Influences of Animal Mucins on Peroxidase Activity in Solution and on the Surface of Hydroxyapatite

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Animal mucins have structural characteristics similar to human salivary mucins. Animal mucins have been regarded as suitable substances for saliva substitutes. Since animal mucin molecules in saliva substitutes and host-derived antimicrobial salivary molecules exist simultaneously in whole saliva and the pellicles of patients with dry mouth, interactions may occur between these molecules. The purpose of this study was to investigate the influence of animal mucins on peroxidase activity in solution and on the surface of hydroxyapatite (HA) surfaces. The effects of animal mucins on peroxidase activity were examined by incubating porcine gastric mucin (PGM) or bovine submaxillary mucin (BSM) with either bovine lactoperoxidase (bLPO) or saliva samples. For solid-phase assays, immobilized animal mucins or peroxidase on three different HA surfaces (HA beads, HA disc, and bovine tooth) were used. Peroxidase activity was determined with an NbsSCN assay.

The obtained results were as follows:

1. PGM enhanced the enzymatic activity of bLPO in solution phase. PGM did not affect the enzymatic activity of peroxidase in saliva sample (POS).
2. BSM did not affect the enzymatic activities of both bLPO and POS in solution phase.
3. HA-adsorbed PGM increased subsequent bLPO adsorption in all three HA phases. The activity of POS was increased on both the HA beads and bovine tooth.
4. The peroxidase activities on the HA beads and disc were increased when the HA surfaces were exposed to a mixture of bLPO and PGM.
5. The binding affinity of bLPO to PGM was greater than that of bLPO to BSM.

Collectively, our results suggest that animal mucins affects the enzymatic activity of peroxidase on the HA surfaces as well as in solution. Saliva substitutes containing animal mucins may affect the function of antimicrobial components in natural saliva and saliva substitutes.

Key words: Porcine gastric mucin, Bovine submaxillary mucin, Peroxidase, Saliva

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Received: 2008-05-07
Accepted: 2008-06-20

* This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea, A050054.

Ⅰ. INTRODUCTION

Many of the protective functions of saliva can be attributed to the biological, physical, structural, and rheological characteristics of salivary glycoproteins.[1] Among the salivary glycoproteins, mucus glycoproteins, or mucins, are primarily responsible for the lubricating and film-forming properties of saliva, as well as for the oral mucosal defense.[2-5] For this reason, animal mucin-containing substitutes are preferred by patients suffering from dry mouth, as they are more beneficial than carboxymethylcellulose-containing substitutes.[6-10]

Salivary mucins play an important role in the maintenance of oral health by presenting multiple host defense functions. One such function includes concentrating antimicrobial molecules onto oral surfaces.[11] Animal mucins used for saliva substitutes, such as porcine gastric mucin (PGM) or bovine submaxillary mucin (BSM), have structural characteristics similar to salivary mucins.[12-15] As human salivary mucins, animal mucins adsorb onto the surface of hydroxyapatite (HA).[16-18] When animal mucins are bound to HA surfaces, they can increase subsequent adsorption of lysozyme.[18]

There is no doubt that pellicles formed by the selective adsorption of salivary and bacterial molecules onto tooth surfaces play a significant role in the maintenance and microbial colonization of oral surfaces. High-molecular-weight salivary mucin (MG1), IgA, α-amylase, lysozyme, peroxidase, acidic proline-rich proteins (PRPs), and cystatins have been identified as components of salivary pellicles.[19-22] Of the antimicrobial molecules identified in salivary pellicles, peroxidase is a prominent antibacterial component widely distributed in various biological fluids including saliva, tears, milk, and cervical secretions.[23] Two species of peroxidase are present in human saliva. Salivary peroxidase (SPO) is secreted by the salivary glands, whereas myeloperoxidase (MPO) emerges from leucocytes reaching the oral cavity. Peroxidase provides antimicrobial activity and protection of oral tissues from oxygen toxicity through oxidation of SCN^- and consumption of H_2O_2.[23,24] Adsorbed peroxidase molecules incorporated as pellicle components display bactericidal activity and reduce the adherence of *Streptococcus mutans* to the HA surface.[25] In patients with dry mouth, bovine lactoperoxidase, either alone or in combination with other molecules, has been incorporated in oral health care products to restore the antimicrobial capacity of saliva.[26]

