Remineralization of Enamel and Dentin by A Chewing Gum Containing Phosphoryl-Oligosaccharide Calcium (POs-Ca) in situ

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Abstract : It was previously shown that phosphoryl-oligosaccharide calcium (POs-Ca) can be prepared from potato starch hydrolysates and substantially promote the solubility of calcium. The aim of this study was to investigate the effects of a sugar-free chewing gum containing POs-Ca on remineralization of enamel and dentin in situ. Twelve healthy volunteers (6 males and 6 females ; mean age＝22 y old) participated in a double-blind cross-over design intraoral study. Each participant wore a removable palatal appliance containing both of demineralized enamel and dentin disks and chewed a xylitol gum or xylitol plus 2.6% POs-Ca gum 4 times a day (period : 2 w per gum). The mineral distributions of the specimens were quantified by microradiography. The lesion depth (ld) of enamel (Mean±SD＝70±11 μm) and dentin (71±13 μm) in the POs-Ca group were significantly reduced by 25-31% compared with the xylitol gum (101±21 μm in enamel and 95±13 μm in dentin ; $p<0.001$). The proposed mechanism of mineral accumulation by POs-Ca is that they contribute to increase salivary Ca/P ratio and maintain a state of supersaturation of calcium with respect to tooth substances enhancing remineralization. In conclusion, it was suggested that daily use of a sugar-free chewing gum containing 2.6% POs-Ca enhances remineralization both in enamel and dentin lesions considerably.

Key words : Phosphoryl Oligosaccharides Calcium (POs-Ca), Remineralization, Enamel, Dentin, Chewing gum

Introduction

Phosphoryl-oligosaccharides (POs) are enzymatic hydrolysates of potato starch, and potato amylopectin is known to contains 200-1,000 ppm of the ester phosphorus[1]. Takeda and Hizukuri[2] demonstrated that the phosphate groups mainly located in the B-chain of amylopectin, and it was revealed that 60-70% of the phosphate groups were bound to C-6 of the glucosyl residues, with most of the rest being C-3[3]. The detailed structures of PO-1 fraction, which is the main

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component of POs and has one phosphate group in each molecule, were analyzed by Kamasaka et al[4]. The average polymerization degree of dephosphorylated PO-1 was 4.02 and the POs were evaluated to be phosphoryl α-1,4 -maltooligosaccharides.

From nutritional point of view, it was reported that POs have an inhibitory effect on the formation of a calcium phosphate precipitate[5]. In addition, it was suggested that calcium bound POs (POs-Ca) would be an advantageous foodstuff as a soluble Ca source, because POs-Ca themselves are substantially soluble in water. Relating to dental caries, it was suggested that POs-Ca can not be metabolized by cariogenic microorganisms, and reduces the plaque pH fall in vitro[6]. Moreover, POs-Ca was suggested to increase remineralization of enamel in our intraoral pilot study[7,8]. The aim of this study was to investigate the effects of a sugar-free chewing gum containing POs-Ca on remineralization of demineralized bovine enamel and dentin by a cross-over intraoral experiments.

Materials and methods

POs-Ca were prepared from potato starch hydrolysates as reported in detail by Kamasaka et al[5], and then desalted by cation-exchange resin (Dowex 50Wx4, 20-50 mesh). Ca-bound POs were prepared by neutralizing the desalted POs solution (pH 2) with $Ca(OH)_2$ to pH 6.5. For the present experiment, the following two types of chewing gums were prepared:

(1) sugar-free gum containing 48.5% xylitol (xylitol gum),

(2) sugar free gum containing 46% xylitol plus 2.6% POs-Ca (POs gum)

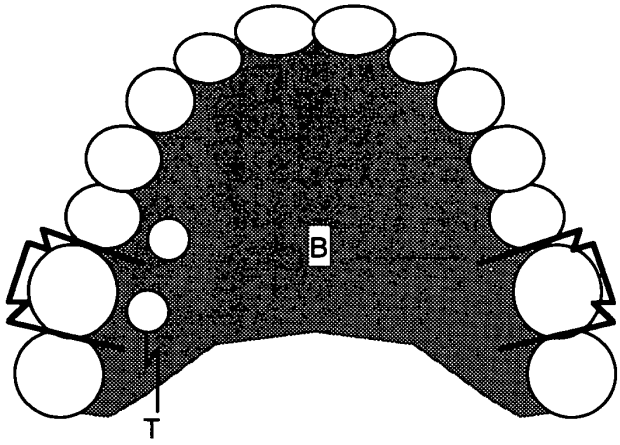

Fig. 1. Schematical draw of a removable palatal appliance containing demineralized enamel and dentin disks. T=Tooth disk ; B=Resin plate.

