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Cytoplasmic Dehydroepiandrosterone Sulfate-Binding Protein in Rabbit Uterine Cervix

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Dehydroepiandrosterone 3-sulfate (DHAS) binding to a cytoplasmic protein of rabbit uterine cervix was studied *in vitro*. When [^{3}H]DHAS was incubated with the minced uterine cervix obtained at term pregnancy, it was bound specifically to the cytosol fraction. From Scatchard analysis, the binding capacity and dissociation constant were found to be 33 fmol/mg protein and 5.05×10^{-8} M, respectively. Incubation of the prelabeled cytosol with isolated nuclei resulted in temperature-dependent transfer of DHAS to the nuclei. The cytoplasmic DHAS-binding protein after concentration with $(NH_4)_2SO_4$ showed a sedimentation constant of 3.6—4.0S, while the DHAS-binding protein in the rabbit serum gave a value of 4.6S. The cytoplasmic DHAS-binding protein was eluted from Sephacryl S-300 mainly in the fractions corresponding to the molecular weight of 1.7×10^5, and in the void volume to a small extent in the presence of molybdate and glycerol. Dehydroepiandrosterone (DHA) inhibited approx. 50% of [^{3}H]DHAS-specific binding, whereas 17β-estradiol 3-sulfate, estriol 3-sulfate, estrone 3-sulfate, 17β-estradiol, estriol, and 5α-dihydrotestosterone showed lower or no affinity for the DHAS-specific binding site. The content of the DHAS-binding protein in the cervix was found to increase with the progress of pregnancy.

These observations suggest that rabbit uterine cervix contains a receptor-like DHAS-specific binding protein in the cytosol fraction which seems to be closely associated with the progress of pregnancy.

Keywords——dehydroepiandrosterone sulfate; dehydroepiandrosterone sulfate binding protein; cervical ripening; rabbit uterine cervix

Dehydroepiandrosterone 3-sulfate (DHAS) is an androgen produced by the adrenal gland,[1] and is converted into estrogens in human placenta.[2] The levels of DHAS in baboon serum[3] and human umbilical cord plasma[4] were found to increase in parallel to gestational stage. Further, in women in a late gestational stage, about 80% of the administered DHAS was converted into estrogens.[5,6] From these findings, it is conjectured that DHAS is concerned directly or indirectly with uterine cervical dilatation at term pregnancy. In fact, DHAS administered to pregnant monkeys induced additional cervical dilatation.[7] Recently, Mochizuki and Tojo have demonstrated that the injection of DHAS into women in a late gestational stage increases the Bishop score and promotes collagenase release into the culture medium of uterine cervical explants.[8,9] Our previous reports indicated that increases in collagenase[10] and alkaline proteinase[11] were important factors which induce dramatic changes in the human uterine cervix during pregnancy at term, and that DHAS administration to rabbits in the middle gestational stage accelerates the cervical ripening and increases the activities of the above enzymes.[12] Details of the mechanism of these DHAS actions, however, have not been clarified. The present studies were designed to clarify the possibility of direct action of DHAS on cervical ripening, and in this paper, the presence and properties of a cytoplasmic DHAS-binding protein in the rabbit uterine cervix are reported.

Materials and Methods

Materials——The following reagents were commercially obtained; [7-^{3}H(N)]dehydroepiandrosterone 3-sulfate ([^{3}H]DHAS) ammonium salt (24 Ci/mmol), which was checked for purity (more than 99%) by thin layer chromatography within 2 months before use, from New England Nuclear, Boston, Mass., U.S.A.; deoxyribonuclease I (DNase) (bovine pancreas, DN-CL), ribonuclease A (RNase) (bovine pancreas type I-A), 17β-estradiol (E_2), E_2 3-sulfate, estriol (E_3), E_3 3-sulfate, estrone (E_1), E_1 3-sulfate, DHAS, dehydroepiandrosterone (DHA), 5α-dihydrotestosterone (DHT), testosterone (T), and human γ-globulin (γ-G) (Cohn fraction II) from Sigma, St. Louis, Mo., U.S.A.; bovine serum albumin (BSA) (5×, crystallized) from ICN Pharmaceuticals, Cleveland, Oh., U.S.A.; pronase E (one million tyrosine units/g) from Kaken Seiyaku, Tokyo, Japan; Sephadex G-50, blue dextran 2000, ferritin, catalase, and Sephacryl S-300 superfine from Pharmacia, Uppsala, Sweden. Other reagents used were of analytical reagent grade.

