Initial studies on the behavior of salivary proteins at liquid/air interfaces

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The mode of adsorption of salivary proteins at air/liquid interfaces was studied by using the drop volume technique to measure the kinetics of surface tension decay of aqueous salivary solutions. Adsorption of salivary proteins from whole saliva was fast, with a plateau value of the surface tension of 43 (±2) mNm^{-1}. As the concentration of saliva was reduced, the plateau value of surface tension increased and was achieved more slowly. The reduction in surface tension of aqueous solutions was larger for salivary proteins than for many other proteins reported. □ *Adsorption; interfacial tension; proteins; saliva*

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Saliva is a very complex fluid, containing many proteinaceous molecules (1). It has two major functional roles (2): 1) Digestive: salivary glycoproteins interact with the food and moisten it as a result of their high water content and also solubilize many food components, such as in the emulsification of fats (3). 2) Protective: the salivary components interact with the oral surfaces and act as a protective film and lubricant. The preferential adsorption of the salivary glycoproteins to the tooth surface results in the formation of the acquired pellicle (4, 5). The proteins probably also bind to the bacterial flora. These interactions have important implications in the aggregation and adhesion of bacteria (6, 7) and in defence mechanisms against infection.

Both the digestive and protective functions involve the adsorption of the proteins at interfaces in the oral cavity. Adsorption of proteins onto surfaces is a very complex process determined by the balance of interactions among protein (containing several different chemical groups), surface, and medium (8, 9). Adding to the complexity of salivary protein adsorption is the fact that saliva contains many proteinaceous molecules whose composition is constantly changing and varies for each individual (1). Information about the adsorption of salivary proteins is therefore limited.

The adsorption of the salivary glycoproteins to the tooth surface is a biologically selective process, the acidic proline-rich proteins being the main component of the acquired pellicle (5, 10). Adsorption of acidic proteins (despite their ionic nature) onto apatite has been suggested to be entropically driven (11). Further, the interfacial behavior of protein molecules at hydrophobic solid surfaces can be related to their behavior at the air/water interface (12).

The drop volume technique has been developed to study the surface tension decay of protein solutions with time (13, 14). Measuring the surface tension decay of saliva will give an insight into the mechanisms of adsorption of the salivary proteins and therefore also into the formation of the acquired pellicle and emulsification processes.

Materials and methods

The saliva samples consisted of stimulated whole saliva from a subjectively healthy 29-year-old woman, who maintained a normal healthy diet and oral hygiene program, over the 2- to 3-month study (avoiding menstrual

periods). The secretion was stimulated by masticatory movements only. Two to 5 ml was collected over 5–10 min during mid-morning periods. No food or drink had been consumed for at least 2 h beforehand. The saliva samples were diluted to various concentrations in 0.005 mol/l phosphate buffer, 0.10 mol/l NaCl, pH 7. The density of the buffer solution was measured with a hydrometer at 25°C and 37°C. All saliva solutions were freshly made each day and used within 2 h of collection. All glassware was washed in concentrated sulfuric acid/nitric acid solution (1:1 (v:v)) and then with double-distilled water.

The time-dependent surface tension of the saliva solutions was measured by the drop volume method described by Tornberg (13), which was automated in accordance with Arnebrant & Nylander (15). By this method a drop of solution of a certain volume, corresponding to a certain surface tension, was formed rapidly, and the time taken for the surface tension in mNm^{-1} (milliNewton per meter) to decrease to a value at which the drop could detach was measured. This was repeated for differing drop sizes, and the surface tension (γ) was plotted versus the detachment time up to 2000 sec. From the experimental γ versus time curves the value of γ at 2000 sec was determined (γ_{2000}). Equation 1 gives the value of the surface tension reduction after 2000 sec (π_{2000})

$$\pi_{2000} = \gamma_0 - \gamma_{2000} \quad [1]$$

where γ_0 denotes the surface tension of the pure buffer solution. On the basis of this equation π_{2000} was then plotted against the log of the concentration (percentage weight) of saliva. Also from the fitted curves $\log(d\pi/dt)$ was plotted versus π.

The experiments were repeated with different concentrations of saliva (1–100% by weight) in buffer. Five measurements were made on the same saliva sample and six samples of the same concentration, to assess reproducibility.