POs-Ca contains 5 wt% Ca and all the gums were supplied as a sheet form (2.7 g per piece) with the same appearance and flavor.

Sample preparation

Enamel and dentin disks (5 mm in diameter ; 1.5 mm in thickness) were cut from the crown parts of bovine incisors using a core-drill (SDH-01, Meiwa, Japan) and the buccal surfaces were polished on a wet abrasive paper (800 grit) to expose a fresh and flat plane of dental hard tissues. The disks were then immersed in a 0.1M lactic acid gel containing 6 wt % carboxymethylcellulose (pH=4.5 for enamel and pH= 5 for dentin) at 37℃ for 2 w to produce artificial caries-like lesions[9]. After demineralization, the dentin and enamel disks were attached at the palatal region of upper right molars in a removable maxillary appliance as shown in Fig. 1.

Intraoral experiment

The experimental protocols of the present intraoral study were applied to and accepted by the ethics committee of the School of

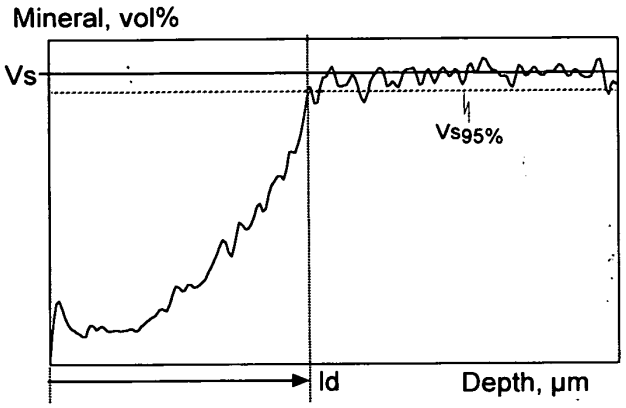

Fig. 2. Schematical mineral distribution and the mineral parameter measured. Vs = mineral vol% level of sound tissue, ld= the lesion depth (μm) defined as the distance from the outer surface position to the location in the lesion at which the mineral content reaches to the 95% level of mineral content in sound tissue ($Vs_{95\%}$), ΔZ=integrated mineral loss value in vol%•μm.

Dentistry, Iwate Medical University. Twelve healthy adult volunteers (6 males and 6 females; mean age=22 y old; mean DMFT= 5.5, mean salivary flow rate=1.6 ml/min, mean pH of parafin-stimulated whole saliva = 7.2) participated in a randomized double-blind cross-over design intraoral study. Prior to this study, informed consent to the intraoral experiments was obtained from the participants, and it was confirmed that they were free from any chronic diseases and medications.

Each volunteer wore a palatal appliance containing demineralized dentin and enamel disks, and chewed one of the experimental gums 4 times a day (after every meal and before bed time) for 2 w. They were instructed to wear the appliances, chew 1 sheet of the gum for 10 min, spit the gum out, and keep the appliances for additional 10 min in the oral cavity. Except for this time (total of 80 min per day), the palatal appliances were removed and stored in a plastic container with 100% humidity. After a rest period for 1 w, the intraoral experiments were restarted with different gums. Thus, all the participants experienced both types of the gum finally.

Microradiography and data analysis.

After intraoral experiments, planoparallel sections of about 200 μm thickness were cut from the enamel and dentin samples. The sections were fitted on a high resolution positive film (Fuji, Japan) together with an aluminum step wedge (15 μm x 15 steps) and microradiographed (PW-1830, Philips, The Netherlands) by Cu-Kα radiation generated at 25 kV and 25 mA for 24 sec. The films were developed, fixed, and rinsed under standardized conditions.

The mineral distributions were quantified on digitally captured microradiographic images by combined means of computer-assisted videodensitometry (CAV) and a mineral distribution analysis software (MDA) developed by Inaba, et al[9,10]. The 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.

