ARCHIVES OF ORAL BIOLOGY 60 (2015) 998–1006

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

The effects of xylitol and sorbitol on lysozyme- and peroxidase-related enzymatic and candidacidal activities

Bum-Soo Kim [a], Ji-Youn Chang [a], Yoon-Young Kim [a], Hong-Seop Kho [b,*]

[a] Department of Oral Medicine and Oral Diagnosis, School of Dentistry, Seoul National University, Seoul, Republic of Korea

[b] Department of Oral Medicine and Oral Diagnosis, School of Dentistry and Dental Research Institute, Seoul National University, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Accepted 20 March 2015

Keywords:
Xylitol
Sorbitol
Lysozyme
Peroxidase
Glucose oxidase
Candidacidal activity

ABSTRACT

Objective: To investigate whether xylitol and sorbitol affect enzymatic and candidacidal activities of lysozyme, the peroxidase system, and the glucose oxidase-mediated peroxidase system.

Design: Xylitol and sorbitol were added to hen egg-white lysozyme, bovine lactoperoxidase, glucose oxidase-mediated peroxidase, and whole saliva in solution and on hydroxyapatite surfaces. The enzymatic activities of lysozyme, peroxidase, and glucose oxidase-mediated peroxidase were determined by the turbidimetric method, the NbsSCN assay, and production of oxidized o-dianisidine, respectively. Candidacidal activities were determined by comparing colony forming units using *Candida albicans* ATCC strains 10231, 11006, and 18804.

Results: While xylitol and sorbitol did not affect the enzymatic activity of hen egg-white lysozyme both in solution and on hydroxyapatite surfaces, they did inhibit the enzymatic activity of salivary lysozyme significantly in solution, but not on the surfaces. Xylitol and sorbitol enhanced the enzymatic activities of both bovine lactoperoxidase and salivary peroxidase significantly in a dose-dependent manner in solution, but not on the surfaces. Sorbitol, but not xylitol, inhibited the enzymatic activity of glucose oxidase-mediated peroxidase significantly. Both xylitol and sorbitol did not affect candidacidal activities of hen egg-white lysozyme, the bovine lactoperoxidase system, or the glucose oxidase-mediated bovine lactoperoxidase system.

Conclusions: Xylitol and sorbitol inhibited salivary lysozyme activity, but enhanced both bovine lactoperoxidase and salivary peroxidase activities significantly in solution. Xylitol and sorbitol did not augment lysozyme- and peroxidase-related candidacidal activities.

* *Corresponding author at*: Dept. of Oral Medicine and Oral Diagnosis, School of Dentistry and Dental Research Institute, Seoul National University, Yunkeun-Dong 28, Chongro-Ku, Seoul 110-749, Republic of Korea. Tel.: +82 2 2072 3989; fax: +82 2 744 9135.
E-mail address: hkho@snu.ac.kr (H.-S. Kho).

http://dx.doi.org/10.1016/j.archoralbio.2015.03.011
0003–9969/

1. Introduction

As the geriatric population expands, the prevalence of dry mouth has also increased.[1] Those with dry mouth usually experience a variety of signs and symptoms, such as difficulty in chewing and swallowing, pain in the oral mucosa, rampant dental caries, and recurrent oral candidal infections, all of which deteriorate the quality of life.[2] Artificial saliva or mouth rinses containing antimicrobials are usually recommended for the recovery of reduced antimicrobial activity.[3]

The most widely used antimicrobial host proteins are lysozyme and peroxidase, mainly of animal origin. Lysozyme provides antimicrobial activity through muramidase-, cation-, and structure-dependent mechanisms.[4,5] Peroxidase provides antimicrobial activity and prevents oral tissue damage by consuming hydrogen peroxide (H_2O_2) and producing hypothiocyanite ($OSCN^-$).[6] Peroxidase can be activated with the peroxidase system and a commercially usable form is the glucose oxidase-mediated peroxidase system.[7]

Because these antimicrobial molecules are in the same environment, interactions must occur with the components of oral health care products and human saliva.[8] These molecular interactions could occur not only in solutions like saliva, but also on surfaces like tooth. Immobilized proteins on surfaces could cause conformational changes of the compounds, potentially altering enzymatic activities.[9–11] Accordingly, the interactions on surfaces may behave in a distinct manner from those in solution. There have also been reports of interactions between antimicrobial supplements and candidate substances of artificial saliva such as animal mucins,[12,13] hyaluronic acid,[14–16] and yam tuber mucilage.[17,18] The results of interactions could be additive, synergistic, or inhibitory, and surface interactions may differ from those in solution.

