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Estrogen Receptors in the Rat Uterus. Multiple Forms Produced by Concentration-Dependent Aggregation†

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ABSTRACT: The sedimentation coefficient of the estradiol receptor proteins from rat uterine cytosol is highly variable and concentration dependent. In dilute buffer solutions without added salt the value of the sedimentation coefficient varies from 7 to 9 S, in 0.15 M KCl solutions the values vary from 3.8 to 7 S, and in 0.4 M KCl solutions the values vary from 3.8 to 5.2 S. The observed concentration dependence seems to result from a slow aggregation of estradiol binding proteins following tissue homogenization. The aggregation does not appear to be self-association of estrogen binding proteins, but seems rather to represent an interaction of estrogen binding units with other proteins present in uterine cytosol. Protein present in the soluble fraction of nontarget tissues appears to interact with uterine estradiol receptors to a more limited extent. The time-dependent aggregation can be minimized by working with dilute solutions. This suggests that *in vivo* the uterine estradiol receptor may exist as a 3.8–4.8S species, rather than 8 S as previously believed.

It is well established that estrogen responsive tissues contain a macromolecular fraction, at least partially protein, which is capable of binding estrogenic compounds tightly and specifically (Gorski *et al.*, 1968; Jensen *et al.*, 1971). This macromolecular fraction has been extensively studied because it appears to be the initial site at which estrogens interact with target tissues. In the uterus and other target tissues the initial interaction of estrogens with these tissue-specific "receptors" is thought to initiate the sequence of events which produces the characteristic growth response of the uterus to estrogenic hormones.

When estradiol enters the uterine cell, it is initially found in the cytosol as part of a macromolecular complex which sediments in sucrose density gradients at approximately 8 S in dilute buffer solutions (Toft and Gorski, 1966; Toft *et al.*, 1967), and at about 4 S in solutions containing 0.4 M KCl (Korenman and Rao, 1968; Erdos, 1968; Rochefort and Baulieu, 1968). This macromolecular complex, containing the bound hormone, is then transferred to the nucleus by a temperature-dependent process which is not clearly understood (Jensen *et al.*, 1971). This nuclear form of the hormone receptor complex sediments at approximately 5 S (Jensen *et al.*, 1968; Shyamala and Gorski, 1969) or 6 S (Giannopoulos and Gorski, 1971b) in 0.4 M KCl.

Currently, sucrose density gradients provide the most widely used method for distinguishing between the cytoplasmic and nuclear forms of the estrogen receptor. In addition, sucrose gradient centrifugation provides a tool for comparing receptors from various species. Despite widespread use of this technique, reported sedimentation coefficients from different laboratories vary considerably (Chamness and McGuire, 1972). Furthermore, the gradient profiles

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often observed in the literature appear broad and diffuse, in contrast to the profiles expected for a single, discrete, noninteracting protein species.

In this article we report results from a careful reexamination of the sedimentation behavior of the cytoplasmic estrogen receptor, using internal ^{14}C-labeled protein markers with varying conditions of protein concentration, ionic strengths, and times. These studies suggest that the cytoplasmic form of the estrogen receptor is initially present in uterine cells as a small form, with a sedimentation coefficient of 3.8–4.8 S. Following homogenization, this small form then appears to undergo a slow aggregation to yield more rapidly sedimenting species. This aggregation does not appear to be self-association of estrogen binding units, but rather aggregation with other proteins present in uterine homogenates.

Materials and Methods

Reagents and Buffers. [6,7-^3H]17β-Estradiol (tritiated estradiol) (48 Ci/mmol) and [^{14}C]formaldehyde (10 Ci/mol) were obtained from New England Nuclear. Unlabeled 17β-estradiol was obtained from Calibiochem.

Proteins used in these studies were obtained from the following sources: ovalbumin (Worthington Biochemical, 2× crystallized and lyophilized), bovine γ-globulin (Nutritional Biochemicals, fraction II, crystallized and lyophilized), bovine serum albumin (Sigma, fraction V), and beef heart lactic dehydrogenase (Sigma, type III, crystalline suspension). These proteins were used without further treatment.

Buffers routinely used in this work were: TE buffer[1] (0.01 M Tris–1.5 mM EDTA, pH 7.4), TKE buffer (0.01 M Tris–0.4 M KCl–1.5 mM EDTA, pH 7.4), TKES buffer (0.05 M Tris–0.15 M KCl–1.5 mM EDTA–1.5 mM $NaHSO_3$, pH 7.4).

All other chemicals were the highest reagent grades commercially available.