Results

Initial demineralization formed caries-like lesions with ld=94±4 μm (mean±SD) and ΔZ=2,107±250 vol%•μm in dentin, and ld= 93±17 μm and ΔZ=2,660±683 vol%•μm in enamel. Main results were presented in Fig. 3. The ΔZ value of dentin samples in the POs-Ca group (1,828±332 vol%•μm) was significantly lower by 13-15% with respect to the xylitol group (2,143±258 μm ; $p<0.001$) and just after demineralization. The ld in the POs-Ca group (71±13 μm for dentin and 70±

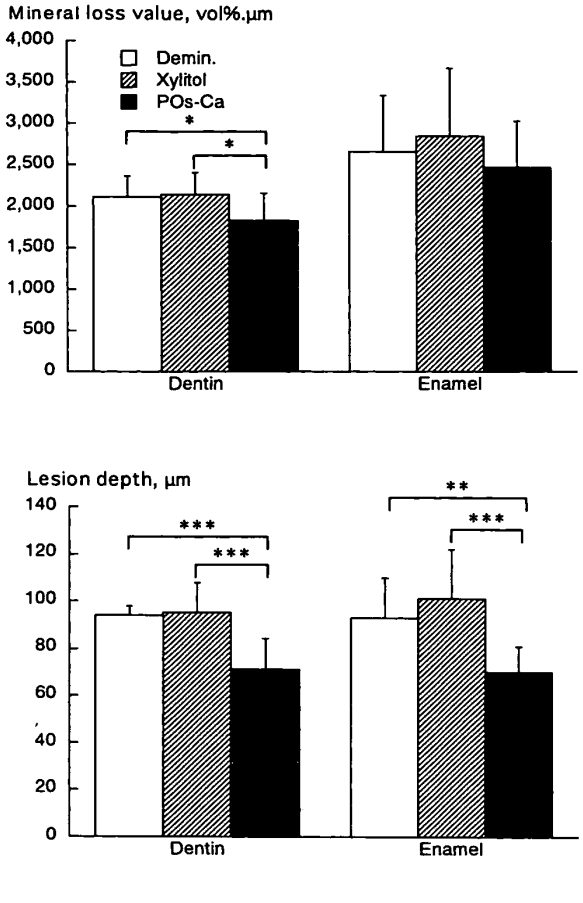

Fig. 3. Comparison of the mineral parameter values. *p<0.05, **p<0.01, ***p<0.001

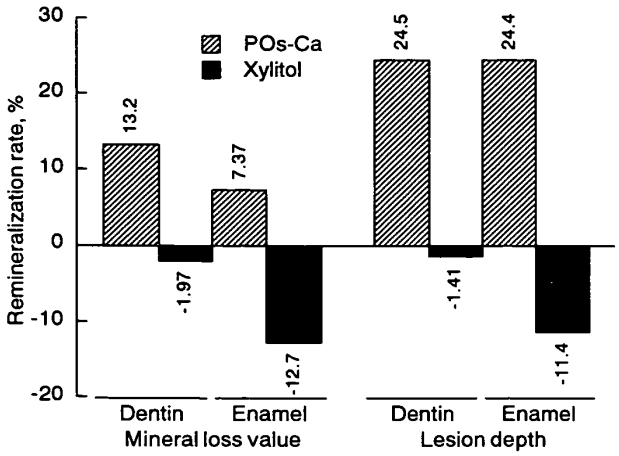

Fig. 4. Comparison of remineralization rate (ld or ΔZ reduction percentage with respect to the initial values after demineralization).

11 μm for enamel) were significantly lower by 24-31% compared both with the xylitol group (95±13 μm for dentin with $p<0.001$ and 101±21 μm for enamel with $p<0.001$) and just after demineralization ($p<0.001$ for dentin and $p<0.01$ for enamel).

Remineralization rates (percentage of ld or ΔZ reduction with respect to the values just after initial demineralization) in the POs-Ca group were in the order of 7.3-24.5% exceeding these in the xylitol group ranging from -1.41% to -12.7% (Fig. 4).

