J Med Dent Sci 2010; 57: 25–33

Original Article

Relationship between Oral Malodor and Glycosylated Salivary Proteins

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Volatile sulfur compounds (VSCs), which are major sources of oral malodor, are produced by putrefactive activities of bacteria. Saliva provides easily degradable protein substrates, and most proteins are glycosylated. We hypothesized that oral malodor would be associated with enhanced proteolysis or deglycosylation in saliva. The purpose of this study was to evaluate properties of glycoproteins in saliva and assess their association with VSC levels. Subjects were 88 patients who visited "the Fresh Breath Clinic", Dental Hospital, Tokyo Medical and Dental University. They were classified into malodor (n = 67) and non-malodor (n = 21) groups. After collecting saliva, the amounts of the total proteins and carbohydrate were determined. Molecular size distributions of salivary proteins/glycoproteins were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The amount of the total salivary proteins was significantly higher in the malodor group. Major proteins/glycoproteins observed in SDS-PAGE analyses showed similar distributions between the two groups. In the malodor group, the salivary protein concentrations were positively correlated with the CH_3SH levels ($p < 0.05$), and the carbohydrate contents were negatively correlated with the H_2S levels ($p < 0.05$). These results indicated the possibility that salivary proteins/glycoproteins might be related to the malodor generation.

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Received September 17 ; Accepted November 13, 2009

Key words: oral malodor, volatile sulfur compounds, resting whole saliva, glycoprotein, SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Introduction

Oral malodor or bad breath is now a concern of millions of people[1]. The oral cavity is the origin of malodor in nearly 90% of cases[2], although there are some extra oral causes of malodor. Oral malodor is closely associated with disorders such as periodontal diseases, poor oral hygiene, tongue coating, dry mouth, dental caries, oral carcinoma, and several forms of stomatitis.

Metabolic products of anaerobic bacteria within the oral cavity are the major causes of oral malodor. They degrade sulfur-containing amino acids such as cysteine and methionine into the foul smelling volatile sulfur compounds (VSCs), namely hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide ($(CH_3)_2S$). VSCs are mainly produced through the putrefactive activities of bacteria in saliva, in the gingival crevice, and on the tongue surface[3]. Saliva is mixed together with bacteria and shed epithelial cells[4]. Therefore, incubation of whole saliva *in vitro* at body temperature can result in the generation of a malodor similar to the VSCs of mouth air[5].

Most of the salivary proteins available in the oral cavity are glycoproteins, whose carbohydrate components could generally interfere with their proteolysis[6]. Therefore, removal of carbohydrate side chains might make them more susceptible to proteolysis[6]. Cleavage of the carbohydrate side chains is catalyzed by a series of glycosidic enzymes, whose

types and activities vary depending on bacterial species and some of their activities are reported to be correlated with oral malodor[7].

We hypothesized that deglycosylation of salivary glycoproteins accelerates the generation of VSCs, and accordingly degraded or reduced amounts of glycoproteins may be observed in the saliva of patients with oral malodor. The purpose of this study was to evaluate properties of glycoproteins in saliva and assess their association with VSC levels.

Materials and Methods

Subjects

Subjects were sequentially selected from 385 patients who visited "the Fresh Breath Clinic", Dental Hospital, Tokyo Medical and Dental University from June, 2007 to March, 2008. Subjects who were on medication or had been diagnosed as having any systemic disease were excluded. Furthermore, those with salivary flow rate lower than approximately 0.06 mL/min were excluded in order to ensure sufficient amount of saliva for completing biochemical analyses. Finally, 88 subjects (33 males and 55 females) were selected. The study protocol was approved by the Ethics Committee for Human Research, Tokyo Medical and Dental University (No. 270), and subjects enrolled in the study received verbal and written information about the study, and signed an informed consent form.

Assessment of oral malodor

Subjects were asked to follow pre-assessment instructions before the oral malodor assessment in order to avoid confounding odors and to maximize oral VSC levels[8]. The measurement was conducted between 9:00 and 11:00 AM. Subjects were instructed to close their mouths for 3 minutes in an upright chair position prior to each assessment and breathe through their nose during the measurements.

Organoleptic test (OT)

The OT score was determined by two trained dentists. They were trained to describe malodor by referring to an odor solution kit for measuring the olfactory sense (T&T Olfactometer, Daiich Yakuhin Sangyo Co., Tokyo, Japan)[9]. Judges subjectively rated the strength of the odor on a scale of 0 to 5 based on Rosenberg's criteria[10]. If the two judges gave different scores, a mean score was used as the representative score for that subject.

