Ferritin in Turkey Muscle Tissue: A Source of Catalytic Iron Ions for Lipid Peroxidation†

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Ferritins from horse spleen (Sigma Chemical Co.) and those obtained from turkey liver and dark and light muscles were separated by gel chromatography and SDS gel electrophoresis. The results indicated that the basic subunits of 20 000–22 000 Da are similar between the ferritins extracted from liver or turkey muscles. The amount of iron/ferritin molecules in muscles is very low, only ~5% of that in the liver. Dark and light muscles during storage at 4 °C released iron from ferritin. Ferritin extracted from turkey muscles stimulated muscle membrane lipid peroxidation. It seems that ferritin plays an important role in the process of muscle lipid peroxidation by being one of the main sources for "free" iron for this reaction.

INTRODUCTION

Ferritins are the main molecule-containing proteins that store iron in cells. They consist of a shell of subunits surrounding a hollow core in which ferric oxyhydroxide is deposited (Clegg et al., 1980). Most biological tissues were found to contain one major ferritin, but heart and other muscle tissues of rat (Linder et al., 1984), and human heart (Linder et al., 1981), which contain two major forms. It seems that muscle cells contain only one ferritin, which migrates rapidly on gel electrophoresis. The other ferritin from muscle tissue was isolated from parenchymal nonmuscle cells. The ferritins were found mostly in the cytosol and were much more concentrated in the nonmuscle cells (Linder et al., 1984).

In vivo, the synthesis of both forms of ferritin is stimulated by iron, and iron administration results in ferritin accumulation (La Cross and Linder, 1980).

Iron can be released from ferritin and utilized by mitochondria for the synthesis of heme proteins. In muscle cells, mitochondria synthesize myoglobin (Ulvik, 1981).

Ferrous ions can be released from ferritin by reductants small enough to pass through channels in the protein shell that surrounds the central iron core of the ferritin molecule. Such systems are FMN-dependent ferrireductase (Ulvik, 1981), dithionite, thioglycollate (Funk et al., 1985), superoxide (Biemond et al., 1986), and anthracycline antibiotics (Thomas and Aust, 1986). Recently, it was found that superoxide ion is the primary reductant in ascorbate-mediated ferritin iron release (Boyer and McCleary, 1987).

Ferritin has been reported to stimulate lipid peroxidation in the presence of ascorbate (Wills, 1966). The role of iron in ferritin and hemosiderin-mediated lipid peroxidation was demonstrated by several authors (Gutteridge et al., 1983; O'Connell et al., 1985; Thomas et al., 1985; Koster and Slee, 1986; Reif et al., 1989).

The aim of this study was to isolate and determine ferritin from turkey muscles and its potential to activate membranal lipid peroxidation.

MATERIALS AND METHODS

Materials. Ascorbic acid, dithiothreitol, and ammonium persulfate were purchased from Merck (Darmstadt, FRG). Ferritin (horse spleen), sodium dodecyl sulfate, polyacrylamide, EDTA, *N,N,N,N*-tetramethylenediamide, Tris, and thiobarbituric acid (TBA) were all obtained from Sigma Chemical Co. (St. Louis, MO). Sepharose 6B was purchased from Pharmacia Fine Chemicals (Freiburg, FRG). All reagents were of Analar grade. The water was deionized, double-distilled, and treated with Chelex 100.

Ferritin Isolation. Ferritin was isolated according to the method of Crichton et al. (1973). Fresh (1 h after slaughtering) turkey muscle tissues (200 g) without connective or fat tissues were homogenized with 200 mL of H_2O for 2 min at room temperature. The homogenate was heated to 72 °C and held constant for 10 min, cooled to 4 °C, and centrifuged at 27000*g* for 3 min. The supernatant was again heated to 75 °C and held constant for 10 min, cooled at 4 °C for 2 h, and centrifuged at 27000*g* for 30 min. The clear supernatant was dialyzed against double-distilled water for 48 h. After dialysis, the dialysate was centrifuged at 30000*g* for 30 min. The supernatant containing the ferritin was precipitated by ammonium sulfate at 50% saturation and centrifuged at 30000*g* for 30 min. The pellet, containing ferritin, was resuspended in 10 mL of phosphate buffer (0.02 M, pH 7.4) containing 0.1 M NaCl.

Resuspended ferritin (1 mL) was applied to a Sepharose 6B column (25 × 50 cm), equilibrated with buffer at pH 7.4, and eluted by the same buffer at a flow of 1 mL/min.

