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Interaction between estrogen receptor and Pit-1 protein is influenced by estrogen in pituitary cells

Chingwen Ying[a,*], Don-Hei Lin[a], Dipak K. Sarkar[b], Tai-Tsung Chen[a]

[a]*Department of Microbiology, Soochow University, Taipei 111, Taiwan, ROC*
[b]*Department of Veterinary and Comparative Anatomy, College of Veterinary Medicine, Washington State University, Pullman, WA 996164-6520, USA*

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Abstract

The estrogen responsiveness of the rat prolactin gene expression requires the presence of both the estrogen receptor (ER) and the tissue-specific transcription factor, Pit-1 protein. We performed protein interaction assays using anti-rat Pit-1 antiserum (a-rPit-1) to investigate the physical interactions which occur between ER and Pit-1 proteins following estrogen treatment. After fusing maltose binding protein (MBP) and Pit-1 protein, we used the resulting MBP–Pit-1 fusion protein to prepare a-rPit-1. Our results show that the estrogen receptor readily co-precipitated with the Pit-1 protein drawn from the lysates of two prolactin-expressing pituitary cell lines GH_3 and PR1. The rate of precipitation appears to be both estrogen- and time-dependent. Cellular levels of estrogen receptors and Pit-1 proteins did not show significant changes during the time of estrogen treatment. We therefore suggest that an estrogen-dependent physical interaction between ER and Pit-1 protein exists in vivo, and that this interaction may play an important role in the regulation of prolactin gene expression. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Estrogen receptor; Pit-1; Protein interaction

1. Introduction

The rat prolactin (Prl) gene is normally only expressed in a specific subset of anterior pituitary cells. Its expression is subject to a complicated and multi-hormonal regulation which includes the effects of estradiol, thyrotropin-releasing hormone (TRH), epidermal growth factor and dopamine [1–3]. Both the tissue-specific expression and the hormonal regulation of the Prl gene involve interactions among multiple transacting factors with DNA elements of the Prl gene. Previous studies have demonstrated that estrogen through the activation of an intracellular estrogen receptor (ER) increases Prl gene expression at the transcriptional level [4,5]. The estrogen–ER complex interacts directly with a distal enhancer element, an estrogen response element (ERE) in the 5′ flanking region approximately 1.7–1.5 kilobases upstream from the transcription initiation site of Prl mRNA [6,7]. Mutations in this upstream region abolish estrogen responsiveness of the Prl gene [8].

Recently, the ability of estrogen to activate transcription of the Prl gene was shown to require the presence of both ER and the pituitary-specific transcription factor Pit-1 protein [9,10], which is also called growth hormone factor-1 (GHF-1). The Pit-1 protein, which plays a fundamental role in mammalian development, is a pituitary specific homeodomain protein belonging to the POU subclass [11,12]. This family of transcriptional regulators shares a conserved DNA binding motif, the POU domain, composed of a 60 amino acid homeodomain and a second region of 75 amino acids located N-terminally to the homeodomain, the POU-specific domain [13–15]. Both domains

* Corresponding author. Tel.: +886-2-2881-9471, ext. 6858; fax: +886-2-2883-1193.
E-mail address: cying@mbm1.scu.edu.tw (C. Ying)

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are necessary for high-affinity DNA binding on the Prl and growth hormone (GH) genes. Mutations of the Pit-1 gene have been found in dwarf mouse strains displaying hypoplasia of GH, Prl and TSH-secreting cells of the anterior pituitary, demonstrating the importance of Pit-1 in the development of certain anterior deficiency of hormonal secretion from these cell types [16].

