Journal of Non-Crystalline Solids 356 (2010) 1908–1913

Contents lists available at ScienceDirect

Journal of Non-Crystalline Solids

journal homepage: www.elsevier.com/locate/jnoncrysol

XAFS studies of the synthetic substitutes of hemozoin

M.S. Walczak [a], K. Lawniczak-Jablonska [a,*], A. Sienkiewicz [a,b], M.T. Klepka [a], L. Suarez [c], A.J. Kosar [c], M.J. Bellemare [c], D.S. Bohle [c]

[a] Institute of Physics, Polish Academy of Sciences, Al. Lotnikow 32/46 PL-02-668 Warsaw, Poland
[b] Institute of Condensed Matter Physics, Ecole Polytechnique Fédérale de Lausanne, Lausanne, CH-1015, Switzerland
[c] Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, Canada

ARTICLE INFO

Article history:
Received 12 May 2010
Available online 9 June 2010

Keywords:
XAFS;
Hemozoin

ABSTRACT

The local atomic structure around iron atoms of two synthetic analogues of malarial pigment product is characterized by iron K-edge X-ray absorption fine structure spectroscopy (XAFS). Malarial pigment, also termed hemozoin, is a condensed phase of ferriprotoporphyrin-IX dimers, and is structurally identical to the synthetic material hematin anhydride, which is also termed β-hematin. Synthetic hemozoin analogues might serve as surrogates or models of malaria pigment for a range of fundamental laboratory methods, for example the design of new antimalarials. Two new iron(III)(protoporphyrin-IX) analogues, based on meso- and deuteroporphyrin are considered. Both new condensed phases are slightly soluble in many organic solvents. In the present work, the detailed examination of X-ray absorption spectra of monomer- and dimer-ferriprotoporphyrin-IX-based compounds is discussed. Parameters obtained by quantitative spectra analysis allow us to classify the new synthetic equivalents of the parasite product as dimer materials. X-ray absorption technique was able to distinguish dimer from monomer structures based on the structural disorder parameters of oxygen ligand bonding.

1. Introduction

The crystalline malaria pigment termed hemozoin is the product of digestion of the red blood cell protein, hemoglobin, by the malaria parasite, *Plasmodium*. Hemozoin is synthesized from heme Fe(III) present in the parasite food vacuole after host's hemoglobin degradation by *Plasmodium*. The initial suggestion in 1717 by Luncisi [1] that crystalline hemozoin observed in erythrocytes was melanin, explains its traditional name — malarial pigment. Fitch and Kanjananggulpan [2] were the first to demonstrate that hemozoin consists only of ferriprotoporphyrin-IX molecules with iron in a ferric state (Fe(III)PPIX). Later on, Slater at al. [3] published preliminary results of the X-ray diffraction, and X-ray absorption, and vibrational spectroscopy suggesting the similarity between hemozoin and hematin anhydride, also called β-hematin. High resolution powder XRD measurements performed at a synchrotron confirmed the identity of the structure of hemozoin and hematin anhydride [4]. Detailed analysis of XRD patterns revealed that both condensed phases are organized in the ferriprotoporphyrin-IX dimers [5]. Such a dimer consists of two ferriprotoporphyrin-IX molecules linked by two propionate side chains of Fe(III)PPIX, and is schematically shown in Fig. 1. Dimers crystallize into chains linked by hydrogen-bonded propionic groups. The identity of hemozoin and hematin anhydride was confirmed also by EXAFS analysis of photoelectron backscattering up to distance of 3.5 Å from the central Fe atoms [6]. XAFS also pointed to a higher disorder in the natural pigment. As a consequence of these prior results the relationship between hemozoin and hematin anhydride is well established. Nevertheless, as a part of our efforts to find new anti-malarial drugs, we searched for other synthetic substitutes of hemozoin with better solubility than hematin anhydride. In the present paper we consider two newly synthesized materials, mesohematin anhydride and deuterohematin anhydride, to check their similarity to hematin anhydride. Here in we report the local atomic order around iron atoms in these materials, and the extended X-ray absorption fine structure (EXAFS) spectra. The results are compared to those of hematin anhydride and hematin, and the data analysis focuses on the detection of the oxygen moiety atoms characteristic for the dimer structure observed for hemozoin and hematin anhydride.