Gas chromatography (GC)

The GC analysis of mouth air was carried out using a GC-8A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame photometric detector. The chromatographic conditions followed the criteria described previously[11]. The Teflon tube connected to the auto-injector was inserted into the center of the oral cavity between the lips and teeth while the mouth remained closed. Following aspiration of 10 mL of mouth air with a syringe connected to the outlet of the auto-injector, the sample air was transferred to the column and chromatographed. Results were shown as the concentrations (ng/10 mL) of H_2S, CH_3SH, and $(CH_3)_2S$.

Oral examination

Dental status including teeth present and decayed teeth of the subjects was recorded. Periodontal status was evaluated by exploring all gingival margins of each tooth with a manual periodontal probe, PCP UNC15 Hu-Friedy (Hu-Friedy Mfg. Co., Inc., Illinois, USA), and the deepest pocket depth was recorded. The numbers of periodontal pockets 4 mm or greater and 6 mm or greater were counted. Gingival bleeding was assessed by recording the presence of bleeding sites of the tooth after periodontal probing. The percentage of teeth with bleeding sites was calculated. Oral hygiene of six selected teeth was assessed using the plaque index of Silness and Loe[12]. The average score of the four surfaces of the selected teeth was calculated. The area and the thickness of the tongue coating were determined by visual inspection. They were recorded as a score of 0 to 3 based on the evaluation criteria[11,13].

Assessment of saliva

Collection of resting whole saliva

Before the oral examination, resting whole saliva samples were collected. Saliva was collected for 5 minutes into a pre-weighed paper cup and re-weighed. Saliva samples were frozen and stored at − 80℃ until protein analyses.

Quantification of total salivary proteins and carbohydrate content

Saliva samples were clarified by centrifugation at 600 × g for 10 minutes at 4℃. The amount of total proteins in saliva was determined spectrophotometrically at 562 nm with a BCA Protein Assay Kit (Pierce Biotechnology, Inc., Rockford, Illinois, USA). Carbohydrate content in salivary glycosylated proteins

was determined spectrophotometrically at 550 nm with a Glycoprotein Carbohydrate Estimation Kit (Pierce Biotechnology, Inc., Illinois, USA).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Each sample was reconstituted in two protein concentrations (10 μg/15 μL and 50 μg/10 μL) in sodium dodecyl sulfate (SDS) sample buffer and subjected to SDS-polyacrylamide gradient (4 - 20%) gel (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) electrophoresis (SDS-PAGE) according to the Laemmli method[14]. Samples in two different protein concentrations were run simultaneously in separated gels using the same apparatus. Molecular weight standards (Bio-Rad Laboratories, Inc., Hercules, California, USA) were used in all electrophoresis. After the electrophoresis, one gel (samples with 10 μg/15 μL protein concentration) was stained for proteins using GelCode Blue (Pierce Biotechnology, Inc., Illinois, USA) and the other (samples with 50 μg/10 μL protein concentration) for glycosylated proteins by GelCode Glycoprotein Staining Kit (Pierce Biotechnology, Inc., Illinois, USA). After staining, gels were scanned with a color image scanner (Seiko Epson Corporation, Nagano, Japan), and digital image analyses were done with the Scion Image Program (Scion Corporation, Frederick, Maryland, USA). The program provided the band densities in 256 gray scale values.

Statistical analysis

Based on the olfactory threshold levels ($H_2S \geq 1.5$ ng, $CH_3SH \geq 0.5$ ng, and $(CH_3)_2S \geq 0.3$ ng in 10 mL of mouth air)[15] and OT scores (score ≥ 2), subjects were classified into the malodor and non-malodor groups. The malodor group consisted of subjects whose VSC levels surpassed one or more of the threshold criteria or the OT score was 2 or greater. Chi-square test was used to analyze any difference between the distributions of male/female to the malodor/non-malodor groups. Inter-evaluator variation for OT scores between two judges was assessed by Cohen's kappa statistics. The Mann-Whitney U test was used to analyze the difference of tongue coating (area and thickness) and the plaque index between the two groups. Student's t-test was used for the analyses of the mean differences of other results. Pearson correlation coefficients were used to explore the association between amounts of salivary proteins vs. carbohydrates, amounts of salivary proteins vs. VSC levels, and carbohydrate contents vs. VSC levels. Because of the non-normal distribution of VSCs, the amounts of proteins, and carbohydrate contents, logarithmic transformation was applied to these values before the calculation of the t-test and correlation coefficients. The distributions of those values after logarithmic transformation were diagnosed as normal with the Kolmogorov-Smirnov test. Data were analyzed using the Statistical Package for Social Science (version 11, Tokyo, Japan). P values less than 0.05 were considered statistically significant.