While the Pit-1 protein binds to the Prl gene at multiple sites, the most important appears to be adjacent to the ER binding site; however, it must be noted that other Pit-1 binding sites also contribute to the response of the Prl gene to estrogen [17–19]. Deletion analysis demonstrated that Pit-1 binding sites in the distal enhancer are required to permit a response to estrogen [9]. Results also showed that the distal enhancer of the Prl gene in heterologous cells is capable of mediating estrogen response only when both ER and Pit-1 are present [9,10]. These studies indicate that a functional complex consisting of several factor-binding sites is essential for the estrogen response of the Prl gene expression. Furthermore, Nowakowski and Maurer [20] found that in vitro transcribed/translated ER was retained on a glutathione agarose bead column containing immobilized glutathione-S-transferase (GST)–Pit-1 fusion protein synthesized in bacteria. Their results imply the possible interaction between Pit-1 and ER in solution. Such interaction between Pit-1 and ER proteins may account for, at least partially, for the role of Pit-1 in facilitating the estrogen response of the Prl gene.

To further analyze the functional roles of Pit-1 and ER in regulating the gene expression of Prl in response to estrogen, we studied the possible physical interaction between ER and Pit-1 in two pituitary cell lines following exposure to estrogen using a protein interaction assay. Our results demonstrate that the requirement of both ER and Pit-1 in the estrogen responsiveness in Prl gene expression may involve the estrogen-dependent changes in the interaction between Pit-1 and ER.

2. Materials and methods

2.1. Chemicals

Plasmid pMAL-P2 and factor Xa protease were obtained from New England Biolabs (Beverly, MA). Phenol red-free Dulbecco's modified Eagle's medium (DMEM), Hank's balanced salt solution (HBSS), fetal bovine serum (FBS), antibiotic/antimycotic mix, protein A agarose and *Taq* DNA polymerase were purchased from Gibco BRL (Gaithersburg, MD).

Anti-Pit-1 antibody was purchased from Transduction Laboratories (Lexington, KY). The New Zealand albino rabbit used in this study was purchased from the laboratory animal center of the National Taiwan University (Taipei, Taiwan, ROC). Diethylstilbestrol (DES) and antiestrogen ICI 182,780 were purchased from Sigma (St. Louis, MO) and Tocris Cookson (Bristol, UK), respectively. All other chemicals and biochemicals were of the highest quality available from commercial sources.

2.2. Bacterial strain and cell lines

The *Escherichia coli* strain used in this research was XL1-BlueMRF′ (Δ(*mcrA*)183Δ(*mcrCB-hsdSMR-mrr*)173*end*A1*supE*44*thirecA*1*gyr*A96*lac*[F′*proABlacI*$^{q-}$*Z*ΔM15 Tn10 (tet^{r}]). The GH_3 rat pituitary cell line was obtained from American type cell culture (ATCC). The PR1 cell line was derived from the pituitary tumor of an F344, ovariectomized rat which had been treated with estrogen for 3 months [21].

2.3. Cell culture conditions

Rat pituitary cells GH_3 and PR1 were maintained in phenol red-free DMEM containing a 1× antibiotic/antimycotic mixture, 5 mM *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid, and 0.37% sodium bicarbonate. The medium was supplemented with either 10% FBS or 3× dextran/charcoal-stripped FBS [22]. Cultures were maintained at 37°C in a humidified atmosphere of 95% air/5% CO_2 and fed every 2 days.

2.4. Construction of Pit-1 protein expression vector MBP-Pit-1

The coding sequence of pit-1 cDNA was amplified from a plasmid pit-1 T7-7 (obtained from R. Maurer), by polymerase chain reaction (PCR), digested with restriction endonuclease *BamH*1 and gel purified. The resulting DNA fragment containing the pit-1 cDNA was then subcloned into the *BamH*1-treated expression vector pMAL-P2 downstream from the *malE* gene which encodes maltose binding protein (MBP). Following ligation, the recombinant plasmid was introduced into the competent *E. coli* XL1-BlueMRF′ strain. Transformants were selected on LB agar plates (1.5% agar, 1% tryptone, 0.5% yeast extract and 0.5% NaCl in H_2O) containing 50 μg/ml ampicillin, 40 mM of isopropyl-β-D-thiogalactose (IPTG) and 1 mg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). Expression of the resultant MBP–Pit-1 fusion protein was driven by the P*tac* promoter and MBP translational initiation signals.

