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Isolation and Characterization of Chicken Muscle Ferritin

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Two ferritins (fast and slow) have been found to exist in the chicken muscle. Ferritin was isolated from the muscle by means of a method based on pH changes and saline fractioning, followed by purification in Ultrogel AcA-3A and ultracentrifugation at 100,000 *g*. Identification of the two ferritins shown in the chromatogram was carried out by electrophoresis in polyacrylamide gel, the typical Prussian Blue band with ferrocyamide appearing in both cases.

Ferritin characterization was carried out by means of molecular weight determination, amino acid analysis, number of Fe atoms linked by ferritin molecule and other parameters.

Key words: Iron, Ferritin, Birds, Chicken muscle ferritin.

Ferritin is a typical iron-accumulating protein in animals and vegetables. Ferritin isolation and characterization in mammals has been thoroughly studied. However, information about ferritin in birds is very scarce.

Other research work has had ferritin in birds as the main subject of their investigation (3, 12).

This study tries to increase the knowledge of this protein in birds and to verify, also, whether we can find in their muscular tissue the two different monomeric ferritin types (fast and slow) often quoted in recent bibliography in the case of skeletal and heart muscles in mammals.

This protein has been isolated from the skeletal muscular tissue using various methods based on its thermostability, water solubility, precipitation by saturation and isoelectric point properties.

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Materials and Methods

The technique used for the isolation and purification of chicken muscle has been basically the same as one used by LINDER and MURO (4), although with some variations in pH precipitation.

After the homogenization of the muscle tissue and 70° C bath for 10 min, the mixture undergoes two pH variations. The first one up to pH 4.5 by adding AcH 1 M, which will precipitate the ferritin. Once this precipitation is dissolved in buffer phosphate (pH 7.4). it decreases

again with the same reagent, this time up to pH 5.5 which produces the required ferritin in dissolution.

The dissolution later undergoes a precipitation reprecipitation with $(NH_4)_2SO_4$ at 50 % and 60 % saturation respectively.

The chromatography was carried out in a 50 ml column with Ultrogel AcA-34, using buffer phosphate 0.2 mM pH 7.4. The fractions of the peaks obtained (fig. 1) were purified and concentrated separately by ultracentrifugation for 3 h at 100,000 *g*.

Electrophoretical techniques were used for ferritin identification, and for its molecular weight determination as well (fig. 2) in polyacrylamide gel at 5 %, 7 % and 9 % acrylamide, using pattern proteins of known molecular weight. This technique has also been used for electrophoretical movement calculation.

The amount of ferritin obtained was determined using Lowry's method (7), and the number of Fe atoms linked by ferritin molecules according to RAMSAY'S method (11).

The amino acid analysis was carried out using the classical methods for protein degradation and Beckman amino acid analyser.

Results

Ferritin fractioning through chromatography in a 50 ml column by Ultrogel AcA-34 showed two components (fig. 1) which when analysed through electrophoresis in a 7 % polyacrylamide gel were identified as genuine ferritin, one slower than the other in its migration.

The following parameters, among others, were later determined for each of them: molecular weight, protein and iron concentration, number of Fe atoms linked by ferritin molecules and amino acid analysis (tables I and II).

The slow ferritin molecular weight

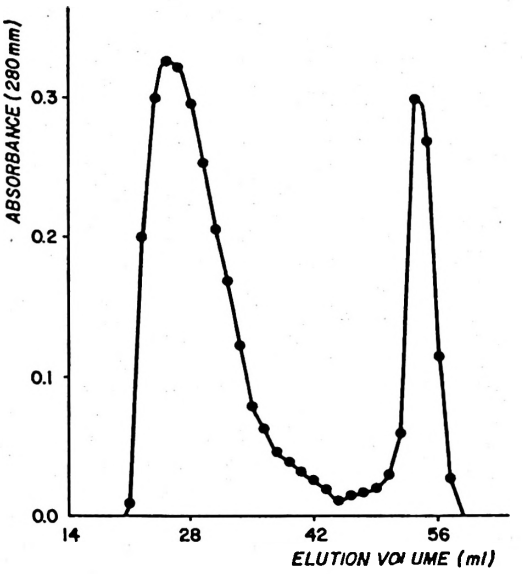

Fig. 1. *Chromatogram obtained in the ferritin isolation and purifying process from the muscle of chicken.*

Table I. *Characterization of ferritin from chicken muscle.*

	Ferritin Slow	Ferritin Fast
Ultrogel AcA-.		
Elution Vol/mi	25.3	53.2
Rf (5 % T)	0.113	0.123
Rf (7 % T)	0.028	0.039
Rf (9 % T)	0.0093	0.0162
Y_O	2.44	1.49
K_R	0.271	0.220
Conc. Fe (μg/ml)	35.2	12.0
Conc. Ferritin (mg/ml)	2.47	0.62
No. at. Fe bounds (at. Fe/mol. ferritin)	165	194
Electrophoretic mobility ($cm^2/v.s$)	3.2×10^{-6}	4.3×10^{-6}
No. Subunits	32	28
Yield (mg ferritin /g tissue)	6.7×10^{-3}	1.7×10^{-3}
Mol wt. (Electrophoresis)	645,000	555,000

