The Molecular Mechanism of the in Vitro 4 S to 5 S Transformation of the Uterine Estrogen Receptor*

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SUMMARY

The rat uterine cytosol contains two estrogen-binding proteins (EBP) referred to as the "4 S" and "5 S" EBP. The 5 S EBP was produced by incubating the uterine cytosol-[3H]estradiol mixture at 28° for 30 min to promote the "4 S to 5 S transformation" as described by Jensen et al. ((1971) Biochem. Soc. Symp. 32, 133). These two EBP species were characterized in high ionic strength buffers using sucrose gradient centrifugation and Sephadex G-200 gel chromatography. With these analytical methods the sedimentation coefficient, molecular Stokes radii, and molecular weights were determined.

In the presence of 0.4 M KCl at pH 7.4 the 4 S EBP has a sedimentation coefficient of 4.2 ± 0.04 S, a molecular Stokes radius of 44.0 ± 0.4 A, and a molecular weight of 76,200. While in the identical buffers the 5 S EBP has a sedimentation coefficient of 5.5 ± 0.02 S, a molecular Stokes radius of 58.5 ± 0.5 A, and a molecular weight of 132,700.

In the presence of 0.4 M KCl and 3 M urea at pH 7.4 the 4 S EBP shows a decrease in its sedimentation to 3.6 ± 0.04 S, but an increase in its molecular Stokes radius to 53.8 ± 0.9 A. The estimated molecular weight is 79,900. The 5 S EBP has a sedimentation coefficient of 4.6 ± 0.9 S and a molecular Stokes radius of 70.6 ± 1.0 A. The molecular weight is 133,900. In addition, sucrose gradient centrifugation analyses, in the presence of 0.4 M KCl, 3 M urea at pH 7.4 showed that a moderate fraction (25 to 50%) of the 5 S EBP is dissociated to the 4 S EBP.

In the presence of 0.4 M KCl at pH 6.8 the 4 S and 5 S EBPs sediment at 4.7 ± 0.04 and 5.6 ± 0.05 S, respectively. The 5 S EBP also shows a tendency to revert to the 4 S EBP. Consequently, it was observed that a combination of 0.4 M KCl and 3 M urea at pH 6.8 is extremely effective, in contrast to using each of these reagents alone, in dissociating the 5 S EBP to the 4 S EBP without causing a loss of [3H]estradiol binding. The dissociation process is first order with a half-life of approximately 5 hours.

These results indicate that the in vitro "4 S to 5 S transformation" is an association of the 4 S EBP, having a molecular weight of ~80,000, with a second component or subunit of ~50,000 to form the 5 S EBP (~130,000). Concurrent with the association process, the 4 S and 5 S EBPs are also capable of marked conformational changes that were discerned when the EBPs were compared to the protein standards under identical conditions. The protein standards used were myoglobin, trypsin, ovalbumin, serum albumin, alkaline phosphatase, aldolase, γ-globulin, and ferritin. The conformational changes of the EBPs were shown by their sedimentation coefficients and molecular Stokes radii varying in a reciprocating manner while their molecular weights remained constant.

The target tissues of the estrogenic hormones contain within their cytoplasm specific estrogen-binding proteins (EBPs) referred to as "receptors" (1). The association of estradiol with the receptor is accompanied by a temperature-dependent redistribution of the estrogen receptor from the cytoplasm to the nucleus (2-5). Subsequently, with the localization of the estrogen receptor complex in the nucleus, enhanced nuclear biosynthetic activities are observed (6-9). However, the exact molecular activity of the estrogen receptor has not been shown. Numerous investigators have shown that the cytoplasmic estrogen receptor sediments in sucrose gradients at approximately 4 S in the presence of 0.4 M KCl, while the estrogen receptor isolated from the nucleus sediments at approximately 5 S (10-12). Several studies have centered upon resolving the molecular basis for the differences in the sedimentation behavior of the cytoplasmic and nuclear forms of the estrogen receptor based upon the supposed that the molecular mechanism for the estrogen receptor's action may then be resolved.