Results

Clinical characteristics of the subjects

Among the 88 subjects, 67 were classified into the malodor group (26 males and 41 females) and the others into the non-malodor group (7 males and 14 females). Differences of the average age and the male/female ratio of the two groups were negligible. Cohen's kappa statistics value, between the two dentists for the OT score, was 0.62.

The average VSC (H_2S, CH_3SH, and $(CH_3)_2S$) levels and OT scores are shown in Table 1. Table 2 indicates average oral conditions of the subjects. In malodor group, the results were higher in the number of decayed teeth ($p < 0.05$), the plaque index ($p < 0.05$), and tongue coating (area, $p < 0.001$; thickness, $p < 0.001$). No statistically significant difference was found in the number of teeth present and periodontal conditions between the two groups.

Results of salivary analyses

In the malodor group, flow rate of saliva and the concentration of salivary proteins were higher than those of the non-malodor group ($p < 0.001$, $p < 0.05$, respectively) (Table 3). Carbohydrate contents in saliva (mg/mL), in salivary proteins (mg/mg protein), flow rate of carbohydrates (mg/min) were 0.28 ± 0.02 (mean ± SEM), 0.16 ± 0.00, and 0.07 ± 0.01 in the malodor group, and 0.22 ± 0.03, 0.15 ± 0.00, and 0.09 ± 0.01 in the non-malodor group, respectively. No statistically significant difference was observed in carbohydrate contents in saliva. In both groups, the amounts of carbohydrate in saliva were correlated with the amount of total salivary proteins (malodor group, $r = 0.69$, $p < 0.01$; non-malodor group, $r = 0.87$, $p < 0.01$) (Figure 1).

In the malodor group, the amount of total salivary protein was correlated with CH_3SH levels ($r = 0.31$, $p < 0.05$) (Figure 2). The amount of total salivary protein was also positively, but not statistically significantly, correlated with H_2S ($r = 0.15$) and $(CH_3)_2S$ ($r = 0.13$) levels. In addition, the carbohydrate contents in proteins were correlated with H_2S levels ($r = -0.26$, $p < 0.05$)

Table 1. Results of oral malodor assesssment
Subjects were classified into the malodor group when the VSC levels by GC surpassed one or more of the threshold criteria or the OT score was 2 or greater.

Parameters	*Malodor (n = 67) mean ± SD*	*Non-malodor (n = 21) mean ± SD*
Age	42.3 ± 11.8	39.0 ± 11.1
Gas chromatography		
H_2S (ng/10 mL)	6.7 ± 7.3	0.8 ± 0.4
CH_3SH (ng/10 mL)	2.9 ± 4.7	0.2 ± 0.2
$(CH_3)_2S$ (ng/10 mL)	0.6 ± 0.5	0.1 ± 0.1
Organoleptic test score	2.0 ± 0.6	1.2 ± 0.3

Table 2. Clinical characteristics of the malodor and non-malodor groups
Subjects were classified into the malodor group when the VSC levels by GC surpassed one or more of the threshold criteria or the OT score was 2 or greater.

Parameters	*Malodor (n = 67) mean ± SD*	*Non-malodor (n = 21) mean ± SD*	*P value*
Age	42.3 ± 11.8	39.0 ± 11.1	0.249
Oral conditions			
Number of teeth present	26.7 ± 1.8	26.0 ± 2.5	0.126
Number of decayed teeth	0.1 ± 0.4	0.4 ± 0.6	0.017
Plaque index	0.5 ± 0.4	0.3 ± 0.4	0.021
Number of teeth with periodontal pockets ≥ 4mm	1.5 ± 2.8	1.3 ± 2.6	0.724
Number of teeth with periodontal pockets ≥ 6mm	0.2 ± 0.8	0.0 ± 0.0	0.115
% of teeth with bleeding sites	20.9 ± 18.8	11.8 ± 18.8	0.066
Area of tongue coating	2.3 ± 0.7	1.6 ± 1.0	< 0.001
Thickness of tongue coating	1.8 ± 0.7	1.0 ± 0.6	< 0.001

Table 3. Results of salivary analyses of the malodor and non-malodor groups

Parameters	*Malodor (n = 67) mean ± SEM*	*Non-malodor (n = 21) mean ± SEM*	*P value*
Saliva			
Flow rate of saliva (mL/min)	0.30 ± 0.03	0.48 ± 0.07	< 0.001
Salivary proteins			
Concentration of proteins (mg/mL)	1.89 ± 0.13	1.45 ± 0.16	0.047
Flow rate of proteins (mg/min)	0.49 ± 0.04	0.60 ± 0.08	0.154
Salivary glycoproteins			
Carbohydrate contents in saliva (mg/mL)	0.28 ± 0.02	0.22 ± 0.03	0.081
Carbohydrate contents in salivary proteins (mg/mg protein)	0.16 ± 0.00	0.15 ± 0.00	0.712
Flow rate of carbohydrates (mg/min)	0.07 ± 0.01	0.09 ± 0.01	0.172