The oral cavity provides an environment for molecular interactions on surfaces as well as in solution. When proteins adsorb to a surface, they undergo conformational changes.[27-29] These structural changes may occur as enzymes become immobilized onto a solid surface, an event which almost certainly results in a modification of the enzymes′ active sites.[30] Indeed, such changes are reported to induce an increase or decrease in enzymatic activities of α-amylase, lysozyme, and glucosyltransferase immobilized onto HA or enamel surfaces.[31-35]

Since animal-derived mucin and antimicrobial molecules in saliva substitutes and host-derived antimicrobial salivary molecules exist simultaneously in whole saliva and pellicles of patients with salivary hypofunction, interactions between these molecules may occur. Such interactions may modify the antimicrobial activity of the innate salivary defense molecule in distinct ways in solution or on surface phase. We have recently reported the results of interactions between animal mucins and lysozyme in solution and on HA surfaces.[18] However, the results of the study had limited value because of differences in surface characteristic between tooth mineral and synthetic HA[36,37] and limitations caused by bead-shaped HA surfaces in substrate exposure to immobilized lysozyme on the surfaces.

In the present study, to overcome these limitations of previous studies, we have investigated the effects of PGM and BSM on peroxidase activity in solution and on HA surfaces using HA beads, HA disc, and bovine tooth.

Ⅱ. MATERIALS AND METHODS

1. Collection of saliva

Saliva was collected from ten healthy non-smokers (5 males and 5 females, mean age 29.4±5.5 yrs) for solution phase assays and from three healthy non-smokers (28-, 31-, and 41-year-old males) for surface phase assays. Saliva was collected between 8:00 a.m. and 12:00 a.m. to minimize variability in salivary composition. The participants refrained from eating, drinking, and tooth brushing for at least 2 h before saliva collection. Unstimulated whole saliva was collected for 10 min by spitting. Saliva was placed in a chilled centrifuge tube and centrifuged at 3,500 xg for 15 min at 4℃. The resulting clarified supernatant fluid was used immediately for assays. The research protocol was approved by the Institutional Review Board of the University Hospital (#CRI 06008).

2. Animal mucins and peroxidase

Porcine gastric mucin (PGM, type III, Sigma-Aldrich, St Louis, MO, USA) and bovine submaxillary mucin (BSM, Sigma-Aldrich, St Louis, MO, USA) were used. PGM and BSM were dissolved in phosphate-buffered saline (PBS). Bovine lactoperoxidase (bLPO, Sigma-Aldrich, St Louis, MO, USA) dissolved in PBS, as well as clarified whole saliva samples, served as peroxidase sources.

3. Solid-phase

Five milligrams of ceramic HA beads (Macro-prep, HA type I, Bio-Rad, Hercules, CA, USA), HA disc (0.5 inch in diameter x 0.05 inch in thickness, Clarkson Chromatography Products Inc., South Williamsport, PA, USA), and bovine tooth (second, third, and fourth incisors) were used as solid phases in each assay. The bovine tooth was polished with a rubber cup and fine-grade pumice after use and incubated with PBS.

4. Peroxidase activity in solution and on HA surface

Peroxidase activity was determined by measuring the rate of oxidation of 5-thio-2-nitrobenzoic acid (Nbs) to 5,5-dithiobis(2-nitrobenzoic acid) $(Nbs)_2$ by $OSCN^-$ ions generated during the oxidation of SCN^- by bLPO and peroxidase in saliva (POS).[38] The POS activity includes the activity of both SPO and MPO. Peroxidase activity was expressed as units/mL for the solution assay and total units for the surface assay. PBS was used as a blank for the solution sample, while equal amounts of HA beads, HA disc, and bovine tooth incubated with PBS were used as surface blank samples.