Jensen et al. (13, 14) have reported that a cell-free "4 S to 5 S transformation" of the estrogen receptor produces a 5 S EBP similar to the isolated nuclear 5 S estrogen receptor. The exact molecular basis for difference between the 4 S and 5 S EBPs has not been elucidated, although numerous models have been proposed (4, 15-22).

Based upon sucrose gradient analysis and gel chromatography

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studies of the 4 S EBP and the 5 S EBP produced by the in vivo "4 S to 5 S transformation" procedure, we report in this paper the molecular characteristics of, and relationship between, the 4 S and 5 S EBPs. The data are consistent with a model indicating that the 4 S EBP (~80,000) associates with a second component of ~50,000 to form the 5 S EBP with a molecular weight of approximately 130,000.

**EXPERIMENTAL PROCEDURES**

**Preparation of Rat Uterine Cytosol and 4 S to 5 S EBP Transformation—**Uteri from 20- to 24-day-old Holtzman rats were excised and collected in 40 mM Tris, pH 7.4, 0°C. The uteri were rinsed in a fresh quantity of the same buffer and homogenized (five strokes of a Polytron PT-10 (Brinkmann Instruments) at a power setting of 4, at 50-s intervals. The homogenate was centrifuged at 220,000 x g for 30 min to obtain a supernatant fraction that is referred to as the "cytosol." The protein concentration of the cytosol was determined by the method of Lowry et al. (23). The [3H]estradiol-cytosol was further incubated at 28 ± 0.5°C for 30 min to promote the 4 S to 5 S EBP transformation of the estrogen receptor as described previously (13).

**Buffers—**The following buffers were used for chromatographic analyses: 40 mM Tris-2 mM Na2EDTA-0.4 M KCl, pH 7.4 (TEK buffer); 40 mM Tris-0.1 M Na2EDTA-0.4 M KCl-1, 2 or 3 M urea, pH 7.4 (TEK-1, 2 or 3 M urea buffer); 40 mM Hepes-2 mM Nae-EDTA-0.4 M KCl, pH 6.8 (HEK buffer). The buffers containing urea were freshly prepared.

**Sucrose Gradient Centrifugation (45)—**Linear sucrose gradients (3.8 ml) were prepared with a Buchner Gradient Former. The concentrations of the sucrose gradients, the buffers used, and the centrifugation conditions are noted with each figure. The gradients were allowed to stand 2 to 4 hours at 4°C before layering a 0.5-ml sample onto the gradient. All samples contained myoglobin (2.0 S) and alkaline phosphatase (6.2 S) as internal sedimentation markers. Frequently, myoglobin, alkaline phosphatase, and bovine serum albumin (4.9 S) were layered on another gradient as an additional control. The samples were centrifuged in a Beckman L2-65 ultracentrifuge using an SW 50 rotor at 4°C.

**RESULTS**

**Transformation of Estrogen Receptor from 4 S to 5 S EBP—**In sucrose gradient analysis, the rat uterine estrogen receptor appeared as two estrogen-binding proteins (EBPs): 4.2 ± 0.04 S, referred to as the 4 S EBP, and 5.5 ± 0.02 S, referred to as the 5 S EBP. The quantity of the 4 S EBP was always greater than that of the 5 S EBP. Occasionally only the 4 S EBP was seen in the cytosol-[3H]estradiol mixture that was incubated 30 or 60 min at 0°C prior to sucrose gradient centrifugation. Incubation of the cytosol-[3H]estradiol mixture at 0°C for 30 min, followed by 28°C for 30 min, reduced the quantity of the 4 S EBP and increased that of the 5 S EBP to 90 to 100% of the total [3H]estradiol-binding capacity present (Fig. 1A). No significant loss of the [3H]estradiol-binding capacity was observed during the in vitro transformation procedure. The addition of a 200-fold excess of unlabeled estradiol to the cytosol-[3H]estradiol mixture (i.e. 4 S EBP) immediately before the 28°C incubation had little effect on the appearance of the labeled 5 S EBP. A small decrease (10 to 30%) in the total [3H]estradiol-binding capacity was observed, but this was presumably due to an exchange of [3H]estradiol with the unlabeled estradiol during the 28°C incubation. This suggests that most of the [3H]estradiol was still associated with the 4 S EBP (or a modified form of the 4 S EBP) and that the [3H]estradiol did not dissociate from the 4 S EBP to reassociate during the 28°C incubation with a "de novo 5 S EBP." The selective transfer of the [3H]estradiol from the 4 S to the 5 S EBP during incubation was also excluded by the observation that the binding affinity of the 5 S EBP was not greater than that of the 4 S EBP. The similarity in the binding affinities of the different forms of the EBPs has been reported previously (12).

