NOTE

Biochemistry

Iron Content of Rat Serum Ferritin

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ABSTRACT. The serum ferritin concentration was significantly higher in female than in male rats, reflecting higher iron stores in females than in males. The mean iron/protein ratio of serum ferritin was 0.018 ± 0.008 (SD) (µg of Fe/µg of protein) in female rats and 0.011 ± 0.011 in male rats, being much lower than that of liver ferritin (0.233 ± 0.014 in females and 0.227 ± 0.020 in males). Iron loading of rats significantly increased serum ferritin concentration, but did not influence the iron content of serum ferritin. These results indicate that rat serum ferritin contains only a small amount of iron independent of body iron stores.

KEY WORDS: ferritin iron, rat, serum ferritin.

Ferritin is a major iron-storage protein that is composed of 24 subunits of two types, termed H and L, the molecular masses of which are 21 kDa and 20 kDa, respectively [4, 8, 16]. Ferritin is present not only in cells but also in sera, and the serum ferritin level is positively correlated with body iron stores [1–3, 12, 15, 18]. The major function of tissue ferritin is to detoxify and store intracellular iron [8], and it contains a large amount of iron [5, 14, 17]. Serum ferritin has been found to have different amounts of iron in some mammals [6, 9, 13, 20, 22]. The physiological significance of serum ferritin and its iron remains unclear. Although rat serum ferritin was purified by Halliday et al. [7], its iron has yet to be determined. In the present study, the iron content of rat serum ferritin was measured, and compared with that of serum ferritin from other mammals.

Female and male Wistar rats aged 9–12 weeks (Clea Japan, Tokyo, Japan) were used. Male rats were iron-loaded by intraperitoneal injection of iron dextran (100 mg iron/ml; Sigma, St. Louis, MO, U.S.A.) to increase body iron stores. Rats received single doses of 7 mg of iron three times on alternate days. Control rats were injected with an equivalent volume of NaCl. Blood samples and livers were collected 2 days after the final administration from the untreated and iron-loaded rats under pentobarbital-induced anesthesia prior to sacrifice. The serum samples and tissues were stored at –25°C until use.

Ferritin was purified from rat livers as previously described [19]. Protein was determined according to the method of Lowry et al. [11] using bovine serum albumin as a protein standard. Rabbit antisera to rat liver ferritin were prepared, and antibodies to the ferritin were purified from the antiserum by affinity chromatography as described previously [19].

Each rat liver was homogenized in a Waring blender for 3 min with 10 volumes of 10 mM Tris containing 0.2 mM Pefabloc SC (Merck, Darmstadt, Germany) as a serine proteinase inhibitor. Each homogenate was divided into two parts; one was used for measuring non-heme iron; and the other was centrifuged at 24,000 × g for 20 min at 4°C, then the supernatant (liver extract) was used for measuring ferritin protein and ferritin iron. Total non-heme iron in liver homogenates was measured as previously described [21] except that 900 µl of 3 M HCl was added to 100 µl of the liver homogenate. Ferritin iron in the liver extracts was determined essentially by an immunoprecipitation technique described for measuring ferritin iron in fetal bovine serum [9]. Ferritin in rat liver extracts and sera was determined using a sandwich enzyme-linked immunosorbent assay (ELISA) established previously [19].

Serum ferritin iron was determined as follows. First, 5 µg of purified rabbit antibodies specific for rat liver ferritin was added to 1 ml of rat serum, and the mixture was incubated overnight at 4°C. Normal rabbit IgG (Sigma) instead of the specific antibody was used as a control. Next, 20 µl of goat antiserum to rabbit IgG-Fc fragment (Bethyl Laboratories, Montgomery, TX, U.S.A.) was added to the mixture, and incubated overnight at 4°C. The mixture was centrifuged at 16,000 × g for 15 min at 4°C. The resulting pellet was washed twice with 20 mM sodium bicarbonate, pH 8.2, containing 150 mM NaCl, centrifuged each time under the same conditions. To dissolve the immunoprecipitated ferritin iron, 200 µl of 3 M HNO₃ was added to the pellet and incubated overnight at 75°C. Iron in the nitric acid digest was assayed directly using an Atomic Absorption Spectrophotometer (Shimadzu Model AA-650) with a graphite furnace atomizer (Shimadzu Model GFA-2), in an argon atmosphere. Absorbance was measured at 248.3 nm. The data were analyzed using the Student’s t test and statistical significance was established at the P<0.05 level.