2.5. Polymerase chain reaction

We used 10 ng of plasmid pit-1 T7-7 in a PCR reac-

tion mixture consisting of 20 mM Tris–HCl, pH 8.4, 50 mM KCl, 0.2 mM dNTPs, 500 ng/ml PCR primers, 2.5 units of Taq polymerase (BRL Gilbco) and predetermined 5 mM $MgCl_2$ to amplify the coding region of pit-1 gene. Final PCR reaction volume mixture was adjusted to be 100 μl by the addition of diethylpyrocarbonate-treated H_2O. The PCR reaction was overlaid with mineral oil and amplified using an Omingene DNA thermal cycler (Hybraid, UK). Cycling parameters (35 cycles) were as follows: denaturation at 96°C for 1 min, renaturation at 55°C for 1 min and polymerization at 75°C for 1.5 min. The reaction mixture was heated to 96°C for 1 min prior to the start of the cycle, and polymerization was extended for 8 min at 75°C at the end of cycle. Reaction products were extracted with chloroform to remove the mineral oil prior to analysis via 3% agarose gel electrophoresis.

Primers used to amplify rat pit-1 cDNA in this study were 5′-TAAGAAGGATCCATACATATGAGTTGC-3′ (5′ primer) and 5′- ATCGATGATAAGCTTGGGCTG-3′ (3′ primer). The amplified DNA fragment contained the complete coding sequence of rat pit-1 gene and some plasmid sequence extended outside of the 3′ end of the pit-1 cDNA. This DNA fragment contains two sites of *BamH*1; one locates outside the 5′ end originally present in the 5′ primer and the other locates in the 3′ end present in the plasmid sequence. Identification was confirmed by Southern blot analysis using a digoxigenin (DIG) labeled rat pit-1 cDNA probe.

2.6. Purification of MBP–Pit-1 fusion protein

The *E. coli* strain containing the recombinant plasmid was grown in LB broth supplemented with 0.2% glucose (w/v) and 100 μg/ml ampicillin. The optimal condition for the induction of MBP–Pit-1 fusion protein in *E. coli* strain was determined experimentally. Cultures were grown at 37°C to an optical density at 600 nm (OD_{600}) of 0.7 and then induced with 0.3 mM of IPTG. After 1 h incubation, cells were harvested, washed, lysed with sonication 4 times for 15 s each at 30 s intervals on ice and centrifugated at $9000 \times g$ for 20 min at 4°C to prepare cell lysates. Cell lysate was then mixed by means of gentle agitation with precleared amylose resin (5 vol.) for 1 h at 4°C prior to washing with column buffer (20 mM Tris, pH 7.4, 0.35 M NaCl, 1 mM EDTA, 0.015% DTT). Bound proteins were eluted from the amylose resin by incubation with equal volumes of 0.5 M maltose at 4°C for 4 h; MBP–Pit-1 fusion protein present in the supernatant was then collected by centrifugation.

2.7. Preparation of anti-rat Pit-1 antiserum (a-rPit-1)

Column purified MBP–Pit-1 fusion protein (0.6 mg) was mixed with the adjutant and used for the initial injection. As a booster, an additional 0.6 mg of the purified fusion protein was injected into the rabbit very five to seven days. The titer of antiserum was monitored by means of enzyme-linked immunosorbent assay (ELISA) with the MBP–Pit-1 fusion protein and MBP protein (data not shown). Collected antiserum was incubated at 37°C for 1 h and then held at 4°C for 15 h. Following centrifugation at $3000 \times g$ for 10 min at 4°C, a-rPit-1 was recovered and stored at −20°C. Preimmue serum was obtained from the New Zealand rabbit prior to primary injection of the fusion protein.