[^{3}H]DHAS Binding to the Cytosol Fraction of Rabbit Uterine Cervix——Nippon white rabbits of the same age and known stage of gestation, weighing 3.5 to 4.5 kg, were maintained on basal diet and water *ad libitum*. The rabbit was killed at 29 d of gestation, and the cervix was immediately excised. Stroma was completely separated from epithelium, rinsed with cold saline, and stored at −20 °C until use, (the storage period did not exceed two months). The minced cervix (about 0.2 g) was incubated for 16 h at 4 °C with 10 vol of 67 nM [^{3}H]DHAS in 20 mM Tris–HCl/3 mM EDTA/12 mM thioglycerol, pH 7.4 (TETg buffer) in the presence or absence of 13.4 μM unlabeled DHAS. Then the tissue was homogenized at 0 °C in a glass homogenizer. For preparation of cytosol fraction, the homogenate was centrifuged at 4 °C for 60 min at 105000 × *g* in a Hitachi 65P ultracentrifuge using a type 65T rotor. For sucrose density gradient analysis, the cytosol fraction was concentrated 4- to 5-fold in terms of protein concentration, *i.e.*, solid ammonium sulfate was added to the cytosol fraction at 80% saturation, and the precipitate obtained was redissolved in a small amount of TETg buffer. The binding activity of serum components in the rabbit cervix was also examined. The serum from the above pregnant rabbit was diluted 1 : 3 (v/v) with TETg buffer and incubated with [^{3}H]DHAS as described above.

Sephadex G-50 Column Chromatography and Determination of Specific [^{3}H]DHAS Binding——Sephadex G-50 chromatography was performed by the method of Godefroi and Brooks[13] to remove unbound steroid. The cytosol fraction prepared as above was applied to a Sephadex G-50 column (1 × 23 cm) equilibrated and eluted with TETg buffer. Each fraction (1 ml) was collected and the radioactivity was counted in Bray's solution using an Aloka 903 liquid scintillation counter. The counting efficiency was about 30%. Samples of the void volume fractions obtained as above were used for determination of the specific [^{3}H]DHAS binding and analysis of the inhibitory effect of other steroids on [^{3}H]DHAS binding, and the dissociation constant was calculated.

Determination of the specific binding of [^{3}H]DHAS to the cytoplasmic components in rabbit uterine cervix was carried out by the isotope dilution procedure, using Sephadex G-50 column chromatography as described above. Specifically bound [^{3}H]DHAS was estimated by the subtraction of nonspecifically bound [^{3}H]DHAS (*i.e.* the amount of [^{3}H]DHAS bound in the presence of a 200-fold excess of unlabeled DHAS) from total [^{3}H]DHAS binding (*i.e.* [^{3}H]DHAS bound in the absence of unlabeled DHAS).

Sucrose Density Gradient Centrifugation——A linear 5—20% sucrose gradient in 4.0 ml of TETg buffer was prepared, and 0.3 ml of the concentrated cytosol fraction was laid on the gradient, then centrifuged at 40000 rpm for 18 h using a Hitachi RPS 65T swing rotor at 4 °C. The sedimentation constant was determined by the method of Martin and Ames[14] using BSA (4.6 S) and γ-G (6.9 S) as standards.

