Immunochemical analysis of chick phosvitin

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SUMMARY

Phosvitin is a yolk phosphoglycoprotein bound to lipoproteins within the yolk granules. Its absorption and utilization during chick embryogenesis is still unclear.

The presence of phosvitin was investigated by immunodiffusion techniques in the water-soluble fraction (WSF) of the yolk during oocyte maturation and chick development and in the embryonic fluids.

The immunochemical analysis showed that phosvitin consists of two components, one of which is detectable during oocyte maturation, while both are shown in the yolk WSF of the incubated egg. The appearance of the second phosvitin component during incubation is probably dependent on granule dissolution, as suggested by the immunochemical and biochemical determinations on the embryonic fluids.

INTRODUCTION

Phosvitin, a yolk phosphoglycoprotein (Shainkin & Perlmann, 1971), contains about 90% of the yolk protein phosphorus (Burley & Cook, 1961) and possibly represents an energy source during embryonic development (Rosentein & Taborsky, 1970). Phosvitin is synthesized in the liver of the laying hen and transferred to the developing oocyte (Heald & McLachlan, 1963, 1965), where it is bound to lipoproteins (α-, β-lipovitellin and low-density lipoprotein) to form the granules (Joubert & Cook, 1958; Burley & Cook, 1961).

During embryogenesis yolk granules at first decrease slowly and then, in the second part of incubation, show a sudden drop (Biagi & Malaguti, 1964; Saito, Martin & Cook, 1965). The mechanism of granule absorption is still unknown (whether they are previously dissolved with the subsequent absorption of single components and/or are englobed as a whole by the yolk sac).

Current experiments deal with the immunochemical analysis of phosvitin in the water-soluble fraction (WSF) of yolk prepared from ovarian, laid and incubated eggs and in serum, amniotic and subgerminal fluids obtained from incubated eggs.

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The presence of phosvitin in the yolk WSF should indicate granule dissolution; the presence of phosvitin in embryonic fluids should suggest its reabsorption.

MATERIAL AND METHODS

Ovarian eggs were obtained from four laying hens (Ross strain, provided by San Martino, Campo, Perugia, Agricultural Station). The yolks were removed from the ovaries, weighed, measured and arranged into eight classes according to MacKenzie & Martin (1967). Eggs from the same strain were incubated (38 °C, 60 % relative humidity) for 6, 7, 8, 10, 13, 15 and 17 days.

Blood samples were obtained from amniotic vessels of embryos and from the wing axial vein of newly hatched chickens and laying hens. Sera were prepared after clotting by centrifugation (600 g, 30 min).

Amniotic fluid, obtained by puncturing the amniotic sac, was lyophilized and dissolved in NaCl 0-9 %.

Yolk was obtained by puncturing the vitelline membrane or the yolk sac; subgerminal fluid was aspirated, taking care to avoid contamination with the underlying thick yolk. WSF was prepared from both by the method of Martin, Vandegaer & Cook (1957) as previously reported (Carinci, Wegelin & Manzoli-Guidotti, 1966).

Immunoochemical analyses were carried out by means of the immunodiffusion technique (Ouchterlony, 1964) against a rabbit anti-chicken phosvitin antiserum (Calbiochem). The protein phosphorus was determined on 10% trichloroacetic acid precipitates by the method of Thompson, Stricklands & Rossiter (1963) and protein content was evaluated by the Biuret method (Bailey, 1967).

RESULTS

Anti-phosvitin antiserum tested against the rough antigen (phosvitin prepared from egg yolk-Calbiochem) exhibits two precipitation bands corresponding to a major slowly diffusing and a minor rapidly diffusing component (Fig. 1). A single precipitation arch corresponding to the slower component has constantly been detected in the yolk WSF from ovarian and from unincubated eggs (Fig. 1).

Two precipitation bands have been observed in the WSF prepared both from yolks and from the subgerminal fluids of eggs incubated for 6 days. These lines are immunologically identical to those given by rough antigen. The immuno-diffusion pattern does not change in the following incubation stages: 8th and 10th day for subgerminal fluid WSF; 8th, 10th, 13th, 15th and 17th day for yolk WSF (Fig. 2).