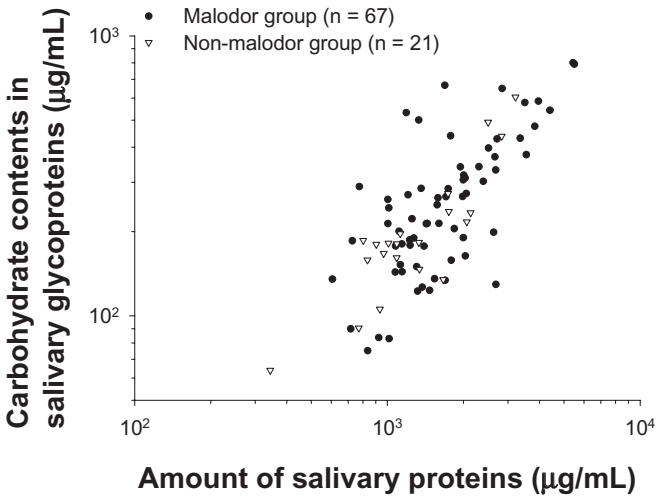


Figure 1 : Relationship between concentrations of salivary proteins and carbohydrates shown in logarithmic coordinates (malodor group, ● ; non-malodor group, ▽). Significant correlations were observed (malodor group, $r = 0.69$, $p < 0.01$; non-malodor group, $r = 0.87$, $p < 0.01$).

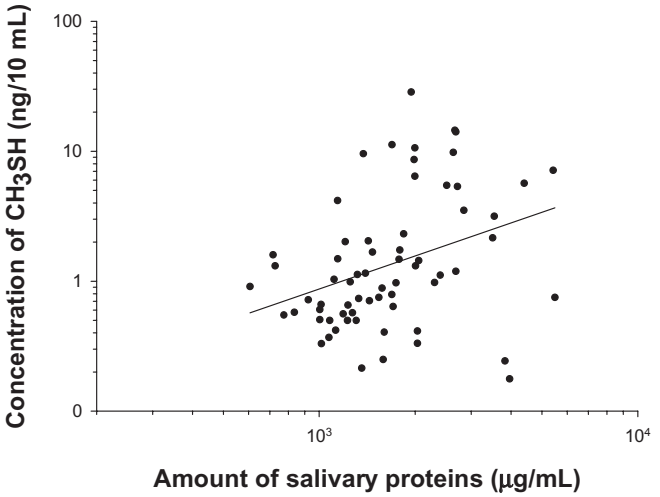


Figure 2 : Relationship between concentrations of salivary proteins and CH_3SH levels in the malodor group (n = 67). A regression line was evaluated ($r = 0.31$, $p = 0.01$).

(Figure 3). The carbohydrate contents in proteins was also negatively, but not statistically significantly, correlated with CH_3SH ($r = -0.19$) and $(CH_3)_2S$ ($r = -0.20$) levels. These statistically significant correlations were only observed in the malodor group.

Results of SDS-PAGE

A representative SDS-PAGE pattern of salivary proteins identified 9 major bands, and that of salivary glycoproteins identified 7 major bands (Figure 4). Figure

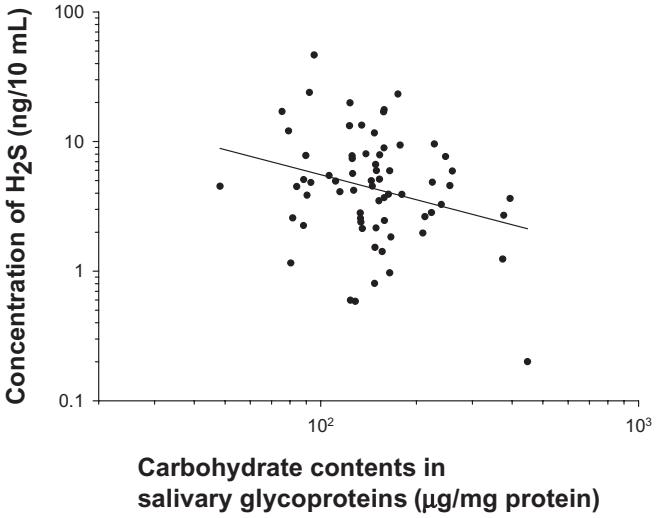


Figure 3 : Relationship between carbohydrate contents and H_2S levels in the malodor group (n = 67). A regression line was evaluated ($r = -0.26$, $p = 0.03$).