2.8. Immunoprecipitation and immunoblotting

Cells lysates of GH_3 and PR1 cells were prepared as described [23]. Briefly, after hormonal treatment, cells were washed three times with ice-cold HBSS before the addition of lysis buffer (0.1% Triton X-100, 1 mM iodoacetamide, 1% bovine hemoglobin, 1 mM PMSF, 0.002 U/ml aprotinin, 20 mM Tris–HCl, 0.14 M NaCl) and incubated at 4°C for 1 h. Following centrifugation at $3000 \times g$ for 10 min at 4°C, protein concentrations of the cell lysate were visualized via SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by staining with coomassie Brilliant blue R-250 dye. Cell lysates of 200 μl containing equal concentrations of protein were immunoprecipitated with 10 μl of a-rPit-1 or preimmune serum at 4°C. Following gently agitation overnight, 50 μl of protein A agarose or protein G agarose previously equilibrated with lysis buffer was added into the reaction mixture and incubated for 12 h at 4°C. The reaction mixture was then centrifuged and washed with ice-cold dilution buffer (0.1% Triton X-100, 1% bovine hemoglobin, 20 mM Tris–HCl, 0.14 M NaCl) three times, with TSA buffer (20 mM Tris–HCl, 0.14 M NaCl) and 0.005 M Tris–HCl (pH 6.8) once each at 4°C. The resulting precipitated immune complexes were solubilized at 100°C for 3–5 min in 20 μl of laemmli sample buffer.

The solubilized proteins were separated by 12% SDS-PAGE and transferred to a nitrocellulose membrane by electroblotting. After blocking overnight at 4°C in 5% skim milk in TBS buffer (20 mM Tris–HCl, pH 7.5, 500 mM NaCl), the membrane was incubated with either ER715, an anti-rat ER antibodies [24] or purchased anti-Pit-1 antibody diluted in TBS buffer containing 5% skim milk for 2 h at room temperature. The purchased anti-Pit-1 antibody can only be applied in western analysis as notified by the supplier since it does not recognize Pit-1 proteins in solution. After washing with TBS buffer, ER or Pit-1 proteins present

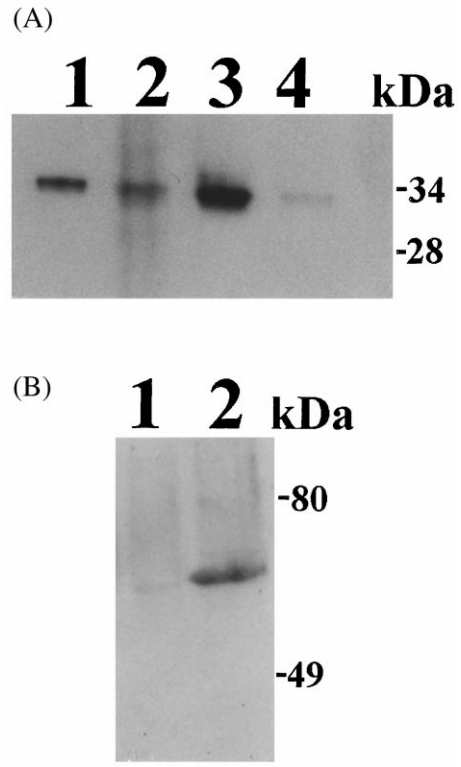

Fig. 1. Immunoprecipitation of Pit-1 proteins with a-rPit-1. (A) Factor Xa-cleaved MBP–Pit-1 protein (1), and GH_3 cell lysate (3) were immunoprecipitated with a-rPit-1 and fractionated in 12% SDS-PAGE. (2) and (4) were factor Xa-cleaved MBP–Pit-1 fusion protein and GH_3 cell lysate prior to immunopercipitation, respectively. (B) GH_3 cell lysate was incubated with a-rPit-1 and precipitated by centrifugation. Proteins remaining in supernatant (1) and precipitated proteins (2) were fractionated in 12% SDS-PAGE, immuno transferred and incubated with a commercially available anti Pit-1 antibody.

in the immune complexes by immunoprecipitation were detected using alkaline phosphatase-conjugated goat anti-rabbit IgG antibodies and an enhanced chemiluminescence (ECL) western blotting system (Amersham).

