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Vitellogenin-Iron and Hemoglobin Synthesis in Avian Reticulocytes

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Avian vitellogenin has been studied as an iron carrier for hemoglobin synthesis by reticulocytes. The Fe-vitellogenin uptake by the immature red cells is progressive with time, following an unspecific iron uptake process. The iron uptake from Fe-vitellogenin was in proportion to the immature red cells present and the radioactive iron was found in the hemoglobin synthesized by these cells. These results open up the possibility of assigning a secondary role to the Fe-vitellogenin in the avian erythropoiesis, added to the classical iron transport function for egg production.

Key words: Iron, Vitellogenin, Phosvitin, Hemoglobin synthesis, Reticulocytes.

Vitellogenin is a plasma protein of oviparous vertebrate females. It comes from the liver, where estrogens induce its synthesis (3, 5). It is also the main storage substance in the egg yolk. It is a lipophosphoprotein complex, in which the phosvitin component plays a role in both the transport and storage of some ions (calcium, phosphorus, iron, etc.), while other protein fractions serve as amino acids store (13).

Plasma iron bound to vitellogenin has been found in estrogenized chickens (4), as well as in laying and estrogenized hens (1, 8), but it has been suggested that this phosphoprotein-iron is only destined for egg formation, while the transferrin-iron will be supplied to erythroid cells (8).

The purpose of this study, however, was to find out if the plasma phosphoprotein-iron, could also be used as an iron source for the heme synthesis by avian erythroid cells.

Materials and Methods

Vitellogenin was purified from male quail plasma after estrogen treatment, following WILEY *et al.* (15). Phenylmethansulfonyl fluoride (50 µg/ml) was added to all buffers to prevent proteolysis. Fowl phosvitin was purchased from Sigma and repurified by ionic exchange chromato-

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graphy on a DEAE-Sephadex (Pharmacia, Sweden) following OSAKI *et al.* (11). Quail plasma transferrin was purified from male plasma following MORGAN (8). Proteins purity was checked by polyacrylamide gel electrophoresis.

Radioactive iron was purchased from Amersham as 59-Fe chloride in 0.1 M HCl (3-20 mC/mg Fe, specific activity). Dulbecco's modified Medium (DMM) came from Gibco (U.K.) Glutamine (500 mM final concentration) was added. Ultrogel AcA 44 was from LKB (Sweden). All other reagents used were analytical grade.

Quail and chicken reticulocytes were taken from phenylhydrazine (Ph) treated animals (single injection of 1.8 mg Ph/kg b.w. intramuscular). After 72 h the Ph injection, blood was taken either from the wing (chicken) or jugular (quail) veins and centrifuged at 2000 × *g* for 15 min to remove plasma and leukocytes. A hemocytometric count for the cell number and Brilliant Cresyl Blue staining for reticulocyte counting were done (7). An average of 60-70 % of reticulocytes were obtained and no further reticulocyte enrichment was achieved. Cells were washed in Hanks solution three times and finally resuspended in DMM.

59-Fe-phosvitin and 59-Fe-vitellogenim were prepared as described by WEBB *et al.* (14). Plasma transferrin was labelled with 59-Fe according to BATES and WERNICKE (2).

A preincubation step, without radioiron labelled proteins, was carried out for 30 min, to remove possible intracellular transferrin as indicated by POLLACK and CAMPANA (12). After preincubation, the cells were washed again as above.

Labelled proteins and cells were incubated at different temperatures (37° and 4 °C) in a shaking bath. Samples were taken at desired times and the iron uptake stopped with ice-cold Hanks solution followed by three washes in the same solution (12).

Washed cells were lysed with distilled water and frozen at −20 °C until analyzed. Lysed cells were centrifuged for 30 min at 30,000 × *g*, at 4 °C and supernatants were brought to an Ultrogel AcA 44 column (1.5 × 120 cm) and the chromatography was developed using phosphate buffered solution as eluent. Aliquots of supernatans were subjected to heme extraction by means of acidified cyclohexanone. Radioactivity was counted with a well type gamma counter.