Xylitol, among sugar alcohols, has particularly been included in many oral health care products because it inhibits the glycolytic pathway for bacterial growth and acid production, making it beneficial for the prevention of dental caries.[19] Xylitol and sorbitol included in artificial saliva can also alleviate dry mouth by stimulating salivary secretions.[20]

Sugar alcohols and antimicrobials could also be present in the same environment as oral health care products and the oral cavity, and so there might also be interactions among them. The consumption of a xylitol diet has been reported to increase salivary peroxidase activity,[21] but mechanism has not been reported to explain the results. Our hypotheses were there might be direct interactions between sugar alcohols and oral antimicrobials. The interactions in solution might also be different from those on hydroxyapatite surfaces. In addition, when sugar alcohols are added in artificial saliva, they might affect antifungal activities of incorporated antimicrobials, which are important for individuals with dry mouth. Our purpose of this study was to investigate the effects of xylitol and sorbitol on the enzymatic and candidacidal activities of lysozyme, the peroxidase system, and the glucose oxidase-mediated peroxidase system.

2. Materials and methods

Reagents were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA) unless stated otherwise.

2.1. Collection of human saliva

Saliva samples were collected from four healthy adult participants (two men and two women) between 8 am and 12 pm to minimize variability in salivary composition. The participants refrained from eating, drinking, and tooth brushing for at least 1 h before saliva collection. Unstimulated whole saliva was collected by the spitting method and placed in a chilled centrifuge tube to which phenylmethylsulphonylfluoride was added immediately to a final concentration of 1.0 mM. The saliva sample was centrifuged at $3500 \times g$ for 15 min at 4 °C, and the resulting clarified supernatant fluid was used immediately for assays. The research protocol was approved by the IRB of the University Hospital (#CRI13005) in 10 May 2013 and the informed consent was obtained from the participants.

2.2. Lysozyme, peroxidase, the peroxidase system, the glucose oxidase-mediated peroxidase system, and sugar alcohols

Hen egg-white lysozyme (HEWL, final concentration of 30 μg/mL) and bovine lactoperoxidase (bLPO, final concentration of 25 μg/mL) served as sources of lysozyme and peroxidase, respectively, for all experiments. For candidacidal assays, the peroxidase system included final concentrations of 25 μg/mL bLPO, 1 mM potassium thiocyanate (KSCN), and 50 μM H_2O_2. The glucose oxidase-mediated peroxidase system included final concentrations of 25 μg/mL bLPO, 1 mM KSCN, 50 μg/mL glucose oxidase from *Aspergillus niger*, and 0.03 mg/mL glucose. Among sugar alcohols, xylitol and sorbitol were used. Concentrations of xylitol and sorbitol (32.5–260 mM) used for experiments were safe and from those of commercial oral health care products. All components were dissolved in simulated salivary buffer (SSB, 0.021 M Na_2HPO_4/NaH_2PO_4, pH 7.0, containing 36 mM NaCl and 0.96 mM $CaCl_2$).[22]

2.3. Measurement of enzymatic activity

Lysozyme activity was determined by the turbidimetric method using *Micrococcus lysodeikticus* ATCC 4698 as a substrate.[23] Peroxidase activity was determined by the NbsSCN assay which measures the rate of oxidation to 5, 5′-dithiobis-2-nitrobenzoic acid by hypothiocyanite.[24] The enzymatic activity of glucose oxidase-mediated peroxidase was evaluated with a glucose assay kit that measured oxidized o-dianisidine production. Experiments for enzymatic activities of lysozyme and peroxidase were performed six times in duplicate. For whole saliva, saliva samples were collected twice in different days from four participants and experiments were performed eight times in duplicate. Experiments for enzymatic activities of glucose oxidase-mediated peroxidase were performed eight times in duplicate.