Mutational analysis of the four a-helix bundle iron loading channel of rat liver ferritin

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摘要

Abstract

We previously reported that the heavy chain of ferritin was required for loading it with iron using ceruloplasmin as a ferroxidase [J.-H. Guo, M. Abedi, and S. D. Aust (1996) Arch. Biochem. Biophys. 335, 197-204], Site-directed mutagenesis, K58E andG61H, on recombinant rat liver L chain ferritin (rL-Ft) was performed to construct a proposed iron-loading channel in the alpha-helix bundle similar to rat liver H chain ferritin (rH-Ft), Conversely, the channel in rH-Ft was closed by mutations E62Kand H65G to form a K62 to E107 salt bridge, which is believed to exist in the L chain. Both variants were expressed in insect cells and were soluble and able to form multi-subunit homopolymers. The rH-Ft mutant homopolymer could not be loaded, whereas the rL-Ft mutant homopolymer could be loaded with iron by ceruloplasmin. However, we found that the initial rate of iron loading into the rL-Ft mutant homopolymer by ceruloplasmin was less than that into the rH-Ft homopolymer. When 500 atoms of iron per ferritin were used for loading, 98% was loaded into the rH-Ft homopolymer by ceruloplasmin in 15 min, but only 30% was loaded into the rL-Ft mutant homopolymer in the same time. Moreover, the ferroxidase activity of ceruloplasmin was enhanced in the presence of the rH-Ft and the rH-Ft mutant homopolymers, but not in the presence of the rL-Ft or the rL-Ft mutant homopolymers. These observations suggested that the four alpha-helix bundle channel of ferritin is required for iron loading, butan additional factor, i.e., a site which stimulate the ferroxidase activity of ceruloplasmin, is also essential. (C) 1998 Academic Press. [References: 35]