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INTERACTION OF SALIVARY PROTEINS WITH HUMAN ENAMEL AND DENTIN POWDER*

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법랑질과 상아질의 타액단백 흡착에 관한 연구

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》국문초록《

법랑질과 상아질의 타액단백의 흡착관계를 구명키 위하여 타액을 법랑질과 상아질 분말의 column에 흡착시킨후 증류수, 3M NaCl, 0.1M phosphate buffer, pH 7.1, 0.2M EDTA 함유한 phosphate buffer와 증류수를 차례로 용출시켜 용출액의 amylase, ribonuclease 및 단백양을 관찰한 결과는 다음과 같다.

1. 타액 amylase와 ribonuclease는 법랑질과 상아질 분말에 모두 흡착하였다.
2. 0.2M EDTA가 함유한 buffer에서는 단백양을 법랑질과 상아질분말에서 모두 33.5%정도 용출되었으나 효소활성은 없었다.

INTRODUCTION

Proteins are generally adsorbed any interface ; liquid/gas, liquid/solid, liquid/liquid. There aretwo significant consequences of this absorption; 1) alteration occur in the secondary and tertiary structure of the adsorbed protein, usually in the direction of a less folded molecule, and 2) the properties, both physical and chemical, of the surface to which the protein is adsorbed are changed.

In the great part of oral pathological process occurring in close connection to a solid/liquid interface, where the solid has so extraordinary surface properties as the apatite, it is likely that concentration gradients are formed between the very interface and the bulk of the solution. Another important consequence of the properties of the surface of the apatite crystals would be the interaction with biologically active compounds, eq. enzymes. Ericson[1] chromatographed samples of saliva proteins on apatite column and observed that a sialic acid-rich fraction was most strongly retained by the apatite. Recently, McGaughey and Stowell,[2] Hay[3] and Ericson[4] haves investigated the possiblity that selective adsorption of some of the saliva protein can be responsible for the early stages of pellicles formation. McGaughey and Stowell,[2] using batch adsorption technic and histochemical

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methods, concluded that some of the salivary sialoproteins were the most strongly adsorbed. However, the results obtained by Hay[3] indicated that, although the saliva sialoproteins are adsorbed at the apatite: saliva ratios used, there is a sialic acid free protein fractin adsorbed at lower apatite: saliva ratios that represents the most selectivelyadsorbed protein.

The present series of experiment were performed to study the adsorption of amylase and ribonuclease on the surface of enamel and dentin powder, using batch adsorption technic. The adsorption to and elution of salivary protein from human enamel and dentin powder was studied under various conditions.

MATERIAL AND METHOD

Preparation of enamel and dentin powder: The enamel and dentin powder were obtained from 200 pooled teeth from dental clinics in seoul area. After removal of carious lesions and fillings, the enamel were mechanically seperated from the dentin, using chisel and carborandum disc, and the seperated enamel and dentin were crushed to powder, using a steel mortar and pestle and then iron contamination removed with a magnet. Thereafter only the enamel or dentin particles that passed through 120 mesh sieve and failed to pass 200 mesh sieve was selected by using Ro-tap sieve shaker. The powdered enamel and dentin between 120—200 mesh sieve size were then dried in the oven at 105°C for 2 hours and stored in desiccator.

Collection of saliva: Whole saliva was collected after the donor had rinsed his mouth thoroughly with water and was stimulated by chewing gum. The first 10 ml. was discarded and the saliva samples were centrifuged for ten minutes at 2,000 xg at 5°C.

Adsorption of salivary protein by enamel and dentin: Columns of enamel and dentin were prepared using 2.5 ml. plastic syringe and 3 gm. of 120—200 mesh sieve enamel or dentin powder supported on a filter paper disk. After flushing the column with water, 3 ml. of whole saliva was run into the column and allowed to equilibrate 45 minutes.

Elution with several eluent: The adsorbed proteins were subsequently eluted with 2 ml. of distilled water, 3 M NaCl, 0.1 M phosphate buffer, pH 7.1, 0.1M phosphate buffer containing 0.2M EDTA, and finally with distilled water. Each collected eluate fractions were dialyzed against water for 24 hours. This dialyzed eluate were evaluated in amylase and ribonuclease activity.

Determination of salivary enzyme activity; 1) *Amylase*[5]: Amylase activity was measured colorimetrically using dinitrosalicylic acid, for determination of the liberated reducing sugar during incubation. One amylase unit was defined as the amount of reducing sugar, liberated after 6 minutes reaction of pH 6.9 at 37°C, which is equivalent to 1 mg maltose. 2) *Ribonuclease*[6]: Ribonuclease activity was determined, as described by Roth, by following the release of acid soluble nucleotide on incubation of 0.25% RNA with or without 2mM PCMB and enzyme solution(eluate fraction) in a final volume of 2ml. Incubation at 37°C were carried out in 0.04 M tris buffer, pH 8.0, for alkaline ribonuclease activity. The reaction was stopped by the addition of 2 ml. of acid ethanol solution. After standing in 0°C for 18 hours, the acid soluble nucleotides were determined by measuring the extinction at 260 mu in a Beckman model DU spectrophotometer. Activities were represented as optical density difference between the final diluted samples

from incubated and controled reaction mixtures. Free ribonuclease activity was calculated by substracting the free activity from the total activity measured in the presence of PCMB.

Protein determination: The protein content was monitored by the method of Lowry et al. in order to obtain specific activity of enzymes. Bovine serum albumin(Nutritional Biochemical Corp.) was used as standard determining the nitrogen content of it by Kjeldahlometry.