5 shows the differences of mean amount of salivary proteins and carbohydrates in the bands identified by the SDS-PAGE analyses. Protein content in band 5 (apparent molecular weight 56 kDa) was higher in the malodor group ($p < 0.05$) (Fig 4a, 5a). Any statistically significant difference was not observed in carbohydrate contents in bands of glycoproteins (Figure 4b, 5b).

Discussion

Proteins and peptides derived from saliva may have a critical impact as a biomarker in diagnosing oral malodor. If VSCs are generated as a result of bacterial degradation of proteins, their level of degradation, represented by deglycosylation and proteolysis, can be a good marker of oral malodor. The goal of our study was to assess extents of deglycosylation of salivary proteins in generation VSCs. The results indicated that the total carbohydrate contents of salivary glycoproteins were not statistically significantly different between the malodor and non-malodor groups. Furthermore, the amount of carbohydrates increased in accordance with the increase of the amount of proteins irrespective of the presence of malodor. These results suggested little appreciable deglycosylation in the malodor group. However, H_2S levels increased as the carbohydrate contents of salivary glycoproteins decreased in the malodor group, which is compatible with the possibility that deglycosylation facilitated proteolysis, resulting in accelerated H_2S generation. It was reported that activity of a glycosidase, namely that

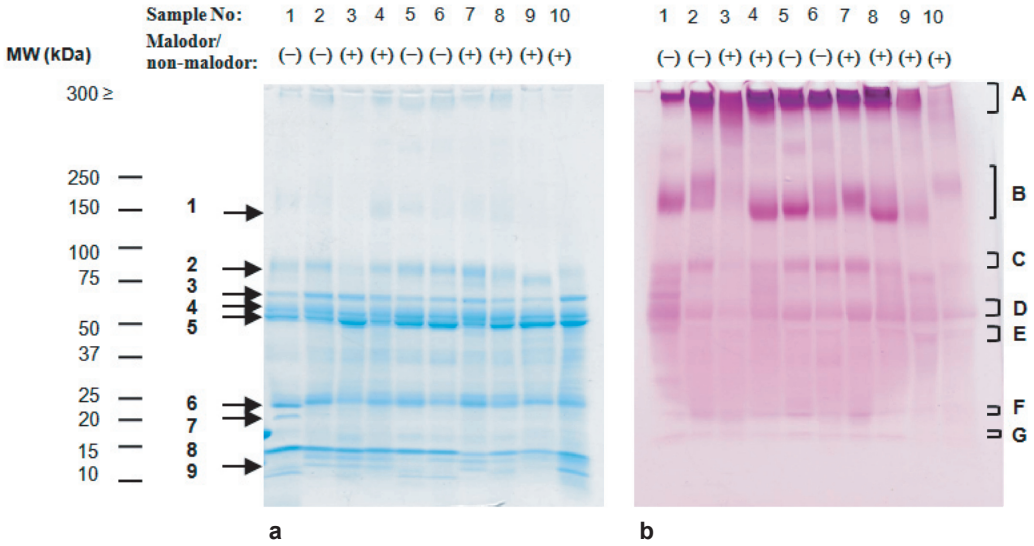
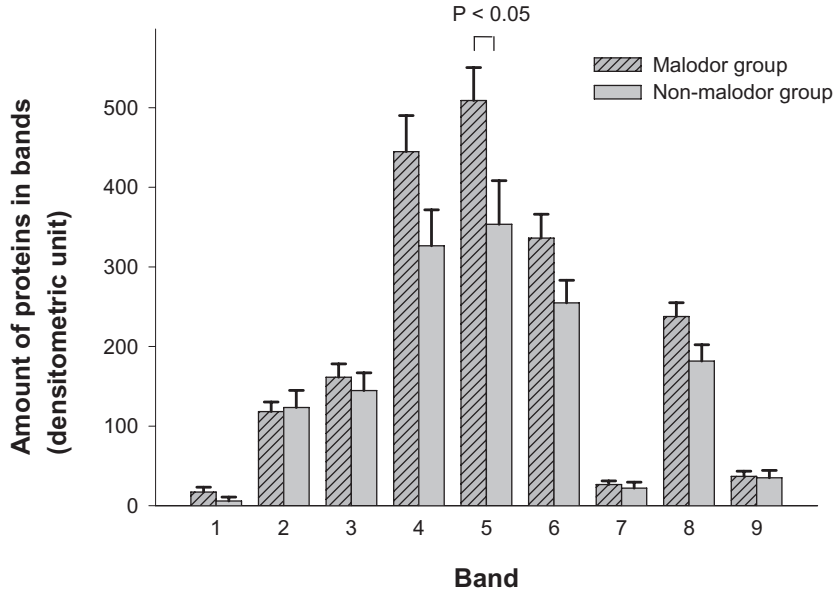


