

Oxidative Consumption Of Root Caries Biomolecules By Ozonated Water

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Introduction

Restorative treatment of primary root caries lesions (PRCLs) represents a major health challenge to the dental professional. In addition to lowered pH values, the profile and concentrations of microbial-derived short-chain organic acids is an important demineralisation parameter regarding the induction, development and progression of dental caries.

The nature, rate and extent of salivary reductant consumption [e.g., that of volatile sulphur compounds (VSCs) responsible for halitosis (predominantly methyl mercaptan and hydrogen sulphide), their amino acid precursors (L-cysteine and L-methionine), thiocyanate, urate and pyruvate] by ozone (O₃) reflects the oxidising capacity of this microbicidal agent, a parameter of much relevance to its therapeutic and aesthetic actions.

High resolution proton (1H) nuclear magnetic resonance (NMR) spectroscopic analysis of human saliva, gingival crevicular fluid (GCF) or appropriate chemical model systems serves as a very useful technique for the *in vitro* evaluation of the O₃-mediated oxidation of such biomolecules, and we have recently found that it also provides much valuable regarding the molecular mechanisms associated with the potential therapeutic actions of this reactive oxygen species (ROS). The aim of this study was to assess a multicomponent evaluation of the oxidative consumption of root caries biomolecules by an ozonated water product [1], TherOzone unit, CA, USA, using a high resolution 1H NMR spectroscopy.

Materials and Methods

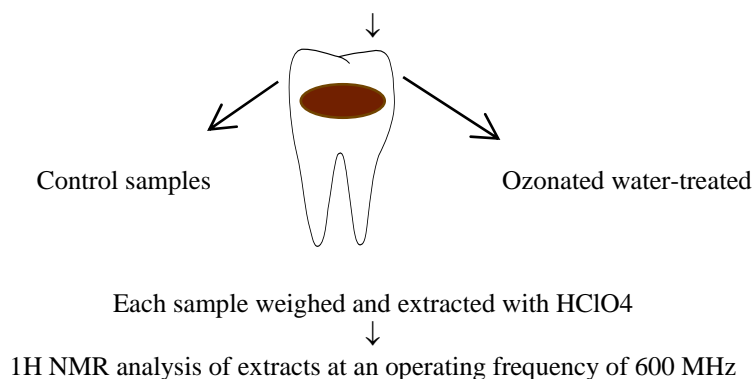
All participants (n=12 for 1H NMR analysis) were attending for routine oral health care (mean age 69.3 ± 3.1 yr. ; range 65-77 yr.). Seven of the patients were smokers. Only patients who had refrained from oral activities (i.e., eating, drinking, tooth-brushing, oral rinsing, smoking, etc.) for at least 2 hr. prior to sample collection were selected.

Subsequent to plaque removal and drying, PRCL specimens were collected using a sterile excavator from each lesion. Samples were then divided into two approximately equivalent portions; a 20 ml volume of ozonated water was applied to the first portion, whilst the second was treated with 20 ml of doubly-distilled (i.e. unozonated) water and hence served as a control. Each sample was then homogenised, lyophilised and the residues obtained accurately weighed on a microbalance. Subsequently, the residues were subjected to a perchloric acid (HClO₄) extraction process involving the stepwise treatment with 0.90 M HClO₄, followed by centrifugation at 3,500 rpm, and neutralisation with KOH followed by removal of the KClO₄ precipitate via further centrifugation (3,500 rpm).

The post-neutralised HClO₄ extracts were then subjected to 1H NMR analysis on a Bruker Avance 600 spectrometer at an operating frequency of 660.13 MHz and probe temperature of 298K.

Study Design

12 leathery primary root carious lesions (PRCLs) samples (following plaque removal and sample drying)



Results

The expanded 0.50 - 4.50 and 5.50 - 9.00 ppm regions of a typical 600 MHz ¹H NMR spectrum of a post-neutralised HClO₄ extract of caries collected from a patient are shown in Figures 1 and 2. The spectra acquired contain many resonances assignable to a wide variety of low-molecular-mass metabolites, and illustrate the multicomponent analytical ability of the technique employed. Indeed, these spectra contain well-resolved, sharp signals assignable to bacterial- or yeast-derived organic acid anions [including formate (Form), acetate (Ace), 3-D-hydroxy-butyrate (Bu) and pyruvate (Pyr)], amino acids (predominantly alanine and glycine), and carbohydrates such as glucose.

Treatment of these samples with ozonated water gave rise to:

- the oxidative decarboxylation of the electron-donor pyruvate (generating acetate and CO₂ as products).
- the oxidative attack of carbohydrates, generating formate.
- the oxidation of the volatile sulphur compound (VSC) precursor methionine (Met-S-CH₃), generating its corresponding sulphoxide (Met-SO-CH₃).
- the oxidation of lactate, urate and glycosaminoglycans, generating acetate (via pyruvate), allantoin and low-molecular-mass saccharide fragments, respectively.
- evidence for the O₃-mediated oxidation of 3-D-hydroxy-butyrate.

