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Propionic acid side chain hydrogen bonding in the malaria pigment β-hematin

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Abstract

Malaria pigment, or β-hematin, the insoluble heme detoxification product resulting from the intraerythrocitic digestion of hemoglobin by young malaria trophozoites has been structurally characterized by X-ray powder diffraction and shown to contain chains of propionic acid linked dimers. Although there is considerable spectroscopic evidence for a monodentate propionate–iron interaction in this crystalline material, the spectroscopic characterization of the propionic acid dimer is limited. Herein we demonstrate the presence of the propionic acid dimer unit by H/D isotope substitution in carboxylic acid dimer. In the Raman spectrum of the deuterium substituted compound there is a circa 12 cm^{-1} shift, H: 1629 cm^{-1} vs. D: 1617 cm^{-1} in the symmetric ring breathing mode for the propionic acid dimer. On the other hand, the IR active asymmetric stretch has a very small shift, <3 cm^{-1}, upon deuteration. These, and other vibrational data, are consistent with the presence of a planar carboxylic acid dimer in the structure of β-hematin.

Keywords: Malaria pigment; Vibrational spectroscopy; Propionic acid dimer; β-Hematin

The recent solution [1] of the structure of the malaria pigment β-hematin by powder diffraction confirms the hypothesis that, in addition to propionate mediated iron–oxygen inter-heme interactions, there are also hydrogen bonds between the free propionic acid side chains. However, unlike prior proposals [2] that malaria pigment is a coordination polymer of propionate linked hemes, the structure, shown in Fig. 1a, is that of a hydrogen bonded chain of reciprocating dimers. During the Rietveld refinement of the structure of β-hematin the propionic acid dimer was found to have discernable deviation from planarity [3]. Although it is most likely that the origin of this deviation is an artifact of the inherent limitations in refining powder diffraction data, we sought to independently confirm the presence of the propionic acid dimer and, if possible, to characterize its geometry. Until a high resolution single crystal structure is available it is unlikely that diffraction alone will be able to unambiguously characterize this interaction. Unfortunately, the current spectroscopic evidence for the presence of propionic acid side chain hydrogen bonding is largely indirect [4] and based on infrared spectroscopy of the synthetic and natural phases and the similarity of some of the propionic acid bands to those in the hemin chloride, whose structure is shown in Fig. 1b [5]. Herein we report the Raman spectra for synthetic β-hematin and isolated malaria pigment, conditions for preparing partially deuterium substituted β-hematin, and finally, the Raman data for these samples. The observed shifts are compared to those calculated with density functional theory and the results confirm the presence of a carboxylic acid dimer in β-hematin and suggest that it has a typical planar geometry.

Materials and methods

Hemin was obtained from Aldrich Chemical company and dried at room temperature in a vacuum oven for 12 h before use. d_1-Deuteromethanol, CH_3OD, was obtained from Cambridge Isotopes and treated with a small chip of sodium before distillation under dry nitrogen. Dimethyl sulfoxide, 2,6-lutidine, and bulk methanol were dried by common methods [6], and stored in an inert-atmosphere box before use. Spectroscopic methods are the same as described elsewhere [4] with all Raman spectra being measured with Krypton laser excitation at 647 nm.

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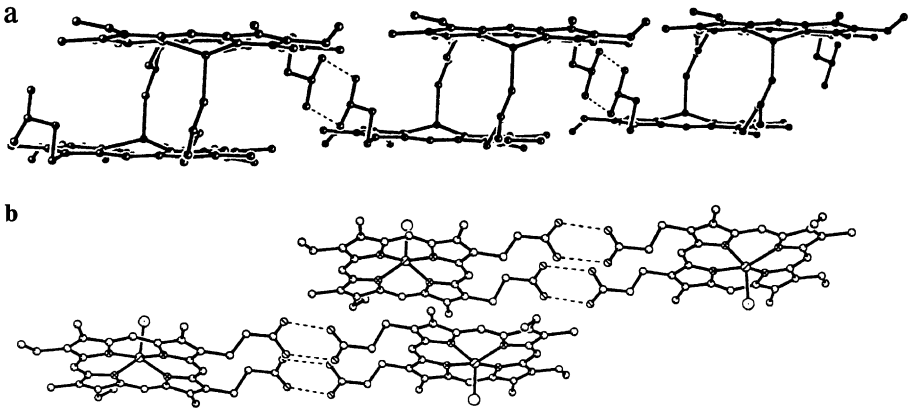

Fig. 1. Crystallographic structures of β-hematin [1] (a) determined by Rietveld analysis of the powder diffraction data and hemin chloride [5]; (b) from single crystal X-ray diffraction. Both structures have propionic acid dimerization.

