Estrogen Receptor Binding in the Oviduct Magnum of Guineafowl Hens: Comparison with White Leghorn Hens

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Estrogen receptor bindings in cytosol and nuclear fractions of the magnum tissue of laying guineafowl and White Leghorn hens were examined by binding assays using [³H]-estradiol-17 β . The value of equilibrium dissociation constant (K_d) in both the cytosol and the nuclear fractions was not different between the two different times, 1.5 h and 23 h after oviposition, and also between the guineafowl and the chicken. The maximum binding capacity (B_{max}) was greater at 1.5 h after oviposition than at 23 h after oviposition in both cytosol and nuclear fractions of White Leghorn hens and in the nuclear fraction of the guineafowl hens, although the B_{max} value was smaller in the guineafowl hens than in White Leghorn hens. The results suggest that the smaller binding capacity of the estrogen receptor in the oviduct magnum may be related to the less amount of albumen secreted from the oviduct magnum in the guineafowl hens than in White Leghorn hens.

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Key words : guineafowl, estrogen receptor, oviduct magnum, albumen secretion

Introduction

The magnum is the albumen-secreting portion of oviduct of birds (GILBERT, 1971). Tubular gland cells of the magnum synthesize ovoalbumin, a major component of egg white (WYBURN *et al.*, 1970), by the action of estrogen through binding to its receptors existing in the cells (O'MALLEY *et al.*, 1969). The amount of albumen in the egg of the guineafowl is less than that in the egg of the chicken (OGAWA *et al.*, 2000). Responsiveness of the oviduct to estrogen for the weight increase is lower in the guineafowl than in the chicken (OGAWA *et al.*, 1998). Whether the less amount of albumen secreted from the magnum of the guineafowl is due to the lower responsiveness to estrogen is obscure. In this study, experiments were performed to elucidate whether the binding property of the estrogen receptor in the magnum tissue of the oviduct is different between guineafowl and White Leghorn hens.

Materials and Method

Birds and tissues

Guineafowl hens (2.3–2.9 kg BW, 25–42 weeks of age ; Galor, France) and White Leghorn hens (1.4–1.8 kg BW, 25–42 weeks of age ; Super Nick) kept in individual cages under 14 h light (0400–1800 h) per day with feed and water given *ad libitum* consumption and laying 4–7 eggs in a sequence were used. In the first experiment, the guineafowl and the White Leghorn hens (3 birds each) were killed at 10 : 00 h of a day of mid-sequence, and the magnum tissue of the oviduct was excised and used to examine binding properties of estrogen receptor. In the second experiment, the guineafowl and White Leghorn hens were killed at 1.5 h (6 birds each) and 23 h (5 birds each) after oviposition of the initial egg of the sequence. After confirming the presence of ovum in the oviduct, the magnum tissue was excised and used for the determination of affinity and capacity of estrogen receptor.

Chemicals and reagents

Estradiol-17 β (E₂), estrone (E₁), estriol (E₃), diethylstilbestrol (DES), testosterone (T), progesterone (P₄) and corticosterone (B) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). [2,4,6,7,16,17-³H(N)]-E₂ (Specific activity : 5218.11 GBq/mmol) was purchased from E. I. Dupont de Nemours Co. Inc., (Boston, MA, U.S.A.). Bovine serum albumin (BSA) fraction V, was obtained from Sigma Chemical Co., or Miles Inc. (Kankakee, IL, U.S.A.). All chemicals used were of analytical grade, obtained from Kanto Chemical Industries, Ltd. (Tokyo, Japan).

Preparation of cytosol and nuclear fractions

Cytosols (soluble fractions in hypotonic buffer solution) and nuclear fractions (insoluble fraction in the hypotonic buffer solution) of the oviduct magnum tissues were prepared using the same method as reported earlier (KAWASHIMA *et al.*, 1984, 1987). After homogenizing in hypotonic Tris-EDTA-dithiothreitol-bacitracin buffer (TEDB ; 10 mM Tris-HCl, 1.5 mM EDTA, 1 mM dithiothreitol, 0.5 mM bacitracin, pH 7.4) containing 10% glycerol at 4°C, the homogenates were centrifuged at 1,000 $\times$ g for 10 min. The precipitated pellets were resuspended and recentrifuged. The supernatants were combined and treated for 30 min with dextran-coated charcoal pellets (0.5% charcoal Norit A and 0.05% dextran T 70) to remove endogenous steroids, and centrifuged at 105,000 $\times$ g for 1 h. The supernatant was designated the cytosol fraction. The 1,000 $\times$ g sediments were washed once in Tris-MgCl₂-dithiothreitol buffer (TMD ; 10 mM Tris-HCl, 1.5 mM MgCl₂, 1 mM dithiothreitol, pH 7.4) containing 0.25% Triton X-100, and then washed twice in the TMD buffer. The washed pellets were suspended in the TMD buffer containing 2.4M sucrose and centrifuged for 1 h. The resultant pellets were washed twice in TEDB buffer, suspended in 0.6M KCl-TEDB buffer, and allowed to stand for 30 min. The suspension was then centrifuged at 10,500 $\times$ g for 1 h ; the final supernatant was used as the nuclear fraction. The remaining pellets were measured for DNA. The cytosol and the nuclear fraction were immediately analyzed for the [³H]-E₂ binding.

