SEQUENTIAL STIMULATION OF SYNTHESIS OF TWO SPECIFIC CYTOPLASMIC PROTEINS IN EARLY ESTROGEN ACTION

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Received March 28,1975

Summary: The early effect of administration of estrogen on the synthesis of cytosol proteins in the uterus of the ovariectomized rat was analyzed. A sequential stimulation of two cytoplasmic proteins, having molecular weights of 44,200 and 70,500, was observed. The former protein is probably the IP protein described by Gorski and his colleagues. The latter and larger protein has a molecular weight equivalent to that of a nonhistone chromosomal protein recently described by our laboratory, and may be the cytoplasmic forerunner of that chromosomal protein.

Several workers have reported the induction of a specific cytoplasmic protein in the uterus of the immature rat at very early times after administration of estrogen (1,2). The synthesis of this protein is detectable at 30 min after hormone treatment, and the protein has been called the "induced protein" (IP) by Gorski and his colleagues (1,2), and the "key intermediary protein" (KIP) by Baulieu and his colleagues (3).

It has also been reported that estrogen stimulates the induction of several nonhistone chromosomal proteins in the uterus of the ovariectomized rat (4-7). Since proteins induced early in hormone action may have important roles in regulating transcription and/or translation, we have examined the relation between the IP protein and the nonhistone chromosomal proteins induced by estrogen. In previous work (5), the molecular weights of the nonhistone chromosomal proteins were determined. In this communication, we report the molecular weights of two cytoplasmic proteins stimulated by estrogen, and describe their temporal patterns of rates of synthesis in response to the hormone.

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Materials and Methods

Female Sprague-Dawley rats, ovariectomized at least 3 weeks, were used. Estradiol-17β (20 μg), dissolved in 0.2 ml of propylene glycol, was injected intraperitoneally, and control animals received only propylene glycol. In experiments with actinomycin D, the inhibitor was dissolved in 0.9% NaCl adjusted to 2% ethanol, and protected from light. Animals received intraperitoneally 800 μg of actinomycin D at 1 hr before treatment with estrogen. The dose of actinomycin D used inhibited RNA synthesis by 70% and protein synthesis by 30%, as indicated by incorporation of labeled precursors.

At the indicated times after treatment, animals (6 per group) were killed by cervical dislocation. The uteri were removed, stripped of fat and mesentery, slit lengthwise, and then rinsed thoroughly in ice-cold Krebs improved Ringer-phosphate buffer II (8) for removal of blood. The uteri were then transferred to Erlenmeyer flasks (25 ml) containing 1 ml of the Krebs-Ringer buffer per uterus, and either 15 μCi per ml of ^{14}C-algal hydrolysate or 130 μCi per ml of ^{3}H-algal hydrolysate (Schwarz Bioresearch). In most experiments the estrogen-treated uteri were labeled with ^{14}C-amino acids, and the control uteri were labeled with ^{3}H-amino acids. In several experiments the isotopes were reversed, and the estrogen-treated uteri were labeled with ^{14}C-amino acids. Control and experimental uteri were incubated at 37°C with gentle shaking for 1 hr. At the end of the incubation period, the two groups of uteri were pooled, rinsed thoroughly with ice-cold 0.9% NaCl, and rapidly frozen on dry ice. The frozen tissues were stored at -30°C. Under these conditions the incorporation of amino acids into uterine protein was linear for incubation periods up to at least 3 hr.

Uterine cytosol was prepared by a modification of the method of Katzenellenbogen and Gorski (2). Pooled frozen uteri were crushed in a stainless steel pulverizer chilled to -80°C, and homogenized in 9 ml of 0.05% Na_2EDTA (pH 7) at 60 volts for 2 min with the Polytron PT 20 homogenizer. The homogenate was centrifuged at 12,000 x g for 10 min. The pellet was discarded and the supernatant fraction centrifuged at 105,000 x g for 1 hr. One aliquot of the supernatant fraction from the second centrifugation was dialyzed against 1% SDS, 1% mercaptoethanol and 0.01 M phosphate buffer (pH 7.1) for 12 hr with 3 changes of buffer, and then dialyzed against the same solution containing 10% glycerol and Na_2EDTA (pH 7). Both aliquots were stored in liquid nitrogen until used.