Results

The surface tension of the buffer, γ_0, was 70.7 (±0.2) mNm^{-1} at 37°C and 72.2 (±0.2) mNm^{-1} at 25°C. Measurements on saliva solutions showed an average standard deviation of ±2 mNm^{-1}. The results of the surface tension decay with time are shown in Fig. 1 for 1%, 2%, 10%, 50%, and 100%

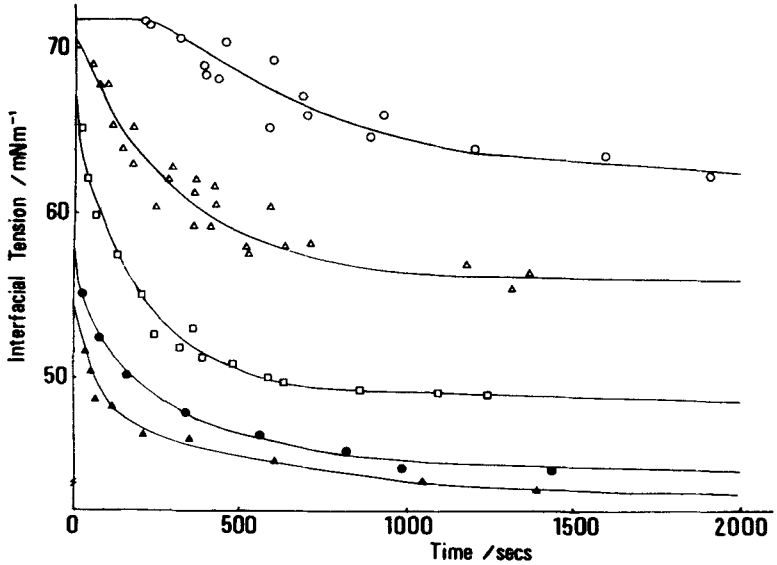

Fig. 1. Time dependence of the interfacial tension of saliva at the air/water interface at different concentrations in pH 7 phosphate buffer. (○) 1%; (△) 2%; (□) 10%; (●) 50%; and (▲) 100% by weight.

saliva solutions. There was no difference in the surface tension reduction at 25°C or 37°C, and both results are therefore included on the same graph.

As the concentration of saliva increased, the surface tension, γ, decreased, and this lowering was achieved more rapidly at the higher concentrations (Fig. 1). At low concentrations there was generally more scatter of the points, and there was also a time lag before any surface tension reduction took place.

To investigate the effect of the concentration of the saliva on the surface tension reduction, the surface tension reduction attained after 2000 sec (π_{2000}) was plotted against the log of the saliva concentration (Fig. 2). In this figure there is a noticeable scatter of points. However, a general trend of surface tension reduction dependence on concentration of saliva could be observed. At high concentrations the surface tension reduction of the salivary proteins decreases slightly as the saliva is diluted. At lower concentrations, <1% saliva, π_{2000} falls off more rapidly.

The graphs of $\log(d\pi/dt)$ versus π were also plotted (Fig. 3). These graphs have a step character. At low concentrations and at low π, $\log(d\pi/dt)$ increased with π, and a plateau was reached at which the rate of the surface tension reduction change became constant. After this, two different steps could be observed, as given by the decreasing straight lines of different slopes. The first one was faster and had a lower slope, whereas the second was slower with a higher slope. At higher concentrations only the latter two steps could be observed. For whole saliva only one step was evident from the data.

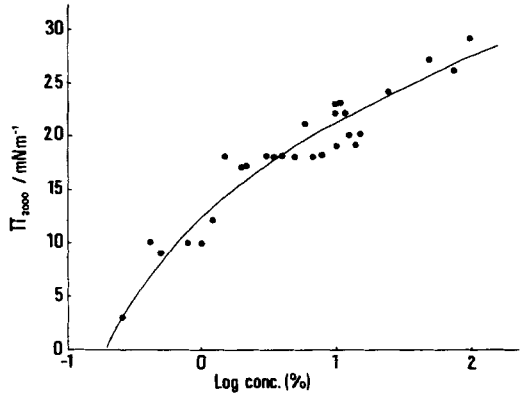

Fig. 2. Dependence of interfacial tension reduction attained after 2000 sec on concentration of saliva.

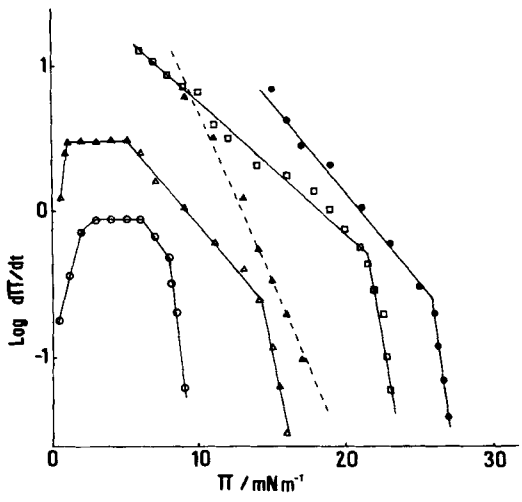

Fig. 3. Log $d\pi/dt$ as a function of π for different concentrations of saliva (symbols as for Fig. 1).

Discussion

The kinetics of protein adsorption in relation to time-dependent surface tension has been discussed by Tornberg (14) and de Feijter & Benjamins (16). In the initial phase of protein adsorption at the air/water interface protein is accumulated on the interface, but the surface tension does not fall until considerable amounts, about half a monolayer, have been adsorbed (16). This initial phase coincided with the 'induction period' reported by Tornberg (14). Increase in the amount adsorbed and rearrangements within the film then increase the surface coverage and thus the surface tension reduction.