Sephacryl S-300 Chromatography——In this experiment, the minced cervix (0.4 g) was incubated for 16 h at 4 °C with 4 vol of 136 nM [^{3}H]DHAS in 20 mM Tris–HCl/3 mM EDTA/12 mM thioglycerol/10 mM Na_2MoO_4/20% (v/v) glycerol, pH 7.4, then the cytosol was prepared as described above. The labeled cytosol (1 ml) was applied to a Sephacryl S-300 column (1.2 × 60 cm) previously equilibrated with 20 mM Tris–HCl/3 mM EDTA/12 mM thioglycerol/10 mM Na_2MoO_4/10% (v/v) glycerol, pH 7.4. Elution was performed with the same buffer at a flow rate of 15 ml/h. Aliquots of 0.5 ml from each fraction (1 ml) were counted for radioactivity. The column was calibrated with blue dextran, ferritin (4.40×10^5), catalase (2.32×10^5), and BSA (6.70×10^4).

Preparation of the Nuclei of Rabbit Uterine Cervix——This was carried out according to the method of Widnell *et al.*[15] with slight modifications. Minced tissues were homogenized in 4 vol of ice-cold 20 mM Tris–HCl/0.32 M sucrose/3 mM $MgCl_2$, pH 7.4 (TSM buffer) with a glass homogenizer. The homogenate was filtered through a single layer of gauze and then through two layers of nylon net. The filtrate was centrifuged at 800 × *g* for 10 min at 4 °C, and the pellet was washed four times with TSM buffer. The washed nuclei were resuspended in 2.2 M sucrose/1 mM $MgCl_2$/20 mM Tris–HCl, pH 7.4, and centrifuged at 100000 × *g* for 30 min at 4 °C using a Hitachi type RPS25-3A rotor. The purified nuclear pellet was washed once with TSM buffer at 3000 × *g* for 10 min at 4 °C and suspended in the same buffer. The purity of the nuclear pellet was evaluated histologically by observing preparations stained with Giemsa under a light microscope.

Transfer of DHAS-Binding Protein Complex from Cytosol to Nucleus——[^{3}H]DHAS-binding protein complex in the cytosol was obtained by Sephadex G-50 chromatography as described above. In this experiment, incubation of minced tissue with [^{3}H]DHAS was performed at 4 °C for 6 h. The prelabeled cytosol (3.8 mg protein) was mixed with

purified nuclear suspension and the buffer system was finally adjusted to 20 mM Tris–HCl/6 mM thioglycerol/1.5 mM EDTA/0.25 M sucrose/2.5 mM $MgCl_2$, pH 7.4. Then, the mixture was incubated at 0 or 25 °C for 6 h. Alternatively, TETg buffer containing [^{3}H]DHAS and BSA (4.0 mg protein) was mixed with purified nuclei, adjusted, and incubated as described above. These incubations were performed in quadruplicate. The incubation mixture was centrifuged at $800 \times g$ for 10 min at 4 °C and the pellet was repeatedly washed with TSM buffer at $3000 \times g$ for 10 min at 4 °C until the radioactivity in the supernatant decreased to the background level. The pellet obtained was resuspended in TSM buffer and aliquots were counted for radioactivity. Other aliquots were used for determination of deoxyribonucleic acid (DNA) in the sample or identification of the radioactive component on thin layer chromatography.

Identification of Bound Radioactivity in Cytoplasmic Protein and Nuclei——DHAS–protein complex chromatographed on Sephadex G-50 or DHAS–nuclei complex was acidified with 0.2 M phosphate–HCl buffer, pH 2.3 and then the [^{3}H]DHAS was extracted with $CHCl_3$ containing methylgreen as the dye–[^{3}H]DHAS complex.[16] The radioactive component in the extract was identified by thin layer chromatography on a silica gel plate with a solvent system of $CHCl_3$–CH_3OH–H_2O (75:22:3, v/v/v). [^{3}H]DHAS, DHAS, DHA, E_2, E_3, DHT, T, E_2 3-sulfate and E_1 3-sulfate were run simultaneously as standards.

Determination of DNA and Protein——DNA was separated from ribonucleic acid (RNA) by the method of Schneider[17] and then determined by the procedure of Burton[18] using herring sperm DNA as a standard. Protein was determined by the method of Lowry *et al.*[19] with BSA as a standard.