5. Influence of animal mucins on peroxidase activity in solution phase

The effects of animal mucins on peroxidase activity in solution phase were examined by incubating 500 μL of PGM or BSM (each to final concentrations of both 0.5 and 1.0 mg/mL) with 500 μL of bLPO (final concentration 12.5 μg/mL) or clarified salivary samples for 10 min at room temperature (RT). To 300 μL of reaction mixture for NbsSCN assay, 15 μL of KSCN (final concentration of 4.2 mM SCN^-) and 15 μL of sample solution were added, and reaction was initiated by the addition of 15 μL of H_2O_2 (final concentrations were 50 μM for bLPO and 100 μM for POS). An incubated mixture of buffer with either bLPO or clarified salivary sample was used as a control. For the blank reaction, an incubated mixture of PGM or BSM with buffer, or an incubated buffer alone was used. The effect of the animal mucin structural integrity on peroxidase activity was also investigated. Animal mucins in solution were denatured by boiling for 10 min and used for assays.

6. Peroxidase activity on different HA surfaces

For surface phase assays, the experimental conditions for each different HA surface were

adjusted such that the peroxidase activities of surface samples would be within detectable ranges. Five milligrams of HA beads, equilibrated with three PBS washes, were incubated with 500 μL bLPO solution (5.0 μg/mL) or clarified salivary samples for 30 min at RT. The supernatant parts were transferred to fresh vials, and the HA beads were washed three times with PBS to remove unbound molecules. Peroxidase activities of the starting bLPO solutions or salivary samples, their supernatant fluids, and HA pellets were then analyzed. For HA pellets, 1.0 mL of reaction mixture for NbsSCN assay and 50 μL of KSCN (final concentration of 4.8 mM SCN^-) were used, and addition of 50 μL of H_2O_2 (final concentration 100 μM) initiated the HA-adsorbed peroxidase to catalyze the substrate.

For HA disc and bovine tooth (the fourth incisors), a 24 well plate containing 2.0 mL of 3.13 μg/mL (3.0 mL of 4.17 μg/mL for bovine tooth) bLPO solution or 2.0 mL of clarified salivary samples (3.0 mL for bovine tooth) was used. A HA disc was suspended in a well of the 24 well plate by a device with two clasp-shaped holding loops made with stainless steel orthodontic wire (standard round, size 0.020 inch, 3M Unitek, Monrovia, CA, USA). The bovine tooth crown was suspended in the 24 well plate by inserting a wire mesiodistally through a hole made below the cementoenamel junction. The same volumes and concentrations of reaction mixture, KSCN, and H_2O_2 that were used for the HA beads were used for the HA disc. In the case of the bovine tooth, the volumes of reaction mixture, KSCN, and H_2O_2 were doubled in order to sufficiently soak the bovine tooth.

7. Influence of animal mucins on surface phase peroxidase activity

To determine the influence of animal mucins on the enzymatic activity of peroxidase adsorbed to HA surfaces, experiments were performed in three ways; 1) the effects of adsorbed animal mucins on subsequent adsorption of peroxidase, 2) the effects of animal mucins on the activity of adsorbed peroxidase, and 3) HA-adsorbed peroxidase activity after pre-incubation with animal mucins.

First, to examine the effects of animal mucins on the adsorption or subsequent activity of peroxidase when present on HA surfaces, solid phases (5 mg of HA beads, HA disc, and the bovine fourth incisors) were coated with animal mucins (1.0 mg/mL, 500 μL for HA beads, 2.0 mL for HA disc, 3.0 mL for bovine tooth) for 30 min at RT. After coating, the HA solid phases were washed 5 times with PBS. The animal mucin-coated HA solid phases were then incubated with bLPO (500 μL of 5.0 μg/mL for HA beads, 2.0 mL of 3.13 μg/mL for HA disc, 3.0 mL of 4.17 μg/mL for bovine tooth) or equal volumes of clarified whole saliva samples for 30 min at RT. Unbound bLPO or salivary molecules were removed with 5 PBS washes. The HA solid phases were used for peroxidase assay as described above. The peroxidase activities of these samples were compared with those of the bare HA sample surfaces coated with bLPO or clarified whole saliva. Second, to examine the effects of animal mucins on HA adsorbed peroxidase enzymatic activity, HA solid phases (HA beads, disc, and the bovine third incisors) were coated with bLPO or clarified whole saliva samples for 30 min at RT, and then washed 5 times with PBS. The coated HA solid phases were subsequently incubated with animal mucins for 30 min at RT. Unbound animal mucins were removed by washing the beads 5 times with PBS. The peroxidase activities of these samples were compared with those of the HA samples coated with bLPO or clarified whole saliva on to bare surfaces and washed in the same manner as described above.