Preparation of Cytosol Fractions. Immature female Holtzman rats (20–24 days old) were used in all experiments. After sacrifice, the uteri were quickly removed, stripped of adhering fat, and placed in chilled (0–4°) TE buffer. In this work, five or ten uteri were then homogenized in 1 ml of TE or TKES buffer using a ground-glass homogenizer. Cytosol was obtained by centrifugation of the homogenate at 45,000 rpm (180,000g) for 45 min in a Beckman Ti50 rotor at 1°.

When cytosol is prepared in this manner 0.6–0.8 mg of soluble protein is obtained from each homogenized uterus, resulting in total protein concentrations of 3–4 mg of protein per ml for five uteri or 6 mg of protein per ml for ten uteri, determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. The cytosol fractions were then incubated with the indicated concentrations of tritiated 17β-estradiol at 0–4° for 3–6 hr. After this incubation, ^{14}C-labeled protein markers were added, the indicated dilutions were performed, and 200 μl of the cytosol-containing solutions was applied to sucrose gradients. The dashed lines in all figures represent the ^{14}C markers, ovalbumin and γ-globulin, and the solid lines represent receptor bound tritiated estradiol.

Sucrose Gradient Centrifugation. Linear 5–20% sucrose gradients were prepared as described by Martin and Ames (1961) using polyallomer centrifuge tubes. Gradients of 3.8 ml were kept at 0–4° for 2–5 hr before use. After sample application, the gradients were centrifuged in a Beckman SW-56 rotor in a Beckman L3-50 preparative ultracentrifuge at 1° for the indicated lengths of time. All runs were performed at 50,000 rpm. No differences were observed between gradients containing reagent grade sucrose or Mann ultrapure sucrose. After the centrifuge run, fractions were collected and counted as previously described by Giannopoulos and Gorski (1971b).

^{14}C-Labeled Marker Proteins. Ovalbumin and γ-globulin were methylated with ^{14}C-labeled formaldehyde and sodium borohydride exactly as described by Means and Feeney (1968). Specific activities of 2–4 × 10^6 cpm/mg of protein were obtained by this method. Unreacted formaldehyde was then removed by passage of the reaction mixture over a column of Sephadex G-25 equilibrated with TE buffer. The labeled proteins were stored at −20°.

The sedimentation coefficients of the markers were taken as 3.6 (ovalbumin) and 7.0 (γ-globulin) (Sober, 1970). There was no measurable change in sedimentation coefficients of the markers with any of the ionic strengths or protein concentrations employed in this work.

Results

Effect of Concentration on the Sedimentation Coefficient of the Cytoplasmic Estrogen Receptor. Our initial studies indicated that the sedimentation coefficient of the cytoplasmic estrogen receptor in low salt gradients was concentration dependent. To reproducibly examine this effect, it was necessary to run all gradients with internal ^{14}C-labeled marker proteins.

An experiment demonstrating a change in sedimentation with dilution is illustrated in Figure 1. Uterine cytosol was prepared and incubated with 5 × 10^{-9} M tritiated estradiol as described in Methods. One aliquot was applied directly to a gradient prepared with TE buffer (Figure 1B); a second aliquot was first diluted 1:10 with TE buffer and then immediately applied to a TE gradient (Figure 1A). Undiluted receptor sediments at approximately 8 S while that in the diluted aliquot sediments at about 7 S. The magnitude of this change in sedimentation coefficient is highly variable from one experiment to the next, but qualitatively a decrease in sedimentation coefficient is always observed with dilution. Appropriate dilution always reduces the value to about 7 S (in TE buffer), but the values for our undiluted cytosol preparations vary from 8 to 9.5 S.

It is also interesting to compare the observed profiles of the estrogen receptors to those of the ^{14}C-labeled markers. Note that at both concentrations employed (Figure 1A and B), the receptor peak is quite diffuse relative to the markers. This observation, coupled with the decrease in sedimentation coefficient upon dilution, suggests that the estrogen receptor is involved in some type of concentration-dependent interaction.

The cytoplasmic estrogen receptor exhibits a similar concentration-dependent decrease in sedimentation coefficient in gradients containing 0.4 M KCl. This is illustrated in Figure 2. Labeled cytosol was prepared (see Methods); undiluted (Figure 2A) and diluted (Figure 2B) aliquots of the same sample were applied to gradients prepared with TKE buffer, which contains 0.4 M KCl.

The undiluted sample sediments as a broad peak with an approximate sedimentation coefficient of 5.2 S (Figure 2A).

[1] Abbreviations for buffer systems are: TE, 0.01 M Tris–1.5 mM EDTA, pH 7.4; TKE, 0.01 M Tris–0.4 M KCl–1.5 mM EDTA, pH 7.4; TKES, 0.05 M Tris–0.15 M KCl–1.5 mM EDTA–1.5 mM $NaHSO_3$, pH 7.4.