Results

Specific Binding of DHAS to Cytoplasmic Component in Rabbit Uterine Cervix

After the incubation of minced cervical stroma obtained at 29 d of gestation with various amounts of [^{3}H]DHAS (0—67 nM) in the presence or absence of a 200-fold excess of unlabeled DHAS, the specific binding in the cytosol fraction was estimated as described in Materials and Methods. As shown in Fig. 1A, specific [^{3}H]DHAS binding increased with increasing concentration of [^{3}H]DHAS in the incubation mixture, reaching a plateau at about 60 nM added [^{3}H]DHAS. The specific binding at 67 nM, which was usually 40—60% of total binding, was not appreciably altered by exposure to a 200- or 1000-fold excess of unlabeled DHAS (data not shown). From Scatchard analysis[20] of these data, the dissociation constant and the number of binding sites were calculated to be 5.05×10^{-8} M and 33 fmol/mg protein, respectively (Fig. 1B).

Time dependency of this specific binding at 4 °C was also examined. The maximal binding was reached at 2 to 4 h of incubation and was steady for at least 24 h. In addition, the radioactive material in the cytosol after incubation for 16 h at 4 °C was found to be only [^{3}H]DHAS by thin layer chromatography (data not shown). The specific binding was also observed when the isolated cytosol was incubated with [^{3}H]DHAS, but was less than that obtained by using the minced cervix. When [^{3}H]DHAS was incubated with the serum prepared from a rabbit of the same gestational stage, the binding of DHAS to the serum component was not depressed by the excess of unlabeled DHAS. The results described above

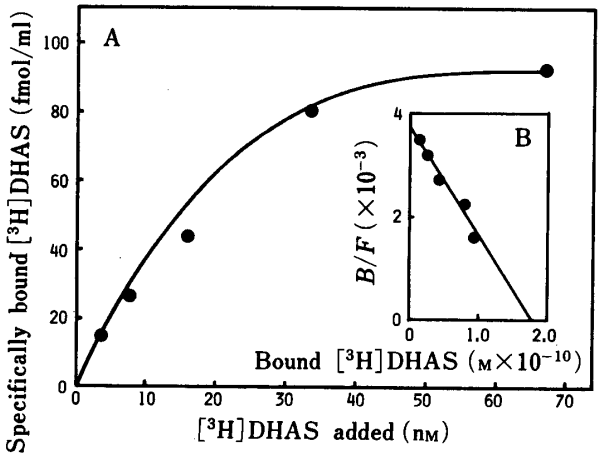

Fig. 1. Specific Binding of DHAS to Rabbit Uterine Cervix Cytosol

A. Specific binding of [^{3}H]DHAS as a function of the concentration of [^{3}H]DHAS. Minced cervical tissue (0.2 g) was incubated with various amounts of [^{3}H]DHAS in the presence or absence of a 200-fold excess of unlabeled DHAS, and aliquots (0.5 ml) of the prepared cytosol were applied to a Sephadex G-50 column to remove unbound steroid. Specific binding was calculated from the difference in binding of [^{3}H]DHAS in the presence and absence of unlabeled DHAS. Other experimental details are described in the text.

B. Scatchard analysis for the cytoplasmic DHAS binding protein in the rabbit uterine cervix. *B/F*, bound-to-free ratio. Data are representative of three experiments.

indicate that rabbit uterine cervix contains a saturable and specific DHAS-binding cytoplasmic component.

Transfer of [^{3}H]DHAS–Cytoplasmic Protein Complex to the Nuclei of Rabbit Uterine Cervix

When the prelabeled [^{3}H]DHAS-binding component complex was incubated with isolated purified nuclei, the radioactivity transferred to the nuclei at 25 °C was 2-fold higher than that at 0 °C. Incubation of the isolated nuclei with [^{3}H]DHAS in the absence of cytosol did not result in binding (Table I). The homogeneity of bound [^{3}H]DHAS was also examined by thin layer chromatography as described in Materials and Methods. The radioactivity extracted from the [^{3}H]DHAS-nuclei fraction was confirmed to appear in a single spot corresponding to standard [^{3}H]DHAS and DHAS (data not shown). These results indicate that DHAS can be translocated to the nuclei in a temperature-dependent process without being metabolized to other steroids, and the cytoplasmic component–DHAS complex might be essential to form the complex of DHAS–nuclei.