Third, to examine the effects of pre-incubation of animal mucins with peroxidase on the adsorption or subsequent activity of peroxidase on HA surface, animal mucins were incubated with bLPO solution or clarified whole saliva samples for 10 min at RT. HA solid phases (HA beads, disc, and the bovine second incisors) were incubated with the mixture for 30 min at RT and then washed 5 times with PBS to remove any unbound molecules. The peroxidase

activities of these samples were compared with those of the HA samples coated with the pre-incubated mixture of bLPO or clarified whole saliva with buffer.

8. Binding of peroxidase to animal mucins

Each microtiter well was coated with 100 μL of animal mucins (100 μg/mL) dissolved with PBS. The plate was then incubated for 1 h at 37℃. The wells were aspirated and washed 3 times with 200 μL of PBS with 0.02% Tween 20. Unoccupied binding sites were blocked with 1% bovine serum albumin in 300 μL for 30 min at 37℃. Volumes of 100 μL of bLPO, dissolved in blocking buffer and serially diluted from 25.0 μg/mL to 3.1 μg/mL, were dispensed into each well and the plate was then incubated for 1 h at 37℃. Next, the wells were aspirated and washed 3 times. Volumes of 100 μL of sheep anti-bLPO horse radish peroxidase conjugate (Abcam, Cambridge, UK) diluted 1:5000 with blocking buffer were added to each well and the plate was incubated for 1 h at 37℃. After 3 washes, 100 μL of 3,3′,5,5 tetramethylbenzidine substrate solution (Sigma-Aldrich, St Louis, MO, USA) was added to each well. Reactions were terminated by the addition of 100 μL of 1 N HCl. Absorbance values were determined at 450 nm. The mean absorbencies from wells with no animal mucin and no bLPO were subtracted from the experimental values for background correction. All experiments were duplicated and performed 4 times.

9. Statistics

The Wilcoxon signed rank test was used to analyze the effects of animal mucins compared with their controls. *P*-values less than 0.05 were considered statistically significant.

Ⅲ. RESULTS

1. Influence of animal mucins on peroxidase activity in solution phase

The effects of PGM at 0.5 mg/mL on bLPO and POS were negligible. PGM at 1.0 mg/mL enhanced the enzymatic activities of bLPO in solution phase ($P < 0.01$). PGM did not affect POS activity; however, BSM did not affect the enzymatic activities of both bLPO and POS in solution. In contrast with intact PGM, boiled PGM showed inhibitory effects on both bLPO and POS (Fig. 1).