Nielsen and A. Notides, unpublished data.
Radius--The 4 S and the 5 S EBPs had separate and highly re-
and 4.6 ± 0.09 S with moderate (-25 to 50%) reversion of the
larger EBP to the smaller EBP (Fig. 1B).

In sucrose gradients containing TEK-1
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proteins on sucrose gradients with or without 3
M urea buffer the EBPs were 3.6 ± 0.04 S
and 5.0 ± 0.05 S with slight (~10 to 20%) reversion of the larger EBP to the smaller EBP.
Using TEK-2
m urea buffer the EBPs were 3.7 ± 0.04 S and 5.6 ± 0.05 S with slight (~10 to 20%) reversion of the larger EBP to the smaller EBP.
Using TEK-3
m urea buffer the EBPs were 3.6 ± 0.04 S and 4.6 ± 0.09 S with moderate (~25 to 50%) reversion of the larger EBP to the smaller EBP (Fig. 1B).

Sedimentation Behavior in Urea—Sucrose gradient analysis of the two forms of the estrogen receptor (4 S EBP and 5 S EBP) in the presence of urea showed decreased sedimentation coefficients, while gel chromatography using identical urea solutions showed an increase in their molecular radii; urea has altered the con-
formations brought about by heating the estrogen receptor without es-
tradiol. Incubation of the cytosol at 28° for 30 min in the absence of
the estradiol, followed by [3H]estradiol at 0° for 30 min, showed
insignificant transformation of 4 S to 5 S EBP during warming, as previously reported by Jensen et al. (13). Sephadex G-200 chromatography provided identical results: with prior incubation at 28° before the addition of the [3H]estradiol, the estrogen re-
ceptor remained predominantly the 4 A EBP (i.e. 4 S) with only a trace of the 5.5 A EBP (i.e. 5 S) (Fig. 2, Curve C). In several experiments, not illustrated, a greater fraction of the labeled EBP appeared in the void volume, presumably due to aggregation brought about by heating the estrogen receptor without es-
tradiol.

The addition of 10% sucrose to the TEK buffer, the increase
Experimental data obtained with or without urea can be made. With minor changes in buffer pH, the sedimentation coefficient can be significantly greater.

In KCl concentration to 1 M, or the dilution of the cytosol samples did not change the elution behavior of the two EBPs during Sephadex G-200 chromatography. This suggests that the sedimentation determination of the estrogen receptor in sucrose gradients was not altered by the sucrose, that the elution positions of the 4 S and 5 S EBPs from the Sephadex G-200 in 0.4 M KCl were not due to a differential retention of the proteins by the gel, or that a concentration-dependent association of proteins was not occurring on gel chromatography.

Gel Chromatography with 3 M Urea—Sephadex G-200 chromatography with TEK-3 M urea buffer revealed that the 4 S and 5 S EBPs were eluted as separable proteins distinguished by a marked increase in their molecular radii. Upon removal of the 3 M urea the 4 S and 5 S EBPs reassumed their original molecular parameters: the 4 S EBP was eluted with a $V_v$ of 1.40 (Fig. 4A, Curve b) and a molecular Stokes radius of 53.8 ± 0.9 A (Fig. 3B). Peak b, after dialysis with TEK buffer and concentration by ultrafiltration, revealed an EBP on sucrose gradient analysis with a sedimentation coefficient of 4.4 S (Fig. 4B, Curve b). Sephadex G-200 chromatography with TEK-3 M urea buffer of the 5 S EBP indicated a $V_v$ of 1.16 (Fig. 4A, Curve a) and a molecular Stokes radius of 70.6 ± 1.0 A (Fig. 3D). Peak a, after dialysis and concentration, showed an EBP with a sedimentation coefficient of 5.5 S (Fig. 4B, Curve a).

