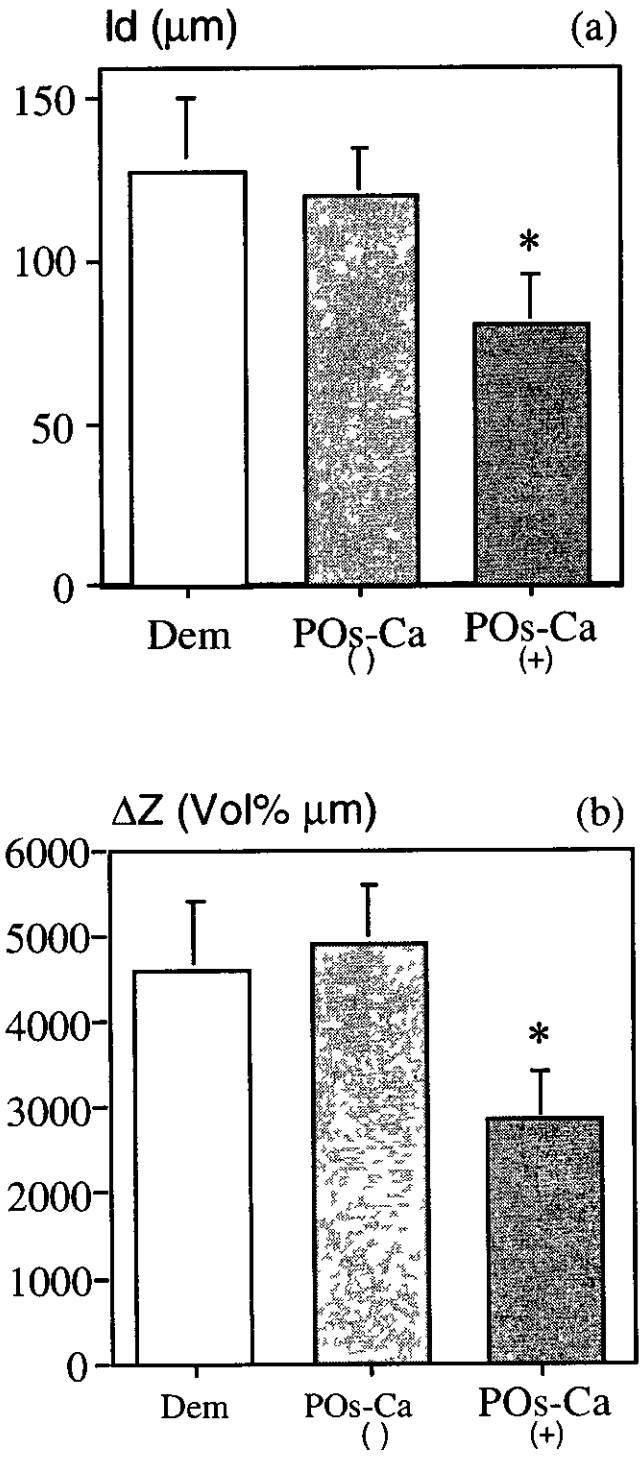

Fig 3

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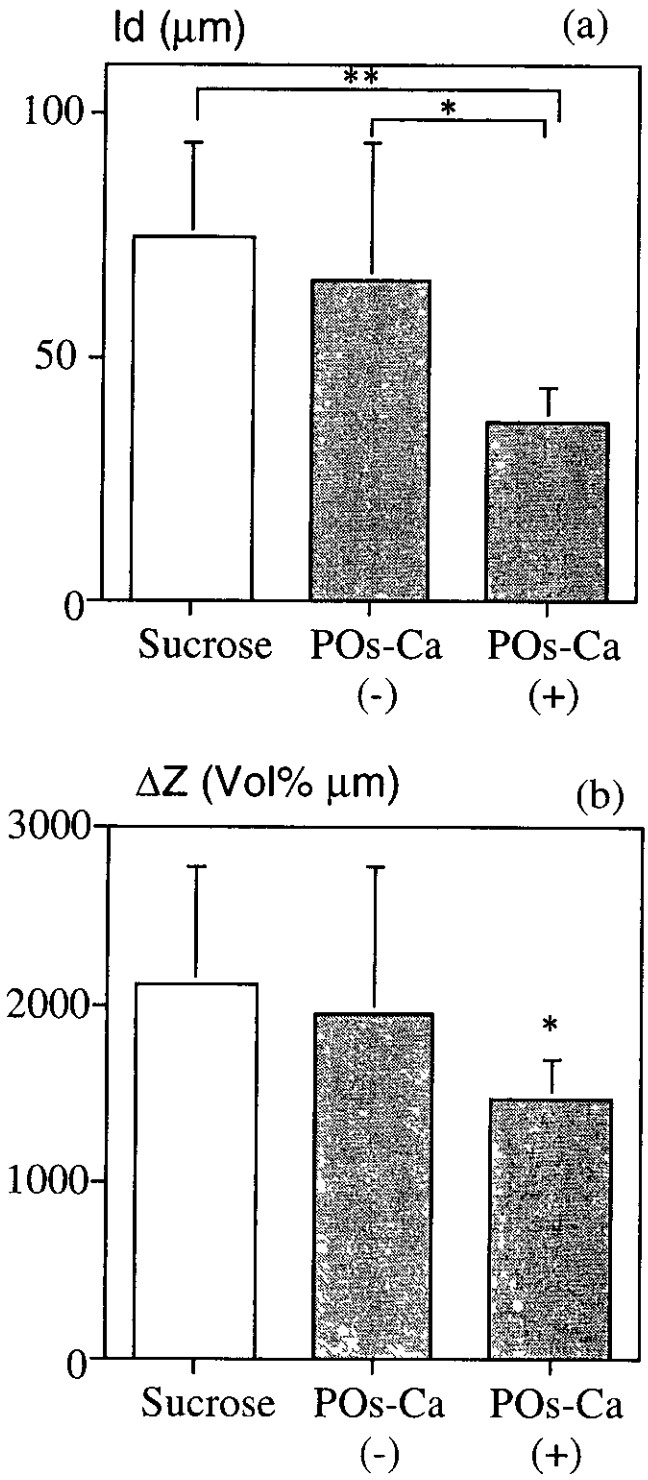

Fig 4

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Original

Intraoral Effects of Phosphoryl-Oligosaccharide Calcium on Remineralization of Enamel Lesions

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Abstract The authors previously reported that phosphoryl oligosaccharide calcium salt (POs-Ca) can be extracted from potato starch hydrolysates and markedly increases the solubility of calcium This study examined the effects of a sugar free chewing gum containing POs Ca on remineralization of enamel in situ Twelve healthy volunteers (6 males and 6 females mean age=21 y old) were randomly divided into 3 groups (n=4 per group) and participated in an intraoral study using a double blind cross over design Each participant wore a removable palatal appliance containing demineralized enamel disks and chewed a xylitol gum a xylitol plus 2 45% POs-Ca gum or a sucrose gum 4 times a day (period 2 w per type of gum) During the study period there was no fluoride agent used and great care was taken not to dry the enamel disks The enamel disks were microradiographed to quantify mineral distributions The lesion depth (ld) in the POs group (Mean±SD=37±7 μm) significantly reduced by 51% compared with that in the sucrose gum group (75±19 μm $p<0$ 01) and by 44% compared with that in the xylitol gum group (66±28 μm $p<0$ 05) The proposed mechanism