Estrogen Stimulates the Transient Association of Calmodulin and Myosin Light Chain Kinase with the Chicken Liver Nuclear Matrix

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ABSTRACT Previous work has demonstrated that estrogen administration to immature chickens results in a rapid but transient increase in nuclear estrogen receptor content, a large portion of which is associated with the nuclear matrix. The present studies were undertaken to determine whether estrogen produced a more generalized change in the protein composition of the nuclear matrix. High-resolution two-dimensional gel analysis of the matrix revealed a very complex protein pattern, but several major qualitative differences were observed after estrogen treatment. To simplify the number of proteins evaluated, we examined the effects of estrogen on a subset of matrix proteins, namely, calmodulin and its binding proteins. Calmodulin was measured by radioimmunoassay and the binding proteins were detected by interaction of ^{125}I-calmodulin with matrix proteins distributed on one-dimensional polyacrylamide gels. Calmodulin and two specific Ca^{2+}-dependent calmodulin-binding proteins were found to be associated with matrix preparations. The two binding proteins exhibited apparent M_r of 200,000 and 130,000. The M_r 130,000 protein was identified as myosin light chain kinase on the basis of enzymatic activity and immunoreactivity with a specific antibody to this enzyme. Estrogen treatment of immature chickens did not alter the hepatic content of calmodulin. However, the steroid did result in an enrichment of the proportion of calmodulin and its two binding proteins associated with the nuclear matrix within 4 h after injection. The time course of these changes paralleled those previously documented for estrogen receptor. Taken together, these data are compatible with a role for calmodulin and myosin light chain kinase in the response of chicken liver cells to steroid hormones.

The liver of the immature chicken constitutes a useful system for the study of estrogen-regulated gene expression in the absence of cell growth and differentiation. Whereas studies designed to evaluate the presence of cytoplasmic estrogen receptors have proven to be negative, administration of estrogen to immature chickens results in a rapid but transient increase in nuclear receptor content (1). Much of the increased nuclear receptor is associated with the nuclear matrix (2), and these changes in receptor occupancy and distribution precede stimulation of gene expression (3–5). Inasmuch as receptor seems to undergo an intranuclear redistribution in response to steroid, it seemed possible that other nuclear proteins might also demonstrate transient association with the nuclear matrix during the early stages of estrogen action.

Because of the extreme complexity of the nuclear matrix protein composition, it seemed likely that qualitative changes in various two-dimensional gel spots might be difficult to interpret. It was desirable, therefore, to focus on a subset of nuclear proteins. Estrogen alters the net cellular Ca^{2+} content of target tissues (6), and Ca^{2+} is known to regulate a variety of functions including contractility and mitosis (7, 8). Calmodulin (CaM[1]) has been shown to occupy an intranuclear domain (9) as has one of the key enzymes regulated by this Ca^{2+} receptor, myosin light chain kinase (10). Immunofluorescence studies have been interpreted to indicate an increase in the nuclear content of calmodulin in the rat uterus within 12 h after estrogen administration (11), and calmodulin is

[1] *Abbreviations used in this paper:* CaM, calmodulin; MLCK, myosin light chain kinase.

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synthesized before DNA replication in eucaryotic cells (12). For these reasons, we have examined the ability of estrogen to promote changes in the intranuclear distribution of calmodulin, quantitated by radioimmunoassay (13), and calmodulin-binding proteins evaluated by a ^{125}I-calmodulin gel overlay procedure (14). The data presented herein reveal that calmodulin and a number of its binding proteins become transiently associated with the chicken liver nuclear matrix in response to estrogen. One of the binding proteins has been identified as myosin light chain kinase. These changes parallel those previously reported to occur for the estrogen receptor (15). These results suggest that calmodulin and myosin light chain kinase may play a role in the response of chicken liver cells to estrogen.

MATERIALS AND METHODS

Animals: Immature, female chickens (3–5 wk old) were obtained from Texas Animal Specialties, Humble, TX. Each chick was injected subcutaneously with a single dose of 2 mg of diethylstilbestrol (Sigma Chemical Co., St. Louis, MO) in sesame oil. Chicks were sacrificed 4 or 24 h after a single dose of the steroid.

