An oocytic membrane receptor for biotin-binding protein

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Abstract The chicken oocyte accumulates a biotin-binding protein (BBP) in the yolk that is distinct from the avidin in the 'egg white'. An identical BBP to that of yolk is also present in the circulation of the laying hen. We report the first evidence for the existence of a BBP receptor in the oocyte vitelline membrane. Reduction of the 100 kDa receptor results in loss of BBP-binding activity; this suggests that a disulfide-bonded region of the receptor is necessary for maintaining BBP-binding activity. We show further that the levels of serum BBP are strictly dependent on the presence of estrogen. As expected, BBP is not detected in significant quantities in rooster serum. Thus, these results suggest that circulatory BBP, like other estrogen-dependent components of serum, has a cognate binding activity on the oocyte membrane that may mediate its endocytosis.

Key words: Chicken; Biotin-binding protein; Estrogen; Oocyte membrane receptor

1. Introduction

The fact that the shell-enclosed eggs characteristic of birds, most reptiles, and a few mammals support embryogenesis, implies that they are nutritionally complete and that a full set of nutrient-delivery systems participate in their formation. Most of the nutritional resources of an egg reside in the yolk [1]. Among yolk precursors in chicken, several isoforms of vitellogenin, an estrogen-induced plasma protein, provide most of the amino acids, considerable amounts of protein-bound phosphate and associated trace minerals, some lipid, and several lipid-soluble vitamins [2]. In addition, substoichiometric amounts of riboflavin-binding protein [3], vitamin D-binding protein [4], and a couple of hormones [5], associate with vitellogenin in blood plasma. A specific vitellogenin receptor in the oocyte plasma membrane [6] mediates the recognition and subsequent endocytosis of vitellogenin with its entourage of nutritional and regulatory components.

Convergence of nutrients on vitellogenin to gain entry to the oocyte appears to be paralleled by convergence on the vitellogenin receptor as well. The major very low density lipoprotein of the chicken oocyte does not associate with vitellogenin but binds directly to the vitellogenin receptor [7].

Some nutrients do not depend on vitellogenin and may not depend on the vitellogenin receptor for deposition in the oocyte. In this poorly studied group are folate, retinol, and biotin. Folate, as 5-methyltetrahydrofolate, does not appear to be protein-bound in the plasma or oocyte yolk [8,9]. Retinol binds to retinol-binding protein which in turn is complexed with transthyretin in the plasma and enters the yolk via a receptor-mediated pathway different from that for vitellogenin [10,11]. Biotin, like retinol, binds to a specific plasma protein [12–14] but the mechanism of its deposition in yolk remains obscure.

During oogenesis, the plasma concentration of biotin [15] and biotin-binding protein (BBP) [16] rise sharply, and an immunologically and structurally similar vitamin–protein complex appears in the yolk [17]. The partition ratio of BBP in yolk to BBP in plasma of 25 to 35 compared to the ratio 5 or 6 for vitellogenin [16], implies that the accumulation of BBP and its associated biotin in yolk is independent of vitellogenin. In contrast to other yolk vitamin-binding proteins, BBP exists in two different forms, BBP-I and BBP-II [16,18,19]. The tetrameric BBP-II is derived from the monomeric BBP-I by proteolytic cleavage between each of the four biotin-binding domains of BBP-I [20,21]. The relative amounts of the two forms and their partitioning between plasma and yolk is coupled to biotin status. BBP-II formation and transport is favored in the normal dietary range [16]. We have detected the first known BBP receptor; we show that this 100 kDa receptor on the oocyte vitelline membrane interacts with the estrogen-induced BBP-II that is normally present in the circulation of the laying hen.

2. Materials and methods

2.1. Materials and experimental animals

Protein A, phenylmethyl-sulfonylfluoride, leupeptin and Ponceau S were obtained from Sigma. Iodo-Gen oxidative radiolabelling reagent was obtained from Pierce. Chicken oocyte membranes were prepared by solubilization in 1% (w/v) Triton X-100 as described [7]. Sources of other reagents have been previously reported [11]. White Leghorn laying hens and roosters, approximately 1 year old, were maintained with a 14 h light period, and free access to food and water. For estrogen treatment, roosters were injected in the leg muscle with 20 mg/kg of 17β-estradiol; after 3 days the treatment was repeated and blood was collected 2 days after the second injection. The rabbit anti-chicken BBP-II antibody preparation was previously described [18]. Protein concentration was determined by the method of Lowry et al. [22].