of mineral accumulation by POs-Ca is the elevation of the salivary Ca/P ratio toward the level in hydroxyapatite (1 67) thus maintaining a state of supersaturation of calcium facilitating enamel enhancing remineralization In conclusion it was suggested that daily use of a sugar free chewing gum containing 2 45% POs Ca (4 times a day) enhances remineralization in enamel lesions considerably

Key words **Phosphoryl-oligosaccharide calcium Remineralization Enamel Chewing gum Intraoral experiment**

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Introduction

The authors previously reported in detail about the features of phosphoryl oligosaccharide calcium salt (POs-Ca) which can be prepared from potato starch hydrolysates and markedly increases the solubility of calcium[1-6] In relation to the etiology of dental caries it was suggested that POs can not be metabolized by cariogenic microorganisms such as mutans strepto cocci and reduces the fall in the pH of plaque in vitro[7] In addition POs-Ca was suggested to increase remineralization of enamel by optimizing salivary conditions[8 9] This study further investigated the effects of a sugar free chewing gum containing POs-Ca on remineralization of demineralized bovine enamel by intraoral experiments using a cross over design

Materials and Methods

POs-Ca were prepared from potato starch hydrolysates as described in detail by Kamasaka et al[3] and the following 3 types of chewing gums were prepared for the present experiments

