Synchrotron X-ray microscopy and spectroscopy analysis of iron in hemochromatosis liver and intestines

J Y Peter Ko1,4, Tsun-Kong Sham1,4, Subrata Chakrabarti2, and Paul C Adams2,3

1 Department of Chemistry, The University of Western Ontario, London, ON N6A 5B7, Canada
2 Department of Pathology, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON N6A 5C2, Canada
3 Department of Medicine, Schulich School of Medicine and Dentistry, London Health Sciences Centre-University Hospital, The University of Western Ontario, 339 Windermere Road, London, ON N6A 5A5, Canada

E-mail: jyko@uwo.ca; tsham@uwo.ca

Abstract. Hemochromatosis is a genetic disorder that causes body to store excess iron in organs such as heart or liver. Distribution of iron, as well as copper, zinc and calcium, and chemical identity of iron in hemochromatosis liver and intestine were investigated by X-ray microprobe experiments, which consist of X-ray microscopy and micro-X-ray absorption fine structure. Our results show that iron concentration in hemochromatosis liver tissue is high, while much less Fe is found in intestinal tissue. Moreover, chemical identity of Fe in hemochromatosis liver can be identified. X-ray microprobe experiments allows for examining elemental distribution at an excellent spatial resolution. Moreover, chemical identity of element of interest can be obtained.

1. Introduction
Third-generation synchrotron X-ray sources, coupled with Kirkpatrick-Baez (KB) mirror or zone plates have been used as a powerful imaging and spectroscopic technique with micron or submicron spatial resolution [1-3]. One of the most common applications for combined X-ray microscopy/spectroscopy is the analysis of metal contents in biological samples, such as tissues and cells [4, 5]. Due to the brightness and tunability of synchrotron light source, and its excellent spatial resolution achievable, synchrotron X-ray microbe analysis (imaging and micro-XAFS spectroscopy) provides valuable chemical information not easily obtainable via conventional methods.

Hemochromatosis is a genetic disorder that causes body to absorb and retain excess iron, affecting approximately one in 300 people of Northern European descent. [6, 7] Excess iron in liver leads to cirrhosis and hepatocellular carcinoma [6]. Liver cells are overloaded with iron in hemochromatosis, whereas iron concentration within intestinal cells is normal or decreased. By means of elemental mapping and micro-X-ray absorption fine structure (µ-XAFS), one can obtain useful information on elemental distribution (in this case, iron distribution) and chemical identity of iron in
hemochromatosis liver and intestine tissues. In this report, we will present distribution and chemical characteristics of elements present in the tissue samples, focusing mainly on iron.

2. Experimental

Three human biopsy liver and three intestinal tissues were obtained from the laboratories of Dr. Adams and Dr. Chakrabarti. For liver tissues, a hemochromatosis liver tissue showing cirrhosis (henceforth labelled Lv1), a hemochromatosis liver tissue with cancer (henceforth labelled Lv2), and a normal liver tissue (henceforth labelled Lv3) were examined. For intestinal tissues, two hemochromatosis intestinal tissues (henceforth labelled I1, and I2) and one normal intestinal tissue (henceforth labelled I3) were examined. These tissues were prepared as thin slices (~10 µm) and covered with a thin layer of paraffin on a substrate. Substrates were silicon wafers for liver tissues and high purity quartz (fused silica) slides for intestine tissues; neither substrates show detectable Fe, Cu or Zn impurities [8].

X-ray microscopy and spectroscopy were performed at the microprobe station of the Pacific Northwest Consortium – X-ray Operations and Research (PNC/XOR), Sector 20ID line at the Advanced Photon Source (APS), Argonne National Laboratory. Schematic layout of microprobe experiment is shown in Figure 1. More details about the microprobe station at PNC/XOR are reported elsewhere [9].

Photon beam from the undulator was monochromatized with a cryogenically cooled Si (111) double crystal monochromator, and focused with a set of Kirkpatrick-Baez (KB) mirror. The monochromator was tuned to energies above Fe K-edge, and the size of the beam was approximately 2 µm × 2 µm. Beam size was calibrated and optimized with a thin Pt wire by adjusting horizontal and vertical KB mirrors prior to mounting the sample on a motorized sample stage. This spatial resolution allows us to track the metal content and chemical identity within a single pixel. Sample was placed on the sample stage at 45° with respect to the incoming beam. The sample stage was scanned in x,y direction in a pre-determined increment. Elemental maps were constructed pixel-by-pixel by setting an energy window to collect fluorescence photons characteristics of elements of interest while scanning a region of the sample. Measurements were made with APS running at 100 mA in a top-up mode, providing a typical flux of $10^{11}$ photons/sec with the microbeam. A typical fluorescence spectrum of Lv1 specimen is shown in Figure 2. Once a map was generated, µ-XAFS experiment was performed on spots of interest.
3. Results and discussion

3.1. Elemental distribution in liver tissue samples

3.1.1. Elemental distribution
Elemental maps of Lv1 (cirrhosis), Lv2 (cancer), and Lv3 (normal) showing distribution of Fe, Cu, Zn and Ca are displayed from left to right in Figure 3. The Fe map of Lv1 and Lv2 (Figures 3a and 3e respectively) show high Fe distribution throughout the sampling area. Moreover, Fe hot spots of Lv1 are much more prominent compared to those Lv3 (a factor of 5 on average judging from the scale). The Fe map also tracks the texture of the optical image (not shown). In addition, the presence of Cu, Zn and Ca are generally concomitant with Fe; i.e. that hot spots for other elements that were examined (Figure 3b, c, and d for Cu, Zn, and Ca distribution respectively in Lv1 and Figure 3f, g, and h for Cu, Zn, and Ca distribution respectively in Lv2) are found where hot spots of Fe are located.