Enzymes: Bovine pancreatic deoxyribonuclease I and ribonuclease A (type III-A) were obtained from Sigma Chemical Co. ^{125}I-labeled Bolton-Hunter reagent was purchased from Amersham Corp, Arlington Heights, IL. Molecular weight markers for gel electrophoresis were purchased from Pharmacia Fine Chemicals, Piscataway, NJ. Rat testis calmodulin was prepared as previously described (16).

Isolation of Liver Nuclei and Nuclear Matrix: Liver nuclei were isolated as described by Snow et al. (1) with modifications. The finely minced liver was suspended in homogenization buffer (10 mM Tris, 10 mM NaCl, 1.5 mM $MgCl_2$, 50% (vol/vol) glycerol, pH 7.0) at a weight/volume ratio of 1:2. The Triton X-100 treatment was carried out in 100 ml of the homogenization buffer adjusted to 0.1% (vol/vol) of Triton X-100. The purified nuclei, obtained after two Triton X-100 treatments were further washed three times in TM buffer (10 mM Tris, 5 mM $MgCl_2$, pH 7.4). All steps in the isolation were carried out at 4°C and all buffers contained 1 mM phenylmethylsulfonyl fluoride.

A modification of the method of Barrack and Coffey (2) was used to prepare liver nuclear matrix. Freshly prepared nuclei were washed once in TM buffer, resuspended in 20 ml of the same buffer, and digested with bovine pancreatic DNase I (200 μg/ml) for 30 min at 22°C. The suspension was centrifuged at 750 *g* for 15 min, washed once in TM buffer, and sequentially extracted with LM buffer (10 mM Tris, 0.2 mM $MgCl_2$, pH 7.4), 1 M NaCl in LM buffer, and 2 M NaCl in LM buffer. The LM buffer extraction was carried out at 4°C for 15 min with centrifugation at 750 *g* for 15 min, the subsequent extractions with NaCl were performed at 4°C for 30 min with centrifugation at 750 *g* for 45 min. This resulting nuclear matrix pellet was extracted with 0.5% Triton X-100 in LM buffer (4°C, 10 min) and washed three times in TM buffer. The final pellet, defined as the nuclear matrix, was determined to contain 12.1 ± 1.3% (n = 25) and 8.0 ± 0.9% (n = 19) of the total nuclear protein and DNA, respectively. The purity and integrity of the matrix preparations were confirmed by electron microscopic examination.

Radioimmunoassay for Calmodulin: Crude homogenates, nuclei, and nuclear matrix fractions were assayed for CaM by a radioimmunoassay previously described (13).

^{125}I-CaM Gel Overlay: SDS-polyacrylamide gel electrophoresis was performed as described by Laemmli using 10% acrylamide (17). CaM was labeled with ^{125}I using the Bolton-Hunter reagent (13). The binding of ^{125}I-CaM to proteins, separated in SDS PAGE gels was performed according to the procedure of Glenney and Weber (14), except that incubation of the gels with the labeled CaM was carried out in sealed polyethylene bags overnight at 4°C with shaking, and bovine serum albumin was used instead of gelatin. ^{125}I-CaM protein complexes were detected in dried gels by autoradiography at −20°C using Kodak XRP X-ray film (Eastman Kodak Co., Rochester, NY) and Cronex Lightning Plus intensifying screens (DuPont Instruments, Wilmington, DE). The amount of radioactive CaM bound by each protein in the gel was quantitated by densitometric scanning of autoradiograms of various exposure length, using a Kontes fiber optic scanner (Kontes Co., Vineland, NJ) attached to a Hewlett-Packard integrator (Hewlett-Packard Co., Palo Alto, CA). Molecular weights of CaM-binding proteins were estimated by reference to molecular weight markers run on the same gel.

Protein and DNA Determination: Protein content of samples was quantitated by the method of Lowry et al. (18). DNA was measured by the Burton method (19) using calf thymus DNA as standard.