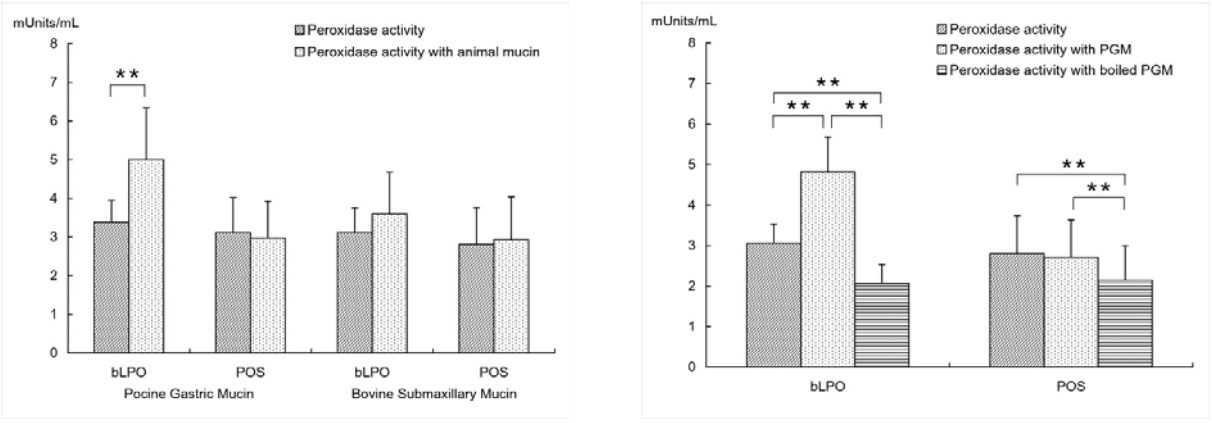

Fig. 1. Influence of animal mucins on peroxidase activity in solution phase. Porcine gastric mucin (PGM) at 1.0 mg/mL enhanced bLPO activity in solution phase. Both animal mucins did not affect POS activity. Boiled PGM inhibited both bLPO and POS activities in solution phase. The assay was performed 10 times for bLPO and POS.
bLPO, bovine lactoperoxidase; POS, peroxidase in saliva
** $P < 0.01$

Table 1. Enzymatic activities of bovine lactoperoxidase and peroxidase in saliva on different hydroxyapatite surfaces

mUnits	Bovine lactoperoxidase n = 7	Peroxidase in saliva n = 9
Hydroxyapatite beads	0.067 ± 0.014	0.071 ± 0.021
Hydroxyapatite disc	0.054 ± 0.015	0.015 ± 0.006
Bovine tooth	0.037 ± 0.013	0.029 ± 0.011

2. Peroxidase activity on different HA surfaces

Under the experimental conditions indicated previously, both bLPO and POS showed detectable enzymatic activity on the three different HA surfaces. Considering the relative surface areas of the solid phases, HA beads did not show significant enzymatic activity compared with HA disc and bovine tooth (Table 1).

3. Influence of animal mucins on peroxidase activity on surface phase

1) Effect of adsorbed animal mucins on peroxidase activity

Adsorbed PGM enhanced the adsorption and subsequent enzymatic activity of bLPO and POS on the surfaces of HA beads and bovine tooth. In the case of the HA disc, the adsorbed PGM enhanced enzymatic activity of bLPO only. The adsorbed BSM did not affect the activity of bLPO and POS (Fig. 2).

2) Effect of animal mucins on adsorbed peroxidase

Both PGM and BSM did not affect the enzymatic activities of immobilized bLPO and POS on the three different kinds of HA surfaces (Data not shown).

3) Effect of pre-incubation of animal mucins with peroxidase on surface peroxidase activity

When the HA surfaces were exposed to the pre-incubated mixture of PGM and bLPO or

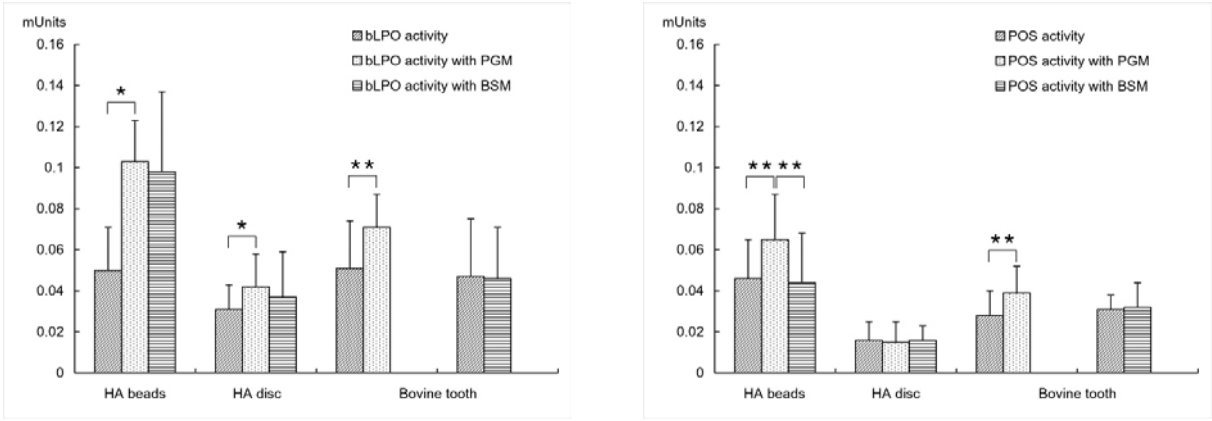

Fig. 2. Effects of hydroxyapatite (HA)-adsorbed animal mucins on peroxidase activity. Adsorbed PGM enhanced adsorption and subsequent enzymatic activity of bLPO on the surfaces of HA beads, HA disc, and bovine tooth. Adsorbed PGM enhanced POS activity on the surfaces of HA beads and bovine tooth. The assay was performed 6 times for bLPO and 9 times for POS.
PGM, porcine gastric mucin; BSM, bovine submaxillary mucin; bLPO, bovine lactoperoxidase; POS, peroxidase in saliva
* $P < 0.05$, ** $P < 0.01$