3. Results

3.1. Immunoprecipitation of Pit-1 protein

The capability of a-rPit-1 to recognize Pit-1 protein in solution was crucial to analyzing the in vivo interaction between Pit-1 and ER protein. The cell lysate of GH_3 cells were first incubated with the a-rPit-1, resolved by SDS-PAGE and then immunoblotted with a commercially available anti-Pit-1 antibody (Fig. 1). Results show that the a-rPit-1 prepared as part of this study was able to specifically precipitate Pit-1 protein from the GH_3 cell lysate (Fig. 1A, 3). Likewise, the a-rPit-1 was capable of immunoprecipitating Pit-1 protein from a reaction mixture containing factor X_a-digested MBP–Pit-1 protein (Fig. 1A, 1). To determine the effectiveness of this a-rPit-1 to precipitate Pit-1 protein, we compared the amounts of Pit-1 protein remaining in the supernatant with that of which was

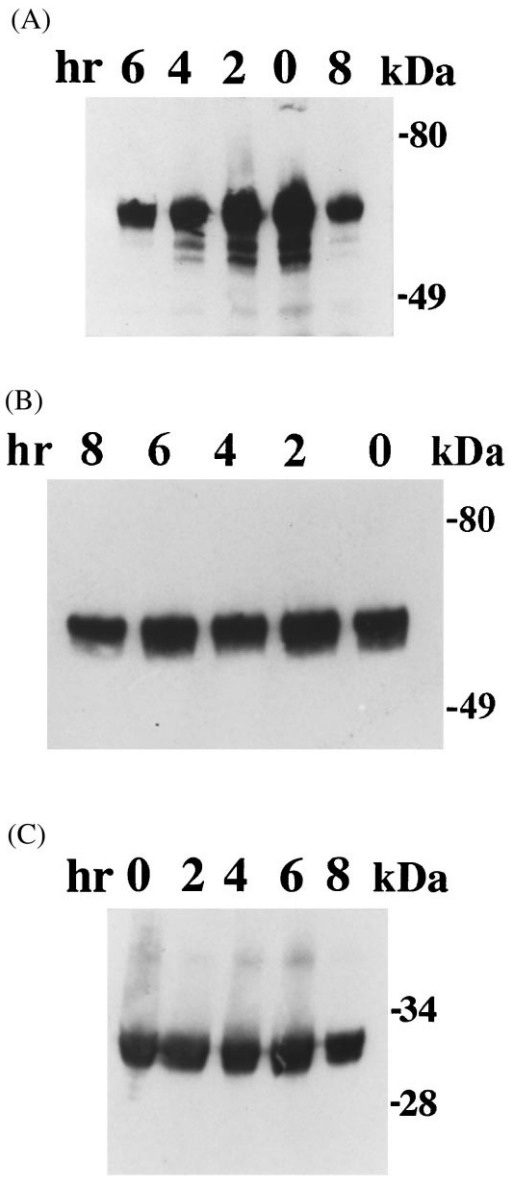

Fig. 2. Effects of estrogen treatment on the interaction between ER and Pit-1 proteins in pituitary cells GH_3. The GH_3 cells were treated with 10 nM DES for 2, 4, 6, or 8 h and cell lysates were prepared. In parallel, the same amount of ethanol was administrated to the GH_3 cells and incubated for 8 h (designated as 0). (A) Prepared GH_3 cell lysates were immunoprecipitated with a-rPit-1, fractionated in 12% SDS-PAGE and western transferred. The amount of ER co-precipitated with Pit-1 protein was recognized by ER715 antibody. (B) Prepared cell lysates were immunoprecipitated with ER715 antibody, fractionated in SDS-PAGE and subsequently western blotted with ER715 antibody to detect the amount of precipitated ER. (C) Prepared cell lysates were immunoprecipitated with a-rPit-1, fractionated in SDS-PAGE and western hybridized with purchased anti-Pit-1 antibody to determine the amount of precipitated Pit-1 protein.