2.2. Gel electrophoresis, Western and ligand blotting

One-dimensional gradient (4.5–18%) SDS-PAGE was performed using a minigel system (Bio-Rad) [23]. Protein samples (see Figure legends) in the presence or absence of 50 mM dithiothreitol were heated at 95°C for 5 min. Proteins were electrophoretically transferred to nitrocellulose and stained with 0.2% Ponceau S in 3% (w/v) TCA. After destaining and blocking (4% milk, 50 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1 mM $CaCl_2$, 0.05% Triton X-100), Western blotting [24] was performed using rabbit anti-chicken BBP-II IgG (1:500) followed by [^{125}I]protein A. For ligand blotting, the nitrocellulose containing the transferred oocyte membrane proteins was incubated with laying hen or rooster serum (diluted 1:2 in blocking buffer) for 2 h at room temperature followed by anti-BBP-II IgG and [^{125}I]protein A.

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Abbreviations: BBP, biotin-binding protein; DTT, dithiothreitol; TCA, trichloroacetic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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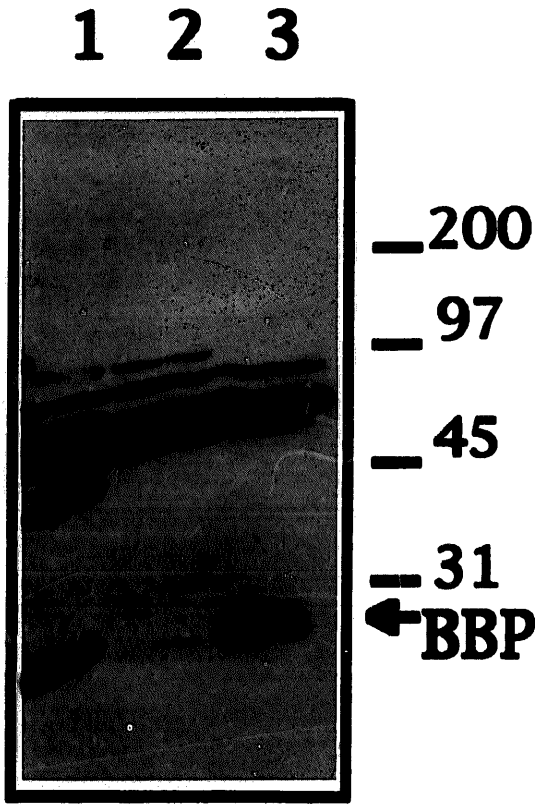

Fig. 1. BBP is detectable in the serum of laying hens and estrogen-treated roosters. Serum samples (3 μl) were reduced, heated and subjected to Western blot analysis as described in section 2. Proteins corresponding to the size of the BBP-II monomer were detected in estrogen-treated rooster serum (lane 1) and laying hen serum (lane 3) but not normal rooster serum (lane 2). Molecular mass standards are indicated in kDa to the right of lane 3.

3. Results

The ~20 kDa BBP-II subunit has been identified by an anti-chicken BBP antibody in both serum and oocytic yolk of the laying hen [21]. In Fig. 1, the presence of the BBP monomer is confirmed in laying hen serum using the same anti-chicken BBP antibody. Estrogen dependence of BBP serum levels is also revealed by its presence in estrogen-treated roosters (Fig. 1, lane 1) and virtual absence from normal rooster serum (Fig. 1, lane 2). In the serum of the estrogen-treated rooster, a 40 kDa species which corresponds to a two-domain fragment of BBP-I [21] was observed in addition to the BBP-II monomer (Fig. 1, lane 1).

In the case of transthyretin, a known laying hen serum yolk precursor, it has been shown that addition of the ligand in its native serum complex form, followed by an anti-transthyretin antibody, results in the detection of the oocyte membrane transthyretin receptor [11]. Based on the differential expression of BBP in laying hen and rooster serum (Fig. 1), we could test for the presence of an oocyte membrane BBP receptor by a similar ligand blotting experiment. The results (Fig. 2) reveal that a 100 kDa receptor interacts either directly or indirectly with the BBP that is present in laying hen serum (Fig. 2, lane 3) and absent from rooster serum (Fig. 2, lane 4). Controls show that the identification of the receptor is dependent on the anti-BBP antibody (Fig. 2, lanes 1 and 2). Interestingly, the receptor's BBP-binding activity is sensitive to disulfide bond reduction by DTT. These data suggest that in the denaturing conditions of SDS-PAGE the BBP receptor retains enough structure to interact with BBP and that this critical amount of structure that it retains involves disulfide bonds. In addition to BBP, the antibody appears to crossreact with 200 kDa species present in the oocyte membrane preparation (Fig. 2, lane 5). Although this large species remains unidentified (and likely represents a contaminant in the antigen preparation), it appears to be present in rooster and laying hen serum as evidenced by the competition of the 200 kDa signal by both sera (Fig. 2, compare lane 5 with lanes 3 and 4).