Immunological Detection of Myosin Light Chain Kinase (MLCK) on Nitrocellulose Sheets: Total nuclear matrix proteins (150 μg) were solubilized in sample buffer and subjected to electrophoresis in 10% polyacrylamide gels containing SDS as described by Laemmli (17). Transfer of the proteins onto nitrocellulose sheets was then carried out according to Towbin et al. (20), as modified by Guerriero et al. (10). The transfer buffer used was 25 mM Tris–192 mM glycine, pH 8.3. Transfer of the proteins to nitrocellulose was performed for 3 h at room temperature at constant voltage (30 V). After the transfer, the nitrocellulose sheet was rinsed twice with Tris saline (10 mM Tris, 160 mM NaCl, pH 7.4) and incubated overnight with 3% bovine serum albumin in the cold with shaking. The sheet was next incubated with affinity-purified antibody to chicken gizzard MLCK (1 μg/ml in 20 ml) for 3 h with shaking at room temperature. After the incubation, the paper was washed three times in Tris-saline containing 3% bovine serum albumin and then incubated with ^{125}I-protein A (3 × 10^6 cpm/20 ml) for another 2 h. The paper was then washed with Tris-saline to remove nonspecific-bound radioactivity, dried, and exposed to XRP x-ray film for 1–3 d.

Assay of MLCK Activity: MLCK activity of nuclear matrix fractions was determined as described by Guerriero et al. (10) using myosin light chain isolated from chicken gizzard as substrate. Enzyme reactions were carried out at 30°C for 8 min. CaM when present was added to a final concentration of 1 μM. The reaction was terminated by solubilization in Laemmli sample buffer (17) and proteins were separated on 12.5% polyacrylamide gels containing SDS. Protein bands radiolabeled with ^{32}P were visualized by radioautography. A 2-d exposure to XRP x-ray film was adequate to resolve the signal.

Two-dimensional Electrophoresis: The methods used for electrophoresis were those described by O'Farrell (21) as modified by Anderson and Anderson (22). Nuclear matrix fractions were dialyzed into TE buffer (10 mM Tris, 1.5 mM EDTA, [pH 7.5]) at 4°C in the presence of phenylmethylsulfonylfluoride and adjusted to a final concentration of 20 mg/ml. The suspension was diluted 1:1 with sample buffer (2% SDS—2% β-mercaptoethanol—500 mM cyclohexylaminoethane sulfonic acid [Calbiochem-Behring Corp., LaJolla, CA], pH 9.5) and boiled for 5 min in glass tubes. Samples were then centrifuged at 200,000 *g* for 3 h in a Beckman Ti60 rotor (Beckman Instruments, Inc., Fullerton, CA) to remove nucleic acids. 5–10 μl of the supernate was applied to the gels and focused for 16 h at 600 V at 25°C. Electrophoresis in the second dimension was carried out using 10–20% gradient polyacrylamide slab gels cast using the DALT System (Electronucleonics, Inc., Oak Ridge, TN) as described by Anderson and Anderson (22). Proteins were visualized using the color-based GELCODE Silver Stain (Health Products, Inc., South Haven, MI) as described by Sammons et al. (23) or by Coomassie Blue Stain.

Determination of Estrogen Receptor Concentration: Binding of [^{3}H]estradiol to nuclear matrix fractions (200–500 μg of protein/tube) over a wide range of labeled steroid concentration (0.4–40 nM) was performed as previously described (2). Nuclear matrix levels of estrogen receptor were calculated from Scatchard analysis of saturation curves (24).

Statistical Analysis: The data in Table I were statistically analyzed with the Student's paired t-test.

RESULTS

Nuclear matrix-associated proteins were analyzed using two-dimensional PAGE followed by GELCODE silver staining (23, 25). Fig. 1 shows a region of two gels containing proteins in the 35–100-kd range for control (*A*) and estrogen-stimulated (*B*) matrices. Comparison of the protein profiles in this area demonstrates that a number of proteins, whether present as predominant or minor species, are common to both unstimulated and estrogen-treated fractions. These proteins exhibited not only the same M_rs and apparent charges, but also the same staining intensities (for example, proteins 1–4, 9, and 10).

Examination of the gels revealed that some proteins of the nuclear matrix do vary with estrogen. A set of proteins (No. 7) is present in unstimulated but is not detected in estrogen-treated matrices. Similarly, a group of proteins (No. 8) appears to be enriched in unstimulated matrix fractions. A number of proteins shown here also exhibited increases in staining intensities after estrogen induction (Nos. 5, 6, and 11, for

example). These changes suggest that the protein composition of the nuclear matrix is hormonally altered. However, because of this complexity of the nuclear matrix, we have limited analysis of estrogen-induced changes in matrix composition to a subset of proteins, namely, CaM and its binding proteins.

