Coexpression of heterodimeric subunits improves iron storage capability of recombinant ferritin in yeast

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Abstract

Saccharomyces cerevisiae was engineered to express different amount of H- and L-chain subunits of human ferritin using a low copy integrative vector (YIp) and a high copy episomal vector (YEp). In addition to *pep4::HIS3* allele, the expression host strain was bred to have the selection markers *leu2-* and *ura3-* for YIplac128 and YEp352, respectively. The heterologous expression of phytase was used to determine the expression capability of the host strain. Expression in the new host strain (2805-a7) was as high as the parental strain (2805), which expresses high levels of several foreign genes. Following transformation, Northern and Western blot analyses demonstrated the expression of H- and L-chain genes. The recombinant yeast was more iron-tolerant, in that transformed cells formed colonies on plates containing more than 25 mM ferric citrate, whereas none of the recipient strain cells did. Prussian blue staining indicated that the expressed isoferritins were assembled *in vivo* into a complex that bound iron. The expressed subunits showed a clear preference for the formation of heteropolymer over homopolymers. The molar ratio of H- to L-chains was estimated to be 1:6.8. The gel-purified heteropolymer took up iron faster than the L-homopolymer, and it took up more iron than the H-homopolymer did. The iron concentration in transformants expressing the heteropolymer, L-homopolymer, and H-homopolymer was 1004, 760, 500 μg per gram dry cell weight of recombinant yeast, respectively. The results indicate that heterologously expressed H- and L-subunits co-assemble into a heteropolymer *in vivo*, and that the iron-carrying capacity of yeast is further enhanced by the expression of heteropolymeric isoferritin.

Iron is an essential element in most living organisms. However, its availability is limited by the low solubility of Fe(III) and the ability of intracellular free iron to produce toxic radicals. Consequently, once iron enters a cell, it must be stored in an intracellular form that

is soluble, non-toxic, and bioavailable [1, 2].

Ferritin (apoferritin-Fe complex) is an iron-storage protein found in most living organisms [3]. It is a spherical macromolecule with a protein coat made of 24 structurally equivalent subunits, which can hold up to 4500 iron atoms as a ferric oxyhydroxide polymer in its central core [4]. The major role of ferritin is to provide iron for the synthesis of iron-containing proteins and to prevent damage by the free radicals produced in iron/dioxygen interactions [3, 5]. Ferritin has two main subunits: heavy (heart: H) and light (liver: L). Various combinations of the two subunits give rise to isoferritins, many of which are associated with specific pathologies or are located in discrete tissues [6]. Generally, L-rich ferritins are characteristics of organs that store relatively high amount of iron ($\geq$1,500 Fe atoms/molecule), while H-rich ferritins are found in organs with a low average iron content ($\leq$1,000 Fe atoms/molecule). No H-homopolymers have been isolated in nature whereas human serum ferritin is devoid of H-chains [7] and an L-homopolymer fraction has been extracted from horse spleen ferritin [8]. Since H-homopolymer is a relatively poor iron core-former and L-homopolymer lacks intra-subunit ferroxidase center, heteropolymeric ferritin stores and releases iron more efficiently than the homopolymer. Natural isoferritins are mostly heteropolymers formed by the co-assembly of the two subunits. The subunits can be joined in various ratios, and only a limited number of H chains are required to maximize the iron uptake [9]. Native horse spleen ferritin, one of the best-characterized and most common sources of ferritin, appears to average about 3 H-chains/molecule.

The food yeast *Saccharomyces cerevisae*, which is known as a GRAS (generally recognized as safe) organism, is grown to produce biomass rich in high quality proteins and vitamins. As a consequence, it is used in feeds for fish, poultry, and fur-bearing animals and as a food supplement for human consumption [10]. Increasing the nutritional quality of feed additives and food supplements is an efficient way to enrich feed and food with specific nutrients. In *S. cerevisiae*, extracellular Fe3+ is first reduced to the more soluble Fe2+ form by a plasma membrane Fe3+-reductase and the resulting Fe2+ product is then taken up by either of two Fe2+-specific transport systems, depending on the extracellular iron concentration [11]. The intracellular iron, which is extremely toxic at high concentrations, must be compartmented or stored as a safe, bioavailable form in the cytosolic ferritin.

The yeast ferritin-like protein has a very low binding affinity to cytosolic iron, which prevents yeast from utilizing it as an efficient source of bioavailable iron. In a previous

study, we showed that *S. cerevisiae* can express the gene encoding the heavy subunit of tadpole ferritin and the protein product can assemble into iron-storing apoferritin, improving the iron-storage capability of the recombinant yeast [12].

In this study, we further increased the iron-storing capability of the recombinant yeast via the expression of heteropolymeric apoferritin. The recombinant was designed to differentially express the heavy and light subunits of human ferritin in order to obtain heteropolymers that contained a limited number of H-chains by co-expressing a low-copy integrative vector and a high-copy episomal vector for the heavy and light subunits, respectively. We also expressed homopolymers consisting of either heavy or light subunits as controls to compare the biochemical characteristics, and demonstrated that the improved iron-storing capability of the recombinant yeast is mainly due to an expression of heteropolymeric ferritin.

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