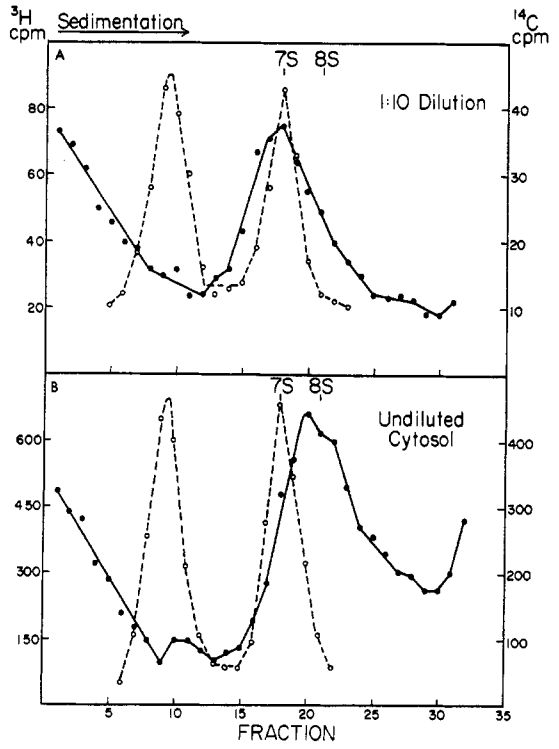

FIGURE 1: Effect of dilution on the sedimentation coefficient of estrogen receptor in TE gradients. Cytosol (5 uteri/ml in TE buffer) was prepared as described in Methods and incubated for 3 hr with 5×10^{-9} M tritiated estradiol. One aliquot of cytosol was diluted 1:10 with TE buffer (A) and a second aliquot was used without dilution (B). Centrifugation was for 12 hr.

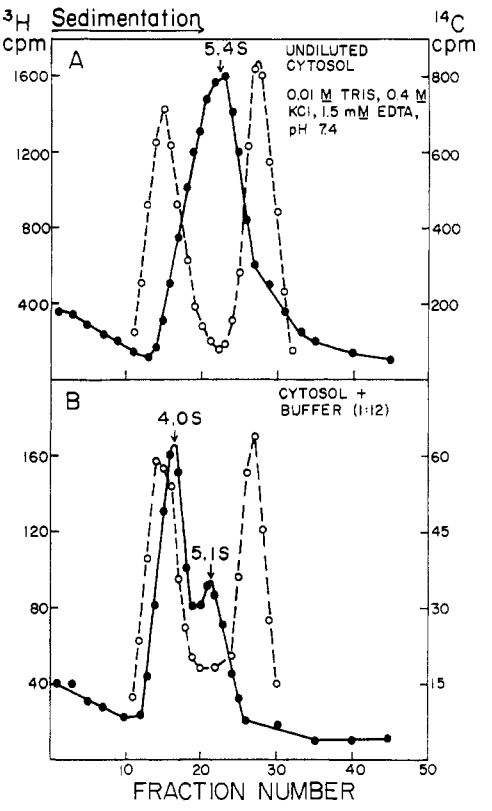

FIGURE 2: Effect of dilution on sedimentation coefficient of estrogen receptors in TKE gradients. Cytosol (10 uteri/ml) was prepared as described in Methods (using TE buffer) and incubated at 0° for 3 hr with 5×10^{-9} M tritiated estradiol. An undiluted aliquot (A) and an aliquot diluted 1:12 with TE buffer (B) were centrifuged for 14 hr.

The diluted sample exhibits a somewhat sharper peak at about 4.0 S, and a smaller peak, or shoulder, at 5.2 S (Figure 2B). This smaller peak is not observed in all experiments, but in some instances dilution simply yields a single, sharp peak at approximately 4 S. As with the "8S" form of the receptor, values for the sedimentation coefficient of different undiluted cytosols vary widely, but dilution always yields a peak which approaches a value of 4 S or slightly less.

Initially, it seemed possible that the decreases in sedimentation coefficient observed upon dilution might be due to a general effect of lowering the total protein concentration. This does not appear to be the case, however, as dilution with buffer solutions containing commercially obtained proteins, either ovalbumin or lactic dehydrogenase, resulted in gradient profiles similar to those observed after dilution with buffer alone.

Figure 3, for example, illustrates the sedimentation behavior of an aliquot of cytosol diluted with a buffered solution of ovalbumin (3 mg/ml), followed by sedimentation in a TKE gradient. The initial cytosol used for this experiment was the same preparation used for the experiment in Figure 2. Hence, the profile in Figure 3 may be compared directly to the profile in Figure 2B. The two profiles are indistinguishable.

