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Biochemistry

Estradiol receptor has proteolytic activity that is responsible for its own transformation

(serine protease/aprotinin/steroid hormone action)

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ABSTRACT **We have investigated the effect of various protease inhibitors and substrates on the hormone- and temperature-dependent binding of partially purified estradiol–receptor complex to isolated nuclei. Only serine protease substrates and inhibitors significantly depressed estradiol receptor transformation. At 20°C, we observed 50% inhibition with about 3 μM aprotinin or with 1.4 mM diisopropyl fluorophosphate. Aprotinin also blocked those size and charge modifications of receptor that are characteristic of the transformation process. The estradiol receptor was able to bind to aprotinin-agarose only under transforming conditions; i.e., the interaction was hormone- and temperature-dependent and inhibited by molybdate. Diisopropyl fluorophosphate, a covalent reagent for serine esterases, competitively inhibited the binding and specifically eluted the estradiol–receptor complex that had been bound to aprotinin-agarose. These results indicate that estradiol receptor transformation is due to the effect of a serine protease and that the receptor itself is endowed with this catalytic activity, which is triggered by the steroid.**

Transformation of steroid receptors has been widely studied in cell-free systems and has been defined as that process which confers affinity to the steroid–receptor complex for nuclei and DNA. In all systems so far investigated, transformation is accompanied by a decrease in the molecular size and the net electric charge of the receptor. Receptor transformation is an irreversible first-order reaction, inhibited by millimolar concentration of molybdate, and is triggered by the specific steroid (for review, see refs. 1–3). Evidence for the physiological role of this reaction (4–7) supports the theory that transformation is the critical step in steroid hormone action. Numerous mechanisms have been proposed to explain the molecular basis of this event, but none has been definitively accepted.

We report here that the receptor-transforming activity of exogenous alkaline phosphatase is due to a contaminating proteolytic activity of the enzyme preparation, that serine protease inhibitors and substrates impair transformation of the estradiol receptor, and that estradiol receptor acquires upon transformation a serine binding site for aprotinin. Therefore, we suggest that estradiol receptor transformation is due to the action of a serine protease activated by the hormone and that the catalytic protein is the hormone receptor itself.

MATERIALS AND METHODS

[2,4,6,7-^{3}H]Estradiol-17β (84–111 Ci/mmol; 1 Ci = 37 GBq) was from New England Nuclear. Nonradioactive estradiol-17β was from Calbiochem. Antipain dihydrochloride, soybean trypsin inhibitor (SBTI), pepstatin A, trypsin inhibitor type II-0 (ovomucoid) from chicken egg white, leupeptin hemisulfate, α_1-antitrypsin, tryptophan methyl ester (Trp-OMe), phosphoramidone, phenylmethylsulfonyl fluoride, aprotinin, calf intestinal alkaline phosphatase types I-S and VII-S, and *p*-nitrophenyl phosphate were from Sigma. *N*-benzoyl-L-arginine methyl ester (Bz-Arg-OMe), diisopropyl fluorophosphate (iPr_2*P*-F), and *N*-benzoyl-L-tyrosinamide were from Serva (Heidelberg). *N*-benzoyl-L-arginine ethyl ester (Bz-Arg-OEt) was from Becton Dickinson. Sodium molybdate was from Baker. DEAE-cellulose (DE-52) was from Whatman. Superose 6B and Sepharose 4B-CL came from Pharmacia. Values of molecular weight (M_r) and Stokes radius of standard proteins were from the literature (8).

The following buffers were used: (A) 7.5 mM phosphate buffer, pH 7.4 at 20°C, containing 1 mM dithioerythritol, 2 mM EGTA, and additional 10 mM molybdate, as specified; (B) 50 mM phosphate buffer, pH 7.4 at 20°C, containing 1 mM dithioerythritol, 2 mM EGTA, 10 mM molybdate, and additional salts as specified; (C) 7.5 mM phosphate buffer, pH 7.4 at 20°C, containing 250 mM sucrose, 25 mM KCl, 1 mM dithioerythritol, and 0.2 mM phenylmethylsulfonyl fluoride.

Frozen (−70°C) calf uteri were used for cytosol preparation. Tissue was homogenized by an Ultraturrax homogenizer (Janke and Kunkel, TP 18/10) in 3 volumes of buffer B. The homogenate was centrifuged at 105,000 × *g* for 60 min, and the supernatant was collected. Estradiol-binding activity of cytosol and of partially purified receptor was measured by the dextran-coated charcoal (DCC) method (9). Nonspecific binding was determined by parallel incubation in the presence of a 300-fold excess of unlabeled estradiol.

