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Isolation and Purification of Duck Liver Ferritin

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A rapid method of purifying duck liver ferritin using high speed centrifugation and chromatography on Sephadex G-200 and Sepharose 6B is described. Protein and iron concentration for each step of purification is given. This method yields 0.12 mg of pure ferritin per gram of wet tissue.

Key words: Duck liver ferritin.

Ferritin is the major iron-storage protein found in most mammalian tissues and widely distributed in nature, from plant (11) and invertebrates (1) to human cells (5).

In the mammal, the liver, spleen and bone marrow have the largest deposits of stored iron, but ferritin can be detected in all tissues (8). If ferritin is of great interest as iron storage in mammalian, it is more important as a regulator of the iron content in birds because the iron levels in serum suffer important variations with laying.

Methods of the purification of ferritin have mainly been described for mammalian ferritins. The present paper describes a rapid procedure of the purification of duck liver ferritin.

Materials and Methods

Isolation of ferritin. Duck liver ferritin was prepared as described by CALVO *et al.* (2) with modifications. Duck liver (450 g) was homogenized with 600 ml of distilled water, diluted with water to 1,800 ml, and heated at 70°C for 10 min. The homogenate was cooled to 4°C and centrifuged at 1,500 *g* for 15 min. The supernatant was adjusted with 1 mol/l acetic acid to pH 4.8, and centrifuged at 1,500 *g* for 15 min. The centrifugate was dissolved in 150 ml of sodium phosphate buffer (0.1 mol/l, pH 7.5) and re-centrifuged at 100,000 *g* for 2 h. The deposit was dissolved in 10 ml of the same buffer containing 0.2 mg of sodium azide per litre, and chromatographed on a column containing Sephadex G-200 (2.5 × 100 cm) pre-equilibrated with some of the same phosphate buffer. The fraction emerging at the void volume was collected and concentrated by centrifuging at 100,000 *g* for 2 h. The deposit was redissolved in 10 ml of buffer and passed through a column containing Sepharose 6B (2.5 × 50 cm) pre-equili-

brated with phosphate buffer. Elution was monitored at 280 nm with a Varian Techtron 634 spectrophotometer. Fractions were analysed in the eluate by disc polyacrylamide gel electrophoresis, stained for protein with amidoblack and for iron with the Prussian blue reaction. Ferritin-containing fractions were pooled and freeze-dried.

Analytical procedures. Polyacrylamide gel electrophoresis Gel electrophoresis was performed as described by ORNSTEIN (9) and DAVIS (3) in a 7 % gel. Tris-glycine buffer (192 mmol/l, pH 8.3) was present in both chambers and a current of 8 mA per tube was applied with bromphenol blue as an internal migration control.

Protein concentration was measured by the method of LOWRY *et al.* (7), using a standard solution prepared from bovine serum albumin (Sigma).

The iron content was measured by the method of o-phenantroline as outlined by the International Standardization Committee (6).

Ferritin immunoreactivity was analysed by Ouchterlony-type immunodiffusion against rabbit anti duck liver ferritin with 1.5 % agar in sodium phosphate buffer (0.1 mol/l, pH 7.5) as supporting medium.

Each rabbit was first injected subcutaneously on both sides with 200 μg of ferritin in complete Freund's adjuvant. Booster doses of 500 μg of ferritin without adjuvant were administered intramuscularly each week. Animals were bled out the seventh week, and the antiserum was stored frozen.

Results

The procedures described above and employed for the purification of duck liver ferritin make use of the heat stability and of the high molecular weight of its molecule.

The step of pH adjust to pH 4.8 of the heated extract eliminated nearly 86 % of the contaminating proteins, and by centrifugation at 100,000 *g* for 2 h, about 94 % of remaining proteins. So the columns of Sephadex G-200 and Sepharose 6B only act as eliminating contaminating proteins to get highly purified ferritin. Gel filtration on Sephadex G-200 gave two main peaks. The peak corresponding to ferritin appeared immediately after the void volume. The final purification of ferritin was obtained by gel filtration on Sepharose 6B, which separated in two peaks the ferritin's one of Sephadex G-200. Table 1 summarizes the yield obtained at each purification step.

Immunodiffusion. When serially diluted anti duck liver ferritin solutions were allowed to diffuse against duck liver ferritin, a single band of reactivity was revealed. No precipitate lines were formed against horse spleen ferritin (Sigma). Using chicken liver ferritin, purified as the procedure described above, a single band was found against serially

Table I. *Purification steps of duck liver ferritin.*

Procedure	Volume ml	Protein mg/ml	Total prot. mg	Iron µg/ml	Total Iron mg	Iron/Protein $\times 10^3$
Heated extract	1,800	5.0	9,000	16.3	29.3	3.3
Change of pH	150	8.6	1,290	62.0	9.3	7.2
Centrifugation at 100,000 g	10	7.2	72	242.2	2.4	33.3
Sepharose 6B	65	0.9	59	36.5	2.4	40.7

diluted anti duck liver ferritin solutions, showing a high degree of cross reactivity.