Results and Discussion

Iron uptake by quail immature red cells from both Fe-vitellogenin and Fe-transferrin complexes is linear with time (fig. 1). Although a much lower iron uptake was obtained when Fe-vitellogenin was used, the amount of iron incorporated is related to the specific presence of reticulocytes (fig. 2).

Since phosvitin is the iron-carrier por-

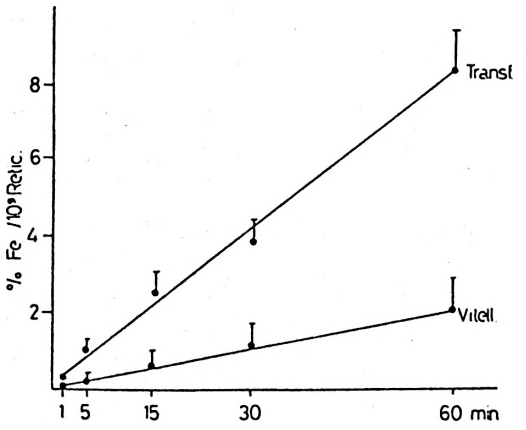

Fig. 1. *The time course variations of iron uptake by quail reticulocytes from Fe-vitellogenin or Fe-transferrin at 37 °C.*
Ordinates are the percentage of total radioactive iron added, taken up by 10^9 reticulocytes. Protein concentration was 100 µg/ml and iron concentration 0.1 µg/ml. Each point gives the mean of 12 determinations and bars are standard deviations.

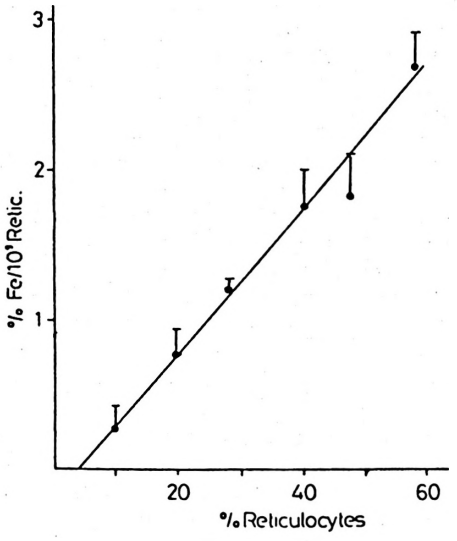

Fig. 2. *The influence of reticulocyte percentage present in the red cell suspension on the iron uptake by quail reticulocytes from the Fe-vitellogenin.*
Ordinates as in fig. 1. Time of the experiments, 1 h at 37° C. Carrier concentration, 100 µg/ml. Iron concentration, 0.1 µg/ml. Each point gives the average of 5 determinations and bars are standard deviations.