2. Experimental

2.1. Investigated materials

Iron(III) (protoporphyrin–IX anhydride)–$C_{68}H_{62}N_8O_8Fe_2$*–hematin anhydride (β-hematin)*, was synthesized from Iron(III) (protoporphyrin–IX chloride), $C_{34}H_{32}N_4O_4FeCl$, following literature methods, [7].

Iron(III) (mesoporphyrin–IX anhydride)–$C_{68}H_{70}N_8O_8Fe_2$ *(*$(CH_3)_2SO$*)*–mesohematin anhydride, was synthesized from Iron(III)

---

* Corresponding author.
E-mail address: jablo@ifpan.edu.pl (K. Lawniczak-Jablonska).

0022-3093/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jnoncrysol.2010.05.023

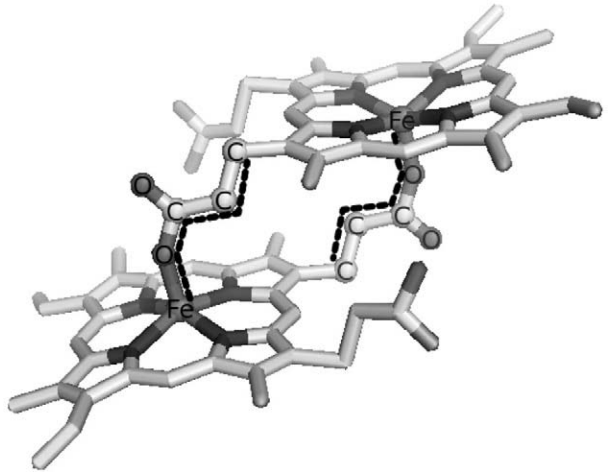

Fig. 1. The stick model of the structure of the dimer unit of hemozoin with marked oxygen moiety bonds.

(mesoporphyrin–IX chloride)–$C_{34}H_{36}N_4O_4FeCl$ by the methods used for hematin anhydride and characterized as its DMSO solvate.

Iron(III) (deuteroporphyrin–IX anhydride)–$C_{60}H_{54}N_8O_8Fe_2$–deuterohematin anhydride, was synthesized from Iron(III) (deuteroporphyrin–IX chloride)–$C_{30}H_{28}N_4O_4FeCl$, following the techniques used for hematin anhydride.

All syntheses were performed in an inert atmosphere box and followed by a careful purification in the open, [7]. The crystallographic data of hematin anhydride obtained by Pagola et al. [5] have been included in the Cambridge Crystallographic Data Centre CCDC database, the XETXUP entry. Crystallographic data of the mesohematin anhydride were kindly provided by Professor Peter W. Stephens.

Iron(III) (protoporphyrin–IX hydroxide)–$C_{34}H_{33}N_4O_5Fe$–hematin (α–hematin) was purchased from Alfa Aesar (Ward Hill, MA, USA) and used as received.

2.2. Experimental technique

Iron K-edge X-ray absorption spectroscopy measurements were performed at BM26 beamline at the ESRF synchrotron in Grenoble using transmission mode of detection. The ionization chambers were used to measure the incident and transmitted X-ray flux intensity. Samples were cooled down to 10–12 K to limit the temperature-induced atomic disorder and avoid sample degradation under irradiation. Spectra were registered in the range of 6950 eV to 8100 eV. In order to control the reproducibility and to gain a good signal-to-noise ratio several spectra were collected and averaged. To ensure the energy calibration, the reference iron foil was measured simultaneously. The background was corrected and normalized using Viper software [8]. Extended X-ray absorption fine structure spectra were analyzed by EXCURVE 9.27 software [9] starting from structural model based on the crystallographic data. The theoretical calculations of scattered wave amplitude and phases employed the fast spherical wave approximation [10–12].