Similar results were also observed using cytosol prepared in TE buffer followed by sedimentation through TE gradients. In this case, cytosol samples diluted with buffer alone or with buffered solutions of lactic dehydrogenase yielded similar gradient profiles.

Ovalbumin (s = 3.6) and lactic dehydrogenase (s = 7.5) were specifically used in these experiments because they sediment in the same regions of the gradients as the "4S" and "8S" forms of the receptor. Therefore, the receptor will be in a region of high protein concentration during the entire course of the experiment.

The possibility that a hormone receptor might be involved in either protein–protein or protein–ligand interactions which affect its structure is very attractive from a physiological viewpoint, and we therefore examined the observed concentration dependence when uteri were homogenized and centrifuged in buffer containing 0.15 M KCl, which should approximate the intracellular ionic strength. The results of these experiments are illustrated in Figure 4.

Once again we observe a broad profile with two peaks in the undiluted cytosol (Figure 4A), while the diluted sample (Figure 4B) sediments as a sharper, more symmetrical band with a lower sedimentation coefficient.

The observance of the two peaks of radioactivity seen in Figure 4A is not the result of homogenizing the uteri in 0.15 M KCl. When the cytosol prepared in the 0.15 M KCl is sedimented through a low ionic concentration TE gradient the receptor sediments as a broad peak at about 9 S, and as a broad peak in the 4–5S region of a high ionic concentration TKE gradient.

The observed changes in receptor sedimentation coefficient are not the result of decreasing the concentration of estradiol or other low molecular weight ligands present in the cytosol preparation before dilution. This conclusion is based upon the following observations. (1) When incubated with varying concentrations of estradiol, aliquots of cytosol containing the same protein concentrations exhibit similar

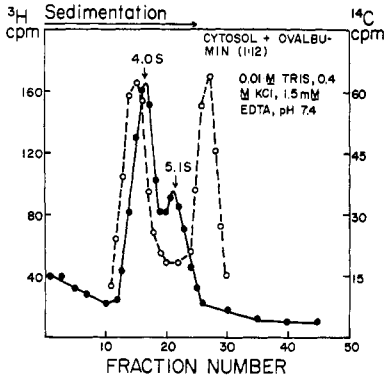

FIGURE 3: Effect of total protein concentration on sedimentation coefficients of estrogen receptor in TKE gradients. The cytosol preparation used in Figure 2 was diluted 1:12 with a solution of ovalbumin, 3 mg/ml, in TE buffer, and centrifuged for 14 hr.

sedimentation behavior. We have observed this in our studies, and other workers have reported similar findings (Giannopoulos and Gorski, 1971b; Jensen *et al.*, 1971). (2) We have observed that passage of cytosol preparations over a column of Sephadex G-25 prior to sedimentation does not alter the observed gradient profiles, and similarly Korenman and Rao (1968) observed that removal of low molecular weight solutes by dialysis did not alter sedimentation rates of the estrogen receptor. Thus, it seems that the observed changes in sedimentation coefficient result from changes in the concentration estrogen receptor itself and/or other uterine proteins.

Since buffers containing 0.15 M KCl should approximate *in vivo* ionic strengths, and since the observed concentration dependence of the sedimentation coefficients of the receptor protein was greatest at that ionic strength, we have focused our attention on determining the nature of the concentration dependence in experiments performed in 0.15 M KCl.

Multiple Forms of the Estrogen Receptor Produced by Aggregation. The concentration dependence of the sedimentation coefficients seems to arise from a rather slow aggregation phenomenon involving the estrogen receptor. It appears that the receptor is initially present in uterine homogenates as a relatively small form, 4–5 S, in 0.15 M KCl. During *in vitro*, cell-free incubations and during the centrifuge run this small form apparently aggregates to larger forms, about 7 S. The effect of dilution, therefore, is to prevent or greatly retard this aggregation phenomenon. This is seen in the data presented in Figures 5 and 6, which were all obtained from different treatments of a single sample of uterine cytosol prepared as described in Methods, so the profiles may be directly compared.

Tritiated estradiol (5×10^{-9} M) was added to one aliquot of the cytosol, and a small amount was removed after a brief incubation (5 min) and layered on a gradient (0.15 M KCl) which was spun immediately at 50,000 rpm for 7 hr. This gradient profile exhibited a sharp peak at 4.8 S with a slight shoulder in the 7 S region (Figure 5A). It is clear that the receptor is predominantly in a 4.8S form.