Discussion

Consumption of methionine by O₃ is of great importance to oral hygiene and clinical periodontology since both CH₃SH and H₂S are generated from this amino acid via metabolic pathways operational in gram-negative microorganisms. Hence, our data indicate that O₃ has the capacity to clinically alleviate oral malodour via the direct oxidative inactivation of VSCs and their amino acid precursors.

As demonstrated here, the technique is of much value concerning multicomponent assessments of the interactions of O₃ with human caries biomolecules, and the oxidative decarboxylation of pyruvate by this oxidant evaluated in this study serves as an important fundamental example of this which may be of some relevance to its mechanisms of action. Indeed, pyruvic acid is a very powerful proton donor (K_a = 3.20 mM) being much stronger in this capacity than lactic acid (K_a = 0.14 mM), and hence may play an important role in promoting tooth demineralisation processes.

Conclusion

Multicomponent analysis of root caries by high field ¹H -NMR spectroscopy provides useful information regarding the oxidation of PRCL biomolecules by O₃ using this Ozonated water. For example, the consumption of pyruvic acid by O₃ may assist the remineralisation process in view of its powerful proton donating properties. Moreover,

oxidation of the volatile sulphur compound precursor methionine to its corresponding sulphoxide may contribute to a reduction in oral malodour using this Ozonated water.

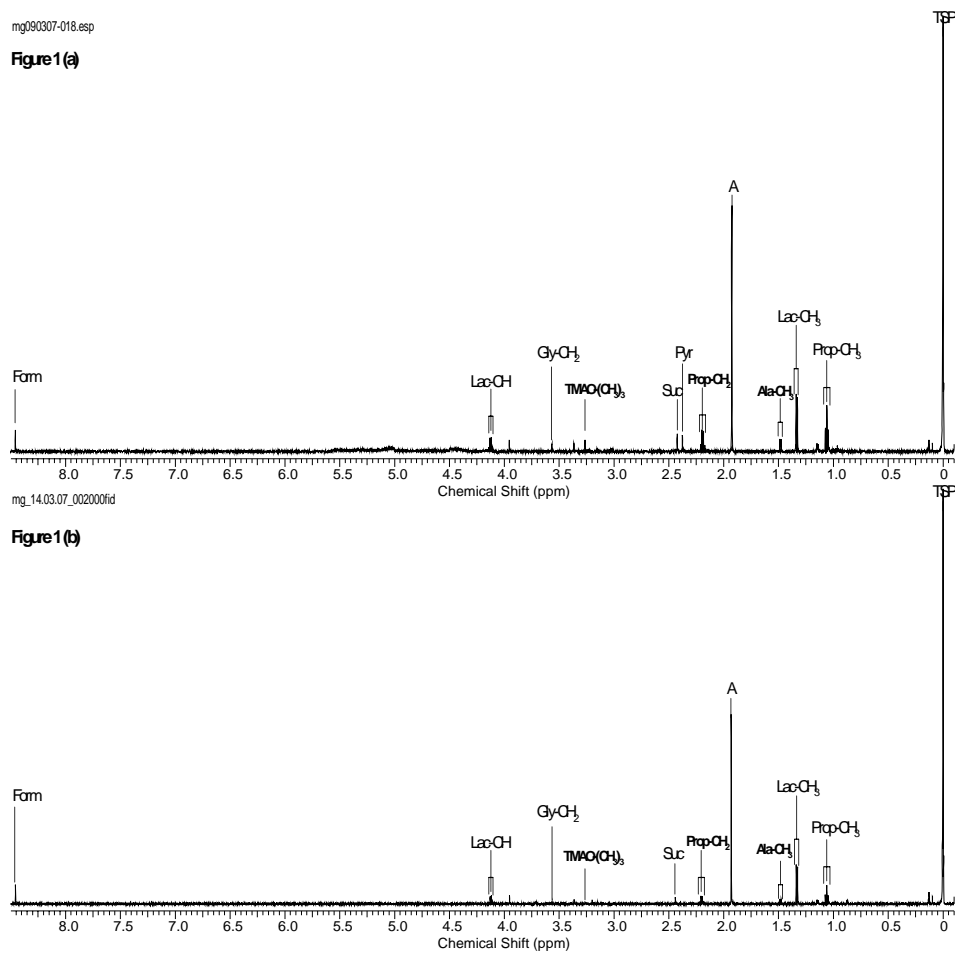


Figure 1. (a) expanded 0.50-8.50 ppm region of the 600 MHz ^1H NMR spectrum of a post-neutralised HClO_4 extract of a caries specimen, (b) of the same specimen treated with O_3 . A typical spectrum is shown. Abbreviations. A, Acetate- CH_3 ; Form, formate- H ; Gly, glycine- CH_2 ; Lac- CH_3 and Lac- CH , lactate- CH_3 and $-\text{CH}$ protons respectively; Prop CH_3 and $-\text{CH}_2$, propionate- CH_3 and $-\text{CH}_2$ group protons respectively; Pyr, pyruvate- CH_3 ; Suc, succinate- CH_2 ; TMAO, trimethylamine oxide $\text{ON}(\text{CH}_3)_3$.