Table I presents the CaM concentration in total homogenate, nuclear, and nuclear matrix fractions of livers isolated from estrogen-treated and control chicks. Total tissue CaM appears to remain constant upon estrogen stimulation. This is true as well for nuclear CaM, where no significant difference ($P > 0.1$) in the amount of the protein was detected after estrogen stimulation. Based on a ratio of 5.4 μg of nuclear DNA per microgram of nuclear protein (our determination) and a value of 2.4 pg of DNA per nucleus (2), the amount of CaM per nucleus of control and estrogen-treated chicken liver is 0.81 and 0.83 pg, respectively. These values are comparable to the total nuclear CaM for Chinese hamster ovary and 3T3 cells, as deduced from the CaM per cell reported for these cells (12). CaM in the nuclear matrix, however, is significantly elevated ($P < 0.01$) after estrogen stimulation. Based on a yield of 12% total nuclear protein in the matrix, the amount of CaM in the matrix is increased from 0.07 pg in control chickens to 0.11 pg in chickens 4 h after estrogen treatment. This represents a 57% change in the amount of nuclear CaM associated with the nuclear matrix after acute (4 h) estrogen stimulation.

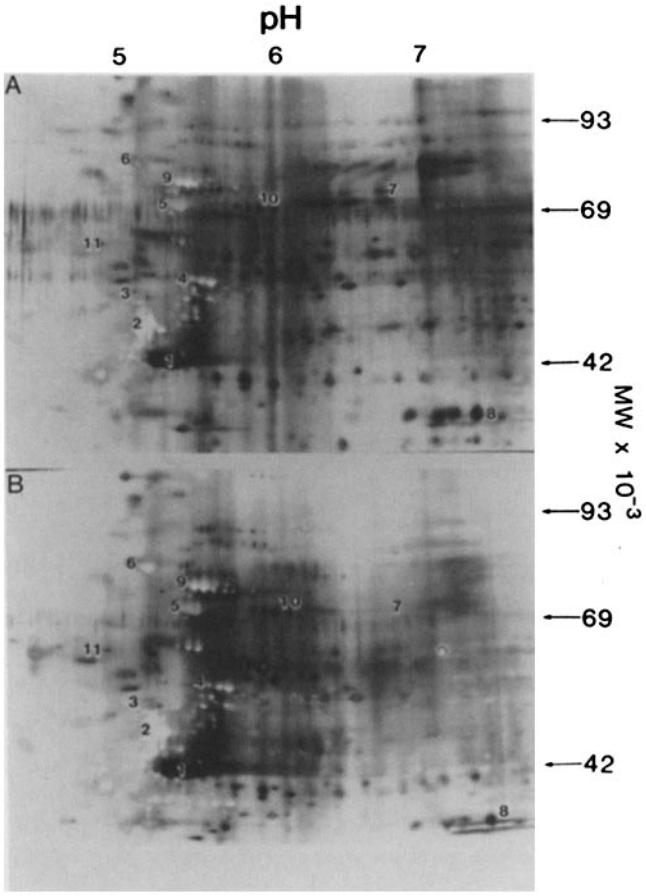

FIGURE 1 Two-dimensional polyacrylamide gels of liver nuclear matrix proteins from control and 4-h estrogen-stimulated chickens. 100 μg of protein was subjected to two-dimensional-PAGE as described in Materials and Methods using a pH range of 4–8. (*A*) Control; (*B*) estrogen-stimulated nuclear matrix proteins.

Because the percentage of total tissue CaM in the nuclear matrix is low, it was essential to establish that the increase in the amount of nuclear matrix CaM described above was not due merely to preparation variability. Three proteins have previously been identified by one-dimensional gel electrophoresis to be ubiquitous components of nuclear matrix preparations. These are an HnRNA-associated protein (36,000), actin (43,000) and the lamin-associated 70,000 protein (26–28). Therefore, we distributed matrix proteins from control and 4-h diethylstilbestrol treated animals on one-dimensional gels. The proteins were identified by Coomassie Blue staining and representative gels are depicted in Fig. 2, lanes *e* and *f*. The gels were then scanned by densitometry. Using this method, standard proteins show a linear relationship between the densitometric signal area and the amount of protein present. Table II shows that the relative amounts of the three constitutive proteins in control and 4-h estrogen-stimulated matrices do not vary in a statistically significant manner, indicating that the enrichment of nuclear CaM in the matrix with estrogen is selective and cannot be solely due to preparation variability. Moreover, the extensive washings in high salt and detergent during isolation of nuclear matrices would preclude nonspecific contamination of matrix fractions with cytosolic proteins.

