INVESTIGATION OF WHOLE PAROTID SALIVA FROM SMOKERS AND NONSMOKERS WITH ESR SPECTROSCOPY

Feride Severcan
Department of Electrical and Electronic Engineering
Dokuz Eylul University, Bornova, Izmir, Turkey

Metin Yavuz
Physics Department
Ondokuz Mayis University, Samsun, Turkey

ABSTRACT. Whole parotid saliva from smokers and nonsmokers have been investigated by Electron Spin Resonance (ESR) Spectroscopy using perdeutero-di-t-butyl nitroxide (PDDTBN) as a spin probe. It is observed that although smoking does not alter the polarity of the medium it alters the spin probe mobility. Rotational correlation time of the PDDTBN in parotid saliva from smokers is found to be smaller than that of the nonsmokers. This implies that habitual smoking increases the spin probe mobility and decreases microviscosity of the medium.

INTRODUCTION

Theoretically saliva can affect caries in different ways such as mechanical cleansing, reducing solubility of anamel, buffering and neutralizing the acids produced within the plaque and by anti-bacterial activity provided by secretory IgA, lysozyme, lactoferrin and lactoperoxidase [1]. Also variations in the composition and in the chemical and physical properties of saliva are objective measures of some basic physiologic processes important in health and disease. For this reason it is interesting to investigate the factors that change the physical and chemical properties of the saliva. Smoking habit is one of these factors. Despite the importance and pervasiveness of cigarette smoking as a risk factor, only a limited number of studies have been performed to understand its effect on saliva. These studies mainly investigated the effect of cigarette smoking on flow rate [2], plaque accumulation and/or mineralization [3] and thiocyanate level[4] in saliva.

In the present work effect of smoking in the polarity and fluidity of the whole human parotid saliva has been investigated with ESR spectroscopy using spin labeling technique.

Spin labelling ESR is a powerful technique to investigate the biological systems [5]. Although it has a wide application in biology and biomedicine [5], it received little attention in periodontal research [6]. By using this technique, in the present work, we were able to monitor the microenvironment of the saliva and showed that habitual smoking does not alter the polarity, but alters the fluidity of the medium.

MATERIALS AND METHODS

The subject of the study were 50 dental studens aged 18-22 yr, equally divided into group of habitual cigarette smokers and nonsmokers with almost identical sex distribution. All samples were collected at the same time of day (midmorning), at least 2 h after the last intake of food or drink.

Saliva was collected by placing a parotid cup (Carlson-Crittenden) over the orifice of the right parotid duct. Samples were achieved by stimulating salivary flow using citric acid of 10 % concentration. Situmulus were applied at 30 seconds

intervals to the tongue untill 4 cc of saliva had been collected.

PDDTBN was kindly provided by Prof. W. Plachy, San Francisco State University. All samples were labelled with spin probe dissolved in acetone to give final probe concentration of 0.0001M. Acetone was removed by N_2 flux before addition of saliva. Samples were contained in 1 mm i.d. 100 μl glass capilaries within standart 4 mm diameter quartz tubes.

ESR experiments were carried out on a X-band Varian E109 spectrometer at room temperature. A 100-kHz modulation frequency was used for conventional, first harmonic, absorption ESR spectra. ESR spectra were recorded at 2 mW microwave power.

Rotational correlation time[7] was calculated by the equation:

$$\tau_c = 6.5 \times 10^{-10} W_0 [(h_0/h_{-1})^{1/2} - 1]$$

where W_0=midfield linewidth in Gauss,h_0=midfield line height and h_{-1}=highfield line height as shown in Fig.1.

RESULTS AND DISCUSSION

Fig.1. shows the ESR spectrum of PDDTBN in parotid saliva. The spectrum consists of three lines corresponding to the nitrogen quantum numbers M=-1, 0, +1. The center of these lines are separated by the nitrogen hyperfine splitting constant A_N which gives information about the polarity of the medium. In the present work we did not observe any change in the polarity (or hydrophobicity) of the parotid saliva for smokers and nonsmokers.