If incubation of the *same aliquot* with tritiated estradiol was continued for 12 hr prior to centrifugation and followed by centrifugation for 11 hr, the profile seen in Figure 5B was observed. In this case the sample (which is the same sample used for the data in Figure 5A) contained receptor which sedimented primarily in the 7S region of the gradient with a smaller amount of material in the 5S region (Figure 5B).

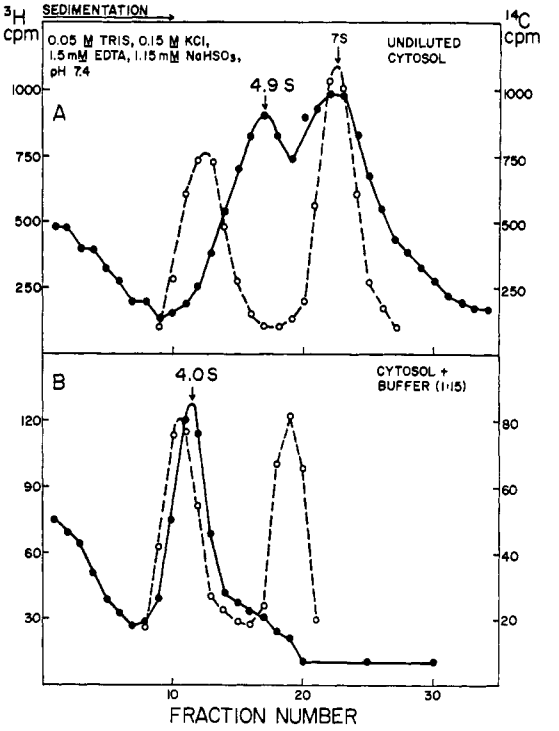

FIGURE 4: Effect of dilution on the sedimentation coefficient of estrogen receptor in TKES gradients. Uteri were homogenized (5 uteri/ml) in TKES buffer; cytosol was prepared as described in Methods and incubated for 3 hr with 5×10^{-9} M tritiated estradiol. Undiluted (A) and diluted (B) 1:15 with TKES buffer aliquots were centrifuged for 14 hr.

The data in Figure 6 suggest that the observed aggregation may be taking place both during the centrifuge run and during the prior incubation with tritiated estradiol. In this experiment the cytosol used in Figure 5 was first incubated with 5×10^{-9} M tritiated estradiol for 2 hr and diluted 1:10 with buffer containing 0.15 M KCl, and the incubation continued for 10 hr (12 hr total incubation). A sample was then sedimented in a 0.15 M KCl gradient for 11 hr (Figure 6). Following this treatment the receptor sediments at about 3.8 S (rather than 4.8 S as in Figure 5A) even though the sample was incubated for a longer time and centrifuged for a longer time than the sample in Figure 5A.

Effect of Cytoplasmic Proteins on the Aggregation of the Estrogen Receptor. The observed aggregation could be due to either of two possibilities; self-association of estrogen binding proteins or association of estrogen binding proteins with other uterine proteins. These two possibilities could be experimentally distinguished by performing mixing experiments using cytosol incubated with tritiated estradiol, if we could obtain cytosol selectively depleted of estrogen receptor relative to other soluble uterine proteins. Such a cytosol preparation, which we will refer to as "depleted cytosol," can be obtained from uteri previously exposed to cold estradiol at 37°, conditions which cause nuclear localization of approximately 90% of the cytoplasmic receptor (Giannopoulos and Gorski, 1971a). Also, we can prepare a cytosol *containing cytoplasmic receptor* labeled with cold estradiol. When the original cytosol–tritiated estradiol is mixed with the "depleted cytosol" the concentration of the estrogen receptor will be lowered, but the concentration of most other soluble uterine proteins will remain unaltered. However, if the original cytosol–tritiated estradiol is mixed with cytosol in-