Discussion

Oligosaccharides have been known as beneficial foodstuffs to improve the intestinal microflora and calcium absorption, and to reduce calorie effects of diet[11-14]. In addition, the present intraoral study indicated an novel function of the substance to promote remineralization in both of enamel and dentin significantly without any fluoride application. The mineral recovery (remineralization) seemed to take place mainly in the lesion bottom since reduction of ld values was more remarkable than that of ΔZ values in the POs-Ca group (Figs. 3 and 4).

Based on the available information on POs, the promoted remineralization of enamel and dentin lesions by POs-Ca 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[5], it is adequate to consider that POs-Ca as low as 0.1% in the saliva would aid to maintain solubility of mineral ions even at pH 7-7.5 and, thereby, ionized Ca had potential to crystallize onto the residual hydroxyapatite crystals in enamel and dentin lesions. In fact, the stimulated saliva itself had no remarkable effects on remineralization in a short time reaction[8].

Thus, under the presence of POs-Ca, soluble Ca in saliva increases efficiently and, thereby, the salivary Ca/P ratio can increase to the value of hydroxyapatite (1.68).

It was interesting that the ld and ΔZ values in the xylitol group showed no significant differences compared with just after initial demineralization. This result suggested that xylitol released from a chewing gum into the oral cavity may not contribute to remineralization in dentin and enamel directly. A xylitol gum has generally been introduced as a remineralization enhancer[15, 16]. However, Wennerholm et al.[17] and Iijima et al.[18] demonstrated negative intraoral effects of xylitol in promotion of remineralization in enamel. Thus, the intraoral effects of xylitol on remineralization of enamel may be ambiguous so far.

To this point, it was suggested from the in vitro study that tolerable levels of xylitol alone may not show a significant caries inhibiting and remineralizing effects, but may act as a caries inhibitor additively with fluoride[19]. Moreover, Steinberg et al.[20] reported that a xylitol gum can aid the remineralization potential of dental plaque. Although further studies should be encouraged, it was suggested from the present results that POs-Ca may facilitate the possible anti-caries effects of xylitol.

In conclusion, it was suggested that POs-Ca would be a novel and unique substance extracted from potato starch to assist enamel and dentin remineralization by solubilizing environmental Ca, and could be utilized for caries prevention. For prevention practice, it was recommended to use a sugar-free chewing gum containing 2.45% POs-Ca daily (4 times per day) to enhances remineralization in enamel and dentin lesions.

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エナメル質と象牙質の再石灰化におよぼすリン酸化オリゴ糖カルシウム塩の口腔内における効果

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抄録：著者らはリン酸化オリゴ糖カルシウム塩（*POs-Ca*）が馬鈴薯澱粉の加水分解物より抽出でき，カルシウムの溶解性を高めることを先に報告した。本研究の目的は，*POs-Ca* 配合シュガーレスガムのエナメル質および象牙質の再石灰化におよぼす効果を口腔内実験により確認することである。12名のボランティア（男性6名，女性6名；平均22歳）をランダムに2群に分け，二重盲検，クロスオーバーデザインの口腔内実験を行った。各被験者は脱灰した牛歯エナメル質および象牙質ディスクを取り付けた上顎口蓋プレートを装着し，キシリトールガムまたは2.6%*POs-Ca* 配合キシリトールガムのいずれかを，1日4回噛んだ（期間：2週間／ガム）。実験期間中，フッ化物は使用せず，エナメル質ディスクが乾燥しないよう注意した。エナメル質ディスクのマイクロラジオグラフから脱灰深度 *ld* を評価した。*POs-Ca* ガム群のエナメル質および象牙質の *ld* 値は，それぞれ70±11μmならびに71±13μm（*Mean*±*SD*）で，キシリトールガムの値（エナメル質：101±21μm，象牙質：95±13μm）よりも25－31%減少していた（$p<0.001$）。このミネラル蓄積のメカニズムは，*POs-Ca* が唾液の *Ca/P* 比をハイドロキシアパタイトの比率（1.67）に向けて高め，歯質に対して過飽和なミネラル状態を維持させたことによると考えられた。本研究より，2.6%*POs-Ca* 配合シュガーレスガムを毎日利用することは，エナメル質と象牙質，双方の再石灰化を強く促進し，齲蝕予防に有効であることが示唆された。

キーワード：リン酸化オリゴ糖カルシウム塩（POs-Ca），再石灰化，エナメル質，象牙質，チューイングガム