Protein concentrations of the cytosol were determined by the method of LOWRY *et al.* (1951) using BSA as a standard. DNA concentrations of the remaining pellets were determined by the method of BURTON (1956) using calf thymus DNA as a standard.

Binding assays

Binding assays of the cytosol and the nuclear fractions were performed by the use of the same method of KAWASHIMA *et al.* (1984). In the binding assays, 1.5 ml polypropylene microfuge tubes pretreated with TEDB buffer containing 2% BSA for 24 h at 4°C were used. The cytosol (400 µg protein/200 µl per tube) and the nuclear fractions (200 µg protein/200 µl per tube) were incubated at 4°C for 20 h with [³H]-E₂ (0.1 to 3.2 nM) in the absence (total binding) or presence (nonspecific binding) of a 1,000 fold molar excess of unlabeled E₂ in a total volume of 300 µl. The binding specificity was examined using 20, 200 and 2,000 nM of unlabeled E₂, E₁, E₃, DES, and 20 and 2,000 nM of unlabeled P₄, T and B as competitors. After the incubation, bound and free steroids were separated by the use of a protamine sulfate precipitation method (0.25 mg protamine sulfate/0.25 ml TEDB buffer per tube). The precipitated pellet was washed twice with 1 ml of ice-cold TEDB buffer, and the scintillation fluid (Pico-fluor 40, Packard) was added. Radioactivity was measured by a liquid scintillation spectrometer (LSC-5100, Aloka). The counting efficiency for ³H was 52–55%. Specific binding was obtained by subtracting the nonspecific binding from the total binding and expressed as femtomoles (fmol) per milligram protein or fmol per milligram DNA. The equilibrium dissociation constant (K_d) and the maximum binding capacity (B_{max}) were determined by the method of SCATCHARD (1949).

Statistical Analyses

For comparison among means of more than two groups, the data were analyzed by one-way ANOVA (SNEDECOR and COCHRAN, 1989). When significant effects were found at 5% level, Duncan's new multiple range test was used to separate means (DUNCAN, 1955).

Results

Relationship of specific [³H]-estradiol-17β binding to concentration of protein

Specific [³H]-E₂ binding increased linearly with the increase of the protein concentration from 50 to 800 µg per tube in the cytosol and from 25 to 400 µg per tube in the nuclear fraction in both the guinea fowl and the chicken (Fig. 1.).

Time course of specific [³H]-estradiol-17β binding during incubation

In the cytosol fractions of both the guinea fowl and White Leghorn hens, the specific [³H]-E₂ binding increased during the first 4 h, and maintained the maximal binding up to 24 h. In the nuclear fractions, the specific [³H]-E₂ binding increased during the first 16 h, and maintained the maximal binding up to 24 h (Fig. 2.).

Binding specificity

The specific [³H]-E₂ binding of cytosol and nuclear fractions was reduced by the presence of a 10 to 1,000 fold molar excess (20 to 2,000 nM) of unlabeled E₁, E₂, E₃ or DES, but not reduced by the presence of a 100 and 1,000 fold molar excess (200 and 2,000 nM) of unlabeled B, P₄ or T (Fig. 3.).

Saturation of specific [³H]-estradiol-17β binding

Specific [³H]-E₂ binding of cytosol and nuclear fractions increased when increasing amounts of [³H]-E₂ were added, and was saturable at about 4 nM (Fig. 4).

Scatchard analysis

Fig. 1. Relationship of specific [^3H]-estradiol-17 β ([^3H]-E $_2$) binding to protein concentration in cytosol and nuclear fractions of the oviduct magnum of guineafowl and White Leghorn. Samples (Cytosol: 50–800 μg protein per tube; Nuclear: 25–400 μg protein per tube) were incubated at 4°C for 20 hours with 2 nM [^3H]-E $_2$ in the presence and absence of 2 μM unlabeled E $_2$. Each point represents the mean of triplicate determinations on three separate pooled samples, and the vertical bars represent SEM. When not shown, SEM falls in the symbol.