Figure 4 : Analyses of salivary proteins and glycoproteins (representative samples) by SDS-PAGE. The same sample numbers indicate identical saliva samples. The migration positions of protein molecular-mass-markers are shown on the left (size in kDa). Samples from malodor subjects are marked as (+), those from non-malodor subjects are as (−).
(a) Analysis of salivary proteins by SDS-PAGE. Major bands were indicated by numbers.
(b) Analysis of salivary glycoproteins by SDS-PAGE. Major bands were indicated by capitalized letters.

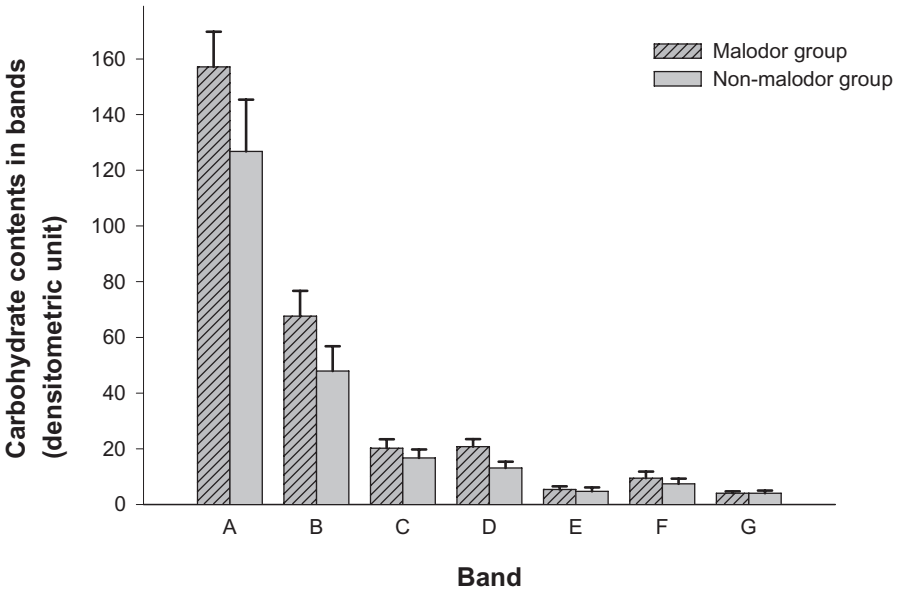
of β-galactosidase, was significantly higher in malodor patients[16]. In order to evaluate the involvement of glycosidases in VSC generation, analysis of β-galactosidase activity alone is not sufficient. There are various kinds of glycosidases including β-galactosidase, sialidase, β-N-acetylglucosaminidase, β-N-acetylgalactosaminidase, and α-fucosidase[17]. β-Galactosidase is produced by *Streptococcus gordonii* and *Actinomyces naeslundii*, not by *Porphyromonas gingivalis* and *Fusobacterium nucleatum*[17]. Sialidase is produced by *Streptococcus oralis* and *Actinomyces naeslundii*, not by *Fusobacterium nucleatum*[17]. Glycosidases, proteases[17] and metabolic status of bacteria can contribute variably to the generation of VSCs. Further studies are required to explore the relationship among glycosidic degradation of glycoproteins, subsequent proteolysis and VSC generation.

The total concentration of salivary proteins, providing substrates for VSC production, was statistically significantly higher in the malodor group. In the malodor group, the association of the total amount of salivary proteins vs. CH_3SH levels, and reduced salivary flow rate were observed. It was reported that CH_3SH level is higher in the malodor patients with periodontitis[3]. In addition, patients with periodontitis show changes in the concentrations of endogenous immune-reactive proteins such as cystatin and secretory IgA, and increased gingival crevicular fluid (GCF) secretion[18,19]. In this study, periodontal conditions of the subjects evaluated by pocket depth and gingival bleeding were not statistically significantly different. While not statistically significantly different, the mean percentage of teeth with bleeding sites in the malodor group was greater than the non-malodor group. This may indicate that gingival bleeding and the amounts of GCF derived-proteins or immune-reactive proteins might increase in the malodor group, which may result in higher concentrations of salivary proteins. Our finding is compatible with the possibility that salivary proteins can be one of the sources of malodor and accelerate VSC levels. It was reported that decrease in salivary flow could also result in increased protein concentrations[20]. Lower flow rate of saliva could affect oral conditions by leading to accumulation of debris, plaque and bacteria[21], potentially generating an environment to elevate VSCs. The result of this study agrees with findings that reduction in flow rate of saliva can be one of the causes of the VSC production[22]. More detailed study of their relationships will be necessary in the future research.