Effects of Urea on Estimations of Molecular Parameters—It was important to determine whether the effects of urea on the estrogen receptor were caused by the increased density or denaturing activity of the urea, or by alteration of the conformational state, or dissociation of the estrogen receptor, or both. In contrast to the estrogen receptor, various protein standards showed excellent agreement in their sedimentation behavior and distribution coefficients in chromatographic experiments in the absence or presence of 3 M urea. The molecular parameters of the protein standards were invariably with or without urea; under identical conditions the observed changes in the estrogen receptor's molecular parameters relative to the protein standards were significantly greater.

Several observations suggest that valid comparisons of the experimental data obtained with or without urea can be made. (a) All sucrose gradient analyses contained two or three internal protein standards which sedimented in a linear relationship, as described by Martin and Ames (24); estimates of sedimentation coefficients were reliable in linear sucrose gradients of 5 to 20%, 5 to 20% with 3 M urea, 10 to 30%, or 12 to 33%. The absolute distances of migration of the standards were a function of the density of the gradients, in the order: 5 to 20% < 5 to 20% + 3 M urea = 10 to 30% < 12 to 33% sucrose gradients (Fig. 3). (b) The distribution coefficients ($K_d$) of the protein standards in Sephadex G-200 chromatography with or without 3 M urea were identical, indicating that the 3 M urea did not alter the molecular Stokes radius (Fig. 3). (c) The alkaline phosphate standards, after sucrose gradient centrifugation with or without 3 M urea, showed identical enzymatic activities. (d) The [3H]estradiol-binding capacity of the estrogen receptor was not decreased in 1 to 3 M urea (Table I).

Effect of pH on Molecular Parameters of Estrogen Receptor—With minor changes in buffer pH, the sedimentation coefficient of the estrogen receptor was readily altered. These changes re-
flect conformational changes rather than significant changes in mass. Sucrose gradient analyses of the 4 S and 5 S EBPs with TEK buffer, pH 9.0, indicated the two EBPs were 3.9 S and 5.3 S (not illustrated); using HEK buffer, pH 6.8, the two EBPs were 4.7 ± 0.04 S and 5.6 ± 0.05 S (Fig. 6). During sucrose gradient analysis with HEK buffer the 5 S EBP showed less change in its sedimentation coefficient than the 4 S EBP. In addition, the 5 S EBP was observed to show a tendency to revert from the 5 S to the 4 S EBP on HEK gradients (Fig. 6; compare A and B). Sephadex G-200 chromatography in HEK buffer indicated that the 4 S EBP has a molecular Stokes radius of 43.2 ± 0.6 Å. The 5 S EBP on Sephadex G-200 chromatography with HEK buffer showed a [3H]estradiol-binding peak equivalent to a molecular Stokes radius of approximately 55 to 60 Å, but 20 to 50% of the radioactivity appeared in the void volume. Because the radioactivity in the void volume may indicate a tendency of the labeled EBP to aggregate under these conditions, the analysis of this 5 S EBP chromatography data was not included in Table II.

**Estimation of Molecular Weight of 4 S and 5 S EBP**—The 4 S EBP showed a decrease in its sedimentation coefficient from 4.7 S (in HEK gradients) to 3.6 S (in TEK-3 M urea gradients), while under identical buffer conditions a corresponding increase in its molecular Stokes radius from 43.2 to 53.8 Å was observed by gel chromatography. However, the molecular weight of the 4 S EBP (estimated with Equation 2) remained approximately 80,000 in each medium, indicating that the change occurred in the conformational state and not in the mass of the 4 S EBP. The large values of the frictional ratio (f/f0, estimated with Equation 3) indicated that the 4 S EBP is an asymmetrical protein whose shape increased to a more asymmetrical form with conditions (3 M urea) favoring a decreased sedimentation coefficient. The estimated molecular weight of the 5 S EBP was 133,000 (with or without 3 M urea), a 65% increase in molecular weight as compared to the 4 S EBP (≈80,000), Table II. The data suggest that the 5 S EBP increased its molecular weight by association with a second component or subunit of approximately 50,000. The calculation of the molecular weights of the 4 S and 5 S EBP with Equation 2 was based upon the assumption that 8 was 0.725 cm³ per g for both EBP species.

