High-Resolution Solid-State NMR Studies of Dentin Collagen-HEMA Interaction

Teresa G. Nunes*, Sónia Gama Pereira
Departamento de Engenharia de Materiais/IST-Universidade Técnica de Lisboa e ICTPOL, Lisboa, Portugal. e-mail: Teresa.Nunes@ist.utl.pt

Sofia G. Nunes
Departamento de Química da FCT- UNL, 2825 –114 Caparica, Portugal

Jorge Perdigão
Division of Operative Dentistry, University of Minnesota MN, USA

Keywords: Human dentin, Collagen; HEMA; ^{13}C CP/MAS; Shielding tensor.

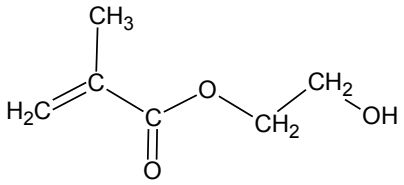
Abstract: *The present study is a preliminary investigation of the eventual interaction of hydroxyethylmethacrylate (HEMA) with glycine and with dentin, using high-resolution solid-state ^{13}C and ^{1}H techniques. Evidences are shown for an interaction glycine-HEMA, which are based on the analysis of the principal elements of the ^{13}CO chemical shielding tensor of glycine and on the detection of HEMA molecules with restricted motion. Significant spectral changes were observed in the ^{13}C spectra of human dentin in the presence of HEMA.*

Resumo: *O presente estudo constitui uma investigação preliminar da eventual interacção do hidroxi etil metacrilato (HEMA) com a glicina e com a dentina, por observação do ^{13}C e do ^{1}H, no estado sólido, utilizando técnicas de alta resolução. Mostram-se evidências de uma interacção glicina-HEMA, baseadas na análise dos componentes principais do tensor de blindagem química do ^{13}CO da glicina e na detecção de moléculas de HEMA com pouca mobilidade. Observam-se diferenças significativas no espectro de ^{13}C da dentina humana em presença de HEMA.*

Introduction

In Restorative Dentistry, the tooth's surface is usually etched with an acid, in order to expose the dentinal collagen fibrils, and subsequently pre-treated with a primer solution, which contains hydrophilic monomers like hydroxyethylmethacrylate (HEMA), before the application of the final restorative material.

Hydroxyethyl methacrylate - HEMA



The role of the primer is to promote adhesion of dentin to the final restorative material. However, adhesion remains a challenge.[1]

Dentin contains a great percentage of organic material, mainly Type I collagen, and water. The major constituents of collagen (~65%) are Gly, Pro, Hyp and Ala residues; the content of Gly, the most abundant amino acid residue, is 33±1%.[2] Thus, it is reasonable to expect that an interaction with HEMA, if any, may involve mainly Gly residues. However, mineralized tissue collagens, like dentin collagen, have unique properties compared to unmineralized tissue collagen, for example, higher resistance to solubilization.[3] Consequently, the characteristics of solid-state NMR make this a suitable technique to study these proteins.

The goal of the present study was to perform a preliminary investigation of the eventual interaction of HEMA with glycine and with dentin, using high-resolution solid-state ^{13}C and ^{1}H techniques.

Experimental

Glycine and HEMA, respectively purchased from Aldrich and Sigma, were used without any treatment. Human dentin was obtained as a powder from a fresh-extracted molar. A Bruker MSL300P NMR spectrometer was used for hydrogen (300.13 MHz) and carbon (75.47 MHz) observations.

Standard RF pulse sequences were selected to record ^{13}C spectra under CP/DD-MAS conditions with contact time 2 ms, B1 50 kHz, relaxation delay 10 s and MAS rate as indicated in the text.