4. Discussion

Vitellogenin is the major yolk precursor for both vertebrate and invertebrate eggs [2]. Given that the receptor-mediated endocytic pathway for vitellogenin probably was present early in the evolution of shell-enclosed eggs, it is reasonable that new nutrient delivery systems would build on the preexisting receptor-mediated transport pathway for vitellogenin. This has occurred in two ways. Some proteins, such as riboflavin-binding protein, bind to vitellogenin in blood plasma and are deposited in yolk as a macromolecular complex [3] while another, plasma very-low-density lipoprotein, intercepts the transport system by binding to the same receptor that

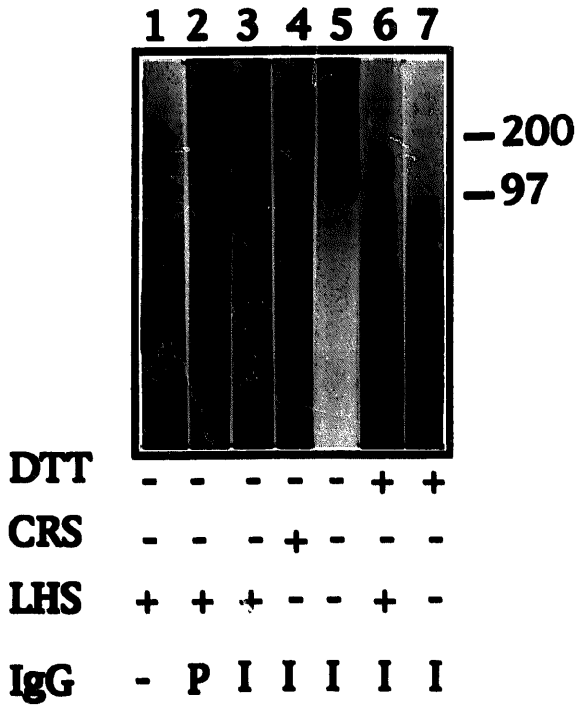

Fig. 2. Identification of a 100 kDa oocytic membrane BBP receptor. An oocyte membrane detergent extract (80 μg of protein) was subjected to SDS-PAGE in the absence (lanes 1–5) or presence (lanes 6–7) of DTT, transferred to nitrocellulose and used for ligand blotting as described in section 2. Control rooster serum (CRS; lane 4) or laying hen serum (LHS; lanes 1–3, 6) was used as the potential source of ligand followed by preimmune (P; lane 2) or immune anti-BBP-II (I; lanes 3–7) IgG. The specific 100 kDa BBP-binding component is detected only in the presence of LHS BBP followed by anti-BBP-II antibody. Two molecular mass markers (kDa) are indicated to the right of lane 7.

recognizes vitellogenin [7]. This convergence on components of the vitellogenin transport system, however, does not apply to all oocytic yolk components. Whether this convergence can be generalized to include biotin remains to be determined.

While the role of biotin-binding protein in delivering biotin to the oocyte is well established [14], the route of entry into the yolk has not been studied previously. With the identification of an oocyte membrane component to which BBP associates, our results provide the first insight into the mechanism of how circulatory BBP becomes yolk BBP. Our results suggest that BBP, like certain other lipoproteins and vitamin transporters, enters the oocyte via a receptor-mediated process. These data are consistent with the observation of a high density of endocytic clathrin-coated pits in the oocyte vitelline membranes of birds and other oviparous species which, in turn, indicates that receptor-mediated endocytosis is the major process involved in oocyte growth [25–27].

From the evidence presented in Figs. 1 and 2, it is clear that we have identified an oocyte membrane component which is specifically interacting with BBP: BBP is the only laying hen serum-specific protein recognized by the antibody. Our data, however, do not exclude the possibility that BBP is interacting indirectly, via another serum component, with the oocyte receptor. Future experiments will reveal the mechanism by which this oocyte membrane receptor interacts with BBP and its relation, if any, to components of the vitellogenin transport system.

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