Gel Electrophoresis. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed on samples after separation by ammonium sulfate and Sepharose 6B column chromatography, according to the method of Porzio and Pearson (1977). The electrophoresis was performed on a vertical-slab system (Protean II, Bio-Rad) at a constant current of 50 mA. Protein samples and marker proteins were dissolved in a solution of 8 M urea, 2.5% SDS, 5 mM EDTA, 5 mM dithiothreitol, and 100 mM Tris–glycine at pH 8.8 and heated for 5 min at 100 °C. Protein samples of 30–50 μg were applied to the gels. The samples were stained overnight by Coomassie blue (0.01%) and washed with acetic acid (10%) and methanol (5%).

Iron Ferritin. The concentration of total iron in ferritin was determined by using a wet digestion procedure of organic compounds by nitric acid and perchloric acid. The digested samples were determined for iron by an atomic spectrophotometer (Perkin-Elmer Model 460) (Ulvik, 1981).

Protein Determination. The modified Lowry procedure (Markwell et al., 1978) was used with BSA as the standard.

Lipid Peroxidation. Isolation of the microsomal fraction from muscle tissues were done by a procedure described previously (Kanner and Harel, 1985).

Microsomes for lipid peroxidation assay were incubated in air in a shaking water bath at 37 °C. The reaction mixture contained 1 mg of microsomal proteins/mL and 4 mL of 50 mM acetate, pH 6.5.

Thiobarbituric acid reactive substances were determined by a procedure of Bidlack et al. (1973). The results are reported as

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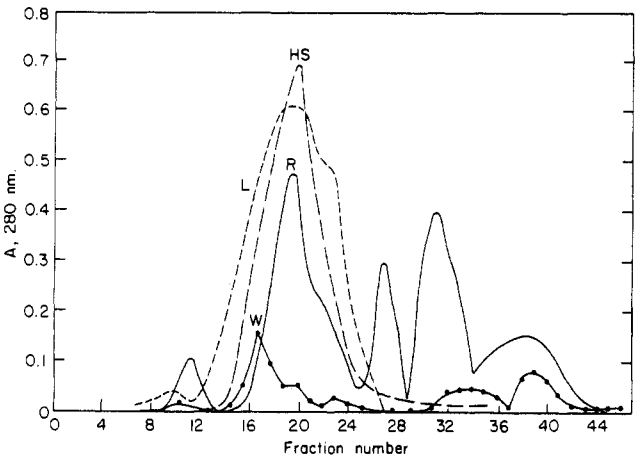

Figure 1. Separation on Sepharose 6B of the ammonium sulfate fraction of ferritin from muscle and liver turkey tissue (control, Sigma horse spleen ferritin). (– –) Control, horse spleen ferritin (HS); (—) dark muscle (R); (●) light muscle (W); (- - -) liver (L).

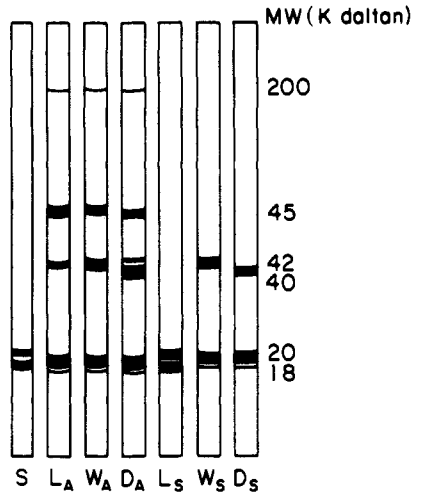

Figure 2. SDS–polyacrylamide gel electrophoresis of ferritin separated previously by ammonium sulfate (A) and Sepharose 6B chromatography (S). S, horse spleen; L, liver; W, light muscle; D, dark muscle.

nanomoles of malondialdehyde (MDA) per milligram of protein, using a molar extinction coefficient of $E_{532} = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

RESULTS AND DISCUSSION

Ferritins from horse spleen (Sigma) and those obtained from turkey liver and dark and light muscles after treatment with ammonium sulfate were separated by Sepharose 6B column chromatography. All the ferritins showed an elution pattern very similar to that from horse spleen; a small peak was eluted between fractions 8 and 12 and the main peak between fractions 26 and 42. The samples obtained from muscles showed several more fractions eluted between fractions 26 and 42 (Figure 1).

Ferritins were also separated by SDS gel electrophoresis. The results showed clearly that dark and light turkey muscles contain ferritin proteins very similar to those of spleen and liver samples. Turkey muscles, similar to heart muscles, contained the main subunit L of a molecular weight of 20 000 and a second subunit of 40 000–42 000 (Figure 2). The results indicated that there was no significant difference between muscle ferritin subunits and those of horse spleen or turkey liver. The subunit of 40 000–42 000 Da probably represents an aggregate of normal-sized subunits (Linder et al., 1981).