Results and Discussion

Figure 1 shows the spectrum of dry glycine. At the slow-MAS rate used for the spectrum acquisition, 980 Hz, it was possible to record an envelope of 12 spinning side bands, as the result of the frequency modulation of the isotropic signal by the rotor spinning frequency. Therefore, the principal values of the chemical shielding tensor of ^{13}CO (Figure 2) in glycine were obtained, using the intensities of the spectral lines as the input of a computer program based on the algorithm by Herzfeld and Berger:[4] σ_{11} -73.1, σ_{22} −3.5 and σ_{33} 76.6 ppm (results previously reported by Haberkorn *et al.* :[5] –70, -4 and 74). Using a similar procedure to treat the spectrum of glycine (80 mg) in the presence of HEMA (100 μl), we have obtained: σ_{11} -68.7, σ_{22} −3.6 and σ_{33} 72.3 ppm.

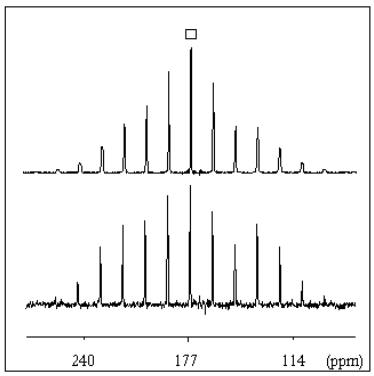


Figure 1. ^{13}CO CP/DD-MAS (980 Hz) signal of dry glycine (bottom) and glycine in the presence of HEMA (top). The isotropic chemical shift is 176.6±0.1 ppm (□).

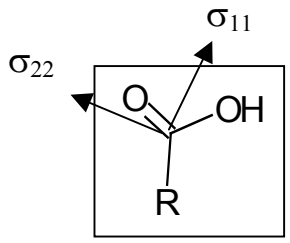


Figure 2. Orientation of the principal axes of the 13C chemical shielding tensor in carboxylic groups. The most shielded element (σ33) is oriented perpendicularly to the 13CO2H plane. In glycine R=NH2CH2.

In the presence of HEMA, it is thus observed that the element σ_{22} of glycine ^{13}CO chemical shielding tensor remains unchanged, while the shielding along the other principal axes were modified by about 5 ppm, corresponding to a shielding increase along σ_{11} and the opposite effect for σ_{33}. This result points to a chemical interaction glycine-HEMA not involving the glycine CO groups, in which case a σ_{22} variation would be detected.

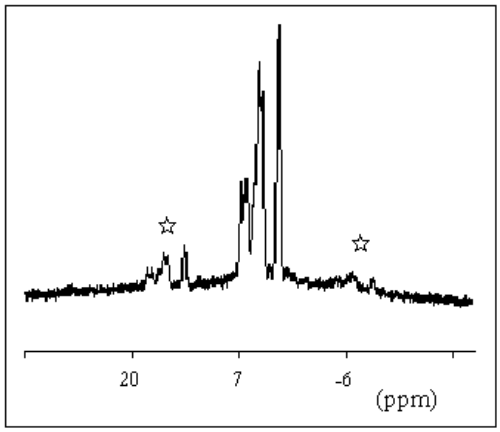


Figure 3. ^{1}H MAS (3.3 kHz) spectrum of glycine in the presence of HEMA. I Spinning side bands.

^{1}H MAS spectra of glycine/HEMA mixtures were also acquired. Figure 3 shows that, beyond the isotropic signals, are also recorded the corresponding first-order spinning side bands, which is in agreement with an induced restricted motion of part of HEMA molecules, in the presence of glycine.

^{13}C CP/DD-MAS spectra obtained from dentin, in the presence and in the absence of HEMA, are shown in Figure 4. The assignment of collagen carbon resonances was accomplished by Saito *et al.*[6] Carbon signals from HEMA molecules with restricted motion are also identified. In particular, significant differences between the spectra of two samples are detected in the chemical shift range 35-50 ppm, where Gly Cα and Hyp C$_\beta$ resonances are observed. [6] Moreover, the spectrum of the sample containing HEMA shows carbonyl signals at frequencies higher than dentin spectrum.