Since the exact partial specific volume (δ) of the 4 S and 5 S EBP was not known, we considered whether the difference in the estimated molecular weights of the 4 S EBP and 5 S EBP (90,000 versus 133,000) resulted from each having a different partial specific volume (δ) induced during the in vitro transformation (e.g. by acetylation, phosphorylation, peptide or lipid release, conformational changes, etc.). Direct estimations of the densities of the labeled 4 S and 5 S EBP, as well as theoretical considerations suggest that this was extremely improbable and support the concept of an increase in mass as a result of an association of two subunits. The sedimentation rates of the 4 S and 5 S EBPs in very dense sucrose gradients (28 to 50%) for 20, 60, and 90 hours showed no change with time, as compared to each other, or to the protein standards, thus indicating that the densities of the 4 S and 5 S EBP were not discernibly different. Similarly, samples of the 4 S or 5 S EBPs layered on a 50% (w/v) sucrose solution in TEK buffer (density of 1.238 g per cm³ at 0°) or on a 75% (w/v) sucrose solution in TEK buffer (density of 1.389 g per cm³ at 0°) and centrifuged for 36 hours showed no difference in their migration into the dense sucrose solutions. The 4 S and 5 S EBPs each migrated 0.7 ml below the meniscus in the 50% sucrose solution and showed no migration below the meniscus in the 75% sucrose solution. These experiments suggest that the 4 S and 5 S EBPs have densities more than 1.338 g per cm³, corresponding to a δ of 0.808 cm³ per g but not less than a density of 1.339 g per cm³, or a δ of 0.720. The partial specific volume (δ) for known proteins is generally found within the narrow range of 0.69 to 0.78 cm³ per g, with lipoproteins having the higher and glycoproteins the lower δ values (29, 31). For one to assume

**Table II**

| Buffer          | Sedimentation coefficient (S) | Vv/Vo | Distribution coefficient (Kd) | Stokes molecular radius (Å) | Molecular weight | Frictional coefficient (f/f0) | Axial ratio |
|-----------------|------------------------------|-------|-------------------------------|-----------------------------|------------------|-------------------------------|------------|
| HEK             | 4.7 ± 0.04                   | 1.56 ± 0.02 | 0.34 ± 0.006                  | 43.2 ± 0.6                  | 83,700           | 1.38                          | 7.1        |
| TEK             | 4.2 ± 0.04                   | 1.50 ± 0.02 | 0.27 ± 0.004                  | 44.0 ± 0.4                  | 76,200           | 1.45                          | 8.3        |
| TEK-3 M urea    | 3.6 ± 0.04                   | 1.40 ± 0.03 | 0.17 ± 0.007                  | 53.8 ± 0.9                  | 79,900           | 1.75                          | 14.1       |
| TEK             | 5.5 ± 0.02                   | 1.30 ± 0.005 | 0.14 ± 0.004                  | 65.8 ± 0.5                  | 123,700          | 1.60                          | 11.1       |
| TEK-3 M urea    | 4.6 ± 0.09                   | 1.16 ± 0.01 | 0.05 ± 0.009                  | 70.6 ± 1.0                  | 133,900          | 1.93                          | 18.4       |

a Experimental values are given as mean ± standard error of the mean of 8 to 30 determinations.

b Distribution coefficient by Sephadex G-200 as determined with Equation 1.

c Determined as described in Fig. 3.
d Determined with Equation 2 using mean values of S and a, and assuming δ of 0.725 cm³ per g.
e Determined with Equation 3, δ is assumed to be 0.2 g of solvent per g of protein.
f Calculated for prolate ellipsoids (37).
that the apparent increase in the molecular weight of the 5 S EBP was solely due to a change in $\tilde{v}$ during the transformation process, a 20 to 30% difference between the $\tilde{v}$ values of the 4 S and 5 S EBPs would have been necessary. Such a marked difference in the $\tilde{v}$ values of the 4 S and 5 S EBPs, extending beyond the normal range of $\tilde{v}$, would have readily been revealed by their sedimentation behavior in high density sucrose. The difference in the sedimentation and chromatography analyses of the two EBPs cannot be accounted for by a difference in the molecular parameters which contribute to their partial specific volumes.