The rotational correlation time equation which is used in the present study can be correctly applied to this system since the spin probe was deuterated. Perdeuteration of the radical decreases the inhomogenous broadening due to the smaller magnetic dipole moment of the deuteron. As a result a significant reduction in the observed linewidth can be achieved. PDDTBN is a very small and almost spherical amphiphilic molecule (r=2 Å) [8] which has a linewidth of less than 0.15 Gauss. For this reason it gives almost isotropic ESR spectrum in liquids. In addition, the narrow linewidth increases the spectral sensitivity so that the mole fraction of probe can be less than 0.001 and any perturbing effect is minimized. This probe was previously applied to lipid membranes [9-12] and parotid saliva [6].

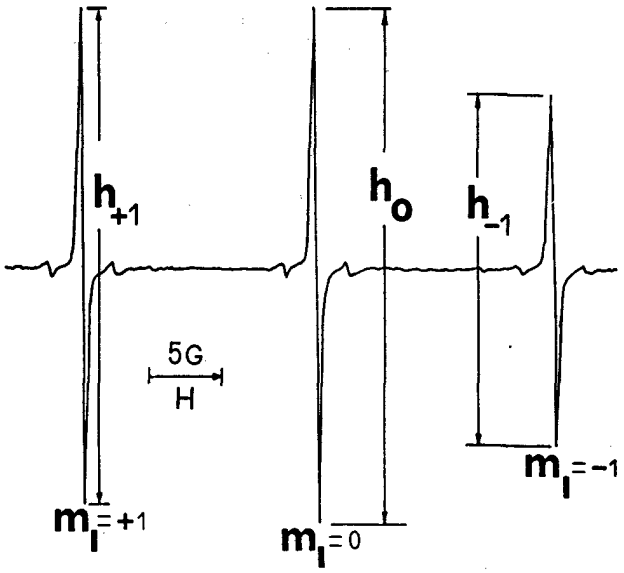

Fig.1. ESR spectrum of PDDTBN in parotid saliva.

TABLE I. Mean rotational correlation time values of PDDTBN spin probe in the parotid saliva of the smokers and nonsmokers.

Group	$\overline{X}$(s)	(SD)
Nonsmokers	1.67×10^{-11}	0.15×10^{-11}
Smokers	1.23×10^{-11}	0.12×10^{-11}

The mean values of rotational correlation times for smokers and nonsmokers were listed in Table I. As seen from Table I., smokers showed a significantly lower mean value of rotational correlation times compared with the nonsmokers. Rotational correlation time is a dynamical parameter. It gives information about the probe mobility, therefore about the fluidity of the medium. The results given above indicate that probe mobility in the saliva (fluidity of the saliva) increases for smokers.

Changes in fluidity are associated with changes in microviscosity. The correlation time, τ_R, of a spherical molecule is related to the microviscosity of the liquid according to the Stokes relationship:

$$\tau_R = 4\pi R^3 \eta / 3kT$$

where R is the tumbling radius of the probe and kT is the thermal energy.

In this expression, if we use the correlation times given in Table I., we obtain the following values for the microviscosity of the saliva at 23°C: η=2.04 cp for the nonsmokers and η=1.50 cp for the smokers. The results obtained can be considered as absolute values of microviscosities. However we should point out that error in the values might arise from oxygen broadening effect in the linewidth since we did not remove the oxygen from the sample. The microviscosity term here implies the microviscosity of the saliva immediately surrounding the probe. The microviscosity can be related to the intermolecular free volume [13]. For this reason fluidity may be related with permeability. If this assumption is true, an increase in fluidity will be indicative of the increase in permeability for smokers. This interpretation may explain the increased mineralization, especially raised Ca concentration, observed in the saliva of smokers which was hypothesized as the reason of plaque accumulation [14].

CONCLUSION

We have shown that PDDTBN spin probe allowed us to obtain information about the effect of smoking on polarity and microviscosity of the parotid saliva. It is found that smoking does not induce a change in polarity but it induces an increase in fluidity or a decrease in microviscosity of the parotid saliva.

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