The bands detected in protein and glycoprotein stainings of SDS-PAGE were of similar molecular weights and amounts in the malodor and non-malodor



a



b

Figure 5 : Mean amounts of salivary proteins and carbohydrates in the malodor and non-malodor groups in bands identified by SDS-PAGE. The shadowed gray bar represents the densitometry analyses (mean ± SEM) of proteins and glycoproteins bands in the malodor group. The gray bar represents that of the non-malodor group.

(a) Mean amount of salivary proteins. The amount of proteins in each band was calculated from the density of each band multiplied by the concentration of proteins. Numbers on x-axis correspond to the bands identified in Figure 4a.

(b) Mean amount of carbohydrates in salivary glycoproteins. The amount of carbohydrates in each band was calculated from the density of each band multiplied by the concentration of carbohydrates. Letters on x-axis correspond to the bands identified in Figure 4b.

groups. The results of this study did not provide sufficient evidence of either enhanced proteolytic or glycosidic activities in the malodor group. If proteolytic or glycosidic activities are accelerated by bacterial enzymes, proteins or glycoproteins distribution would have skewed to those with smaller molecular weights or reduced amounts. Most of the proteins recognized in our SDS-PAGE analyses were present at higher levels in the malodor group reflecting increased total salivary protein concentration. Since the protein and glycoprotein ratios in bands were highly variable, it was difficult to evaluate extent of deglycosylation of a particular glycoprotein in the present method.

Major salivary proteins include amylase, MUC5B, MUC7, and proline rich proteins among various kinds of proteins detected in whole saliva[23]. Most of salivary proteins exist as glycoproteins, and are implicated to have an important role in modulating bacterial colonization in oral cavity[24]. A previous report using mass spectroscopy revealed that band 'A' may correspond to MUC5B and band 'B' may correspond to MUC7 (Figure 4b)[25]. MUC5B, also known as MG1, is a high molecular weight human salivary mucin whose molecular weight ranges between 20 and 40 million Da. On the other hand, MUC7, also known as MG2, is a low molecular weight human salivary mucin whose molecular weight ranges approximately between 130 and 180 kDa. MUC5B and MUC7 were tentatively identified as prominent proteins in bands 'A' and 'B' in the results of glycoprotein staining (Figure 4b). Both are heavily glycosylated[24], which accounts for broadness of the bands because of microheterogeneity. Proteins contained in other bands are difficult to characterize only from their molecular weights, because each band may not be homogeneous or may be partially degraded. Identities of those other bands are currently under investigation.

Degradation of mucins was reported to cause VSCs generation in vitro study[26]. Sterer *et al.* reported that deglycosylation facilitated proteolysis of mucins, and resulted in VSC elevation[26]. The results of the present study did not support Sterer's findings. We could not clarify if either substantial deglycosylation or proteolysis was involved in VSC generation. More specific analyses focusing on salivary glycoproteins such as MUC5B and MUC7, and metabolic clearance by their degradations are necessary in the future studies.

Saliva is constantly secreted and swallowed, while its amount and compositions are influenced by various factors including food intake, the degree of systemic hydration, and circadian rhythms. In other words, saliva is constantly replaced by newly secreted saliva. If VSCs are generated through degradation of salivary proteins and glycoproteins by various glycosidases and proteolytic enzymes, the degradation of salivary proteins and glycoproteins must take place before saliva is swallowed. Considerable accumulation of proteins, positive correlation between concentrations of proteins vs. CH_3SH level, and negative correlation between carbohydrate contents vs. H_2S level in the malodor group were observed, which indicate the possibility that salivary proteins and glycoproteins might be related to the generation of VSCs. This finding is in conflict with the data showing unenhanced proteolysis or deglycosylation. These correlations were only observed in the malodor group, not in the non-malodor. Malodor generation is multi-factorial, only a small change of salivary proteins can generate VSCs, changes may be so small that they are easily missed when proteins are analyzed as a whole, or changes in salivary proteins may be compensated by newly secreted saliva.

In this study, subjects with any systemic diseases were excluded to avoid the possible effect on GC analyses and salivary analyses. Those with insufficient salivary flow were also excluded in order to complete electrophoretic analyses of saliva. Because of these limitations, careful generalization is necessary when applying our results to all the patients with oral malodor.