Embryonic serum tested against anti-phosvitin antiserum on the 6th, 8th and 10th days of incubation gives a single line, which corresponds to the rapid component of the rough antigen. In contrast, two lines corresponding to the
Fig. 1. Ouchterlony test. The central wells contain the anti-phosvitin antiserum. In peripheral wells are different preparations from yolk WSF of developing oocytes arranged into eight classes (I–VIII; the 1st class corresponds to mature oocyte) and phosvitin (Ph).

Fig. 2. Ouchterlony test. The central wells contain anti-phosvitin antiserum, the peripheral wells different preparations from yolk WSF for the indicated incubation days.
Fig. 3. Ouchterlony test. The central wells contain anti-phosvitin antiserum, the peripheral wells phosvitin (Ph) and serum (S) for the indicated incubation days.
Chick phosvitin

Fig. 4. Ouchterlony test. The central wells contain anti-phosvitin antiserum, the peripheral wells amniotic fluid (Am) for the indicated incubation days.

homologous phosvitin fractions are detectable in the sera obtained during later periods (13, 15, 17 days) (Fig. 3). The same two lines are present in the serum of newly hatched chickens and laying hens.

Similar patterns are offered by amniotic fluid: a single on the 6th, 8th and 10th day of incubation; two arcs in the subsequent stages (Fig. 4).

The protein phosphorus determinations as referred to total protein are reported in Fig. 5. The concentration of amniotic fluid protein phosphorus declines during incubation, while that of WSF protein phosphorus increases.

DISCUSSION

Immunological analysis of phosvitin against the homologous antiserum shows two components, therefore confirming the electrophoretic (Sundararajan, Sampath Kumar & Sarma, 1960) and biochemical (Clark, 1970) heterogeneity of this protein. The same two components are detectable in the laying-hen serum; on the other hand, ovarian egg yolk contains a single component. The presence of a phosvitin water-soluble form during oocyte maturation has been suggested by MacKenzie & Martin (1967) on the basis of electrophoretic analysis; and the possibility that a phosphoprotein may be associated to the livetin fraction of unincubated egg yolk has been considered by Mok & Common (1964).

Our data clearly establish that a phosvitin fraction is, at least partly, soluble in egg yolk. Another component is completely bound into the granules; its
appearance in the yolk WSF during incubation indicates granule dissolution. Dissolution of the granules within the yolk is also demonstrated by the increase of WSF protein P/total protein ratio, which indicates a rise of relative concentration in the yolk of soluble phosphoprotein. This increase closely parallels the decline in granule content of the yolk.

Since phosvitin occurs in the granules as a part of a complex which dissociates in salt solutions at mildly alkaline pH (Burley & Cook, 1961; Radomski & Cook, 1964), and since the yolk physico-chemical properties change with the age of the embryo (e.g. rise of pH) (Romanoff, 1967), it is possible that these factors are in some way involved in the mechanism of granule dissolution.

Phosvitin released from the granules is possibly hydrolysed, at least partly, within the yolk as indicated by acid-soluble organic phosphorus determinations (McIndoe, 1960).

Detection of phosvitin in embryonic serum and amniotic fluid indicates a transfer from the storage compartments. This transfer occurs at different periods for the two components and must involve the yolk-sac membrane. Phosvitin probably reaches the amniotic fluid via the blood circulation.

Fig. 5. Protein P content referred to total protein (A) in the amniotic fluid; (B) in the yolk WSF, for the indicated incubation days.
Chick phosvitin

(Romanoff, 1960). Diminution of amniotic protein P/total protein ratio corresponds to transfer of albumen protein into the amnion after the sero-amniotic connexion perforates (Carinci & Manzoli-Guidotti, 1968).

To summarize, granule absorption probably occurs by dissolution within the yolk followed by an early transfer of phosvitin to the blood, where it is available for the metabolic requirements of the embryo.

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REFERENCES


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