**Conditions for Reversal of 5 S EBP to 4 S EBP**—Incubation of the 5 S EBP in 0.4 M KCl, 3 M urea, and 40 mM Hepes, pH 6.8, readily causes its dissociation to the 4 S EBP without a loss of [H]estradiol-binding activity. Any one of these reagents alone, even after 24-hour incubation, is not sufficient to produce a significant reversal of the labeled 5 S EBP to the 4 S EBP (Fig. 7). Gel chromatography of the EBP that results from incubation of 5 S EBP in 0.4 M KCl, 3 M urea, and 40 mM Hepes, pH 6.8, confirmed that the molecular Stokes radius was 44 A, a value identical with that of the 4 S EBP, and indicated that dissociation rather than a unique conformational change in the 5 S EBP had occurred. The rate of dissociation of the 5 S EBP during incubation with 0.4 M KCl, 3 M urea, and 40 mM Hepes, pH 6.8, was measured by sucrose gradient analysis. The initial dissociation process was strictly first order with a half-life of 5 hours (Fig. 8).

**DISCUSSION**

Measurements of the estrogen receptor with gel chromatography and sucrose gradient centrifugation under carefully controlled and standardized conditions have provided highly reproducible data that show a molecular relationship between the 4 S EBP and 5 S EBP forms of the receptor. The estrogen receptor can readily assume a range of sedimentation coefficients (3.6 S to 5.5 S, Table II) in the presence of high ionic strength buffers as a result of two different and separable processes. (a) The 4 S to 5 S EBP transformation of the estrogen receptor is an association process requiring estradiol and temperature (28°); we have also described the dissociation from the 5 S to the 4 S EBP (Figs. 7 and 8). (b) The estrogen receptor, i.e. the 4 S or 5 S EBP species, is capable of assuming different conformational states when observed with urea or with changes in pH.

The estimated molecular weight of the 4 S EBP is 80,000 and that of the 5 S EBP is 133,000. The 4 S to 5 S transformation must result from association of the 4 S EBP and a second component or subunit of approximately 50,000, not simply from a conformational change of the 4 S EBP. The analysis of the 4 S and 5 S EBPs in urea or HEK buffer indicates that the molecular weight of each EBP is relatively constant within the range of experimental error, although their sedimentation or chromatographic behaviors are readily altered as a result of conformational changes. Numerous investigations (32-34) using purified or crude preparations have shown that sedimentation coefficients and molecular Stokes radii measurements provide molecular parameter data comparable in accuracy to standard methods, e.g. sedimentation equilibrium studies of purified proteins.

A kinetic analysis of the 4 S to 5 S EBP transformation has shown that it is a second order process, consistent with a bi-molecular association model indicated by this report. The necessity of increasing the temperature to observe an association reaction between the 4 S EBP and its subunit indicates that it is an endothermic process. This is consistent with the observation that the estrogen receptor uptake by the nucleus is a temperature-dependent process (2, 3).

The molecular characterization of the 4 S and 5 S EBPs permits proposing a number of models for the molecular events involved in the 4 S to 5 S transformation and excludes others. (a) The subunits of the 5 S EBP, i.e. the 4 S EBP and its 50,000 molecular weight component, are usually weakly associated in dilute or isotonic buffers. Upon [H]estradiol binding and at an elevated temperature (28°), an increased avidity between these two subunits occurs as a result of a conformational change; with sucrose gradient centrifugation in 0.4 M KCl the 5 S EBP is observed intact and is presumably the active form of the estrogen receptor. In the experiments where the estrogen receptor binds estradiol but does not undergo a temperature-dependent...
conformational change, i.e. at 0°, using sucrose gradient centrifugation in the presence of 0.4 M KCl, a 4 S EBP is observed. Although the dissociation of the estrogen receptor by 0.4 M KCl and the appearance of the 4 S EBP are experimentally induced, the dissociation suggests that an "inactive" form of the estrogen receptor was present (Fig. 9). (b) In a simpler sequential model for the molecular events involved in the 4 S to 5 S transformation, the 4 S EBP forms a complex with estradiol and at 25° undergoes a conformational change leading to its "activation." The "activated" 4 S EBP by a random process associates with another specific protein. (c) An alternative possibility is that the temperature-"activated" 4 S EBP is the active or functional form of the estrogen receptor and that its association with another protein to form the 5 S EBP is purely a fortuitous experimental reflection of the fact that the conformation change and "activation" of the 4 S EBP have taken place.