CaM is not known to be active unless complexed with other proteins (7). One way of identifying such CaM-binding proteins is to evaluate the ability of proteins distributed on one-dimensional polyacrylamide gels to bind ^{125}I-CaM in a Ca^{2+}-dependent manner (14). In that such gels had been prepared to quantify the constitutive matrix proteins, replicate ones were also evaluated by gel overlay procedure. As shown in Fig. 2, lane *b*, four major CaM-binding proteins are detected in control matrices with relative M_r of 200,000, 130,000,

TABLE I

CaM Concentrations in Liver Subfractions as a Function of Estrogen

Fraction	Control*		Estrogen-treated‡	
	Protein recovery§	μg CaM/mg protein	Protein recovery§	μg CaM/mg protein
	%		%	
Homogenate	100	2.90 ± 0.65 (3)	100	2.71 ± 0.53 (3)
Nuclear	1.8	1.68 ± 0.34 (8)	1.7	1.73 ± 0.35 (8)‖
Nuclear matrix	0.23	1.25 ± 0.27 (8)	0.22	1.85 ± 0.27 (8)¶

* Values (mean ± SE for *n* determinations) from livers of unstimulated immature female chicks.
‡ Values (mean ± SE for *n* determinations) from livers of immature female chicks injected with diethylstilbestrol 4 h before sacrifice.
§ Percent of homogenate.
‖ $P > 0.1$ vs. control nuclear fractions.
¶ $P < 0.01$ vs. control matrices.

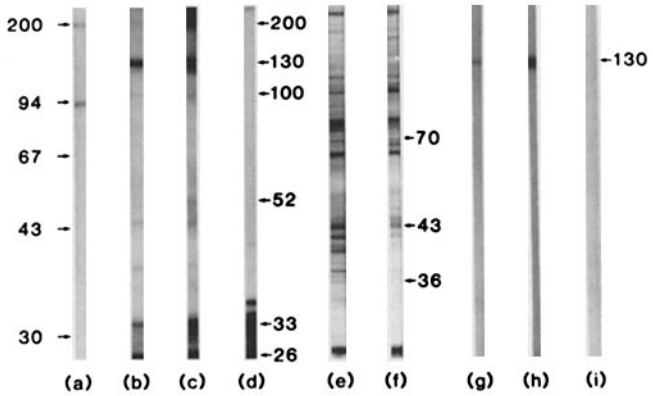

FIGURE 2 Detection of calmodulin binding proteins and identification of MLCK in chicken liver nuclear matrix. Total matrix proteins isolated from unstimulated and 4-h estrogen-stimulated chick livers were separated on a 10% polyacrylamide-SDS gel and evaluated for ^{125}I-CaM binding (lanes *b–d*) or interaction with MLCK antibody (lanes *g–i*). Lane *a*, molecular weight standards after calmodulin overlay; lane *b* and *c*, unstimulated and estrogen stimulated matrices, respectively in the presence of 1 mM calcium; lane *d*, estrogen-stimulated matrix with 1 mM EGTA. Lanes *e* and (*f*) represent Coomassie Blue-stained one-dimensional gels of nuclear matrix from unstimulated and estrogen-stimulated chick livers, respectively. In lanes *g* and (*h*), nuclear matrix proteins from unstimulated and estrogen-treated chick livers, after electrophoresis, were transferred electrophoretically to nitrocellulose and reacted with MLCK antibody and with ^{125}I-labeled protein A. The sample in lane *i* also contains 10 μg of chicken gizzard MLCK during incubation with the antibody. Each lane was loaded with 150 μg of total nuclear matrix proteins except for lanes *e* and *f* which has 100 μg of proteins each. Values are molecular weight × 10^{-3}.