1) sugar free gum containing 48 5% xylitol (xylitol gum)

2) sugar free gum containing 46% xylitol plus 2 45% POs-Ca (POs gum) and

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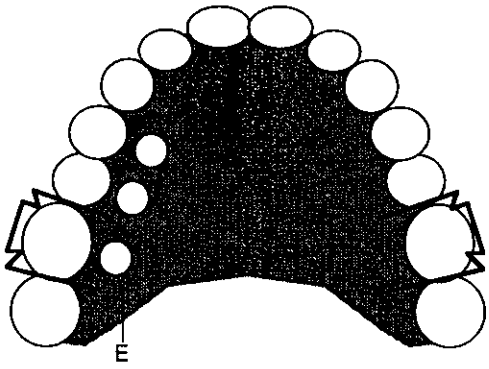

Fig 1 Schematical draw of a removable palatal appliance containing demineralized enamel disks
E=Enamel disk B=Resin plate

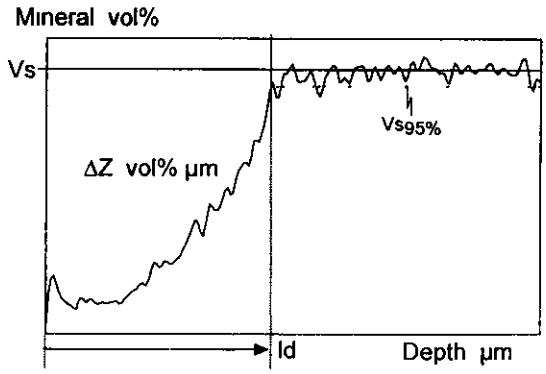

Fig 2 Schematical mineral distribution and the mineral paramter measured
Vs=Mineral vol% level of sound tissue ld=Lesion depth in μm ΔZ=Mineral loss value in vol% μm

3) gum containing 62% sucrose (sucrose gum) POs-Ca contains 5 wt% Ca and all the gums were supplied in a tablet form (1 45 g per piece) with the same appearance and flavor

1 Sample preparation

Enamel disks (5 mm in diameter 1 5 mm in thick ness) were cut from the crown parts of bovine incisors and the buccal surfaces were polished on a wet abra sive paper (800 grit) to expose a fresh and flat plane of enamel The disks were then exposed to a 0 1 M lactic acid gel containing 6 wt% carboxymethylcel lulose (pH=5) at 37°C for 3 w to induce artificially early caries lesions[10] After demineralization the enamel disks were mounted at the palatal region of upper right molars in a removable maxillary appli ance as shown in Fig 1

2 Intraoral experiment

Twelve healthy adult volunteers (6 males and 6 females mean age=21 y old mean DMFT=5 5 mean salivary flow rate=1 6 m*l*/min mean pH of parafin stimulated whole saliva=7 2) participated in the intraoral study using a randomized double blind cross over design Prior to this study informed con sent to the intraoral experiments was obtained from the participants and it was confirmed that they were free from any chronic diseases and were not taking medications

Each volunteer wore a palatal appliance containing demineralized enamel disks and chewed one of the experimental gums 4 times a day (after meals and before bed time) for 2 w They were instructed to wear the appliances chew 2 tablets of the gum for 10 min spit the gum out then keep the appliance in the oral cavity for an additional 10 min When not being worn the palatal appliances were stored in a plastic con tainer with 100% humidity After a rest period for 1 w the intraoral experiments were restarted with differ ent gums Thus all the participants eventually tested each of the 3 types of gum

3 Microradiography and data analysis

After remineralization planoparallel sections about 500 μm thick were cut from the enamel samples using a water cooled diamond coated saw (Isomet Buhler USA) under water supply These sections were ground planoparallely on a wet 800-grit abrasive paper to a thickness of about 200 μm The sections were fitted on a high resolution positive film (Fuji Photo Film Japan) together with an aluminum step wedge (15 μm×15 steps) and microradiographed (PW-1830 Philips The Netherlands) by Cu-Kα x-ray generated at 25 kV and 25 mA for 24 sec The films were devel oped fixed and rinsed under standardized conditions

The mineral distributions were quantified on digit ized microradiographic images by a combination of computer assisted videodensitometry (CAV) and mineral distribution analysis software (MDA)[10,11] Lesion depth (ld) μm and mineral loss value (ΔZ vol% μm) were measured (Fig 2) The data were analyzed statistically by the repeated measure ANOVA followed by the Tukey-Kramer test for multiple comparison