TABLE I. Translocation of [^{3}H]DHAS into Nuclei of Rabbit Uterine Cervix

Incubation of nuclei together with:	Translocation of [^{3}H]DHAS in the nuclei (dpm/μg DNA)	
	0 °C	25 °C
[^{3}H]DHAS bound to cytosol	12.3 ± 1.4 (2.6)	25.3 ± 1.0[a] (5.3)
[^{3}H]DHAS and BSA	0.7 ± 0.2 (0.06)	1.0 ± 0.3 (0.09)

The purified nuclei (69.2 μg DNA) from rabbit uterine cervix were incubated with the prelabeled cytosol (3.3×10^4 dpm; 620 fmol) or a buffer containing [^{3}H]DHAS and BSA (7.8×10^4 dpm; 1464 fmol) at 0 or 25 °C for 6 h. Nuclei were reisolated by centrifugation at $800 \times g$ for 10 min, washed and counted for radioactivity. Other experimental details are described in the text. Each value represents the mean ± S.D. of four determinations. *a*) Significantly different from the value at 0 °C ($p < 0.001$). The percentage of total radioactivity which was taken up by the nuclei is given in parenthesis.

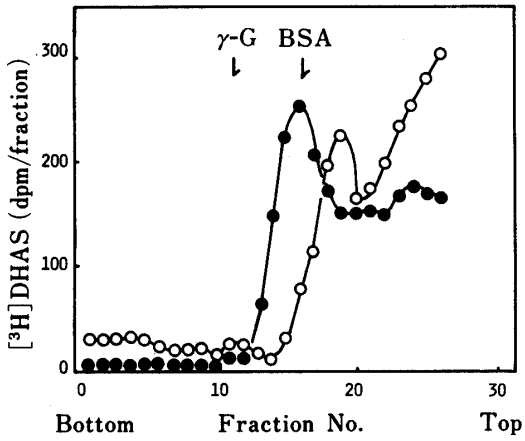

Fig. 2. Sucrose Density Gradient Analysis of [^{3}H]DHAS-Binding Components in the Rabbit Uterine Cervix and Serum

The minced cervix or serum was incubated with 67 nM [^{3}H]DHAS, and after removing unbound [^{3}H]-DHAS by gel filtration, the $(NH_4)_2SO_4$-concentrated cytosol fraction or serum containing about 0.9 mg of protein was laid on a 5—20% sucrose gradient in TETg buffer. Other experimental conditions are described in the text. (○), cytosol; (●), serum; γ-G (6.9 S) and BSA (4.6 S).

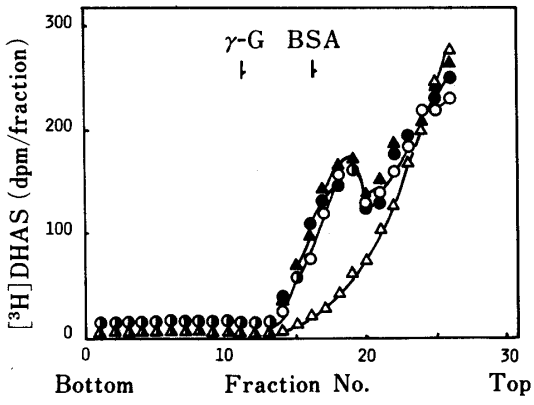

Fig. 3. Sucrose Density Gradient Analysis of the Cytoplasmic [^{3}H]DHAS-Binding Component after Treatment with Pronase E, DNase or RNase

The cytosol fraction containing [^{3}H]DHAS–macromolecule complex and about 0.9 mg of protein was treated with an enzyme (350 μg) in a total volume of 0.35 ml of TETg buffer at 37 °C for 30 min, and then 0.3 ml of the mixture was used for the analysis as described in Fig. 2. (▲), control; (△), pronase E; (○), DNase; (●), RNase.