Dark and light muscles were determined for total protein and total iron before and after separation by ammonium sulfate and column chromatography on Sepharose 6B. The amounts of protein in the raw dark and light muscle were 256 ± 10 and 305 ± 11 mg/g, respectively, and the amounts of total iron were 12.6 ± 0.8 and 8.2 ± 1.5 μg/g, respectively. The amounts of the proteins and iron in purified ferritin from various tissues are given in Table I. The yield of the purified ferritins after column chromatography showed that the concentration of ferritin on a protein basis was approximately 0.03% in dark muscles and 0.008% in light muscles. The amount of ferritin iron was 1.0 and 0.3 μg/g for dark and light raw muscles, respectively.

Table I. Ferritin Fraction, Separated from Several Turkey Tissues after Purification[a]

	treatment				
	ammonium sulfate		gel chromatography[d]		
samples	protein,[b] μg/g	iron,[c] μg/g	protein, μg/g	iron, μg/g	iron, atom/mol
dark muscle	250.2	1.6	75.2	1.0	116
light muscle	128.1	0.5	25.6	0.3	104
liver	1103.3	250.1	1068.0	231.0	1932

[a] Ferritin was isolated after heat treatment, as described under Materials and Methods. [b] Values are given in micrograms of protein/gram of wet meat tissue and are the average of five samples from the same turkey tissue. [c] Values are given in micrograms of iron/g of wet meat tissue and are the average of three samples from the same turkey tissue. [d] Values are given for the main fractions (13–25).

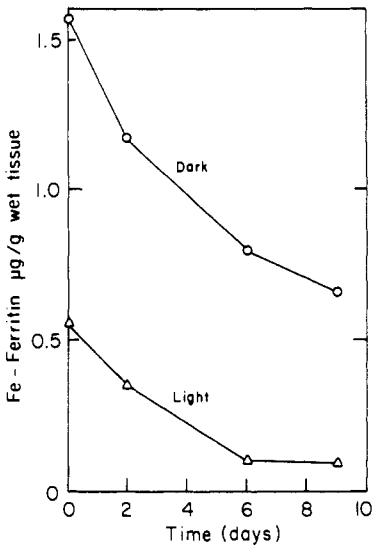

Figure 3. Loss of iron from ferritin isolated from dark and light turkey after storage at 4 °C. (Ferritin was isolated by the standard procedure and ammonium sulfate.)

The role of ferritin as a source of free iron ion in muscle foods was hypothesized by us previously (Kanner et al., 1988).

The amount of iron, as calculated on the basis of atoms of iron per mole of ferritin, was relatively low in muscle ferritins compared with liver ferritins. It is well-known that liver ferritin has a ratio of 2000–4000 iron atoms/mol of ferritin (Ulvik, 1981) but that muscle ferritin is significantly lower (Linder et al., 1981). It may be possible that a part of ferritin iron was released during the process of separation.

Dark and light muscle tissues were incubated at 4 °C, and the amount of ferritin iron was determined during a 10-day storage period (Figure 3). Results showed a decrease in iron of more than 55% in dark muscle and 80% in light muscle. The release of 500 ng of iron/g of muscle will yield a concentration of about 10 μM "free" iron ions.

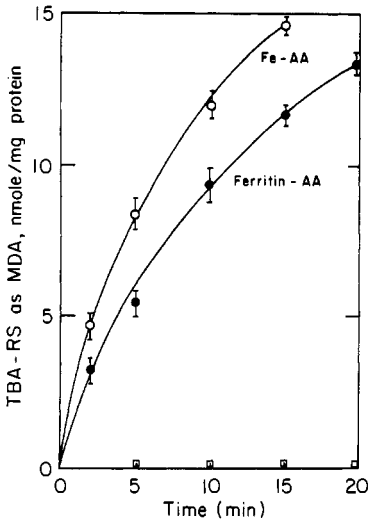

Figure 4. Muscle membranal lipid peroxidation initiated by turkey ferritin-iron (~5.0 μg/mL) in the presence of ascorbic acid (200 μM). (●) Ferritin–ascorbic acid; (○) ascorbic acid and $FeCl_3$ (10 μM). (□) ferritin or $FeCl_3$ without ascorbic acid. Membranal fractions are sarcosomes (1 mg of protein/mL) in 4 mL of 50 mM acetate, pH 6.5, incubated at 37 °C.

Muscle membrane lipid peroxidation was stimulated significantly in the presence of ferritin (isolated from turkey muscles) containing 5 μg/mL iron and ascorbic acid (200 μM) (Figure 4). Ascorbic acid releases iron from ferritin by a reaction that is dependent on superoxide (Boyer and McCleary, 1987). Iron ions in the presence of ascorbic acid develop a system that initiates muscle lipid peroxidation (Kanner et al., 1986).

In conclusion, we believe that muscle ferritin, being one of the main sources of free iron, plays an important role in the process of initiation and propagation of lipid peroxidation in muscle foods. New data on beef muscle ferritin were published recently, during the reviewing process of this paper, by Decker and Welch (1990).

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