Water-Melanin Interactions: An HR-MAS Magnetization Transfer Study

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INTRODUCTION

Melanin pigments are very hydrophilic. They also contain free radicals and have paramagnetic properties, which intensity remains a source of controversy (1). However bound water plays a role in maintaining the structure of the pigment (2). Here we aimed at comparing water-melanin interactions in synthetic and natural melanin pigments from magnetization transfer data.

METHODS

Synthetic melanin (tyrosine chemical melanin) (S) and Natural melanin (Sepiomelanin) (N) were purchased from Sigma Chemicals, St Louis, MO. Before the MR study, melanin granules were allowed to fix water from ambient air for 24 hours.

The MR study was performed on a Bruker DRX 500 magnet equipped with an HR-MAS probe. Samples were set into 4 mm diameter 80 µL Zirconia rotors, then spun at 4 kHz. Spinning may help in the detection of underlying sample compartments. The investigated samples were melanin granules and suspensions of granules (15% in D_2O).

Two kinds of acquisitions were performed : broadband (+/-100 ppm) ^{1}H spectra and magnetization transfer (MT) of water. The MT sequence involved a 5 sec duration MT pulse with B_2 = 15.6 µT, and 32 offset frequencies from 0 to 250 kHz. The offset frequencies were selected in order to avoid direct saturation of rotation bands at 4 kHz and multiples.

MT curves were analyzed similarly to a bound/free water 2-compartment model (3), in which the bound compartment concept was extended to a bound and/or paramagnetically enlarged signal compartment.

RESULTS

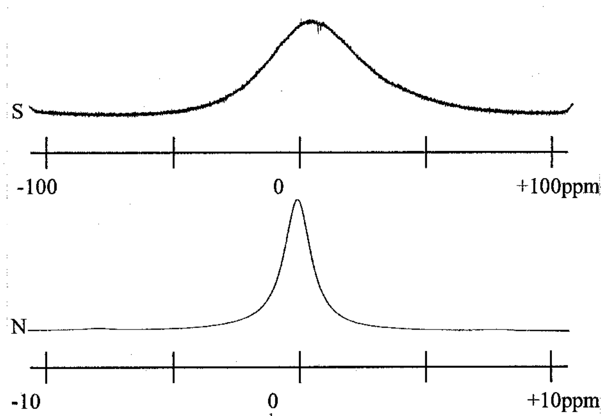

Fig 1: Broadband HR-MAS ^{1}H spectra of melanin granules (S: top, N: bottom). Broad signals of ~20 kHz (S) and ~650 Hz (N) can be identified. They originate from exchanging water molecules undergoing paramagnetic effects. The much less broad signal for N suggests a large compartment with water weakly bound or undergoing moderate paramagnetic effects.

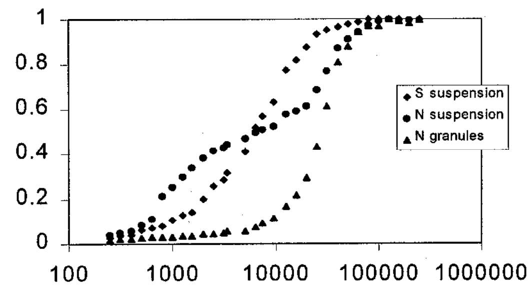

Fig 2: MT curves of S and N suspensions and of N granules. ^{1}H spectra of suspensions display a narrow line (~12 Hz) which signal is plotted against the offset frequency. For N granules the signal in Fig 1 is plotted against the offset frequency.

The analysis of MT curves in terms of a 2-compartment model for water in S suspension and a lorentzian model for the broad signal compartment, yielded a broad signal compartment T_2 of 48 $\pm$ 10 µs, and an exchange rate between compartments of 2900 $\pm$ 400 s^{-1}. For N suspension the broad signal compartment T_2 was 10 $\pm$ 2 µs, and an exchange rate of 700 $\pm$ 100 s^{-1}. For N granules the broader signal compartment T_2 was 12 $\pm$ 3 µs, and the exchange rate of 200 $\pm$ 220 s^{-1}.

DISCUSSION-CONCLUSION

These data suggest different water-melanin interactions for synthetic and natural melanin pigments.

For the synthetic melanin suspension, the interaction between water and melanin can be interpreted as taking place at sites with binding and/or paramagnetic effects of intermediate strength. However, the difference between the broad signal of S granules (~20kHz) and that of S suspension (~7kHz) may suggest a redistribution of strong interactions towards weaker ones, with the possibility of a continuum from strong to weak interactions.

For the natural melanin suspension, we had 2 compartments, one weakly bound and/or undergoing moderate paramagnetic effects, and the other tightly bound and/or undergoing strong paramagnetic effects. The slowest exchange rate takes place between these two compartments. This could be further interpreted as involving deep and superficial sites for water molecules at N.

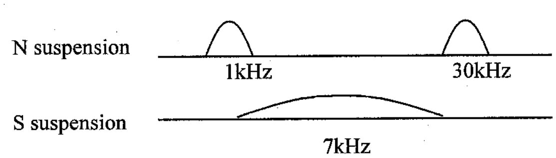

Fig 3: Distribution of water molecules in N and S melanins as can be interpreted from the MT data.

REFERENCES

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2)Ibidem p 74.
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