Characterization of Cytoplasmic DHAS-Binding Component in Rabbit Uterine Cervix

As shown in Fig. 2, a distinct peak of DHAS-binding component was observed on sucrose density gradient centrifugation. Such a clear peak, however, was not obtained when non-concentrated cytosol fraction was applied. The sedimentation constant of the component was found to be 3.6—4.0 S, less than the value of BSA (4.6 S). On the other hand, a non-specific DHAS-binding component in the rabbit serum was sedimented at the same position as BSA. An identical result was obtained when serum concentrated with $(NH_4)_2SO_4$ at 80% saturation was used, indicating that the sedimentation behavior of the serum component was not altered by concentration with $(NH_4)_2SO_4$ (data not shown). To clarify whether the DHAS-binding component in the cervix was protein or nucleic acid, the component was digested with pronase E. DNase and RNase. As shown in Fig. 3, the DHAS-binding peak was eliminated completely by pronase E, while DNase and RNase had no effect on the peak, indicating that the component is composed mainly of protein.

Recently, various reagents and experimental conditions for analyzing the molecular structures have been reported for other well-known steroid receptor systems.[21-24] From this point of view, we examined the possibility that the cytoplasmic DHAS-binding protein had other molecular forms. When labeled cytosol was chromatographed on Sephacryl S-300 in the presence of molybdate and glycerol, two peaks associated with the macromolecules were observed (Fig. 4). The first small peak was found at the void volume and probably represents aggregates of the binding protein. The second main peak had a molecular weight of about 1.7×10^5. This finding suggests that the 3.6—4.0 S form obtained by sucrose density gradient analysis may be a product of dissociation formed either during concentration with $(NH_4)_2SO_4$ or during long-term ultracentrifugation without stabilizing agents. The effects of some steroids on the specific binding of DHAS to the cytoplasmic protein are summarized in Table II. DHA was able to inhibit about 50% of the specific binding at 13.4 μM. This inhibition, however, was still weaker than that by 1.34 μM DHAS (77% inhibition). E_2, E_3 and DHT showed lower or no inhibitory effect. Sulfates of E_2, E_3 and estrone did not effectively compete

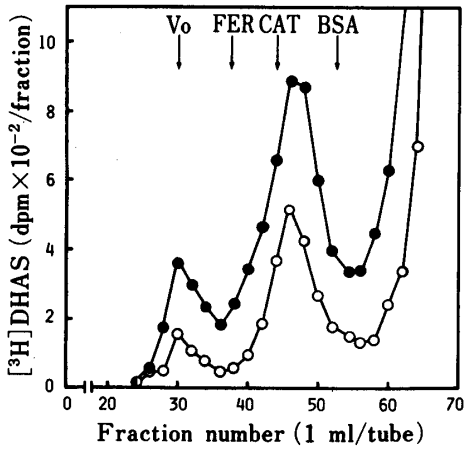

Fig. 4. Elution Pattern of [^{3}H]DHAS-Binding Protein in Rabbit Uterine Cervix from Sephacryl S-300

Minced cervical tissue (0.4 g) was incubated for 16 h at 4 °C with 136 nM [^{3}H]DHAS in the presence (○) or absence (●) of a 200-fold excess of unlabeled DHAS. The cytosol was applied to a Sephacryl S-300 column (1.2 × 60 cm), and eluted with 20 mM Tris–HCl/3 mM EDTA/12 mM thioglycerol/10 mM Na_2MoO_4/10% (v/v) glycerol, pH 7.4. Other experimental conditions are described in the text. The arrows indicate the elution positions of blue dextran (Vo), ferritin (FER), catalase (CAT), and BSA. Data are representative of two identical experiments.