TABLE II

Effects of Estrogen on Proteins Associated with the Nuclear Matrix

Proteins	M_r	NM(4)* / NM(C)	NM(24)* / NM(C)
CaM-binding proteins	200,000	3.80 ± 0.50 (3)	1.10 ± 0.47 (3)
	130,000	2.91 ± 0.34 (3)	1.43 ± 0.38 (3)
	100,000	E* (3)	E* (3)
	52,000	E* (3)	E* (3)
	33,000	2.10 ± 0.15 (3)	2.65 ± 0.32 (3)
	26,000	4.13 ± 1.30 (3)	3.97 ± 0.64 (3)
Estrogen receptor‡		21.6 ± 6.6 (8)	6.22 ± 1.72 (8)
Constitutive proteins§	36,000	1.08 ± 0.01 (3)	ND
	43,000	0.90 ± 0.09 (3)	ND
	70,000	1.23 ± 0.31 (3)	ND

ND, not determined; *NM*, nuclear matrix.

* Values represent the ratio of the amount of radioactive CaM bound by each of the proteins in 4-h [NM(4)] and 24-h [NM(24)] estrogen-stimulated and control (C) matrices. E* indicates present only in estrogen-treated matrix fractions.

‡ Values represent ratios of estrogen receptor in control and estrogen-stimulated matrices quantitated by Scatchard analysis as described in Methods.

§ Values represent ratios of percent area obtained from densitometric scans of Coomassie Blue-stained one-dimensional polyacrylamide gels of nuclear matrix fractions from estrogen-treated and control livers, using a Kontes fiber optic scanner coupled to a Hewlett-Packard integrator. Each gel was loaded with 100 μg of total protein.

33,000, and 26,000. A representative overlay from matrices prepared 4 h after diethylstilbestrol injection is illustrated in Fig. 2, lane *c*. All four proteins present in the control preparations can be seen. In addition, CaM-binding proteins are also apparent at M_r 52,000 and 100,000. Chelation of Ca^{2+} by EGTA abolishes the interaction of CaM with the proteins present in Fig. 2, lane *c* that exhibit M_r of 52,000, 100,000, 130,000, and 200,000. Finally, Fig. 1*A* reveals that two of the M_r standards also bind CaM under the conditions used. These two standards are myosin heavy chain (200,000) and phosphorylase (94,000), respectively.

To examine the changes in the relative amounts of the matrix-associated CaM-binding proteins in response to estrogen stimulation, several preparations of nuclear matrix proteins isolated from control and estrogen-stimulated (4- and 24-h) chicken livers were subjected to ^{125}I-CaM binding. The results of densitometric scanning of the radioautograms are summarized in Table II. Estrogen increases the four CaM-binding proteins of M_rs 200,000, 130,000, 33,000, and 26,000 that are common to control matrices as well as those prepared from 4- and 24-h estrogen-stimulated animals. In matrices isolated 4 h after estrogen stimulation, the amounts of these four proteins are elevated two- to fourfold. However, by 24 h after estrogen administration, the amounts of the 200,000 and 130,000 proteins have declined to the levels of the control, although the proteins of M_r 33,000 and 26,000 remained elevated. In addition, two Ca^{2+}-dependent CaM-binding proteins of M_r 52,000 and 100,000 became apparent in matrices after 4 and 24 h of stimulation with estrogen. These proteins are also depicted in Fig. 2, lane *c*.

The presence of the 200,000 and 130,000 proteins, in both control and estrogen-stimulated matrices, and the Ca^{2+} dependency of their CaM binding suggest that these are the major Ca^{2+}-dependent CaM-binding proteins in the nuclear matrix. The specificity of their interactions with ^{125}I-CaM were evaluated by densitometry of the radioautograms in the presence of unlabeled CaM or the homologous Ca^{2+}-binding protein, troponin C as shown in Table III. A 10-fold excess of unlabeled CaM resulted in 35–42% reduction in ^{125}I-CaM binding to these two matrix proteins; the binding interaction was virtually eliminated with a 100-fold molar excess of CaM. In contrast, a 1,000-fold molar excess of troponin C resulted in only a 25–32% decline in binding to either protein. The proteins of M_r 52,000 and 100,000, which are present only in estrogen-stimulated matrices, were similarly competed by 10- to 100-fold molar excesses of CaM but not by troponin C at even higher concentrations (data not shown).