Figure 2. X-ray fluorescence spectrum of Fe “hotspot” of Lv1 specimen

Figure 3. (a-d): 400 × 400 µm² elemental map of Lv1 specimen (a) Fe, (b) Cu, (c) Zn, (d) Ca; (e-h): 800 × 800 µm² elemental map of sample Lv2 specimen (e) Fe, (f) Cu, (g) Zn, (h) Ca; (i-l): 400 × 400 µm² elemental map of sample Lv3 specimen (i) Fe, (j) Cu, (k) Zn, (l) Ca. The intensity scale is shown in the inset. Arrows in Figure 3a indicates spots on which spectroscopy was performed.
3.1.2. Fe K-edge EXAFS

An iron "hot spot" and a "medium spot" from Lv1 specimen were located from the Fe elemental map (Figure 3a), where µ-XAFS was performed. Figure 4a shows normalized EXAFS from the spots examined together with model compounds (human ferritin and holo-transferrin), with inset showing the XANES region. Although the XANES of both hot and medium spots have similarities, the extended region (~7265 eV and above) shows differences. Results of the Fourier transform of both EXAFS, shown in Figure 4b, exhibit more disorder (weaker magnitude) in the first shell of the medium spot and a noticeable difference in the second coordination shell (shortened by about 0.2 Å and unusually intense) relative to that of the hot spot. Fourier transform of the hot spot EXAFS resemble those of ferritin [10]. The enhanced second coordination peak of the medium spot is most likely due to focusing effect arising from near-linear arrangement of absorbing and scattering atoms, e.g., Fe-O-O, as observed by Kuzmin et al. in the ReO₃ case [11]. Although semi-quantitative at best due to limited statistics, it can suggested that chemical environment between the two spots are very different, a very interesting result observed for the first time. Further experiment at low temperature and modeling are required to gain more details on this observation.

![Figure 4](image-url)

**Figure 4.** (a): Fe K-edge EXAFS of a “hot spot” and a “medium spot” of Lv1 specimen (spots are indicated with arrows in Figure 3a), as well as human ferritin and holo-transferrin. Inset: XANES region of hot and medium spots of Lv1. (b): Fourier transform of the EXAFS of hot and medium spots of Lv1. Inset: chi(k) plot (k-weight = 2)

3.2. Intestine tissue samples

3.2.1. Elemental distribution

Elemental maps of I1, I2 and I3 specimen showing distribution of Fe, Cu and Zn are displayed in Figure 5. Fe concentration, even in I1, I2 (two Fe “overload” samples) is low. Hot spots of elements that were examined cannot easily be located in I1 specimen (Figure 5a-c). In I2 specimen, Fe hot spots can be found in the Fe elemental map (Figure 5d), though not as prominent as in the case for Fe overload liver tissue samples (Figure 3a and e). These hot spots are located near the edge of the tissue. Cu and Zn hot spots can also be seen from respective elemental maps (Figure 5e-f). In I3 specimen, hot spots of Fe, Cu, and Zn are found to be scarce; however, interestingly, Fe elemental map of I3 specimen (Figure 5e) show a slightly higher concentration of Fe than I1 specimen, suggesting Fe concentration in intestine may have decreased in hemochromatosis intestine tissues.
Figure 5j shows a typical fluorescence spectrum of Fe hotspot from I2 specimen. Due to much lower concentration of Fe, Cu and Zn compared to liver specimen, peaks are not as intense; however, these elements are still detectable, and elemental maps can be generated.

Figure 5. (a-c): 400 µm × 600 µm elemental map I1 specimen (a) Fe, (b) Cu, (c) Zn; (d-f): 400 µm × 600 µm elemental map of I2 specimen (d) Fe, (e) Cu, (f) Zn; (g-i): 120 µm x 200 µm elemental map of I3 specimen (g) Fe, (h) Cu, (i) Zn (j): X-ray fluorescence spectrum of Fe “hot spot” of I2 specimen.

3.2.2. Fe K-edge XANES

Iron “hot spot” from I2 specimen was located from the Fe elemental map (Figure 5d), and µ-XAFS was performed. Figure 6 shows normalized XANES of the spot. Due to low concentration Fe, signal to noise ratio in the extended region of the spectrum is not ideal, even with long integration time and multiple scans. However, analysis of the near-edge is possible. Unlike the case for liver specimen, near-edge spectral profile of the hot spot of I2 specimen resembles that of holo-transferrin instead of ferritin.

Figure 6. Fe K-edge EXAFS Fe “hot spot” of I2 specimen, ferritin, and holo-transferrin.
4. Summary and prospects
Synchrotron X-ray microbe experiments routinely allow for obtaining elemental maps at micrometer resolution, as well as chemical information of selected spots from the elemental maps. Hemochromatosis liver tissue samples show high concentration of iron (> a factor of 5), while intestine samples of hemochromatosis patients show normal to lower concentration of iron compared to normal tissue samples. By performing µ-XAFS, we have shown that iron in the tissue samples most likely exists in ferritin form (or similar) while in intestines it resembles that of holo-transferin.

With the methods presented here, one can routinely analyze not only metal contents in biological tissue samples for their distribution and chemical environment, but also trace elemental contents on any samples.

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