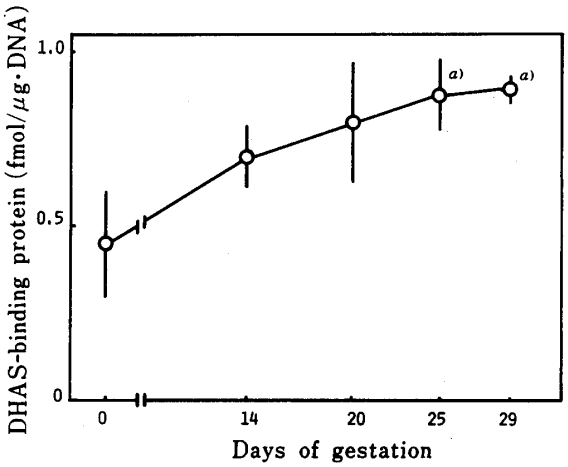

Fig. 5. Changes in the Cytoplasmic DHAS-Binding Protein in Rabbit Uterine Cervix during Pregnancy

Experimental conditions are described in the text. Data are given as mean ± S.D. of 4 or 5 animals. *a*) Significantly different from nonpregnant control ($p < 0.05$).

TABLE II. Effect of Unlabeled Steroids on the Binding of [^{3}H]DHAS to Cytoplasmic Protein in the Rabbit Uterine Cervix

Unlabeled steroid	Concentration (μM)	Inhibition of specific binding (%)
Dehydroepiandrosterone sulfate	13.4	(100)
	1.34	77± 5
Dehydroepiandrosterone	13.4	48± 2
	1.34	29±13
17β-Estradiol	13.4	0±17
Estriol	13.4	19± 3
5α-Dihydrotestosterone	13.4	34± 3
	1.34	34± 9
Estrone 3-sulfate	13.4	18±13
17β-Estradiol 3-sulfate	13.4	29±10
Estriol 3-sulfate	13.4	11± 8

Minced cervical tissue (0.2 g) was incubated at 4 °C for 16 h with [^{3}H]DHAS (67 nM) in the presence or absence of the indicated concentrations of excess unlabeled steroid. Other experimental conditions are described in the text. Bound radioactivity was determined by using gel filtration on Sephadex G-50. The specific binding of [^{3}H]DHAS corresponded to 107 fmol/ml cytosol (5700 dpm). Data are expressed as mean ± S.D. of three experiments.

with [^{3}H]DHAS for the binding sites.

Changes in the Content of Cytoplasmic DHAS-Binding Protein in Uterine Cervix during Pregnancy

We have already reported that the administration of DHAS to rabbits in the middle gestational period accelerates the cervical dilatation *in vivo*[12] and in cervical tissue culture, addition of DHAS to the culture medium promotes collagenase production.[25] These observations encouraged us to investigate the changes in cytoplasmic DHAS binding protein during pregnancy, in order to clarify the physiological role of the protein in the uterine cervix. As shown in Fig. 5, the level of the DHAS-binding protein increased in parallel with the progress of pregnancy and the levels on days 25 and 29 of gestation were twice that of the non-pregnant controls.

Discussion

Although DHA-binding protein has been found in rabbit efferent duct fluid and serum,[26] the presence of cytoplasmic DHAS-binding protein has not been reported yet. In the present study, we confirmed the presence of cytoplasmic DHAS-binding protein distinct from the serum DHAS-binding protein in the rabbit uterine cervix. Therefore, this is the first report of a binding protein of DHAS, which is known to be prohormone.

When DHAS was incubated with the isolated cytosol fraction of the cervix, the specific binding was less than that obtained by using minced tissue. Furthermore, we observed that the amount of specific binding when homogenization was carried out after removing the excess unbound DHAS was the same as that in the presence of the steroid during homogenization. The specific binding, therefore, may not have occurred during homogenization of the mixture of minced tissue and the excess unbound steroid. A similar phenomenon has also been reported for estrogen receptor in mouse skin.[27] The reason might be that the cytoplasmic binding protein was destroyed by mechanical homogenization of cervical tissue or by some neutral proteinases released during the homogenization.

