Iron Loaded Mammalian Tissue: Iron Oxide Nanoparticulate Size and Distribution using Anomalous and Scanning Small Angle X-ray Scattering

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Iron overload diseases such as haemochromatosis and thalassaemia affect a large fraction of the world's population. Excess iron is toxic and accumulates in tissues in the form of iron(III) oxyhydroxide associated with the iron storage compounds ferritin and haemosiderin. The physical form of the iron is of particular importance since it is expected to reflect the biological and environmental conditions of deposition. Size distribution determines the surface to core iron ratio, which is in turn expected to determine the molar toxicity of cellular deposits. Despite its significance, a method for characterising the physical form of iron oxide deposits in bulk tissue samples has been lacking, until now.

We have studied the liver and spleen from a series of rats (n=64), iron-loaded under three different regimes for various durations to result in a variety of iron-loaded conditions. Two methods of sample preparation were utilized for small angle x-ray scattering (SAXS) at ChemMatCARS at Advanced Photon Source; 1) Samples were freeze-dried and ground to form pellets of tissue for bulk analysis by anomalous SAXS (ASAXS), 2) Samples were wax embedded and sectioned to 1 mm thickness to study local variation in the tissue by scanning SAXS.

Using ASAXS at the iron K-edge we have demonstrated the ability to measure iron oxide particle size in bulk tissue samples and applied the technique to a controlled biological system. The most likely particle diameter (±SD) for spherical iron oxide deposits is 6.4 ± 0.3 nm. No appreciable difference could be found with duration of iron loading, loading regime or organ type and the ferritin protein shell is therefore likely to be the primary determinant of particle size.

From radiography we know the distribution of iron is uneven on the mm length scale. We have applied scanning ASAXS to investigate the local variation in iron deposition within a tissue. 2D maps have been generated to reveal element- and dimension-specific images where nanoparticulate iron deposits give rise to image intensity. The SAXS maps compare well with radiography and reveal significant heterogeneity in nanostructure over mm distances, depending on iron loading condition.

KEYWORDS: small angle x-ray scattering, ferritin, haemosiderin, iron overload, liver, spleen