Table 1
The effect of estrogen on the affinity between estrogen receptor and Pit-1 protein in GH_3 cells

Treatment	Total protein (au)[a]	Interaction between ER and Pit-1 (au)[a]	Relative affinity[b] between ER and Pit-1
+ Ethanol			
0 h	28530 (2187)	8043 (836)	0.282
8 h	29390 (2351)	8395 (738)	0.286
+ Estrogen			
2 h	27660 (1704)	6777 (620)	0.245
4 h	29260 (2161)	5426 (544)	0.186
6 h	33830 (3392)	4698 (477)	0.139
8 h	34890 (3023)	4590 (671)	0.132

[a] Arbitrary unit. Values are mean (SD).
[b] Total protein (au)/interaction between ER and Pit-1 (au).

became bounded to protein A agarose after precipitation (Fig. 1B). Results show that the majority of Pit-1 protein bounded to the protein A agarose following immunoprecipitation (Fig. 1B, 2); only a small percentage of Pit-1 protein remained in the supernatant. Indistinguishable results were produced when protein G agarose was used in stead of protein A agarose and no Pit-1 proteins were precipitated when a-rPit-1 was substituted by the rabbit serum or IgG in the immunoprecipitation assays (data not shown). Our results thus clearly indicated that the a-rPit-1 can effectively and specifically recognize Pit-1 protein in vivo in our protein interaction assays.

3.2. Interaction of Pit-1 protein and ER protein in vivo

Cell lysates of pituitary GH_3 cells, previously incubated with the synthetic estrogen diethylstilbestrol (DES) for 0–8 h were immunoprecipitated with a-rPit-1. The immune complexes were then separated by SDS-PAGE and immunoblotted with ER715 (an anti-ER antibody) to study interactions between the Pit-1 and ER proteins (Fig. 2A). As indicated by the resulting cross-reactivity with ER715 in immunoblotting assays, the ER protein co-precipitated with the Pit-1 protein in GH_3 cells. It thus suggested that the ER protein was able to interact either directly or indirectly with the Pit-1 protein in vivo and this potential physical interaction between ER and Pit-1 proteins appears to be estrogen dependent (Fig. 2A). The amount of ER protein co-precipitated with Pit-1 protein in GH_3 cells declined upon exposure to DES in a time dependent fashion with the lowest level observed 8 h following treatment (approximately 50% of ethanol-treated control). In order to determine whether this decrease in ER protein co-precipitation was due to a down regulation in the ER level by DES treatment, the amount of ER protein present in GH_3 cells was measured (Fig. 2B). No notable difference was found in ER protein levels in GH_3 cells incubated with DES for 0–6 h; only a slight decrease was observed upon exposure to DES for 8 h. However, this decrease in the ER protein level in GH_3 cells could not account for the degree of decrease in the level of co-precipitated ER protein. In addition, measurements of Pit-1 protein levels in GH_3 cells also did not reveal any significant changes in response to DES treatment (Fig. 2C). Therefore, the ligand dependent decrease in the physical interaction between ER and Pit-1 protein in GH_3 cells did not appear to be caused by the cellular levels of ER or Pit-1 proteins. To evaluate the affinity of the interaction between ER and Pit-1 in the presence or absence of estrogen, the intensity of each signal was quantitated with a luminescent image analyzer LAS-1000 (Fujifilm, Tokyo, Japan) and normalized to total proteins present in each sample (Table 1). The amount of co-precipitating ER was undistinguishable between zero time control (0 h) and 8 h control. In the absence of estrogen, the relative affinity between ER and Pit-1 did not change during the period of time analyzed Table 1. Estrogen, on the other hand, induced significant decrease up to 2 fold in the relative affinity between ER and Pit-1 protein. Such decrease

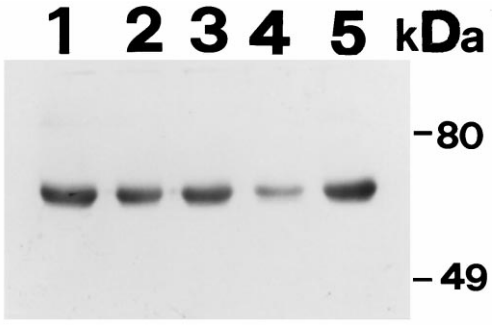

Fig. 3. The effects of antiestrogen on the estrogen induced interaction between ER and Pit-1 protein in GH_3 cells. Cell lysates were incubated with ethanol (1) or DES at the concentrations of 10 (2, 3) or 50 nM (4, 5) at 4°C. The antiestrogen ICI 182,780 at 20 fold molar excess was added simultaneously with DES in (3) and (5). Amount of ER protein co-precipitated with Pit-1 protein was determined by the ER715 antibody following immunoprecipitation with a-rPit-1.