Aknowledgments

We are grateful to Dr. F. A. C. Wright, a visiting professor at Tokyo Medical and Dental University, for suggestions and advice.

References

1. Meskin LH. A breath of fresh air. J Am Dent Assoc. 1996;127:1282-85.
2. Delanghe G, Bollen C, Desloovere C. Halitosis-foetor ex ore. Laryngorhinootologie. 1999;78:521-4.
3. Yaegaki K, Sanada K. Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease. J Periodontal Res. 1992;27:233-8.
4. Kleinberg I, Codipilly M. Modeling of the oral malodor system and methods of analysis. Quintessence Int. 1999;30:357-69.
5. Tonzetich J. Direct gas chromatographic analysis of sulphur compounds in mouth air in man. Arch Oral Biol. 1971;16:587-97.
6. Sterer N, Rosenberg M. Effect of deglycosylation of salivary glycoproteins on oral malodour production. Int Dent J. 2002;52 Suppl 3:229-32.

7. Sterer N, Greenstein RB, Rosenberg M. Beta-galactosidase activity in saliva is associated with oral malodor. J Dent Res. 2002;81:182-5.
8. Sopapornamorn P, Ueno M, Shinada K, et al. Relationship between total salivary protein content and volatile sulfur compounds levels in malodor patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103:655-60.
9. Murata T, Yamaga T, Iida T, et al. Classification and examination of halitosis. Int Dent J. 2002;52 Suppl 3:181-6.
10. Rosenberg M, Kulkarni GV, Bosy A, et al. Reproducibility and sensitivity of oral malodor measurements with a portable sulphide monitor. J Dent Res. 1991;70:1436-40.
11. Oho T, Yoshida Y, Shimazaki Y, et al. Characteristics of patients complaining of halitosis and the usefulness of gas chromatography for diagnosing halitosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;91:531-4.
12. Silness J, Loe H. Periodontal Disease in Pregnancy. Ii. Correlation between Oral Hygiene and Periodontal Condtion. Acta Odontol Scand. 1964;22:121-35.
13. Miyazaki H, Sakao S, Katoh Y, et al. Correlation between volatile sulphur compounds and certain oral health measurements in the general population. J Periodontol. 1995;66:679-84.
14. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227:680-5.
15. Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. J Periodontol. 1977;48:13-20.
16. Sterer N, Shaharabany M, Rosenberg M. Beta-galactosidase activity and H2S production in an experimental oral biofilm. Journal of Breath Research. 2009;3:1-4.
17. Bradshaw DJ, Homer KA, Marsh PD, et al. Metabolic cooperation in oral microbial communities during growth on mucin. Microbiology. 1994;140:3407-12.
18. Henskens YM, van den Keijbus PA, Veerman EC, et al. Protein composition of whole and parotid saliva in healthy and periodontitis subjects. Determination of cystatins, albumin, amylase and IgA. J Periodontal Res. 1996;31:57-65.
19. Hagewald S, Bernimoulin JP, Kottgen E, et al. Salivary IgA subclasses and bacteria-reactive IgA in patients with aggressive periodontitis. J Periodontal Res. 2002;37:333-9.
20. Yarat A, Akyuz S, Koc L, et al. Salivary sialic acid, protein, salivary flow rate, pH, buffering capacity and caries indices in subjects with Down's syndrome. J Dent. 1999;27:115-8.
21. Antoniazzi RP, Miranda LA, Zanatta FB, et al. Periodontal conditions of individuals with Sjogren's syndrome. J Periodontol. 2009;80:429-35.
22. van den Broek AM, Feenstra L, de Baat C. A review of the current literature on aetiology and measurement methods of halitosis. J Dent. 2007;35:627-35.
23. Oppenheim FG, Salih E, Siqueira WL, et al. Salivary proteome and its genetic polymorphisms. Ann N Y Acad Sci. 2007;1098:22-50.
24. Levine MJ, Reddy MS, Tabak LA, et al. Structural aspects of salivary glycoproteins. J Dent Res. 1987;66:436-41.
25. Walz A, Odenbreit S, Stuhler K, et al. Identification of glycoprotein receptors within the human salivary proteome for the lectin-like BabA and SabA adhesins of *Helicobacter pylori* by fluorescence-based 2-D bacterial overlay. Proteomics. 2009;9:1582-92.
26. Sterer N, Rosenberg M. *Streptococcus salivarius promotes* mucin putrefaction and malodor production by *Porphyromonas gingivalis*. J Dent Res. 2006;85:910-4.