Preparation (deuteration) of [Fe(III)(PPIX)(CO_2^-)(CO_2D)]$_n$, (β-hematin). This procedure is an adaptation of the prior published methods [7] and employs d_1-methanol, CH_3OD, as both solvent and deuterium source. In an inert atmosphere box 59 mg, 0.0905 mmol, of dry hemin is stirred overnight in 15 mL CH_3OD at room temperature to affect dissolution and exchange. After this initial period 2,6-lutidine, 0.5 mL, is added and stirred and the mixture is removed from the stirrer and allowed to stand tightly capped for 15 days. The suspension of β-hematin is then isolated by removing the slightly colored liquid from the solids with a pipette, fresh CH_3OD, 2 mL, is then added and the suspension vortexed briefly and then allowed to settle before the CH_3OD is removed. This step was repeated twice so that the final wash was colorless and the final black precipitate of β-hematin was dried in a vacuum over phosphorus pentoxide.

Isolation of malaria pigment. Approximately 4×10^9 infected red blood cells of the 3D7 clone of the NF54 chloroquine resistant strain of *P. falciparum* were brought to 92% parasitemia by a combination of synchronization and sorbitol treatment steps and then frozen and lyophilized to give a free flowing dry black mass. From this stage a modified literature method was employed [8], which involves treatment for two days at 37 °C in media consisting of pancreatin, 1 mg/mL, in 0.1 M Tris–HCl, pH = 7.5, and 20 mg/mL chloramphenicol. The media were changed daily by first centrifugation of the solids, washing the pellet with distilled water, and then resuspending the pellet in fresh media. Control experiments with uninfected red blood cells spiked with β-hematin indicated that this treatment has no effect on the infrared spectrum of the β-hematin. The final isolated yield based on mass/mass figure for malaria pigment/infected dry red blood cell is 4.4%.

Theoretical calculations. Density functional calculations for the formic acid dimer were performed with the Becke 3 parameter hybrid functional [9] coupled to the Lee et al. [10] correlation functional, B3LYP. A triple-ζ quality basis set, 6-311++G** with both diffuse and polarization functions added to the heavy and hydrogen atoms, was employed. The source code used for these calculations was Gaussian98 [11] implemented on a Silicon Graphics Origin platform. The ground state geometry and the selected vibrational modes depicted in Fig. 4 correspond to the optimized ground state stationary point characterized by all positive frequencies.

Results and discussion

While infrared spectroscopy is frequently used to characterize synthetic and natural (malaria pigment) samples of β-hematin, Raman spectroscopy is employed less often [12]. In part this reflects the domination of the Raman scattering spectrum by the porphyrin core [13], while the bands associated with the carboxylate groups, the key groups associated with the inter-heme aggregating interactions, are strongly infrared active. Nevertheless, some of the vibrational modes for the carboxylate groups are Raman active and can be expected to be markedly isotope sensitive with respect to proton/deuterium exchange [14].

The Raman spectrum for the carboxylate stretching region for synthetic β-hematin and the isolated pigment from *P. falciparum* is shown in Fig. 2. The complicated set of observed bands is largely based on porphyrin core modes and the two spectra are similar with minor differences in background and the noise present in the malaria pigment spectrum. There is an excellent match in the energies of the observed bands and their relative intensities. The intense band 1625 cm^{-1} is most likely

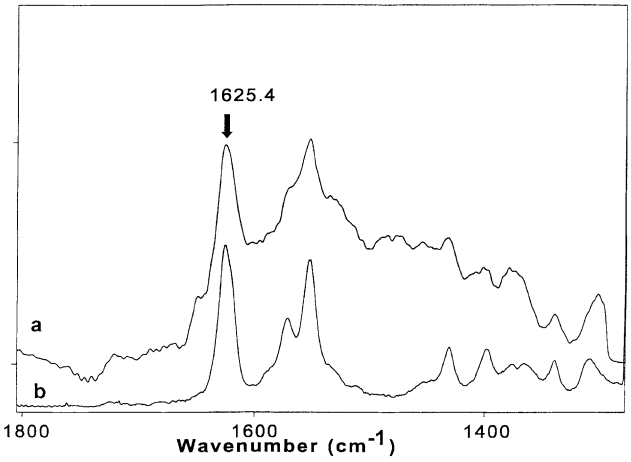

Fig. 2. Raman spectra for β-hematin isolated from (a) *P. falciparum*, malaria pigment, and (b) synthetic β-hematin. Both samples are measured at room temperatures as powders in capillaries with a krypton laser with radiation at 647 nm.

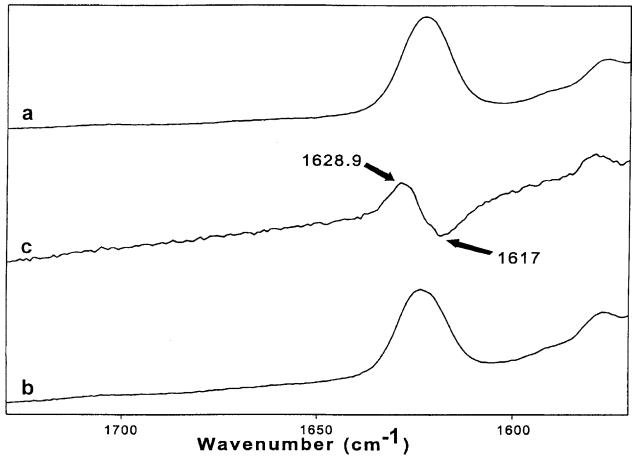

Fig. 3. Raman spectra for synthetic β-hematin as its natural protio form, (a), its deuterated form (b), and finally the difference spectrum shown in (c). For the conditions for these measurements see the caption to Fig. 2.

due to the overlap of a relatively strong band due to the vinyl ν(C=C) [13] with a weaker one due to the non-conjugated propionic acid dimer. The later assignment is based on the observed shift and difference in this band upon deuteration, Fig. 3. Here the difference trace, Fig. 3c, subtracts out the shared vinyl mode and gives a typical difference curve with a peak to trough separation of 11.9 cm^{-1}. The corresponding infrared spectra for the samples in Fig. 3 are almost identical with an upper limit shift in the $\nu(CO_2)_{asym}$ of 3 cm^{-1} upon deuteration.

