

Purification of fusion ferritin from recombinant *E.coli* using two step sonications

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Iron is an essential nutrient for most organisms, which is supplied to them in a protein-iron complex known as ferritin (Bauminger *et al.* 1999). Ferritin, an iron-storage protein, consists of protein shell of similar 24 subunits, resulting in molecular weight of 450 kDa (Hudson *et al.* 1993). It is classified as heavy chain (21 kDa) or light chain (19 kDa) type (Bauminger *et al.* 1994), and is able to accommodate 4500 iron atoms. Ferritins are hetero-polymers combined by H-, L-chain with variable ratio (Theil 1987).

Studies of the homo-polymers of human H- and L-type chains and of some variants indicated that H ferritin oxidizes iron at rates several-fold faster than L ferritin (Levi *et al.* 1988), due to the presence of a particular ferroxidase center in H ferritin that is absent in L ferritin (Santambrogio *et al.* 1993). L ferritin appears to induce iron mineralization with higher efficiency than the H ferritin (Levi *et al.* 1992). L is notably more stable to physical denaturation than H ferritin. Thus, the hybrid molecules composed by H- and L-chains are more efficient in taking up iron than the homo-polymers of H- and L-chain.

Fusion ferritin, combined by heavy chain ferritin(21 kDa) and light chain ferritin(19 kDa), was expressed in recombinant *E. Coli*. The fusion ferritin was easily purified by two step sonications as well as gel filtration chromatography. SDS-gel electrophoresis showed a single protein band of 38 kDa suggesting the presence of heavy and light chains. MALDI-TOF-MS revealed the molecular weight of fusion ferritin was 38 kDa. The specific activity and yield of purified fusion ferritin are $0.41 \text{ Fe}^{3+\text{mg}^{-1}}$ and 66.1 %.

References

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