The mean non-heme iron content of the female rat livers was about two times that of the male rat livers (female: 366 ± 53 (SD) µg/g wet weight (n=7), male: 181 ± 36 µg/g wet weight (n=7), P<0.001). The mean concentrations of ferritin protein and iron in the female rat livers were 997 ± 156 and 232 ± 35 µg/g tissue, respectively, whereas those in the male rat livers were 466 ± 127 and 105 ± 26 µg/g tissue, respectively. The mean iron/protein ratios (µg of Fe/µg of protein) of ferritin in the female and male rat livers were...
The serum ferritin level was significantly (P<0.01) higher in female than in male rats (Fig. 1A). When purified rat liver ferritin with an iron/protein ratio of 0.305 was added to rat serum to give a ferritin iron concentration of 146 ng/ml and immunoprecipitated with rabbit antibodies to rat liver ferritin and goat antibodies to rabbit IgG, the recovery of added ferritin iron was 93.5 ± 7.7% (n=5). The iron/protein ratios of serum ferritin in female and male rats were 0.018 ± 0.008 and 0.011 ± 0.011, respectively (Fig. 1B), representing no significant difference between them.

Iron dextran was injected into male rats to increase body iron stores. The mean liver non-heme iron concentration of iron-loaded rats was about three times that of control rats (iron-loaded: 479 ± 136 µg/g wet weight (n=10), control: 168 ± 19 µg/g wet weight (n=10), P<0.001). The serum ferritin concentration was significantly increased by iron administration (P<0.01) (Fig. 2A), whereas the iron content of serum ferritin unchanged (Fig. 2B).

In the present study, both non-heme iron and ferritin iron concentrations were higher in female than in male rat livers. These results are in keeping with previous data on iron storage in rats [5, 10]. The serum ferritin concentration was found to differ significantly between female and male rats. This seems to reflect the gender difference in iron stores. The serum ferritin level was increased by iron loading, which also appears to reflect the increased iron stores. Immunoblotting of ferritin immunoprecipitated from rat serum showed that it contained three subunit types that were immunoreactive with anti-liver ferritin antibodies: a subunit of 18.1 kDa plus two subunits corresponding to the H and L subunits of liver ferritin (unpublished observation). The source and secretion mechanism of circulating ferritin remain to be elucidated.

**Fig. 1.** Serum ferritin (A) and its iron content (B) in female and male rats. Ferritin iron content is expressed as an iron/protein ratio (µg of Fe/µg of protein). Each value is the mean ± SD of 10 rats. *: Significantly different from male rats (P<0.01).

**Fig. 2.** Effects of iron loading on ferritin protein (A) and ferritin iron content (B) in male rat sera. Iron was given as iron dextran intraperitoneally (21 mg of Fe spread over three injections given on alternate days). Control rats received 0.9% saline. All animals were sacrificed 2 days after the last injection. Ferritin iron content is expressed as an iron/protein ratio (µg of Fe/µg of protein). Each value is the mean ± SD of 10 male rats. *: Significantly different from controls (P<0.01).
The iron/protein ratio of liver ferritin was about 0.23 in female and male rats, essentially agreeing with previous data [5, 10]. In the present study, the iron content of serum ferritin was found to be much lower than that of tissue ferritin. There was no significant difference in the iron content of serum ferritin between female and male rats, or between iron-loaded and unloaded rats. These results indicate that rat serum ferritin contains only a small amount of iron independent of body iron stores. The iron/protein ratio of serum ferritin has been found to be 0.02–0.07 in humans [6, 13, 22], 0.11 in dogs [20], and 0.20 in bovine fetuses [9]. Taking the previous and present data together suggests a species-difference exists in the iron content of serum ferritin. In dogs and bovine fetuses, serum ferritin as well as transferrin may serve as an iron-transporter because of the high iron content of serum ferritin [9, 20]. In rats, however, serum ferritin does not appear to be involved in transporting serum iron because of its low iron content.

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REFERENCES