MLCK is one of the CaM-binding proteins common to all animal cells and, at least in the chicken, exhibits a M_r of 130,000 (10). Replicate one-dimensional gels were prepared from control and 4-h estrogen-treated matrices and the pro-

TABLE III

Protein Specificity of the Major CaM-binding Proteins in Liver Nuclear Matrix

	^{125}I-CaM Bound	
	M_r 130,000	M_r 200,000
	%	%
0 CaM	100	100
10× CaM	58	65
100× CaM	8	23
1,000× CaM	0	0
1,000× troponin C	75	68

Nuclear matrix proteins from 4-h estrogen-stimulated chicken livers were electrophoresed in a 10% polyacrylamide-SDS gel and subjected to ^{125}I-CaM overlay in the presence of various concentrations of unlabeled CaM or 1,000-fold troponin C as described in Materials and Methods. The relative amount of ^{125}I-CaM bound in the presence of the competing proteins was determined by densitometric scans of the autoradiograms.

teins were transferred to nitrocellulose. Fig. 2 demonstrates the presence in both preparations of a single 130,000-protein species that interacts with MLCK antibody (lanes *g* and *h*, respectively). Moreover, authentic chicken gizzard MLCK can compete for the antibody binding observed on the blots (Fig. 2, lane *i*). Confirmation of the identity of MLCK in matrix preparations is shown in Fig. 3. Nuclear matrix from 4-h estrogen-treated chickens was used as a source of MLCK evaluated by the ability to phosphorylate exogenous myosin light chains. Only the 20,000 M_r light chain was phosphorylated (Fig. 3, lane *a*). The reaction was dependent on the presence of Ca^{2+} (lane *b*) nuclear matrix (lane *c*), and light chains (lane *d*).

DISCUSSION

We report in this study that CaM as well as two major Ca^{2+}-dependent CaM-binding proteins of M_r 200,000 and 130,000 are present in the nuclear matrix of chicken liver. Administration of estrogen to the chickens 4 h before matrix isolation resulted in a 1.6- and 2–4-fold increase, respectively, in the amounts of nuclear matrix-associated CaM and of these binding proteins. The 130,000-component has been identified as MLCK on the basis of (*a*) Ca^{2+}-dependent binding to ^{125}I-CaM; (*b*) cross-reactivity with a specific antibody to chicken MLCK; and (*c*) the presence of MLCK enzyme activity in nuclear matrix preparations by the Ca^{2+}-dependent incorporation of ^{32}P into exogenous myosin light chains.

Our identification of nuclear matrix CaM is consistent with earlier reports of the presence of CaM in nuclei of rat liver, rat uterus, and adrenal cells respectively (9, 11). However, in contrast to the latter two studies in which nuclear levels of CaM were shown to increase subsequent to hormone stimulation, we find that estrogen treatment does not alter the cellular or nuclear content of CaM. Rather, enrichment of nuclear CaM to the nuclear matrix was observed to occur within 4 h of estrogen stimulation. Redistribution of total cellular CaM has been demonstrated to occur after gonadotropin-releasing hormone stimulation of pituitary gonadotrophs independent of a change in total content of the regulatory protein (29). In this system, CaM association with the plasma membrane increased concomitant with the depletion of cytoplasmic CaM. The similar redistribution of CaM, albeit in the nucleus, may be related to the changes in the amounts of the 200,000 and 130,000, and the appearance of the 52,000 and 100,000 CaM-binding proteins, respectively.

We (15) and others (2) have reported the enrichment in hormone-responsive tissues of nuclear estrogen receptor in the nuclear matrix. In particular, we found that the quantity of nuclear estrogen receptor associated with the nuclear matrix increased fourfold within 4 h of a single administration of the steroid, after which time (24 h), a notable decline was observed. The changes in the levels of nuclear matrix CaM 4 h after estrogen stimulation and of the 200,000 and the 130,000 binding proteins 4 and 24 h after estrogen treatment are temporally consistent with the changes observed for the estrogen receptor (Table II).

Because CaM is a regulatory molecule that mediates its regulation by binding to and modulating the activities of a number of enzymes and proteins (7, 30), it is not surprising that the magnitude of the estrogen effects we observed here is greater at the level of the CaM-binding proteins than on CaM itself. The significance of the finding that one CaM-binding protein that is influenced by estrogen is MLCK is still unclear.