Acrylamide (5%) gels containing 0.1% SDS were prepared, and run according to the method of Shapiro *et al.* (9). The gels were 12.5 by 0.6 cm. Tris-borate acrylamide gels were prepared by the method of DeAngelo and Gorski (1). The gels contained 6% acrylamide dissolved in 0.02 M boric acid, 0.003 M Na_2-EDTA and 0.066 M tris-HCl (pH 8.6). These gels were 9 by 0.6 cm. All gels were stained with Coomassie brilliant blue R-250 (6).

For determination of radioactivity, individual bands were sliced from the gels, placed in scintillation vials, and dried at 80°C. Then 1 ml of NCS (Nuclear Chicago Solubilizer) and 0.25 ml of H_2O were added to each vial. The capped vials were incubated at 50°C for 8 hr. 10 ml of scintillation solution (4 g of PPO, 50 mg of POPOP in 1 liter of toluene) was then added, and the vials were refrigerated overnight before counting. In some experiments the entire gel was sliced into 2 mm sections, and treated in the same manner as described. The recovery of radioactivity from the gel slices was greater than 90%. No significant difference in the recovery of ^{3}H and ^{14}C was observed in replicate experiments.

Results

Fig. 1 describes the pattern of bands of uterine cytosol proteins separated in 5% acrylamide gels containing 0.1% SDS. As expected, there is a diver-

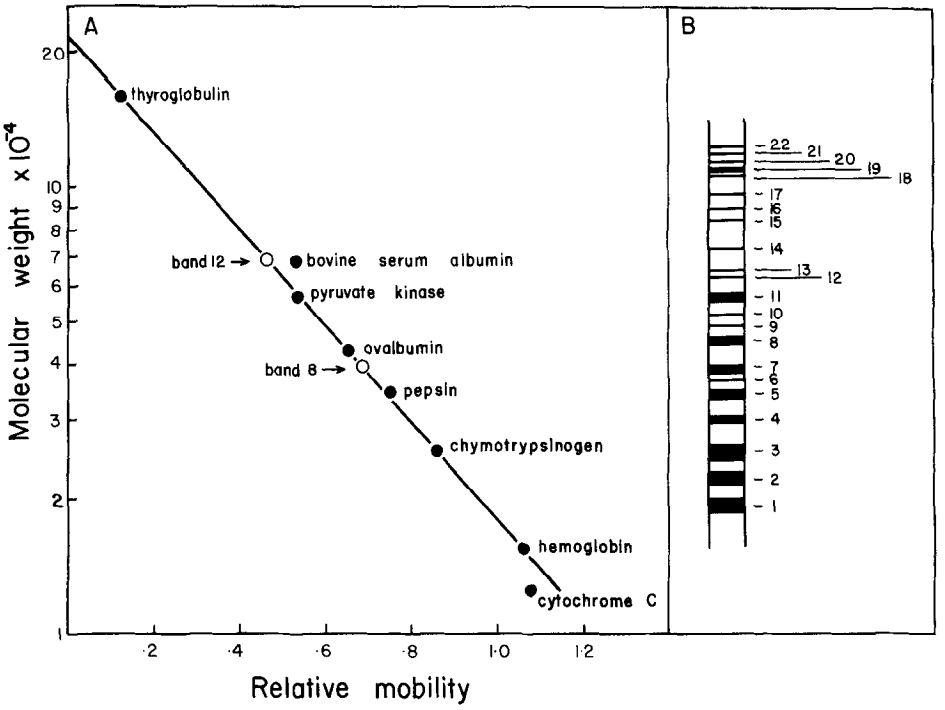

Fig. 1. (A) Calibration curve showing the molecular weights of the cytosol proteins in bands 8 and 12. (B) Schematic representation of uterine cytosol proteins electrophoretically separated in 5% acrylamide gels containing 0.1% SDS. The numbering system for the bands is an arbitrary one.

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sity of protein bands having a wide range of molecular weights. Fig. 1 also shows that the proteins of cytosol bands 8 and 12 have molecular weights of 44,200 and 70,500 respectively. Fig. 2 shows a typical electrophoretic separation in SDS-acrylamide gels of cytosol proteins extracted from uteri of ovariectomized rats treated for 1 hr with estrogen. There was a 25% increase in the rate of synthesis of protein in band 12. At later periods of hormone action, a more significant increase in the rate of synthesis of the protein in band 12 was observed. Equivalent results were obtained when the isotopes of the precursors used in the labeling of experimental and control uteri were reversed.

