SHORT COMMUNICATION

High resolution proton nuclear magnetic resonance studies of human saliva

Kenichi Dan, Keiichi Kawano, Yoshihiro Terada, Fumihiko Iga and Ryozo Hirayasu
Department of Prosthetic Dentistry I, Kyushu University
Maidashi 3-1-1, Fukuoka 812, Japan
(Accepted for publication: December 15, 1988)

Key words: Proton NMR / human saliva

Introduction

High resolution proton Nuclear Magnetic Resonance (NMR) can be used for multi-component analysis of low molecular weight compounds in small amounts of intact biological materials and biological fluids. It can provide useful qualitative and quantitative biochemical information without the necessity of any pretreatment such as extraction, separation, or purification. The NMR spectra of serum, plasma, cerebrospinal fluid, or urine are related to the clinical conditions of the subjects, and have already been shown to be diagnostically valuable1-3. In some cases, the NMR spectrum of human saliva can also be useful clinically4,5. However, the signal of the spectrum of human saliva is not assigned in detail and the limitation of this method is not made clear. In this paper we present the assignments of saliva NMR spectra by one-dimensional and two-dimensional

Fig. 1-1
techniques and show the variability of saliva components with the sampling conditions.

**Materials and Methods**

Specimens of human whole saliva were collected from the oral cavities of twenty healthy subjects, and two specimens were collected directly from the parotid gland by using a canule. All saliva samples were measured either immediately after collection or stored at 4°C and used within 3 days. Prior to the NMR measurements, the samples were allowed to warm slowly to room temperature. All samples (0.4 ml) were placed in 5 mm diameter NMR tubes. $^2$H$_2$O (0.1 ml) was added to each sample as an internal frequency standard.
field-lock. 3-(Trimethylsilyl)-(2H₄) propionate (TSP) was added to all samples as an internal chemical shift reference. One-dimensional proton NMR spectra were obtained by homonuclear irradiation for suppression of the proton signal of water. For each saliva sample, 1000 free induction decays (FIDs) were measured using 90° pulses, each decay represented an acquisition time of 1.360 sec, which was divided into 16,384 data points. Data from all FIDs were accumulated in these bins to improve the signal to noise ratio. Prior to each pulse, the intense water signal was suppressed by irradiation (gated off during data acquisition). The dynamic range problem presented by the H₂O peak were overcome by freeze drying and homonuclear irradiation. For measurements using the two-dimensional (COSY) technique, samples were freeze dried and concentrated by redissolving in

Fig. 3-1
Results and Discussion

We were able to identify the source of many resonances of human whole saliva by considering their chemical shifts and characteristic coupling patterns\textsuperscript{2,7,8}. Acetate, alanine, formate, glycine, lactate, propionate, phenylalanine, succinate, tyrosine and some other substances were identified. The two-dimensional (COSY) NMR spectrum was helpful in the identification. The off-diagonal peaks (Fig. 1) revealed the connectivities between coupled resonances and reduced the signal overlap problems present in the one-dimensional spectrum. The spectra of the whole saliva of normal subjects are similar, but the intensities of the peaks particularly acetate (mean : 9.5mmol/l, SD : 6.0), lactate (mean : 0.51, SD : 0.49), propionate (mean : 2.2, SD : 2.0), and formate (mean : 0.45, SD : 0.33) show a wide variability (Fig. 2). The intensities within a single individual also vary, depending on recent eating history. That is, the concentration of substances in the whole saliva such as glycine, succinate, alanine, and formate may be strongly influenced by the oral condition at the time of sampling (bacterial flora and lactic acid fermentation, tooth brushing, time since previous meal, etc.) (Fig. 3). It has been shown that in some cases the NMR spectrum of human whole saliva can be useful to investigate...
Fig. 4 ¹H NMR spectrum for saliva collected directly from the parotid gland by using a canule.

clinical conditions⁴,⁵.

But the information about certain substance in whole saliva is highly dependent on oral conditions. Fig. 4 shows the NMR spectrum of saliva collected directly from the parotid gland. It has no signal of acetate, propionate, glycine, succinate, and formate. Caution is therefore required when comparing NMR spectra of human whole saliva, and further investigations are needed to establish the quantitative effects of oral conditions, age, sex etc.

Acknowledgements

We are due to thanks to Professor H. Nakayama of Department of Microbiology, Kyushu University for valuable discussion.

References