In the present experiments, there were some possible problems in the analysis of the

DHAS-binding protein interaction. The Scatchard plots, which indicated a single class of binding sites at the concentrations of [^{3}H]DHAS that we employed (Fig. 1), might be curvilinear if analysis had been done by the procedure of Rosenthal[28] with less than 4 nM [^{3}H]DHAS. However, it is impossible at present to determine exactly the binding capacity at less than 4 nM [^{3}H]DHAS, since the specific activity of the commercially obtained [^{3}H]DHAS used in the present study is low. Furthermore, we could not fully optimize the conditions for the measurement of DHAS-binding protein because of its lability and relatively high dissociation constant. In fact, sucrose gradient analysis using non-concentrated cytosol resulted in complete dissociation of [^{3}H]DHAS from the binding protein. Even when concentrated cytosol was used, the presence of free [^{3}H]DHAS on the top of the gradient (Fig. 2) indicates fairly rapid dissociation, which might result in underestimation of the binding. However, since separation of free [^{3}H]DHAS from bound complex by Sephadex G-50 chromatography took only a short time, it is still clear that DHAS-binding protein is present in the rabbit uterine cervix, and that the content of DHAS-binding protein in the cervix increased with the progress of pregnancy, *i.e.*, on days 25 and 29 of gestation, the content was twice that of the non-pregnant controls.

DHA inhibited at most 50% of the [^{3}H]DHAS binding to the protein, and estrogen sulfates did not depress that binding (Table II), indicating that the sulfated form is essential to the specific binding, though the charged sulfated region of DHAS does not cause nonspecific binding of DHAS to the cytoplasmic protein. Furthermore, in a cell-free system, the DHAS–protein complex can translocate to the nuclei (Table I). If this is correct, it is not clear how the DHAS with its charged sulfated region permeates into the cervical cells. However, adrenal gland of human fetus biosynthesizes DHAS and releases it into blood intact[1] and DHAS is metabolized into other steroids without loss of the sulfated region.[29] In addition, as in the case of the cell-free system, we have observed that the incubation of [^{3}H]DHAS with minced tissue resulted in the distribution of the [^{3}H]DHAS in the nuclei without loss of the sulfated region (data not shown). These results may suggest that DHAS can permeate through the cell membrane and translocate to the nuclei *via* binding with cytoplasmic protein.

It is well known that DHAS is changed into androgens by the action of sulfatase and Δ^4-3β-hydroxysteroid dehydrogenase (3β-HSD) and then into E_2 by aromatase, and the primate placenta contains much 3β-HSD and aromatase.[30] However, negligible amounts of these enzymes were found in rabbit placenta.[31] When DHAS was administered to late pregnant rabbit, serum levels of DHAS, DHA and testosterone formed were only 0.1% of DHAS and that of E_2 was 0.0001%.[32] In addition, we have observed that DHAS promotes cervical ripening and increases the production of collagenase and alkaline proteinase in pregnant rabbits having a low conversion activity of DHAS to E_2.[12] On the other hand, we have confirmed that administration of E_2 at 0.1 mg/kg body weight (corresponding to 0.5% of the DHAS dose) frequently induced death and/or abortion of rabbit fetuses (A. Ito, S. Hirakawa and Y. Mori, unpublished data). Recently, Mochizuki and Tojo have proposed the hypothesis that the action mechanism of DHAS on human cervical ripening is due to the production of cervical collagenase induced by the action of E_2 formed from DHAS.[8] However, they presented no evidence that E_2 promotes the production of collagenase in human uterine cervix. On the other hand, E_2 has been reported to inhibit collagenase activity *in vivo* in rat uterus,[33] and to have no effect on the biosynthesis of the enzyme in tissue culture.[34,35] We also observed that DHAS stimulated the production of collagenase in rabbit uterine cervical cell culture.[36] Thus, it is suggested that DHAS itself, at least partially, has hormonal actions in the uterine cervix.

Although the physiological roles of DHAS-binding protein are not known at this time, it is possible that it is able to act as an effective concentrating agent or a hormone receptor. The translocation data (Table I) favor the latter possibility. It remains to be shown that the cervical

DHAS-binding protein has the functional properties of a receptor, *i.e.* that it can induce messenger ribonucleic acid (mRNA) for a specific protein in the nuclei. More detailed experiments are in progress to gain a better understanding of the biological role of DHAS-binding protein in connection with cervical ripening.

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