Recently, Stancel et al. (19) have reported that the isolated nuclear 5 S and the cytoplasmic 4 S estrogen receptor could be dissociated by using 4 M urea, 1 M KCl, 50 mM mercaptoethanol, and 50 mM NaHSO4, to a common 3 S estrogen-binding protein. In agreement with these investigators, our results have shown that the 5 S EBP, produced by the in vitro transformation method, can be dissociated to the 4 S EBP by the addition of 3 M urea, 0.4 M KCl, and 40 mM Hepes, pH 6.8. Under the experimental condition of this study, gel chromatography and sucrose gradient analyses indicate that the 4 S EBP did not dissociate to a 3 S EBP as reported by Stancel et al. (19). We have observed that the 4 S is capable of assuming a lower sedimentation coefficient, but only as a result of a reversible conformational change.

The relationship of the 4 S or 5 S EBP observed using sucrose gradient analysis in high ionic strength buffers to the 8 S estrogen receptor observed with buffers of low ionic strength has not been elucidated here. This report has shown that the structure of the estrogen receptor is capable of assuming different conformational states, and raises the possible speculation that the 8 S EBP is the 8 S form of the estrogen receptor after it has undergone a conformational change due to the low ionic strength buffer. If one assumes that the molecular weight (~133,000) of the 5 S EBP is not altered, but its sedimentation coefficient is increased to 8 S, then the molecular Stokes radius must decrease to approximately 40 A radius. A 40 A radius would give the 8 S estrogen receptor a frictional coefficient (f/f0) of 1.2, typical of most globular proteins (29). However, molecular analysis of the 8 S estrogen receptor will not be possible until the conditions are found which will prevent the estrogen receptor from aggregating during gel chromatography with dilute buffers, or until the estrogen receptor has been purified sufficiently to use other methods. Another possible explanation may be that the 8 S form of the estrogen receptor is a dimer of the 5 S EBP that is formed in low ionic strength buffers.

A previous report from this laboratory (35) has shown the existence of a human uterine protease that is capable of "transformation" the 8 S estrogen receptor to a 4.5 S EBP. This was observed in sucrose gradients without KCl. The 4.5 S EBP produced by the uterine protease has a molecular Stokes radius of 31 A, a molecular weight of 60,400, and a frictional coefficient (f/f0) of 1.2. Puca et al. (15) have reported the presence in calf uteri of a "Ca2+-activated transforming factor" that appears to produce a 4.5 S EBP with molecular properties similar to that produced by the action of the human uterine protease. The molecular mechanism of the "Ca2+-activated transforming factor" has not been described. It is evident that the "transformation" produced by the uterine protease yields a 4.5 S EBP (~60,400 mol wt) observed using sucrose gradients with low ionic strength buffers, which is different in its molecular parameters and behavior from the salt-dissociated 4.2 S EBP (~80,000 mol wt) described here. The action of the uterine protease is inhibited by disopropyl fluorophosphate (35), whereas the 4 S to 5 S EBP transformation is not, further indicating the dissimilarity of the two processes.

The in vitro induced 4 S to 5 S EBP transformation produces a 5 S EBP that is similar to the in vivo form of the nuclear 5 S EBP (13, 36). Recently, the in vitro induced 5 S and the isolated nuclear 5 S EBP have been reported to activate RNA polymerase, while the 4 S EBP does not (9). These measurements of the molecular relationship between the two EBP, their conformational changes, and protein-protein interactions may provide an insight into the molecular mechanisms involved in the activation of the estrogen receptor.

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