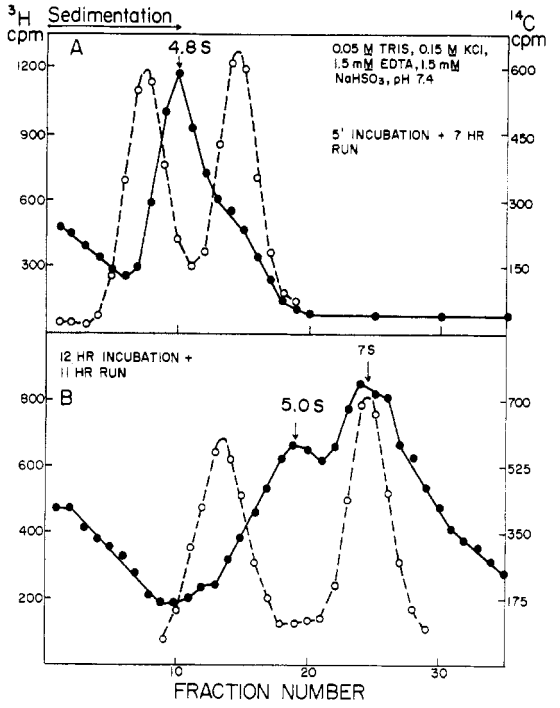

FIGURE 5: Aggregation of estrogen receptor in TKES buffer. Cytosol was prepared in TKES buffer (5 uteri/ml) and incubated at 0° with 5×10^{-9} M tritiated estradiol. (A) After a 5-min incubation with tritiated estradiol, an aliquot was centrifuged in TKES gradient for 7 hr. (B) After a 12-hr incubation with tritiated estradiol, an aliquot was centrifuged in a TKES gradient for 11 hr.

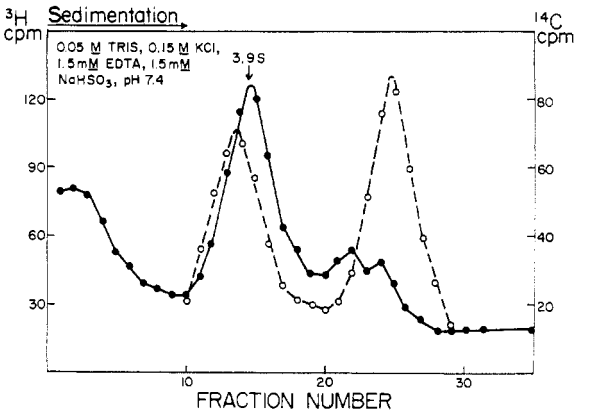

FIGURE 6: Effect of dilution on aggregation of estrogen receptor in TKES buffer. The cytosol used in Figure 5 was incubated for 2 hr with 5×10^{-9} M tritiated estradiol, and diluted 1:10 with TKES buffer. The sample was further incubated for 10 hr and then centrifuged for 11 hr in a TKES gradient.

cubated with cold estradiol, the total receptor concentration (tritiated-estradiol-labeled receptor plus estradiol-labeled receptor) as well as the concentration of other soluble uterine proteins will remain unaltered. In both cases the gradient profiles observed by counting would represent receptor originally present in the same cytosol preparation.[2] If both gradient profiles were similar it would, therefore, suggest that the aggregation involved association of estrogen receptor proteins with other uterine proteins. The results of such an experiment are illustrated in Figure 7.

The profile seen in Figure 7A was obtained from a sample of cytosol–tritiated estradiol which had been mixed with "depleted cytosol," and that in Figure 7B was obtained by mixing the same original cytosol–tritiated estradiol with an identical cytosol sample preincubated with cold estradiol (see legend to Figure 7 for details). The two profiles are very similar, and this suggests that the cytoplasmic estrogen receptor(s) is probably aggregating with other proteins present in uterine cytosol. Since the original cytosol–tritiated estradiol used for this experiment was also used to obtain the data in Figure 4, the profiles in Figures 7A and B may also be directly compared to the profile in Figure 4A, original undiluted cytosol. It is seen that the three profiles are essentially the same. It should be pointed out that the profiles in Figures 4B, 7A, and 7B represent a recovery of 80–100% of the counts observed in the original cytosol (Figure 4A).

At this point, it was of interest to determine if other tissues contained proteins which would associate with uterine estrogen receptor proteins to yield the larger aggregated species previously observed in the 7S region of the gradient. In this experiment a sample of uterine cytosol (5 uteri/ml in 0.15 M KCl) was prepared and incubated with 5×10^{-9} M tritiated estradiol. An aliquot was then removed without further treatment and layered directly on a gradient (Figure 8A). A second aliquot was diluted 1:10 with buffer containing 0.15 M KCl (Figure 8B). A third aliquot was diluted 1:10 with another sample of uterine cytosol (5 uteri/ml), containing 10^{-6} M cold estradiol (Figure 8C), and a final aliquot was diluted 1:10 with a preparation of cytosol from diaphragm, adjusted to the same total protein concentration as the uterine cytosols (Figure 8D). The diaphragm cytosol also contained 10^{-6} M cold estradiol.