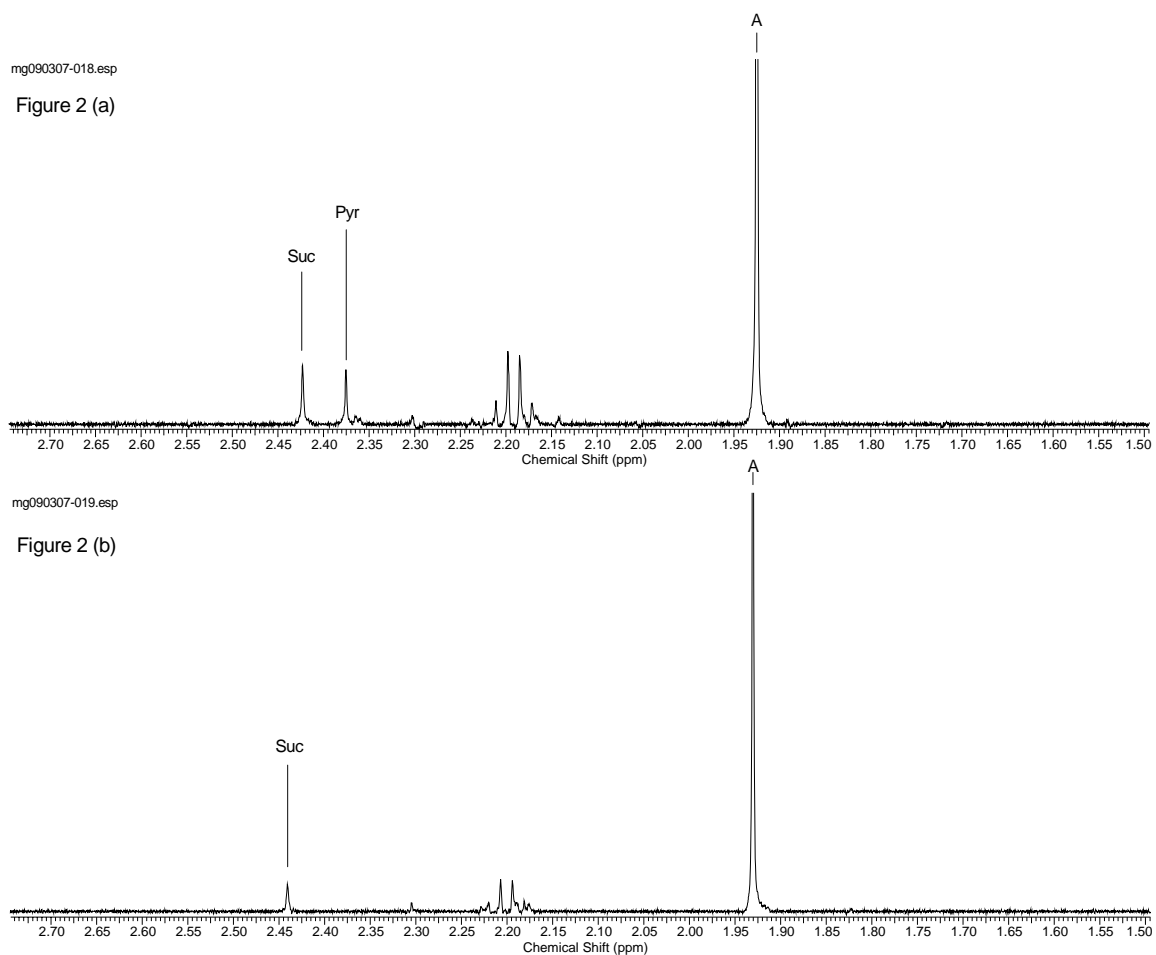


Figure 2. (a) Expanded 1.5-2.75 ppm region region of the 600 MHz ^1H NMR spectrum of a post-neutralised HClO_4 extract of a caries specimen, (b) of the same specimen pre-treated with O_3 showing complete consumption of pyruvate. A typical spectrum is shown. Abbreviations; A, Acetate- CH_3 ; Pyr, pyruvate- CH_3 ; Suc, succinate- CH_2 .

References

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