Scatchard analysis revealed a linear relationship between the amount of specific [^3H]-E $_2$ binding and the ratio (B : F) of specific [^3H]-E $_2$ binding to free [^3H]-E $_2$ (Fig. 4), indicating a single class of binding sites. The value of K_d , B_{max} , and correlation coefficient (r) between B : F and specific [^3H]-E $_2$ binding in the guineafowl was 0.38 nM (K_d), 4.28 fmol/mg protein (B_{max}) and -0.989 (r) in the cytosol fraction, and 0.42 nM (K_d), 0.35 fmol/ μg DNA (B_{max}) and -0.985 (r) in the nuclear fraction. The value of K_d , B_{max} , and correlation coefficient (r) between B : F and specific [^3H]-E $_2$ binding in White Leghorn was 0.44 nM (K_d), 5.25 fmol/mg protein (B_{max}) and -0.979 (r) in the cytosol fraction, and 0.44 nM (K_d), 0.21 fmol/ μg DNA (B_{max}) and -0.962 (r) in the nuclear fraction.

Comparison of K_d and B_{max} between guineafowl and chicken

The K_d value in both the cytosol and the nuclear fractions was not different between the two different times, 1.5 h and 23 h after oviposition, and also between the guineafowl and the chicken (Table 1). The B_{max} value was greater at 1.5 h after oviposition than at 23 h after oviposition in both the cytosol and the nuclear fractions of the White Leghorn hens and in the nuclear fractions of the guineafowl hens (Table 2). The B_{max} value was smaller in the guineafowl hens than in the White Leghorn hens (Table 2).

Fig. 2. Time course of specific [^3H]-estradiol-17 β ([^3H]-E $_2$) binding in cytosol and nuclear fractions of the oviduct magnum of guinea fowl and White Leghorn. Samples (Cytosol: 400 μg protein per tube; Nuclear: 200 μg protein per tube) were incubated at 4°C for various times with 2 nM [^3H]-E $_2$ in the presence and absence of 2 μM unlabeled E $_2$. Each point represents the mean of triplicate determinations on three separate pooled samples, and the vertical bars represent SEM. When not shown, SEM falls in the symbol.

Discussion

The presence of estrogen receptor in cytosol and nuclear fractions of the magnum tissue of the oviduct was demonstrated in guinea fowl hens as well as in White Leghorn hens by the use of radioligand binding assays for the binding property of a receptor such as binding specificity (Fig. 3), saturation (Fig. 2 and Fig. 4), affinity (Fig. 4) and capacity (Fig. 4).

The affinity of the estrogen receptor in the cytosol and nuclear fractions as indicated by the value of the equilibrium dissociation constant, was found to be not different between the birds of the different species, the guinea fowl and the chicken (Table 1). The affinity was very similar to that of estrogen receptor reported on the hypothalamus and the pituitary (KAWASHIMA *et al.*, 1987) of the chicken.

The B_{max} value of the estrogen receptor in both cytosol and nuclear fractions of White Leghorn hens was greater at 1.5 h after oviposition than at 23 h after oviposition (Table 2). In the guinea fowl, the B_{max} value in the nuclear fraction was greater at 1.5 h after oviposition than at 23 h after oviposition (Table 2). The difference in the B_{max} values of the cytosol fraction was not statistically significant ($P > 0.05$). However, the B_{max} value in the cytosol fraction was slightly greater at 1.5 h after oviposition than at 23 h after oviposition. The time, 1.5 h after oviposition is the time of albumen formation in the magnum, and the time, 23 h after oviposition, is the time of no albumen

Fig. 3. Competition for [³H]-estradiol-17β ([³H]-E₂) binding in the cytosol and nuclear fractions of the oviduct magnum of the guineafowl and White Leghorn. Samples (Cytosol: 400 μg protein/tube; Nuclear: 200 μg protein/tube) were incubated at 4°C for with 2 nM [³H]-E₂ in the absence (control) or presence of various fold molar excess of unlabeled competitions, and the [³H]-E₂ binding was measured. The value of [³H]-E₂ binding in the control was 33.18 fmol/mg protein (Cytosol) and 8.94 fmol/mg protein (Nuclear) in the guineafowl, and 30.27 fmol/mg protein (cytosol) and 10.14 fmol/mg protein (Nuclear) in White Leghorn hens, respectively. Each point represents the mean of triplicate determinations of two separate pooled samples. The competitors tested were: estradiol-17β (E₂: ●), estrone (E₁: ○), estriol (E₃: ■), diethylstilbestrol (DES: □), testserone (T: ▲), progesterone (P₄: △) and corticosterone (B: ▼).

formation (GILBERT, 1971). Therefore, this finding suggests that the binding capacity of estrogen receptor may be related to the albumen formation in the magnum of the oviduct.