Fig. 3 demonstrates that an increase in the rate of synthesis of the cytosol protein in band 8 was detectable as early as 30 min after hormone treatment, and declined thereafter. An increase in the rate of synthesis of

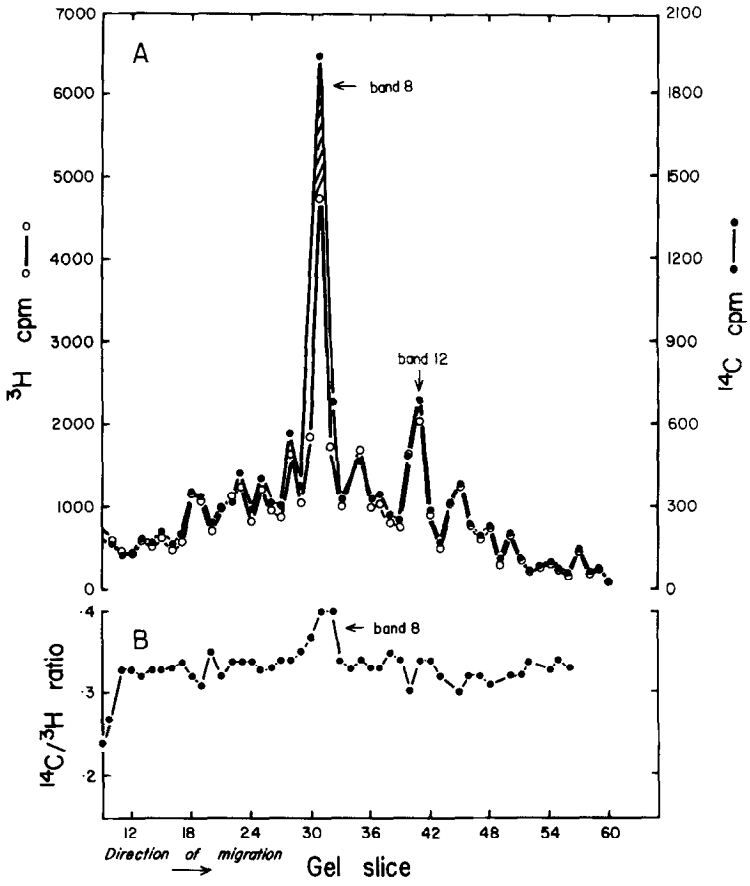

Fig. 2. (A) Electrophoretic separation of cytosol proteins in 5% acrylamide gels containing 0.1% SDS. Rats were treated for 1 hr with estrogen. Estrogen-treated uteri were labeled with ^{14}C-amino acids, and controls were labeled with ^{3}H-amino acids. The shaded area represents a region where increased protein synthesis has taken place. ● ——— ●, ^{14}C cpm; o ——— o, ^{3}H cpm. (B) ^{14}C/^{3}H ratios of the gel slices.

protein in band 12, however, was first repeatedly detectable at 1 hr after hormone treatment, reached a maximum at 3 hr, and then declined.

When an aliquot of the same cytosol fraction used in the experiment described in Fig. 2 was run on tris-borate acrylamide gels, the results observed (Fig. 4) were similar to those reported by DeAngelo and Gorski (1). We found that at 30 min after hormone treatment only one protein band exhibited increased incorporation, whether the cytosol proteins were separated in SDS-acrylamide gels or in tris-borate acrylamide gels. However, we observed a difference in

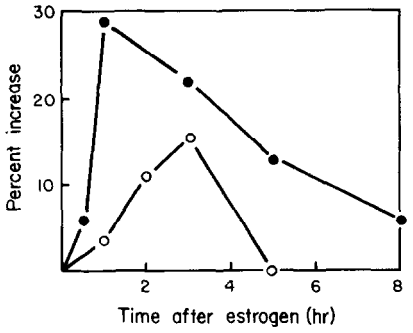

Fig. 3. Change in the rate of synthesis of specific cytosol proteins relative to the rate of synthesis of total cytosol protein, as a function of time after hormone treatment. The values represent changes in the $^{14}C/^{3}H$ ratio of the band relative to the change in the overall cytosol $^{14}C/^{3}H$ ratio expressed as percent above control. ● ——— ●, Band 8; o ——— o, band 12.

