Effect of Estradiol Administration on Plasma Protein Composition in the Male Japanese Quail (*Coturnix coturnix japonica*)¹

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Introduction

Estrogen can stimulate vitellogenesis in immature and mature male and female birds, amphibians, reptiles and fishes^{1,2,3,4)}. In these animals, estradiol treatment produces profound changes in plasma protein, lipid and calcium^{2,5,6,7)}. TANABE and OGAWA⁸⁾ reported the presence of new polymorphic estrogen-dependent proteins in the prealbumin zone of quail and chicken egg yolk plasma and blood plasma by using horizontal polyacrylamide gel electrophoresis. They reported that prealbumin-3 (*Pa*-3) was an estrogen-dependent protein in quail yolk plasma and blood plasma, both of which having six phenotypes of A, B, C, AB, AC and BC, which were controlled by autosomal *Pa*-3 locus with three codominant alleles, *Pa*-3^A, *Pa*-3^B and *Pa*-3^C.

The present study was undertaken to elucidate the effect of estrogen treatment on quail plasma proteins including prealbumin-3 (*Pa*-3) by using horizontal polyacrylamide gel electrophoresis.

Materials and Methods

Hormone treatments and blood sampling: Six male Japanese quail of five months of age, weighing approximately 110 g, were injected intramuscularly with estradiol-17 β -benzoate at 1 mg per day for four consecutive days. Blood samples were collected from the individual quail using heparinized syringe immediately before and 24 hr after estrogen injection. The blood plasma was obtained by centrifugation at 3,000 rpm for 10 min and stored at -20°C until used for electrophoresis. Blood plasma samples were also obtained from laying and non-laying quail.

Electrophoresis: To identify the estrogen-dependent protein bands, horizontal polyacrylamide gradient gel electrophoresis was carried out as described before^{2,9)}. Gel buffer was 0.1875 M Tris-sulphate at pH 9.0. The electrode buffer was 0.13 M Tris-borate at pH 9.2. A step gradient gel of polyacrylamide concentration of 8%, 4% and 10% was used. The blood plasma samples soaked into small pieces of filter paper (6 mm $\times$ 2 mm) and were applied on 4% gel. Electrophoresis was carried out at a constant current of 1.25 mA/cm at 4°C for 2.5 hr. After electrophoresis, the gel was stained with Coomassie brilliant blue R in

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3.5% perchloric acid for 3 hr, and destained in 5% acetic acid for about 30 min. The density of plasma protein bands on the 10% gel of polyacrylamide concentration was scanned by Computing Densitometer (ACD 18, Ato).

Eluting specific protein bands: After electrophoresis, the gel fractions of prealbumin-3 (*Pa-3*) protein bands of blood plasma were sliced into 1 mm sections and eluted with agitation at 37°C into 10 volumes (up to 5 ml) of 5 mM NaHCO₃ containing 0.05% SDS. After 13–15 hrs, polyacrylamide fragments were spun out in a centrifuge and the supernatant was lyophilized. The lyophilized residue was dissolved directly in 0.3% pyridine and applied to a column of Sephadex G 50 (2×110 cm). The column was eluted with a 0.3% pyridine at a room temperature at a flow rate of 0.6 ml/min. The absorbance at 280 nm of secreted chromatographic fractions was monitored to follow the removal of proteins by spectrophotometer. The effluent collected in 5.0 ml fractions was dialyzed against several changes of distilled water at 4°C and lyophilized¹⁰⁾. The lyophilized *Pa-3* protein sample was hydrolyzed and amino acid analyses were carried out.

Results and Discussion

Effect of estradiol benzoate injected at a dose of 1 mg/bird/day for four consecutive days on blood plasma protein composition of adult male quail is shown in Figure 1 and Figure 2. The estradiol treatment brings about profound changes in plasma protein levels in male quail. After electrophoresis, quail plasma proteins were separated into 15 electrophoretic

Fig. 1. Polyacrylamide gel electrophoretograms of five months old male quail plasma before and after the injection of estradiol benzoate at 1 mg/bird/day for four consecutive days.

Fig. 2. Computed densitometric traces of polyacrylamide gel electrophoretograms of male quail plasma before (I) and after (II) the injection of estradiol benzoate.

zones; prealbumin-3 (*Pa-3*), prealbumin-2 (*Pa-2*), prealbumin-1 (*Pa-1*), prealbumin (*Pa*), albumin (*Alb*), postalbumin (*Poa*), pretransferrin (*Ptf*), transferrin (*Tf*), posttransferrin-1 (*Potf-1*), and posttransferrin-2 (*Potf-2*). Estrogen produced the formation of plasma polymorphic prealbumin-3 (*Pa-3*) protein bands in male quail as previously reported by TANABE and HAYASHI³⁾ and TANABE *et al.*⁸⁾ (Fig. 1). The density of *Pa-2* protein bands changed between before and after the injection of estradiol, but no change occurred in *Pa-1* protein bands. After the injection of estradiol benzoate, the intensity of albumin band decreased, whereas that of transferrin and posttransferrin-2 increased.

In globulin zone, posttransferrin protein band was almost disappeared and less intense protein bands newly occurred after the estrogen treatment. TANABE *et al.*¹¹⁾ reported that major phosphoprotein band migrated between postalbumin and transferrin bands in the autoradiogram of laying quail plasma 24 hr after injection of ³²P at a dose of 850 μ Ci³²P (H_3PO_4 , carrier free). It is suggested that the estrogen-dependent major phosphoprotein, vitellogenin¹²⁾ in estrogenized male quail blood plasma occurred as the less intense protein bands in the posttransferrin zone.