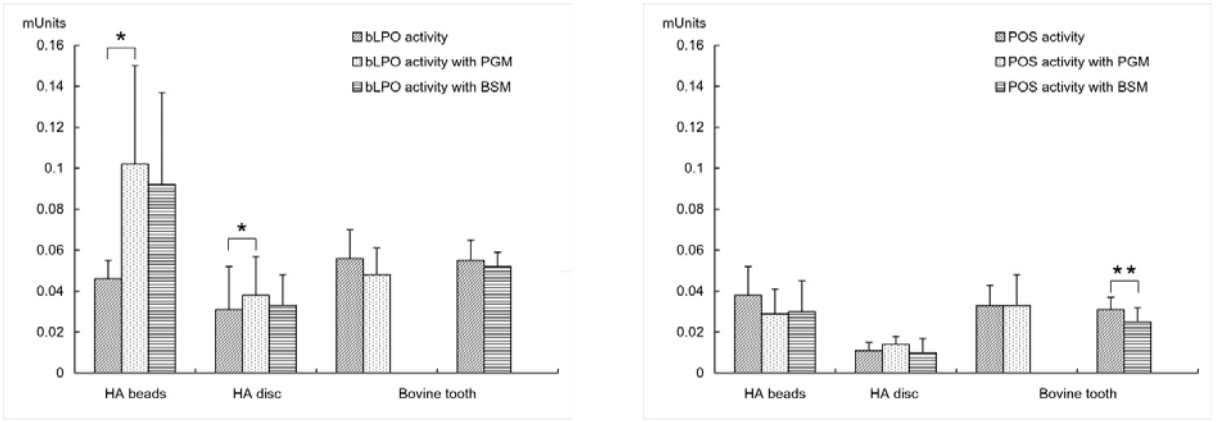

Fig. 3. Effects of pre-incubation of animal mucins with peroxidase on hydroxyapatite (HA) surface peroxidase activity. The enzymatic activities of immobilized bLPO on HA beads and HA disc were significantly increased. The immobilized POS activity on HA surfaces was not affected. In the case of BSM, immobilized POS activity on bovine tooth was decreased. The assay was performed 6 times for bLPO and 9 times for POS.
PGM, porcine gastric mucin; BSM, bovine submaxillary mucin; bLPO, bovine lactoperoxidase; POS, peroxidase in saliva
$^{*}P < 0.05$, $^{**}P < 0.01$

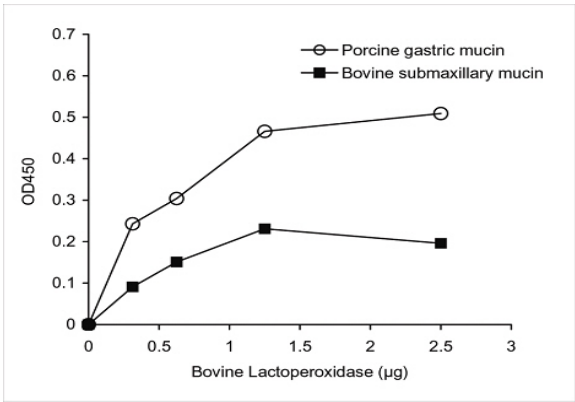

Fig. 4. Binding of bLPO (bovine lactoperoxidase) to animal mucins immobilized on a microtiter plate. The assay was performed 4 times.

clarified whole saliva, the enzymatic activity of immobilized bLPO on HA beads and HA disc was significantly increased; however, immobilized POS activity was not affected. In the case of BSM, immobilized POS activity on the bovine tooth was decreased (Fig. 3).