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the magnitude of incorporation, depending upon the conditions of electrophoresis. In tris-borate acrylamide gels, the IP protein migrated to a region of the gel containing little protein and the incorporation was 190% above the control. In SDS-acrylamide gels, this protein was stimulated only 25% above the control, but migrated to a region containing more protein. This probably masked to a significant degree the actual stimulation of incorporation.

Finally, we note that when rats were administered actinomycin D for 1 hr prior to estrogen treatment, the stimulation of synthesis of the protein in band 8 was inhibited by 55%, and the stimulation of that in band 12 was completely abolished.

Discussion

Our data indicate that after injection of estradiol-17β into the ovariectomized rat there is a sequential change in the rates of synthesis of two cytosol proteins. Separation of the cytosol proteins in SDS-acrylamide gels reveals that the two proteins occur in bands 8 and 12 and have molecular weights of 44,200 and 70,500 respectively. The increased synthesis of the protein of band 8 is first detectable at 30 min after hormone treatment, reaching a peak at 1 hr. Since the temporal pattern of induction of the protein in band 8 is

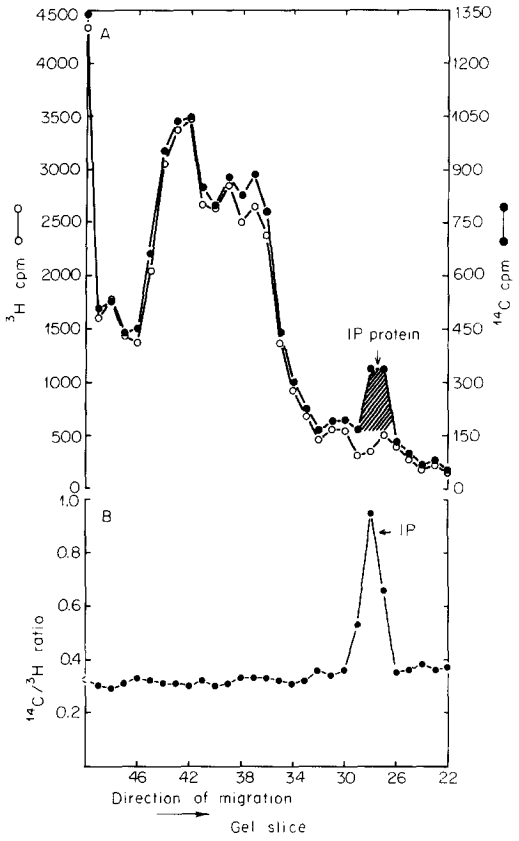

Fig. 4. (A) Electrophoretic separation of cytosol proteins in tris-borate acrylamide gels. The cytosol aliquot used was from the same experiment as described in Fig. 2. The shaded area represents a region where increased protein synthesis has taken place. ● ——— ●, ^{14}C cpm; o ——— o, ^{3}H cpm. (B) ^{14}C/^{3}H ratios of the gel slices.

essentially identical to that described for the IP protein by Gorski et al. (1,2), we assume the two proteins are the same. Also, while this manuscript was in preparation, Jacobelli et al. (11) isolated the IP protein and reported a molecular weight of 45,000.

We (5,6) have detected the induction of several uterine nonhistone chromosomal proteins after estrogen treatment. These proteins had molecular weights of approximately 70,500, 96,000, 29,400, 20,700, and 16,400. No increase in any nonhistone protein with a molecular weight of 44,200 was observed. This suggests, but does not prove, that the first cytoplasmic protein (IP) induced

by estrogen is not a nonhistone chromosomal protein and does not bind to chromatin as suggested by others. In addition, the IP protein does not have a molecular weight corresponding to the values reported for the estrogen receptors (12) One would think that a protein which is synthesized so early after hormone treatment would function in some regulatory role. Unfortunately, it is difficult to surmise what that role might be.

Finally, we emphasize that a nonhistone chromosomal protein (mol. wt. = 70,500) we have previously described (5,6) has several properties in common with the cytoplasmic protein in band 12 (Figs. 1 and 2). Their molecular weights and electrophoretic mobilities are identical. They show a similar temporal pattern of induction at early times (1-4 hr) following administration of estrogen. Furthermore, the degree of estrogen-stimulated synthesis for both proteins is similar. These common features suggest that the cytoplasmic protein in band 12 may be the same as the induced nonhistone chromosomal protein. In particular, their similarities with respect to patterns of induction point to a concomitant cytoplasmic synthesis and transport to the nucleus.

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