Polyacrilamide gel electrophoresis from first supernatant to pure ferritin showed a progressive disappearance of protein bands, as it was expected (fig. 1). Pure ferritin showed a major, fast moving band, and a minor, slow moving band. Staining for protein and for iron was coincident.

Molecular weight determination was performed by polyacrylamide gel electrophoresis with thyroglobulin, horse ferritin, albumin, ovoalbumin, carbonic anhydrase, and lactalbumin as standards. A molecular weight of 447,000 daltons was obtained.

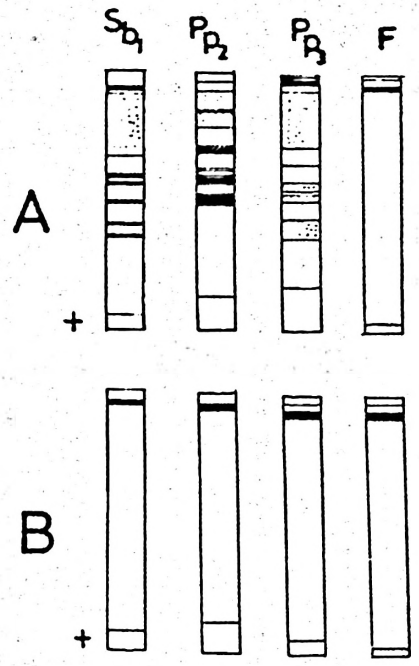

Fig. 1. *Polyacrylamide gel electrophoresis of purification steps of duck liver ferritin.* S_{b1}: heated extract, P_{p2}: change of pH, P_{p3}: centrifugation at 100,000 g, F: Sepharose 6B. A) Staining for protein, B) Staining for iron. (+) indicates the direction of migration. The rapidly migrating band is bromphenol blue.

Discussion

General procedures reported for ferritin isolation usually include a step of precipitation with ammonium sulfate half saturated (8). CALVO *et al.* (2) also include this procedure to isolate chicken ferritin. This step involves posterior dialysis against several changes of water and buffer. Therefore it is time-consuming and its elimination is justified by the results.

The yield obtained with the method described above is 0.12 mg of pure ferritin per gram of wet tissue. The yields obtained with different procedures as compared by GONYEA *et al.* (4) for isolating human spleen ferritin were between 0.06 mg and 0.39 mg of immunoreactive ferritin per gram of wet tissue. However, the material was 75 % and 10 % pure respectively. PAGÉ *et al.* (10) have reported a method for isolating human spleen ferritin with a yield of 0.19 mg per gram of wet tissue and 100 % pure, but SHINJYO *et al.* (12) only obtained 0.02 mg per gram for spleen chicken ferritin.

Chicken liver ferritin has been reported to have a molecular weight of 420,000 daltons (2) by polyacrylamide gel electrophoresis. This paper presents evidence of a molecular weight for duck liver ferritin of 447,000 daltons, which is closely related to that found for chicken ferritin. This is in agreement with the finding that chicken liver ferritin gives cross reactivity with rabbit anti duck liver ferritin.

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Resumen

Se describe un método rápido de purificación de ferritina de hígado de pato usando ultracentrifugación y cromatografía en columna con

Sefadex G-200 y Sefarosa 6B. Se determina la concentración de hierro y proteínas en cada etapa de purificación. Este método proporciona unos rendimientos de 0,12 mg de ferritina pura por gramo de tejido húmedo.

References

1. BOTTKE, W. and I. SINHA: *Wilhelm Roux's Arch.*, **186**, 71-76, 1979.
2. CALVO, J. J., MARTÍN, M., GARCÍA, J. R. and RECIO, J. M.: *Rev. esp. Fisiol.*, **37**, 447-454, 1981.
3. DAVIS, D. J.: *Ann. N.Y. Acad. Sci.*, **121**, 404-409, 1964.
4. GONYEA, L. M., LAMB, C. M., SUNDBERG, R. D. and DEINARD, A. S.: *Clin. Chem.*, **24**, 513-518, 1976.
5. HARRISON, P. M.: *Seminars Hematol.*, **14**, 55-70, 1977.
6. I.C.S.H.: *Brit. J. Haematol.*, **38**, 281-290, 1978.
7. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R.: *J. Biol. Chem.*, **193**, 265-275, 1951.
8. MUNRO, H. N. and LINDER, M. C.: *Physiol. Rev.*, **58**, 318-421, 1978.
9. ORNSTEIN, L.: *Ann. N.Y. Acad. Sci.*, **121**, 321-326, 1964.
10. PAGÉ, M., LAGUEUX, J. and GAUTIER, C.: *Can. J. Biochem.*, **58**, 494-498, 1980.
11. PONCE-ORTIZ, Y. and CRICHTON, R. R.: *Biochem. Soc. Trans.*, **5**, 1128-1134, 1977.
12. SHINJYO, S.: *J. Japan. Biochem. Soc.*, **45**, 289-295, 1973.