The results of this study show that the saliva has the same main features as food proteins (17) and pure proteins (13, 14) with

regard to its time-dependent interfacial properties. At low concentrations there was thus a delay or 'induction period', before the surface tension reduction took place. The rate of surface tension reduction then slowed down as the amount in the film approached its plateau value.

For saliva, which consists of numerous proteinaceous molecules, the smaller, faster-diffusing or the most abundant molecules will arrive first and adsorb. Slower, larger molecules may then arrive and penetrate the film, and these high molecular weight proteins will in general have a larger number of contact points and will therefore be more irreversibly bound. The larger proteins may displace some of the smaller ones at the interface and sequentially replace them until a steady state is reached. This type of exchange process can thus contribute to the observed time dependence of the reported surface tensions of the saliva-containing solutions.

The graph of log (conc.) versus π (Fig. 2) shows the surface tension reduction to decrease gradually on dilution of saliva. The salivary proteins of this test subject do not show signs of any self-association, as would have been given by a sharp increase in π with log (conc) and a leveling off at a discrete concentration (12, 17).

MacRitchie (18) found that at an air/water interface the extent of unfolding of protein molecules is usually large especially at low concentrations, but at high concentrations native protein molecules have been reported to predominate (19). Rearrangements in the adsorbed layer may require compression of the surface film. On coverage of the interface the compressibility of the film will decrease. To visualize the changes in compressibility of the film during this process, the logarithm of the rate of surface tension reduction, $\log(d\pi/dt)$, can be plotted as a function of the surface tension reduction, π (14). The curves obtained may in some cases show 'kinks', indicating abrupt changes in film compressibility (14). From Fig. 3 it is evident that at low concentrations and at low π, $\log d\pi/dt$ increased with π, which may be a consequence of the rapid increase in surface pressure achieved as the packing density of the molecules increased at the interface (16). Further, $\log(d\pi/dt)$ started to decrease at surface tension reductions of 5–7 mNm^{-1} for low concentrations of saliva. This decrease started at a higher surface tension reduction for higher concentrations of saliva. Similar observations for other protein systems were interpreted as a decrease in film compressibility at a lower π for lower concentrations, which was achieved by the protein molecules spreading more easily at the lower concentrations (14, 17). The next change in the slope of the curve took place at π, increasing from 8 to 27 mNm^{-1}, with increasing saliva concentration from 1–50%. Whole saliva did, however, not show any changes in film compressibility.

The saliva used contained approximately 0.2% by weight of protein. The effect of concentration on the interfacial properties of this subject's saliva can be compared with that of the proteins used as emulsifiers in the food industry studied by Tornberg (17). Here it should be noted that the values observed for salivary solutions after 2000 sec are compared with those of Tornberg obtained after 2400 sec. Saliva shows a similarly shaped π versus log (conc.) curve as for the soy proteins. However, a higher surface tension reduction was reached at lower protein concentrations for the saliva, π_{2000} = 29 mNm^{-1} for whole saliva compared with π_{2400} = 22–25 mNm^{-1} for the food proteins at 0.2%. The high surface tension reduction may be inherent in the structure of salivary proteins or might be due to the fact that various protein molecules can pack efficiently at the interface. In this context it should be noted that recent ellipsometric studies of protein adsorption at saliva/air interfaces (20) show much higher amounts of adsorbed material than those reported in the literature for other protein solutions.

For whole saliva the adsorption of the salivary macromolecules to the air/water interface was very fast and probably as fast as to the tooth surface (4). The noted value of 53 mNm^{-1} for the initially measured (~1 min) surface tension coincides with that reported by Glantz (21) and decreases to 43 mNm^{-1} over 30 min. This value is also in good agreement with the results of Water-

man et al. (22), who found a plateau value of 46 mNm^{-1}. It is well known that macromolecules are spontaneously adsorbed to the tooth surface and restorative material and also that pellicle is formed during 2 h in vivo and shows no major further changes in the first 6 h (23–25). These findings are all consistent with the noted surface tension results for whole saliva, which showed that after the first 2 h 99% of the surface tension reduction had been reached (curves not shown).

It must be emphasized that the data discussed here are based on results obtained from one individual, and variations in protein concentrations and their relative proportions may occur between individuals. For all experimental results in which comparisons could be made with our own reference data or data presented in the literature (21, 22), the observed intraindividual variations of surface tension of the donor's saliva did not exceed reported interindividual variations. Further, the results compared favorably with related analytical adsorption studies on salivas from a range of individuals, which showed similar adsorption characteristics between individuals and between test surfaces of similar characteristics (23, 26–28).

This preliminary investigation gives an initial insight into the behavior of whole saliva in the oral cavity. More detailed investigations are required to determine the individual effects of the pure proteinaceous materials, using secretions from the individual glands and from a range of subjects.

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