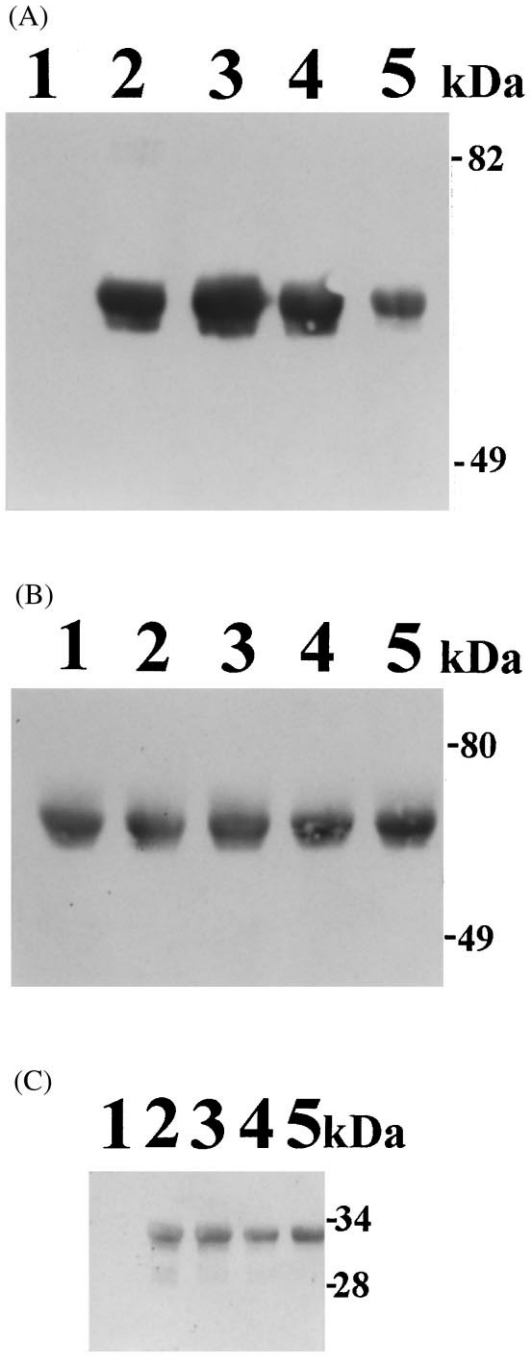

Fig. 4. Interaction of ER and Pit-1 protein in PR1 cells following DES treatment. (A) Amount of ER protein co-precipitated with Pit-1 protein after binding with a-rPit-1 (2–5) or with preimmune serum (1) was recognized by the ER715 antibody. DES was administrated to PR1 cells for 8 h (3), 18 h (4) or 24 h (5) and ethanol was administrated to PR1 cells to the same concentration (0.1%) and incubated for 24 h as a control (2). (B) Amount of ER protein present in PR1 cells treated with DES for 8 h (2), 18 h (3), 24 h (4), 48 h (5) and ethanol (1) was determined by the ER715 antibody. (C) Amount of Pit-1 protein in each prepared cell lysate was determined by precipitating cell lysates with a-rPit-1 (2–5) or preimmune serum (1) and immuno reacted with purchased anti-Pit-1 antibody.

in the relative affinity caused by estrogen also appeared to be time-dependent.

The estrogen induced changes in the interaction between ER and Pit-1 proteins appeared to be sensitive to the addition of antiestrogen (Fig. 3). Simultaneous addition of the "pure" antiestrogen ICI 182,780 blocked the decrease in the amount of co-precipitated ER caused by DES treatment. In the presence of DES and ICI 182,780, the level of co-precipitating ER was similar to that of the control cell lysates. The decrease in the amount of co-precipitating ER also seemed to be dose dependent (Fig. 3). Treatment with higher concentration of DES (50 nM) showed more profound effects on the interaction between ER and Pit-1 proteins than that of lower concentration (10 nM).