The mechanism of the action of estrogen in cells of the oviduct of the chicken in stimulating the biosynthesis of egg-white proteins has been well established (O'MALLEY *et al.*, 1969). The finding that the capacity of the estrogen receptor in the magnum, the albumen secreting portion of the oviduct, was less in the guineafowl than in the chicken (Table 2) suggests that the less capacity may be related to the less amount of albumen in guineafowl eggs than in White Leghorn eggs (OGAWA *et al.*, 2000), and may be also related to the lower responsiveness to estrogen for the increase in the weight of

Fig. 4. Specific bindings of [^3H]-estradiol-17 β ([^3H]-E $_2$) in cytosol and nuclear fractions of the oviduct magnum of the laying guinea fowl (●) and White Leghorn hens (○) without regard to the time of a laying sequence (Left), and their Scatchard plot (Right). Samples (Cytosol: 400 μg protein per tube; Nuclear: 200 μg protein per tube) were incubated at 4°C and 20 hours with various concentrations (0.25 to 8 nM) of [^3H]-E $_2$ in the absence or presence of a 100-fold molar excess of unlabeled E $_2$. Each point represents the mean of duplicate determinations of one pooled sample.

Table 1. The equilibrium dissociation constant of estrogen receptor in the magnum tissue of the guinea fowl (GF) and the chicken (WL)

Hrs after oviposition	Bird	No. of birds	Cytosol (nM)		Nuclear (nM)	
1.5	GF	6	0.38 $\pm$ 0.03 [*]	a ^{**}	0.35 $\pm$ 0.01 [*]	A ^{**}
	WL	6	0.45 $\pm$ 0.03	a	0.36 $\pm$ 0.02	A
23	GF	5	0.38 $\pm$ 0.02	a	0.37 $\pm$ 0.03	A
	WL	5	0.46 $\pm$ 0.04	a	0.40 $\pm$ 0.03	A

^{*} Mean $\pm$ SEM

^{**} Means in the same column with same letter are not significantly different by Duncan's new multiple range test ($P < 0.05$).

whole oviduct in guinea fowl than in White Leghorn chickens (OGAWA *et al.*, 1998).

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Table 2. The maximum binding capacity of estrogen receptor in the magnum tissue of the guinea fowl (GF) and the chicken (WL)

Hrs after oviposition	Bird	No. of birds	Cytosol (fmol/mg protein)		Nuclear (fmol/ μ gDNA)	
1.5	GF	6	6.15 $\pm$ 0.13 [*]	c ^{**}	0.57 $\pm$ 0.00 [*]	C ^{**}
	WL	6	8.67 $\pm$ 0.22	a	0.67 $\pm$ 0.01	A
23	GF	5	5.67 $\pm$ 0.17	c	0.53 $\pm$ 0.01	D
	WL	5	7.83 $\pm$ 0.15	b	0.62 $\pm$ 0.02	B

^{*} Mean $\pm$ SEM

^{**} Means in the same column with same letter are not significantly different by Duncan's new multiple range test ($P < 0.05$).

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ホロホロチョウの卵管膨大部におけるエストロジェンレセプターの結合性：白色レグホーン種のニワトリとの比較

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産卵期のホロホロチョウと白色レグホーン種のニワトリについて，卵白分泌時と非分泌時の卵管膨大部組織のサイトゾール画分及び核画分におけるエストロジェンレセプターの平衡解離定数 (K_d) と最大結合容量 (B_{max}) を [³H]-estradiol-17 β を使用した結合アッセイによって求めた。

K_d 値はサイトゾール画分及び核画分のいずれにおいても卵白分泌時と非分泌時との間及びホロホロチョウとニワトリとの間に差は認められなかった。 B_{max} は，ニワトリのサイトゾール画分及び核画分，ホロホロチョウ

の核画分においては卵白分泌時の方が非分泌時よりも大であった。また，ホロホロチョウの方がニワトリよりも小さかった。それ故，ホロホロチョウの卵の卵白量が少ないことは，卵管膨大部のエストロジェンレセプターの結合容量が少ないことに起因するのであろうと思われる。

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