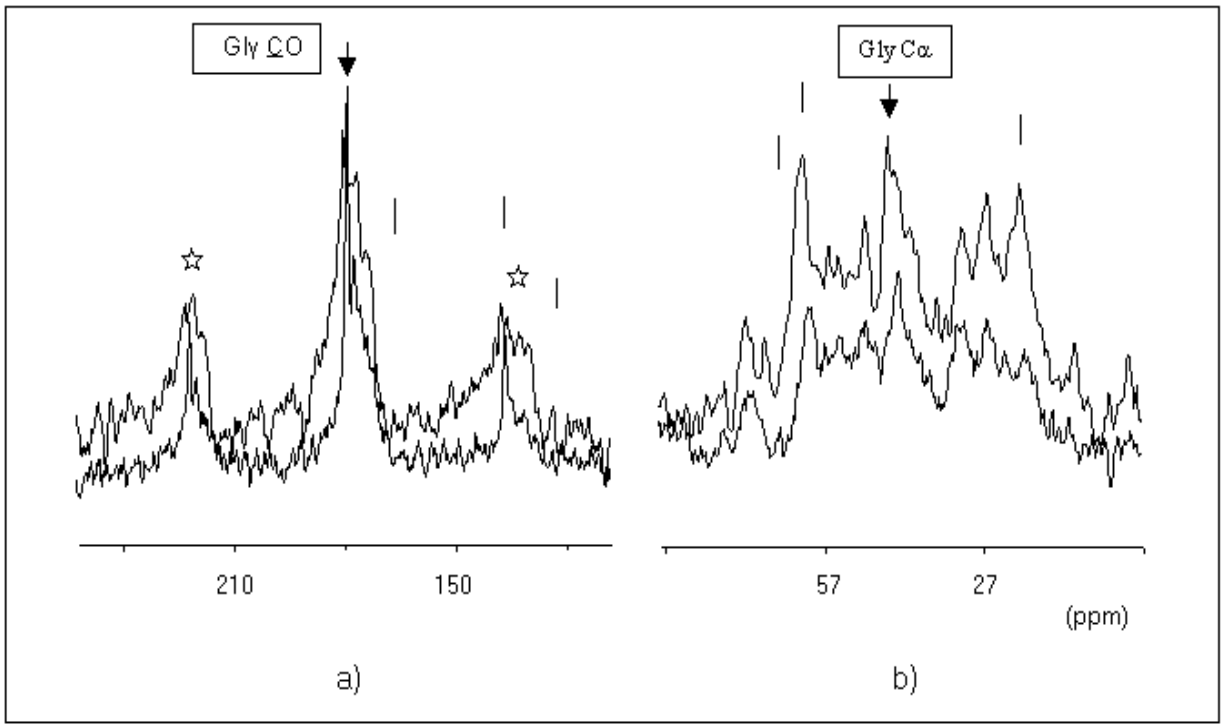


Figure 4. ^{13}C CP/DD-MAS (3.3 kHz) spectra of dentin (bottom) and dentin in the presence of HEMA (top), showing amplifications of carbonyl (a) and aliphatic resonances (b). Dentin was obtained as a powder from a human tooth (molar). Chemical shifts of HEMA are indicated by vertical lines: 167.7, 137.4, 125.6, 67.0, 60.7, 18.4 ppm. [I] Spinning side bands

The spectrum obtained for dentin in the presence of HEMA shows higher-resolved signals for aliphatic groups but broader carbonyl signals, as compared with dentin spectrum.

Conclusions

The present results clearly indicate an interaction glycine-HEMA not involving the glycine CO groups, but most probably concerning OH groups. ^{1}H spectra indicates the presence of HEMA molecules with restricted motion in the presence of glycine and this observation gives additional support to an eventual glycine-HEMA interaction.

As far as collagen is concerned, there are significant spectral changes like in CO, Gly Cα and Hyp C_β ^{13}C resonances. Further investigation is needed in order to explain these observations, which may be related with protein conformational changes and point to interactions involving other amino acid residues as well.

Acknowledgements

This work is part of a research project funded by *Fundação para a Ciência e a Tecnologia* (POCTI 33193/99). SGP and SGN acknowledge the grants PRAXIS/BD/20066/99 and PRAXIS/BD/13879/97.

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