As previously observed the original, undiluted cytosol sample and the sample diluted with a second sample of uterine cytosol gave similar patterns (Figures 8A and C), consisting of major components at 4.8 S with large shoulders in the 6.5–7S regions of the gradient. Also expected was the sharp peak at about 3.9 S for the sample diluted with buffer alone (Figure 8B). The original uterine cytosol diluted with the diaphragm cytosol showed a major peak at 4.4 S and a small shoulder in the 7S region of the gradient (Figure 8D). While the pattern was not quite the same as that obtained by dilution with buffer, it is quantitatively different from that in Figure 8A for the undiluted uterine sample. This quantitative difference is reproducible and appears real, although it is difficult to interpret. Patterns identical with that for the diaphragm solution were also obtained with cytosols from liver and spleen.

Discussion

In order to reproducibly measure the sedimentation coefficient of uterine cytoplasmic estrogen receptors we have found it necessary to use internal ^{14}C-labeled marker proteins, rather than the more commonly used bovine serum albumin standard in a separate gradient, and to carefully control experimental conditions, especially protein concentration. We strongly recommend that future experiments using this technique adhere to these precautions. Even with these precautions there is some variability in values for different cytosol preparations, and, therefore, we further suggest that conclusions concerning structural features of estrogen receptor proteins be based only on comparison of samples

[2] These experiments can be performed because of the exceptional stability of the receptor–estradiol complex. Thus, the half-life for dissociation of the complex is 22 days at 0° (Alberga *et al.*, 1970).

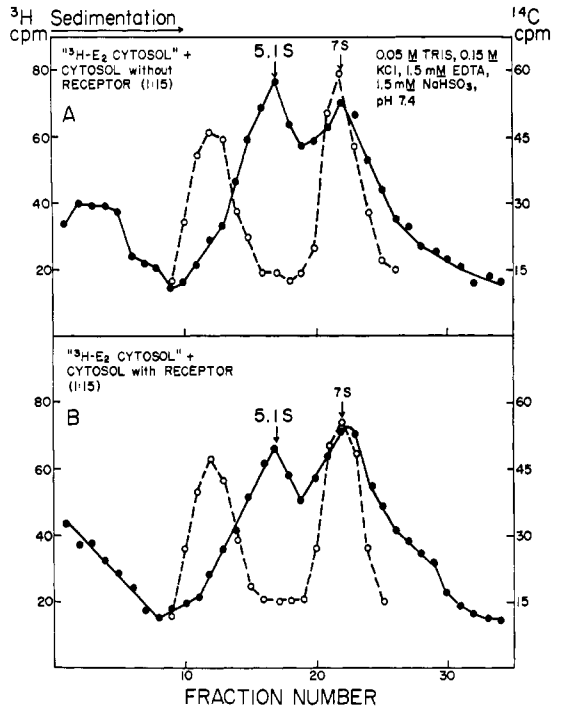

FIGURE 7: Effect of soluble uterine proteins on aggregation of estrogen receptor. The cytosol preparation used for the data in Figure 4, which was incubated with 5×10^{-9} M tritiated estradiol in TKES buffer for 3 hr, was diluted 1:15 with uterine cytosol (5 uteri/ml) which did not contain estrogen receptor (A). This "depleted cytosol" was obtained from uteri that had been incubated in HeLa medium for 1 hr at 37° with 1×10^{-8} M cold estradiol, followed by homogenization in TKES buffer containing 1×10^{-6} M cold estradiol. The cytosol preparation used in Figure 4 was then diluted 1:15 with cytosol containing estrogen receptors, but prelabeled with 10^{-6} M cold estradiol (B). Centrifugation was for 14 hr in TKES gradients.

