

Previous kinetics studies with homopolymer ferritins (bullfrog M-chain, human H-chain & *E. coli* bacterial ferritin) have established that a  $\mu$ -1,2-peroxo diferric intermediate is formed during Fe(II) oxidation by O<sub>2</sub> at the ferroxidase site of the protein. The present study was undertaken to determine whether such an intermediate is also formed during iron oxidation in horse spleen ferritin (HoSF), a naturally occurring heteropolymer ferritin of H- and L-subunits. Stopped-flow spectrophotometry of HoSF demonstrated that a peroxo complex is also produced in this protein, exhibiting an absorbance maximum at 650 nm as for other ferritins. However, the peroxo complex forms at only about one-third of the 3.3 H-chain ferroxidase sites of the 24mer protein when Fe(II) is presented to the protein in a stoichiometry amount of 2 Fe(II)/H-chain. These results indicate that, due to the presence of the L-chain, an alternative mechanism(s) of iron oxidation/deposition takes place. The amount of peroxo complex formation drops significantly to only ~12 % of the H-chains when 20 Fe(II)/H-chain are added to the protein, indicating a declining role of the peroxo complex in iron deposition, presumably due to an increase in the mineral surface reaction mechanism. Multi-wavelength stopped-flow kinetics was analyzed as per human H-chain ferritin (HuHF)<sup>1</sup> to determine reaction sequences and rate constants. The peroxo complex in HoSF is formed about 5-fold slower than human H-chain and decays more slowly (~3-fold) as well. As found for HuHF<sup>1</sup>, evidence is obtained for the formation of a second intermediate in HoSF that is possibly a hydroperoxo complex.

<sup>1</sup> “Origin of the Unusual Kinetics of Iron Deposition in Human H-Chain Ferritin” F. Bou-Abdallah, G. Zhao, H. R. Mayne, P. Arosio and N. D. Chasteen (2005) *J. Am. Chem. Soc.* **127**, 3885-3893.