4. Binding of bLPO to animal mucins

Using a PGM- or BSM-coated on a microtiter plate, we found that the affinity of bLPO to PGM was greater than that of bLPO to BSM (Fig. 4).

Ⅳ. DISCUSSION

Animal mucins affected the enzymatic activity of peroxidase both in solution and on HA surfaces. Binding of bLPO to PGM- or BSM-coated microtiter plates suggested the existence of an animal mucin-peroxidase complex molecule. Previous studies have shown that mucins form heterotypic complexes with other salivary molecules, such as secretory IgA, lysozyme, cystatins, α-amylase, PRPs, statherins, histatins, lactoferrin, and agglutinin.[12,39-45] Although the differing effects of PGM and BSM on peroxidase activity could not be fully explained by the varied structures of the two animal mucins,[13-15,46] PGM, rather than BSM, affected peroxidase activity as well as lysozyme activity.[18]

The enhancement of peroxidase activity by PGM in solution could be explained by the typical

characteristics of mucins. The bLPO and/or substrate could be concentrated in the mucin network with viscoelastic properties, and the bLPO activity could be expressed as an enhanced value. It has been theorized that the boiling step may cause heat degradation and/or aggregation of animal mucin molecules which leads to abrupt change in viscoelastic properties of animal mucin solution and lose normal biological function.[18,47] Differences in the effects of PGM on bLPO and POS in solution could be explained by complex interactions between PGM and various salivary molecules in natural saliva. To substantiate these conjectures, the interactions of animal mucins with major salivary molecules require further studies.

Strong affinity of animal mucins to HA has been reported in previous studies. The amount of adsorbed PGM was nearly equal to that of BSM.[16,18] The enhancement of peroxidase activity in conjunction with a PGM adsorbed surface appears to be due to an increased amount of peroxidase adsorption compared to bare HA surface. Considering the fact that immobilized peroxidase retains 75% of its native activity,[48] the quantity of peroxidase molecules adsorbed on PGM-immmobilized HA surface was more than the increase of peroxidase activity. The low binding affinity of bLPO to the BSM-immobilized microtiter plates was consistent with a subtle increase or no change of peroxidase activity on a BSM- relative to a PGM-adsorbed HA surface. The increase of enzymatic activity on the HA surface after exposure to a mixture of pre-incubated PGM and bLPO could be explained by increased adsorption of the PGM-peroxidase complex to the HA surface. The difference between bLPO and POS could be explained with the same reasoning in the case of solution assay. Differences in the physical and chemical composition of the surface between tooth mineral and synthetic HA may explain the differences in the results according to different solid phases.[36,37] Indeed, the composition of pellicle is also affected by the availability of surface area as well as the amounts of various salivary molecules with different affinity to HA surfaces.[36] Therefore, different relationships between the volume of saliva and the HA surface area in three different solid phases could also explain the differences in the results corresponding to different solid phases.

There are several issues to be considered before the results of the present study can be extrapolated to an *in vivo* situation. It has been reported that the formation of the *in vitro* pellicle does not completely mirror what occurs in the mouth.[30,36,37,49] A specific whole saliva sample used to form an *in vitro* pellicle comprises a closed system, whereas the oral environment is an open system in which there is a constant influx and clearance of oral fluids.[37] Salivary proteins are incorporated into multi-layers in the pellicle.[34,50-52] Peroxidase molecules immobilized on HA beads might not be fully exposed to the substrate compared with those on the HA disc and bovine tooth. It was also suggested that immobilized enzymes in the pellicle tend to desorb spontaneously in reagents used for determination of enzyme activity.[27,34,53] Despite an *in vitro* nature of our study, the results of the present study provide valuable information on the effects of animal mucin components on antimicrobial activities in oral health care products and possibly in the oral cavity.

Collectively, our results suggest that the pattern of interactions between animal mucins and bLPO or POS is different between in solution and on the HA surfaces. The presence of animal mucins affects the enzymatic activity of peroxidase on the HA surfaces as well as in solution. Saliva substitutes containing animal mucins may affect the function of antimicrobial components in natural saliva and saliva substitutes, and the composition of salivary pellicle on tooth surfaces.