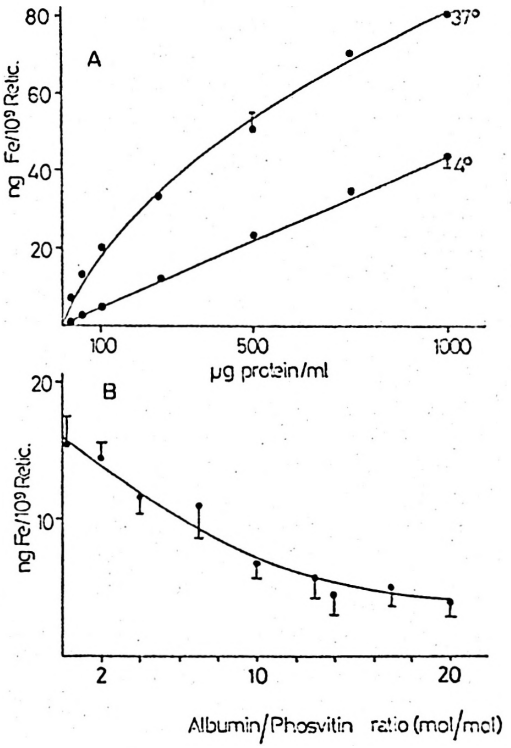

Fig. 3. *Unspecificity in the binding of phosvitin-bound iron to chicken reticulocytes.*
A) The iron uptake from phosvitin-bound iron by chicken reticulocytes versus the carrier concentration, at two different temperatures (4° and 37 °C). Iron concentration of the carrier was 10 µg Fe/mg protein. B) The effect of increasing albumin concentrations in the incubating medium, on the ability of chicken reticulocytes to take up iron from Fe-phosvitin, at 4 °C. Phosvitin concentration was 100 µg/ml, and the iron content, 1 µg/ml Fe/mg protein. Each point gives the average of three independent determinations and the bars give the standard deviations.

tion of vitellogenin, as well as, the part of vitellogenin molecule which is specifically recognized by vitellogenin receptors of developing oocytes (16), this protein was used for the following studies.

The metal delivering capacity of the Fe-phosvitin for chicken reticulocytes at two different incubation temperatures (4° and 37 °C) changed by 50 % (fig. 3A) and did not show a saturating effect although a wide protein concentration range was used. This response clearly differed from transferrin-Fe data, where the reduction in uptake was 90-95 % (data not shown). This effect of the temperature variation was general among non-specific components (9, 10), and the interference of increasing albumin concentrations on the iron uptake by reticulocytes shown in fig. 3B, is in agreement with the absence of specific membrane receptors. Thus, iron transport from Fe-phosvitin to reticulocytes could be considered a non-specific process. These experiments questioned the presence of specific vitellogenin receptors in avian reticulocyte membranes, which are also absent in mature hen erythrocytes (16).

The probable non-specific character of this carrier binding to the red blood cells,

Table I. *Incorporation of iron-phosvitin, in heminic and non-heminic fractions (μg Fe/10^9 Retic.) in chicken reticulocytes, at 37 °C, during a 240 min period.*
Means ± standard deviation from 3 independent determinations.

	Time (minutes)					
	1	15	30	60	120	240
Total Iron	2.3 ± 1.1	5.3 ± 3.1	11.0 ± 3.7	14.0 ± 2.5	19.9 ± 6.2	27.5 ± 3.7
Heminic Iron	0.2 ± 0.1	1.0 ± 0.4	3.7 ± 0.1	4.3 ± 0.1	5.8 ± 0.6	8.8 ± 1.7
Non-heminic	2.2 ± 0.3	4.3 ± 0.3	7.2 ± 0.7	9.8 ± 1.1	14.2 ± 1.2	18.9 ± 0.2

however, does not limit the capacity of the immature erythroid cells to synthesize heme groups with the iron carried. The incorporation of this iron to heme and non heme components was demonstrated by heme extraction with cyclohexanone. The time course pattern of the incorporation of 59-Fe to these compounds is shown in table I. The incorporation of radioactivity to heme was very fast, reaching 33 % after 30 min, and maintaining this percentage throughout experiment.

Present results suggest that Fe-vitellogenin could be used as a complementary source of iron for heme synthesis in the avian bone marrow or in the intravascular space in those species which possess circulating reticulocytes (6).

Resumen

Se estudia la función de la vitelogenina como donante de hierro para la síntesis de hemoglobina en reticulocitos de ave. La incorporación de Fe a las células es progresiva con el tiempo y no depende de receptores específicos. La captación de hierro está ligada a la presencia de células inmaduras. El hierro incorporado por la célula se halla en la hemoglobina sintetizada por estas células. Estos resultados abren la posibilidad de asignar un papel secundario al complejo Fe-vitelogenina en la eritropoyesis de las aves, además de su función transportadora de metal hacia el huevo.

Palabras clave: Hierro, Vitelogenina, Síntesis de hemoglobina, Reticulocitos.

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