RESULT AND DISCUSSION

For the any help in studying of the interaction of salivary protein with enamel and dentin, this present paper was undertaken to observe the adsorption of salivary proteins to human tooth under various condition, and the effect of adsorption on the biological activity of amylase and ribonuclease in saliva. Saliva is difficult to use in adsorption studies, because the mucin tend to precipitate, forming a viscid mass with enamel or dentin powder. Centrifugation of whole saliva reduced the tendency for precipitation. Since the whole saliva samples were obtained by centrifuging out organisms and food debris, it seemed possible that some of the protein were being lost in the rejected whole salivary solids. Robinovitch[8] suggested that it would be relatively easy to use apatite protein.

Table I Relative amounts of salivary proteins desorbed from human enamel and dentin powder by various eluents.

Eluents	Enamel		Dentin	
	mg./ml.	%	mg./ml.	%
saliva	1.73	100	1.73	100
H_2O	0.62	35.8	0.65	37.6
NaCl	0.48	27.7	0.51	29.5
PO_4^{--}	0.41	23.7	0.39	22.5
PO_4EDTA	0.58	33.5	0.58	33.5
H_2O	0.17	9.8	0.19	11.0

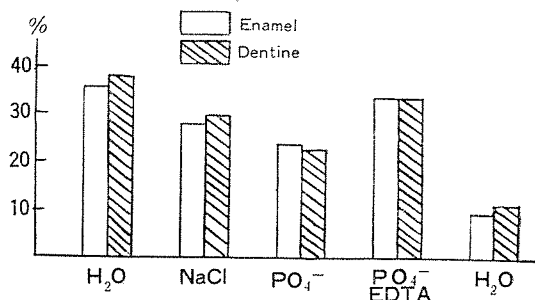

Fig. 1. Percentage of salivary proteins desorbed from human enamel and dentin.

From Table I and Fig 1 it is evident that the protein content in each eluate fraction of saliva mixture is similar, but the last water eluate protein content is lesser than the former eluates. Table I shows the relative amounts of protein in each eluate compared with the total protein content of the initial saliva mixture. Total values higher than 100% in the Lowry procedure are due to interference by traces of EDTA, which could not removed by dialysis.

Table II Adsorption of salivary amylase from centrifuged whole saliva by human enamel and dentin powder.

Eluents	Enamel		Dentin	
	Amylase activity (unit/ml. eluent)	Specific activity (unit/mg. protin)	Amylase activity (unit/ml. eluent)	Specific activity (unit/mg. potein)
saliva	4.00	2.3	4.00	2.3
H_2O	2.88	4.6	2.66	4.09
NaCl	3.29	6.9	3.53	6.9
PO_4^{--}	2.58	6.4	3.08	7.9
PO_4EDTA	—	—	—	—
H_2O	(0.21)	1.2	0.23	1.2

Amylase activity was determinated in whole saliva and in the eluate fractions, following the result as appeared in Table Ⅱ. It is characteristic that amylase activity is not presented in the phosphate buffer containg EDTA, which means that EDTA denaturate this biologic activity of desorbed protein, and that amylase activity of NaCl eluate fraction is higher than any other fractions, which was considered that chloride ion can activate this enzyme activity. Amylase requires chloride for activation, although some other anions can substitute for it.[9]

Table Ⅲ Adsorption of alkaline ribonuclease from centrifuged whole saliva by human enamel and dentin powder (alkaline ribonuclease activity was represented as optical density difference between the final diluted samples from incubated and control reaction mixture)

Eluents	Enamel		Dentin	
	Free RNase	Latent RNase	Free RNase	Latent RNase
	(optical density difference)			
WS	0.645	0.38	0.645	0.38
H_2O	0.432	0.10	0.47	0.15
NaCl	0.55	0.05	0.47	0.13
PO_4^{--}	0.37	0.20	0.51	0.26
PO_4EDTA	—	—	—	—
H_2O	0.22	0.15	0.16	0.07

Alkaline ribonuclease activity was detected in centrifuged whole saliva and in each eluate fractions as seen in Table Ⅲ.

According to Table Ⅲ. there is not any change or alteration of both alkaline ribonuclease in each eluate fraction, because of the presence of biological activity in all eluate fraction, but as same in amylase, the EDTA eluate fraction does not present the ribonuclease activity.

These results indicates that salivary protein is adsorbed to the enamel and dentin powder, and that the tertiary structure has not deformed in the reason of the remaining activity of amylase and alkaline ribonuclease. And it is tempting to speculate that a mechanism may exist that modifies those proteins exhibiting the selective adsorption and desorption behavior.

This results also suggest that it would be relatively easy to use apatite columns or batch adsorption methods to isolate the selectively adsorbed protein. Therefore this technique should prove useful in the further understanding of salivary proteins and their changes in pathological conditions.

CONCLUSION

For the elucidation of the interaction of salivary proteins with human enamel and dentin powder in vitro, salivary protein adsorbed to enamel and dentin powder column from centrifuged human whole saliva were subsequentially eluted with water, 3 M NaCl, phosphate buffer, pH 7.1, 0.1 M, phosphate buffer containing 0.2 M EDTA, and finally water. The collected eluate fraction was assayed in salivary amylase and alkaline ribonuclease activity and evaluated the protein contents. This result indicate that 1) salivary amylase and ribonucelase from centrifuged whole saliva, were all adsorbed to enamel and dentine powder and that 2) they were all desorbed by water, 3 M NaCl, phosphate buffer except with EDTA containing phosphate buffer.

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