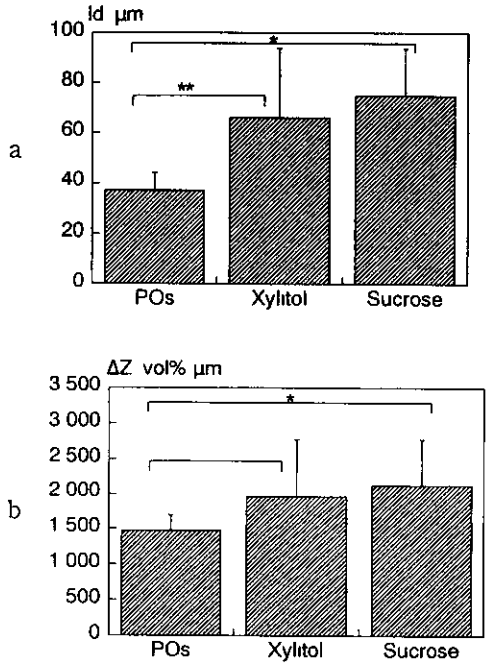

Fig 3 Comparison of the lesion depth (a) and mineral loss values (b) after in situ experiments

Results

Main results were presented in Figs 3 a and 3 b The lesion depth (ld) in the POs group (Mean±SD= 37±7 μm) was significantly redcuced by 51% compared with that in the sucrose gum group (75±19 μm $p<0$ 01) and by 44% compared with that in the xylitol gum group (66±28 μm $p<0$ 05) The mineral loss value (ΔZ) in the POs group (1 474±226 vol% μm) was significantly lower by 30% compared with that in the sucrose gum group (2 120±651 vol% μm $p<$ 0 05) and by 25% compared with that in the xylitol gum group (1 955±818 vol% μm $p<0$ 05)

Discussion

In nutritional science oligosaccharides have been demonstrated to be a ingredient improves the intestinal microflora and calcium absorption reduces acidogenicity and decreases the caloric effects of foods[12-15] In addition to these advantageous features this double blind cross over design intraoral study newly demonstrated unique function of POs-Ca in saliva supplied by chewing gum significantly enhanced remineralization of enamel without fluoride

Although available information on the role of POs-Ca in the caries process is limited at present the enhanced remineralization of enamel lesions by POs-Ca may be explained as follows Based on the measurements of paraffin stimulated saliva samples for the participants and in a previous study[8] the pH of the saliva while chewing gum was estimated to be 7 0-7 5 in this study This pH is suitable for remineralization but is not preferable for Ca and phosphate to be ionized[3] In fact the stimulated saliva itself had no substantial effects on remineralization in the previous study despite the presence of sufficient amounts of Ca and phosphate in saliva[8] Therefore the present results suggested that small amounts of POs-Ca as low as 0 1% in the saliva could maintain the solubility of mineral ions even at pH 7 and therefore contributed to the recrystallization of soluble Ca onto residual hydroxyapatite crystals in enamel lesions Hence POs-Ca may play an important role in caries prevention as an ingredient that regulates mineral solubility in the oral environment (saliva and/or dental plaque)

The inhibitory effect of POs-Ca on the formation of calcium phosphate precipitate was suggested to be derived by the ability of POs-Ca to form a soluble complex with Ca dependent upon the covalently bound phosphoryl groups in the molecule[3-5] In addition to the present findings it was suggested in vitro that POs-Ca is not metabolized by either of *Streptococcus mutans* MT 1848 or *Streptococcus sobrinus* 6715 and inhibition of the pH fall is ascribed to the metabolization of sucrose by these microorganisms in a dose dependent manner[7] The inhibition of pH drop in culture was considered to be facilitated by the buffering action of the phosphoryl groups in POs-Ca Thus POs-Ca is expected not only to enhance remineralization by acting as a mineral ion stabilizer at a neutral pH but also to function as a non cariogenic carbohydrate

It was concluded that POs-Ca would be a novel and unique substance extracted from potato starch to assist enamel remineralization by solubilizing environmental Ca and could be utilized in a dietary approach to caries prevention In practice it was suggested that daily use (4 times per day) as a sugar free chewing gum containing 2 45% POs-Ca enhances remineral