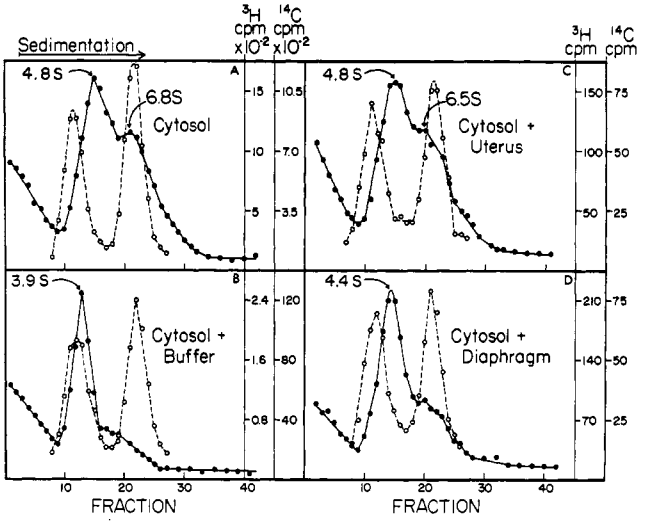

FIGURE 8: Effect of soluble proteins from nontarget tissues on aggregation of the estrogen receptor. A uterine cytosol fraction was prepared in TKES buffer (5 uteri/ml) as described in Methods and incubated with 5×10^{-9} M tritiated estradiol for 3 hr. A series of cytosols in TKES was also prepared from uterus, diaphragm, liver, and spleen. These were adjusted to the same protein concentration as the uterine cytosol incubated with tritiated estradiol, and each cytosol in the series was then incubated with 10^{-6} M cold estradiol. The uterine cytosol incubated with tritiated estradiol was then untreated (A), or diluted 1:10 with TKES buffer (B), uterine cytosol with 10^{-6} M cold estradiol (C), diaphragm cytosol (D), liver cytosol (not shown), or spleen cytosol (not shown). Centrifugation was for 14 hr in TKES gradients.

derived from the same original cytosol preparation. While our conclusions are based on sucrose gradient centrifugation experiments, it seems likely that similar precautions should be taken when utilizing any other type of moving boundary experiment, *e.g.*, electrophoresis or chromatography.

The physiological significance of our results is difficult to assess, but our studies indicate that the true intracellular nature of the cytoplasmic estrogen receptor must remain an open question. If the cytoplasmic receptor is truly present in the soluble cellular fraction, it must be in an environment with a protein concentration roughly an order of magnitude higher than the highest concentration used in any of our studies. Clearly, we are not able to predict with certainty the behavior of the cytoplasmic receptor under such conditions.

Our results from gradients run in 0.15 M KCl, however, suggest that the estrogen receptor is most likely present in uterine cytosol as a 3.8–4.8S species, somewhat smaller than the 8S species observed in low salt gradients and originally thought to represent the native state of the cytoplasmic estrogen receptor (Toft and Gorski, 1966). After homogenization of uterine tissue and preparation of the cytosol fraction in 0.15 M KCl, this small 3.8–4.8S species appears to undergo a slow aggregation involving other soluble uterine proteins to yield more rapidly sedimenting species, although the exact relationship between the 4-, 5-, and 7S species observed in 0.15 M KCl is not precisely clear. It may be that similar aggregation phenomena are also the basis for the smaller variations in sedimentation coefficient observed in gradients containing no KCl (TE) and 0.4 M KCl (TKE).

Several other groups have also studied the uterine receptor in 0.15 M KCl (Chamness and McGuire, 1972; Erdos *et al.*, 1970; Reti and Erdos, 1971), and reported sedimentation coefficients of 6 S. Based on our results it seems likely that this 6S species arises from the smaller species (3.8–4.8S) we have observed in this work as a result of aggregation.

In the following article we report that the uterine cytoplasmic estrogen receptor sediments at about 3.6 S in gradients containing 4 M urea, 1 M KCl, and reducing agents (Stancel *et al.*, 1973). Based on this observation, and the results observed in the work with 0.15 M KCl-containing solutions, we would thus like to raise the possibility that the estrogen receptor protein may exist *in vivo* as a unit with a sedimentation coefficient of about 4 S which contains only a single polypeptide chain. In a number of elegant studies, Puca *et al.* (1971, 1972) have also observed several forms of the receptor from calf uterus. It is difficult to compare results directly because those studies used partially purified preparations which had been treated with Ca^{2+}, but it may be that the "4S" species we observe in 0.15 M KCl is similar to or identical with the smallest species, 4.5 S, observed by these investigators.

Chamness and McGuire (1972) have recently reviewed the values of sedimentation coefficients obtained for the cytoplasmic estrogen receptors in different studies. Values as high as 9.5 S have been observed in dilute buffer (Toft and Gorski, 1966; Jensen *et al.*, 1967), and we report that values of 7 S are obtained under similar conditions by lowering the protein concentration. Numerous studies have reported intermediate values (Chamness and McGuire, 1972). The results presented in this work strongly suggest that this varia-

tion in literature values is due, at least in part, to differences in the protein concentrations at which experiments are performed in different laboratories, differences in times of incubation, and times of centrifugation. These experimental differences probably lead to varying degrees of aggregation.

At present we have very little information concerning aggregation phenomena involving estrogen receptor proteins. While these interactions appear to be time-dependent processes exhibiting a concentration dependence and an ionic strength dependence, we have no data about the nature of the components involved or factors such as reversibility. Recently, Puca *et al.* (1972) have reported on studies of a "receptor transforming factor" in the calf uterus. They also used sucrose gradients to a great extent in their study. Because of our studies reported above as well as those of Chamness and McGuire (1972), we believe that information obtained by techniques such as sucrose gradient centrifugation may not be as readily interpretable as previously believed.

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