3. Results and discussion

3.1. Structural models based on XRD results

To analyze the extended X-ray absorption fine structure (EXAFS) data the construction of a reasonable starting model of the local atomic order around Fe atoms in the investigated materials is necessary. In this case, the crystallographic models for the hematin anhydride dimer, Fig. 2a and b, and related high spin O-atom ligated monomer, Fig. 2c, are useful starting points. These models take into account only atoms enclosed in the sphere of 4.4 Å radius from the central iron of the ferriprotoporphyrin-IX and the axial atoms of the coordinated propionate (axial atoms (a.a.): (O a.a., C1 a.a., C2 a.a., O2 a.a.) in the case of the dimer and a single oxygen ligand (O a.a.) in the case of the monomer Fe(III)PPIX).

The atomic positions for both mesohematin anhydride and hematin anhydride starting models were obtained from crystallographic powder diffraction data, and are presented in Table 1. These atomic positions will be useful to compare later with atomic positions

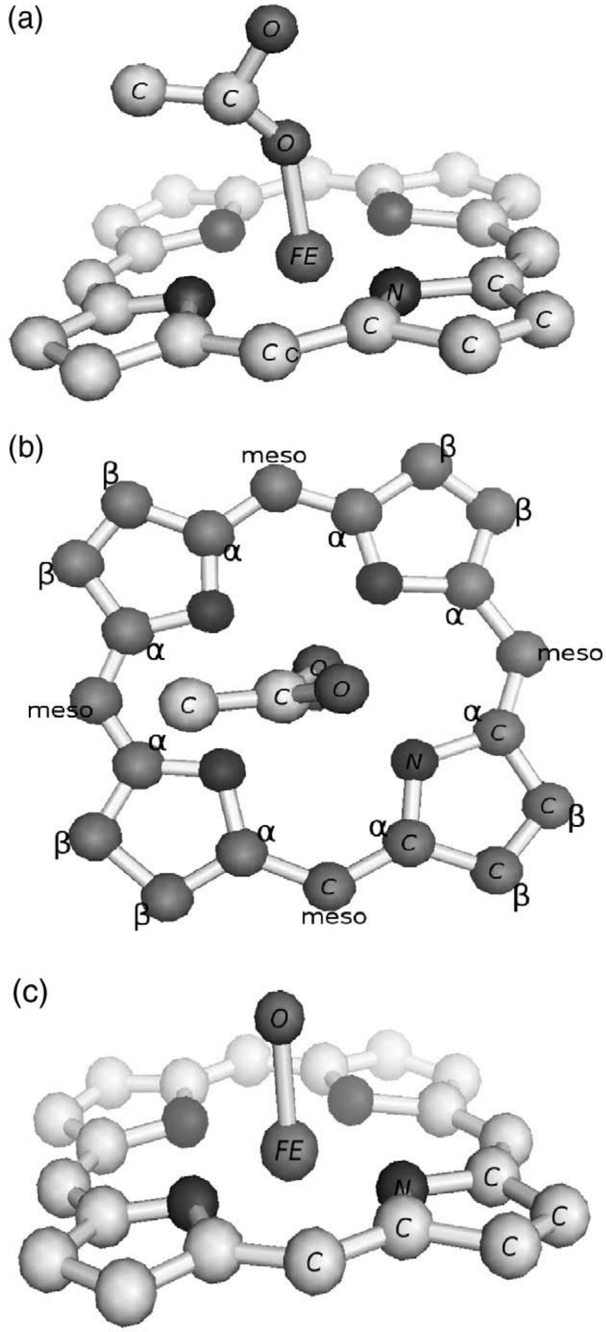

Fig. 2. The structural model of ferriprotoporphyrin-IX ring and oxygen moiety bond atoms used for EXAFS refining procedure of dimer material (a and b) and for monomer material (c). The spheres inside ferriprotoporphyrin-IX ring for dimer as well as monomer model are as follows: sphere of four nitrogen, sphere of eight carbons C_α, sphere of four carbons C_{meso} and spheres of eight carbons C_β. The spheres of ferriprotoporphyrin ring structure were chosen in exactly the same way as for hemozoin and hematin anhydride structure analysed in paper [6]. Groups of carbon atoms were named after IUPAC terminology.