The plasma protein bands were separated into seven densitometric peaks of prealbumin-

Fig. 3. Polyacrylamide gel electrophoretograms of plasma from laying quail (L), and non-laying quail for more than two days (N).

Fig. 4. Computed densitometric trace of polyacrylamide gel electrophoretograms of laying quail plasma.

-3 (*Pa*-3), prealbumins except *Pa*-3 (*Pa*-2, *Pa*-1 and *Pa*), albumin (*Alb*), postalbumin (*Poa*), pretransferrin (*Ptf*), transferrin (*Tf*), posttransferrin (*Potf*) (Fig. 2). In adult normal male quail plasma (just before the estrogen treatment), relative densitometric concentrations of *Pa*-3, prealbumins except *Pa*-3, *Alb*, *Poa*, *Ptf*, *Tf* and *Potf* proteins were 0%, 5.1%, 38.3%, 7.3%, 28.7%, 13% and 8.2%, respectively. They became 4.3%, 3.5%, 29.8%, 8.5%, 7.8%, 28.3% and 18.4%, respectively, after the treatment of estrogen.

Electrophoretograms of blood plasma of laying and non-laying quail are shown in Figure 3. Electrophoretic patterns of laying quail were similar to those of estrogenized male quail. Electrophoretic patterns of the plasma proteins of non-laying quail were similar to those of normal female quail. Densitometric trace of electrophoretogram of laying quail plasma is shown in Figure 4. Relative densitometric concentrations of *Pa*-3, prealbumins except *Pa*-3

(*Pa-2*, *Pa-1*, *Pa*), *Alb* (including *Poa*), *Ptf*, *Tf* and *Potf* in laying quail plasma were 2.0%, 4.2%, 43.6%, 16.1%, 20.4% and 13.6%, respectively.

After electrophoresis, the elution of estrogen-dependent prealbumin-3 (*Pa-3*) protein was performed in pyridine by gel filtration on a Sephadex G 50 column and amino acid compositions of the recovered *Pa-3* protein in the void volume were analysed. The contents of aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine and proline of the protein were 11.5, 4.3, 6.9, 12.5, 8.8, 9.0, 0.5, 6.8, 2.4, 4.4, 8.4, 3.8, 4.0, 6.1, 0.6, 4.3 and 5.5%, respectively. So the polymorphic estrogen-dependent *Pa-3* protein of laying quail plasma newly found by TANABE and OGAWA⁸⁾ is not phosvitin.

SUMMARY

Five month-old male Japanese quail were injected with estradiol benzoate at a dose of 1 mg/bird/day for 4 consecutive days. Horizontal polyacrylamide gradient gel electrophoretograms of plasma proteins were compared before and after the estrogen treatment.

After the estrogen treatment, the intensity of albumin and pretransferrin bands decreased drastically and that of transferrin and posttransferrin-2 (the slowest migrating band) were increased. In pretransferrin zone, new protein bands appeared.

Fastest migrating estrogen dependent prealbumin-3 (*Pa-3*) protein bands occurred in estrogenized male quail plasma, and its amino acid compositions of Asp, Thr, Ser, Glu, Gly, Ala, Cys Val, Met, Ile, Leu, Tyr, Phe, Lys, His, Arg and Pro were 11.5%, 4.3%, 6.9%, 12.5%, 8.8%, 9.0%, 0.5%, 6.8%, 2.4%, 4.4%, 8.4%, 3.8%, 4.0%, 6.1%, 0.6%, 4.3% and 5.5%, respectively.

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雄ウズラ (*Coturnix coturnix japonica*) 血漿蛋白質構成に 対するエストラジオール投与の影響

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成雄ウズラにエストラジオール-17 β -ベンゾエートを1羽当たり1mgずつ4日間連続投与した。血液は投与直前および投与終了24時間後に採取し、血漿を得た。また産卵中および休産中の雌ウズラからも血漿を採取した。この各個体の血漿の蛋白質の泳動像の変化を水平式ポリアクリルアミドゲル電気泳動法(pH 9.0)により調べた。泳動後、CBB-Rで染色し、デンストメトリーにより蛋白質の濃度を比較した。

エストラジオール投与によって成雄ウズラ血漿蛋白質の泳動像が、産卵中の雌ウズラ型に変化した。すなわち成雄ウズラの投与前には、プレトランスフェリン(*Ptf*)の比率が36%であったが、投与後16%と半減した。逆にトランスフェリン(*Tf*)は、投与前は13%であったが、エストラジオール投与によって28%と約2倍に増

加した。最も遅く移動するホストトランスフェリン-2(*Potf-2*)の泳動帯の染色も投与後増加した。アルブミン(*Alb*)は、本実験のエストラジオール処理後は、処理前の2/3に減少した。

エストラジオール投与処理によって、成雄ウズラ血漿に、産卵中の雌血漿に特有の多型をもつエストロゲン依存蛋白質であるプレアルブミン-3(*Pa-3*)の泳動帯が出現した。この泳動帯より蛋白質(*Pa-3*)を抽出し、アミノ酸組成を調べた。その結果、*Pa-3*は、セリンが6.9%であまり多くなく、最も多いのはグルタミン酸で12.5%、つぎに多いのがアスパラギン酸(11.5%)である蛋白質であった。従ってこの蛋白質はホスピチンではないと思われる。

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