V. CONCLUSIONS

Animal mucins have structural characteristics similar to salivary mucins. Animal mucins have been regarded as suitable substances for saliva substitutes. Since animal mucin molecules in saliva

substitutes and host-derived antimicrobial salivary molecules exist simultaneously in whole saliva and the pellicles of patients with dry mouth, interactions may occur between these molecules. The purpose of this study was to investigate the influence of animal mucins on peroxidase activity in solution and on the surface of hydroxyapatite (HA) surfaces. The effects of animal mucins on peroxidase activity were examined by incubating porcine gastric mucin (PGM) or bovine submaxillary mucin (BSM) with either bovine lactoperoxidase (bLPO) or saliva samples. For solid-phase assays, immobilized animal mucins or peroxidase on three different HA surfaces (HA beads, HA disc, and bovine tooth) were used. Peroxidase activity was determined with an NbsSCN assay.

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4. The peroxidase activities on the HA beads and disc were increased when the HA surfaces were exposed to a mixture of bLPO and PGM.
5. The binding affinity of bLPO to PGM was greater than that of bLPO to BSM.

Collectively, our results suggest that animal mucins affects the enzymatic activity of peroxidase on the HA surfaces as well as in solution. Saliva substitutes containing animal mucins may affect the function of antimicrobial components in natural saliva and saliva substitutes.

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국문요약

동물성 Mucin이 용액상태와 Hydroxyapatite표면에서 Peroxidase 활성에 미치는 영향에 관한 연구

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동물성 mucin은 인체 타액 mucin과 유사한 구조적 특성을 가지고 있으므로 효과적인 타액대체제의 개발에 적합한 성분으로 여겨져 왔다. 구강건조증 환자가 동물성 mucin 함유 타액대체제를 사용할 경우, 동물성 mucin과 인체 타액에 존재하는 항균 단백질이 용액상태인 전타액과 구강표면에 형성된 pellicle에 동시에 존재할 수 있으므로 이들 물질사이에 상호작용이 일어 날 수 있을 것이다. 본 연구의 목적은 용액과 hydroxyapatite (HA) 표면에서 동물성 mucin이 peroxidase 활성에 미치는 영향을 평가하기 위하여 시행되었다. 동물성 mucin이 peroxidase 활성에 미치는 영향은 돼지의 위장 mucin (porcine gastric mucin, PGM) 이나 소의 악하선 mucin (bovine submaxillary mucin, BSM)을 소의 lactoperoxidase (bovine lactoperoxidase, bLPO)나 타액검체와 incubation하는 방법을 사용하여 분석하였고, 표면상태에서의 연구를 위해 HA beads, HA disc, 소의 치아와 같은 3가지 종류의 HA 표면을 활용하였다. Peroxidase 활성은 NbsSCN 법을 이용하여 분석하였다.

1. 돼지위장 mucin은 용액상태에서 bLPO 활성을 증가시켰으나 타액검체의 peroxidase (peroxidase in saliva sample, POS) 활성에는 영향을 미치지 않았다.
2. 소 악하선 mucin은 용액상태에서 bLPO와 POS 활성에 영향을 미치지 않았다.
3. HA 표면에 부착된 돼지위장 mucin은 peroxidase의 부착과 활성을 증가시켰고 이러한 효과는 세 종류의 HA 표면 모두에서 일어났으며, POS의 활성증가는 HA beads와 소 치아 표면에서만 나타났다.
4. bLPO와 돼지위장 mucin의 혼합물을 HA 표면에 부착시킬 경우, HA beads와 HA disc 표면에서의 peroxidase 활성은 증가하였다.
5. bLPO의 돼지위장 mucin에 대한 부착친화도는 소 악하선 mucin에 비해 컸다.

이상의 결과를 종합해 볼 때 동물성 mucin은 용액상태와 HA 표면에서 peroxidase 활성에 영향을 미침을 알 수 있으며, 동물성 mucin을 포함하고 있는 타액대체제는 인체타액 및 타액대체제에 있는 peroxidase 활성에 영향을 미칠 수 있을 것이다.

주제어: 돼지 위장 mucin, 소 악하선 mucin, Peroxidase, 타액