To illustrate the origins of these shifts we have calculated the vibrational modes for the formic acid dimer, Fig. 4, with density functional theory. The theoretical frequencies and normal coordinate modes are shown for the optimized gas phase formic acid dimer. The individual atomic contribution to the infrared active B_u stretch, responsible for the strong IR absorption, has little involvement of the proton. And so the isotopic shift on H/D exchange is calculated to be a modest 6.0 cm^{-1}. On the other hand, the Raman active symmetric "ring breathing mode" A_g has a much larger shift, 22.1 cm^{-1}, upon deuteration. Although the absolute energies of these two bands are higher than those observed, in this case circa 50 cm^{-1} higher than those found in β-hematin, the relative energies and most importantly *their shifts* closely match those observed. In this case small differences may in part be due to the involvement of the formic acid C–H moiety. Clearly the remarkable similarity in the energies, isotopic shift magnitudes, and intensities for these bands support the assignment of the presence of a propionic acid dimer in the structure of β-hematin.

Deviations in planarity of the propionic acid dimer can be expected to alter the energies and isotopic shifts in both of the B_u and A_g bands. One key difference however will be the activities of the two bands; any decent in symmetry from the centrosymmetric C_{2h} point group to C_{2v} or C_2, can be expected to lift the mutual exclusion of both bands from the Raman and IR spectra. Thus if the propionic acid group is deformed and non-planar, then a Raman band at 1712 cm^{-1} and an IR band 1625 cm^{-1} would be observed. In both spectra these bands are not in fact observed or are only very weak. Thus we conclude that the propionic acid group is flat and centrosymmetric and does not have significant deviations from planarity.

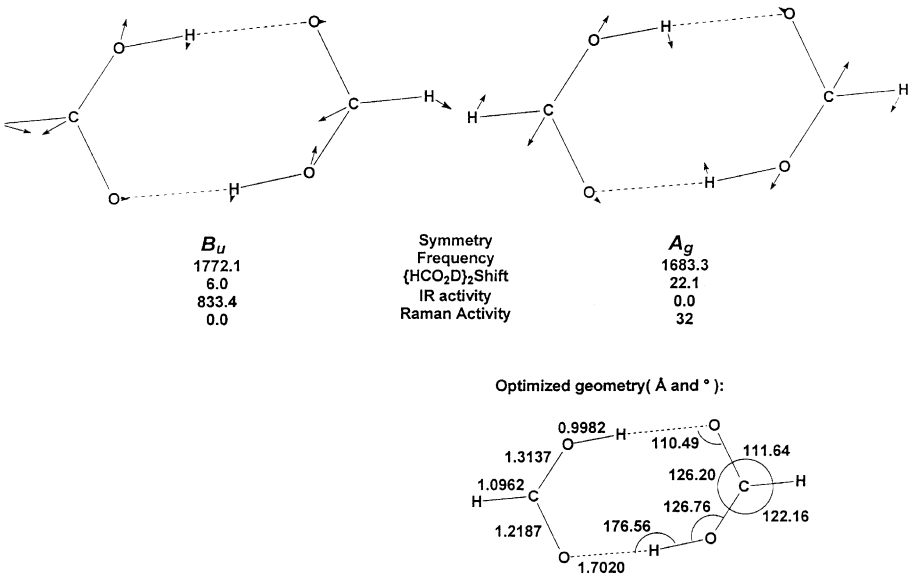

Fig. 4. Density functional theoretical results (B3LYP/6-311++G**) for the gas phase formic acid dimer. The top figures show the normal coordinate motions for the IR allowed B_u and the Raman allowed A_g modes, along with their frequencies, in cm^{-1}, shifts upon deuteration, in cm^{-1}, IR activity in KM/mole, and Raman activity in $Å^4$/amu. The optimized ground state geometry, shown in the lower figure, has C_{2h} symmetry.

Conclusion

This study provides new direct evidence for the presence of a propionic acid dimer in β-hematin. The observed bands and their shifts upon isotopic substitution are consistent with a planar symmetric structure, rather than a free or distorted carboxylic acid. This hydrogen bond is likely to be important in the formation of β-hematin in the parasite and we suggest that it may be the critical interaction in the growth of the pigment crystals. A current model for the drug action of the quinoline antimalarials is for drug/crystal binding on the surface [15] and understanding all of the interactions and chemical structures in this unusual condensed phase becomes critically important.

Acknowledgments

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