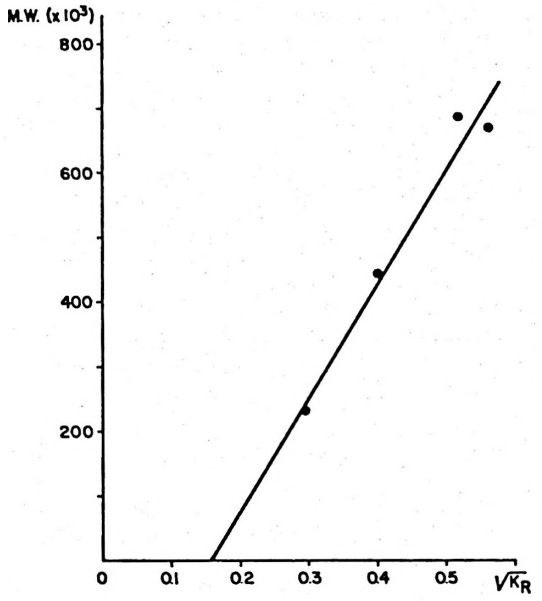

Fig. 2. *Calibration for determining the molecular weight of chicken muscle ferritin.*

(645,000 daltons) and the fast ferritin molecular weight (555,000 daltons) were determined by electrophoresis in polyacrylamide gel. The slope values (Kr) and the ordinate in origin (−1g Yo) of the lines were in connection with the size and molecular charge respectively and are included in the global results table (table I), as well as the Rf values at the rates of acrylamide used.

The calibration line for molecular weight determination (fig. 2), of ferritin has been obtained using the protein pattern molecular weight as seen through their $\sqrt{}$ kr values.

Discussion

This work has been carried out in order to find out whether there are two different types of ferritin in chicken muscle as in the case of mammal skeletal and heart muscles.

Ferritin isolation and characterization

Table II. *Amino Acid composition nmol/*100 nmol) of chicken muscle ferritins.

Amino Acid	Muscle ferritin «Slow»	«Fast»
Lys	7.68	7.41
His	1.65	3.03
Arg	4.60	5.19
Asp	10.88	10.19
Thr	4.79	5.74
Ser	4.01	5.74
Glu	17.76	11.37
Pro	3.92	2.97
Gly	9.67	13.96
Ala	9.52	10.81
Val	6.56	5.81
Met	1.80	3.15
Ile	5.13	4.63
Leu	7.93	7.78
Tyr	0.50	0.49
Phe	3.61	1.73

show two monomeric ferritin types (fast and slow) with different parameters: molecular weight, protein and iron concentration, number of Fe atoms linked, amino acid analysis and electrophoretic mobility, the results of which are discussed now. The identification of the two peaks in the chromatogram (fig. 1), show by electrophoresis in polyacrilamide gel, that they were ferritins, one faster in its migration than the other. These results indicate that these two components (fast and slow) could well be those in higher muscle ferritin found in different mammals, (1, 2, 6, 14).

Although the bibliographic data show the difficulty of splitting these two ferritin components, it was achieved in this case, due perphaps to the low sample concentration; a previously study carried out on chicken heart ferritin (8) showed that in high dilution it was possible to identify two peaks in ferritin fractioning with Ultrogel AcA-3A.

The slow and the fast ferritin molecular weights are of the same magnitude as those measured in the rat heart ferritin

(13). With human beings the difference in molecular weight between the fast one and the slow one is smaller (530,000 daltons and 540,000 daltons) (5).

The electrophoretic mobility in electrophoretic experimental conditions was higher for the fast ferritin than for the slow one (table I). These data for the fast ferritin and the slow one point out that the slow ferritin band is outstanding in adult male rats while the fast one is more abundant in females (14). This study was carried out in adult chickens.

The iron analysis showed the presence of 194Fe atoms per fast ferritin molecule as opposed to the 165 linked by slow molecule. These results agree with others (17) which emphasize the fact that acid ferritins (muscular and cardiac) have a low content, and that the fast one links this element more easily than the slow one (9).

The results of comparative amino acid analysis between the two types of muscle ferritin from chickens showed (table II) a general higher proportion of acid amino acid than the basic ones, not only for fast and slow ferritin in chicken muscle, but also for chicken and dove liver ferritin. This fact agrees with all other ferritin studied so far. In chicken muscle ferritin, although there is a double phenylalanine concentration in the slow component, the amount of histidine and methionine in the fast ferritin is double the amount in the slow one.

Tyrosine is the amino acid found in the lowest proportion, whose content is very low but similar in both ferritins.

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Resumen

Se estudia la existencia de dos ferritinas (rápida y lenta) en el músculo de pollo, aisladas mediante técnicas de fraccionamiento basadas en cambios de pH, fraccionamiento salino y térmico. La purificación se realiza por cromatografía en columna con Ultrogel AcA-34 y posterior ultracentrifugación a 100.000 g y la identificación, por electroforesis en gel de acrilamida. La caracterización de ambas ferritinas se lleva a cabo por medidas de su peso molecular, número de subunidades, análisis de amino ácidos y número de átomos de hierro unidos a la proteína, así como otros parámetros, carga eléctrica y movilidad electroforética.

Palabras clave: Hierro, Ferritina, Aves, Ferritina muscular de pollo.

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