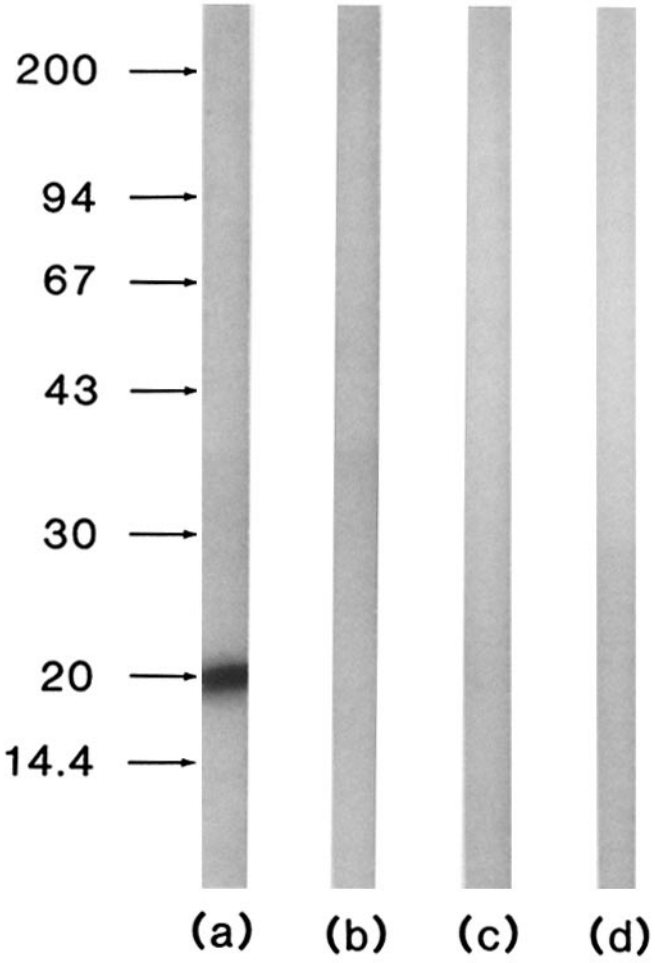

FIGURE 3 Autoradiography demonstrating MLCK activity of nuclear matrix proteins from 4-h estrogen-stimulated chick liver by ability to phosphorylate exogenous myosin light chains (see Materials and Methods). Lane *a*, nuclear matrix (60 μg of total protein) + Ca^{2+} (1 mM) + CaM (1 μM) + skeletal muscle myosin light chains (18 mg protein/ml); lane *b* as in lane *a*, in the absence of Ca^{2+}; lane *c* as in lane *a* in the absence of nuclear matrix proteins; lane *d* as in lane *a* in the absence of exogenous light chains. Values are molecular weight × 10^{-3}.

CaM and MLCK are mandatory components for contractility of nonmuscle and smooth muscle cells (31). Previous studies have shown that changes in the size of the nuclei accompany the onset and cessation of DNA synthesis (32). In addition, structural changes in the nucleoli accompany the synthesis and processing of ribosomal RNA (33, 34), and MLCK has been localized by immunofluorescence in the nucleoli of PtK_2 and Chinese hamster ovary cells (10). The above observations, together with the known estrogen effects on DNA replication and RNA polymerase activity, would be compatible with a role for CaM-mediated activation of MLCK activity in estrogen action. It is tempting to speculate that the 200,000 protein may be myosin since it co-migrates on two-dimensional gels with authentic myosin heavy chain, and myosin heavy chains will bind CaM in a Ca^{2+}-dependent manner assessed by the gel overlay technique (Fig. 2, lane *a*). However, further studies are required to prove the identity of the 200,000 as well as the other CaM-binding proteins.

The observations that the protein composition of the nuclear matrix is very complex and can be altered by estrogen in vivo, are consistent with the apparent multifunctional roles

this structure plays in important nuclear activities (35–37). In steroid hormone action, some nuclear matrix proteins are thought to be involved at the level of receptor-acceptor interactions involving nuclear ribonucleoproteins (38, 39). At the level of transcription, certain hormonally regulated genes preferentially associate with the matrix during their expression (37, 40). Our findings that estrogen changes the concentration of nuclear matrix-associated CaM and a number of its binding proteins raise the possibility that these Ca^{2+}-regulated proteins may also be involved in the action of steroid hormones at the nuclear level.

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