To further reveal whether the physical interaction between ER and Pit-1 proteins also exists among other Prl-expression cells, we used another pituitary derived PR1 cells to measure co-precipitating ER proteins via protein interaction assays (Fig. 4). Our results, which were similar to those presented in Fig. 2, indicate that DES treatment of PR1 cells caused a decrease in ER interaction with Pit-1 protein, as indicated by the smaller amount of ER co-precipitating with Pit-1 protein. The quantities of ER and Pit-1 proteins present in PR1 cells did not change significantly in response to DES treatment (Fig. 4B, C). Therefore, the decrease in the amount of ER co-precipitation is most likely due to the changes in the affinity between ER and Pit-1 proteins upon DES treatment.

4. Discussion

The present study has shown Prl expression pituitary cells in which Pit-1 binds to ER in a ligand dependent fashion. Observation of the physical interactions between ER and Pit-1 protein was previously reported with an in vitro labeled ER protein bound to bacterial expressed Pit-1 protein immobilized on glutathione agarose beads [20]. Our results provide further evidence that the formation of ER and Pit-1 complex in Prl expression pituitary cells is affected by estrogen. Hormonal dependent changes in the physical interaction between ER and Pit-1 proteins may explain why the presence of both proteins is required for the regulation of Prl gene expression as well as the functional role of Pit-1 in facilitating the Prl gene's estrogen response. In their analysis of nuclease sensitivity, Seyfred and Gorski [25] showed that estrogen treatment induced sensitivity in the Prl gene's distal enhancer and proximal promoter, but not in the DNA between these two regions. Nuclear ligation assay further showed that the distal enhancer in chromatin is in close association with the proximal promoter region [26]. Therefore, it is likely that estrogen response

involves functional and physical interactions between the distal and proximal promoter elements of Prl gene.

Multiple factors have been demonstrated to interact with steroid receptors in a ligand-dependent manner [27,28]. These include the mouse bromodomain-containing protein, TIF1 [29], the human homolog of the adaptor Sug1p; TRIP1 [30], and the ER-associated proteins ERAP160, RIP160 and RIP 80 [31,32]. However, none of the these proteins has been shown to enhance receptor-mediated transcriptional activity and so their role as potential cofactor remains undefined. Recently, human $hTAF_{II}30$, a TATA box binding protein (TBP)-associated factor has been shown to interact selectively with the hormone binding domain of ER protein and appears to contribute to transcriptional activity in vitro [33]. However, the interaction between $hTAF_{II}30$ and ER was unaffected by binding of either 17-β-estradiol or anti-estrogens (e.g., 4-hydroxytamoxifen), and is mapped to an inactive region in mammalian cells [34].

The work of Sakai and Gorski [35] indicated that ER in the cells is always associated with a nuclear component. By reporting that ER protein is bound to its specific DNA sequences regardless of the hormonal status of the cell, Murdoch et al. [36] challenged existing explanation of the functional role of hormone in cells. They suggested that the tight nuclear binding observed upon occupation with ligand is not due to receptor–DNA interactions alone but in stead mediated by interaction with another nuclear component. Furlow et al. [37] also proposed that DNA binding may only serve to localize the ER protein to target genes and that proper protein–protein interactions are probably the key function of the ER induced by estrogens. Therefore, an easy explanation would be that the functional role of estrogen in controlling Prl gene expression may involve regulating the association of a large complex, presumably between the ER and Pit-1, in the highly ordered environment of the intact nucleus. The formation of such a complex would be the mechanistic basis for changes in transcriptional activity, which in turn lead to the physiological response to estrogens. However, our results showed that the estrogen is capable of dissociating the formation of ER and Pit-1 complex in pituitary cells. The physiological role of this regulation and its relationship with Prl gene's expression in response to estrogen is intriguing and demands further analysis.

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