# Loading of iron into recombinant rat liver ferritin heteropolymers by ceruloplasmin

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摘要

## Abstract

We have reported previously that the heavy chain of ferritin is required for iron incorporation by ceruloplasmin (J.-H. Guo, M. Abedi, and S. D. Aust (1996) Arch. Biochem. Biophys. 335(1)). The purpose of this study was to determine how many heavy chains were required for ceruloplasmin to interact with ferritin such that iron loading occurred. The cDNA sequences encoding the heavy and light chains of rat liver ferritin were cloned into the baculovirus transfer vector pA-cUW51 under the control of polyhedrin and p10 promoters, respectively, which was then incorporated by homologous recombination into the infections Autographa californica nuclear polyhedrosis virus genome. Both ferritin chains were expressed and assembled into two heteropolymers following the infection of insect cells by recombinant virus, which were separated by DEAE-Sepharose chromatography. The percentage of heavy (H) and light (L) chains making up the two heteropolymers, determined by gel scanning following the resolution of chains on SDS-PAGE, were equivalent to 1 H and 23 L chains and 2 H and 22 L chains. The maximal extent of iron loading was observed using 1 mol of rat ceruloplasmin per mole of H chain in the two heteropolymers. The extent of iron incorporation decreased with additional ceruloplasmin. Iron incorporation into rat liver ferritin, found to contain 10 H chains, increased as the molar ratio of ceruloplasmin to ferritin increased to 4:1 and remained the same up to 8:1. Iron loading into horse spleen ferritin, found to have one H chain, appeared similar to that for recombinant ferritin, having only one H chain. Therefore, we propose that the optimal molar ratio of ceruloplasmin to ferritin depends upon the numbers of H chain making up the ferritin molecule for the maximal incorporation of iron into ferritin. These results also suggest that the iron loading channel is contained within a single H chain subunit.