Estradiol receptor was partially purified by DEAE-cellulose chromatography. Cytosol (200 ml), prepared in buffer B plus 50 mM KCl, was incubated with 30 g of DEAE-cellulose preequilibrated in the same buffer. After 60 min of agitation at 4°C, the slurry was packed in a column (internal diameter, 2 cm) and washed with 150 ml of the equilibration buffer, and bound material was eluted with a 100-ml linear gradient of 50–400 mM KCl in the same buffer. Fractions of 3 ml were collected. Specific estradiol-binding activity in aliquots of each fraction was measured, and the peak fractions of estradiol-binding activity were combined and stored at 4°C after dialysis in buffer A. Protein concentrations of such pools varied from 2 to 3 mg/ml, and estradiol-binding activity from 80,000 to 120,000 cpm/0.1 ml. Recovery of the receptor varied between 40% and 60% of the original binding activity of the cytosol. The continuous presence of 10 mM molybdate and 50 mM phosphate, together with the absence of estradiol, was necessary to decrease spontaneous modifications at 0°C.

Estradiol receptor partially purified by DEAE-cellulose chromatography, which in a sucrose gradient settled at 7.8 S, was eluted as a sharp peak (identically to the cytosolic receptor) from a calibrated Superose 6B column (see *Results*). From a calibration plot of M_r of standard proteins vs.

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Abbreviations: SBTI, soybean trypsin inhibitor; iPr_2*P*-F, diisopropyl fluorophosphate; DCC, dextran-coated charcoal.

elution volumes, the molecular weight of the 7.8S receptor was found to vary from 215,000 to 250,000 (Fig. 4 *Inset*), very similar to the value that can be calculated from the Stokes radius (66.83 ± 1.83 Å; $n = 6$) and sedimentation coefficient (8). This partially purified estradiol receptor is called 8S receptor in the following text. Incubation of 8S receptor at 20°C in buffer A in the presence of estradiol, but not in its absence, induced a decrease in the high M_r complex and the appearance of smaller species, with the predominance of a molecule of apparent M_r 75,000 (Stokes radius = 39.8 ± 0.84 Å; $n = 4$). A shoulder between the two peaks, corresponding to an apparent M_r of 120,000 (Stokes radius = 49 ± 2 Å; $n = 4$) was consistently present. When the nuclei were incubated at 0°C with the preheated estradiol–receptor complex and then centrifuged, the amount of M_r 240,000 component in the supernatant remained unchanged, while the M_r 120,000 and smaller components decreased and could be extracted quantitatively from nuclei with buffer B containing 400 mM KCl. Incubation of 8S receptor with nuclei and hormone in the buffer used for the nuclear extraction did not modify the elution of the estradiol–receptor complex. Analogously, we have reported (10) the estradiol-dependent appearance at 20°C of modified hormone–receptor complexes that were eluted from DEAE-cellulose at lower salt concentration and that were the only forms able to bind to nuclei at 0°C. These more basic forms were also extracted from the uterine nuclei of rats injected with physiological doses of estradiol (11).

Isolated nuclei were prepared (12) from the crude nuclear pellet of calf uterus in buffer C. Concentration of DNA was determined (13) and adjusted to 1.2 mg/ml with the buffer. Nuclei, which were stored in 2- to 3-ml aliquots at −70°C, were thawed to 4°C at the beginning of the experiment. Binding of the [^{3}H]estradiol-8S receptor complex to nuclei (0.1 ml, 120 μg of DNA) was assayed in duplicate. The binding of the complex was time-, temperature-, and estradiol-dependent and was blocked by 10 mM molybdate. Nonspecific binding, obtained by incubating unheated [^{3}H]-estradiol–8S receptor complex with nuclei, was subtracted from values obtained. At the end of the incubation, the nuclei were pelleted in a swinging bucket rotor at 3000 × g for 10 min and washed three times with 4 ml of buffer C. The pellet was resuspended in 1 ml of the same buffer for measurement of radioactivity by liquid scintillation counting.

Aprotinin, SBTI, ovomucoid, and protamine were coupled to CNBr-activated Sepharose CL-4B in 25 mM phosphate buffer (pH 7.6) according to Cuatrecasas (14). Under our conditions, 330 μg of aprotinin and 3 mg of SBTI, ovomucoid, or protamine were covalently bound per g of agarose.

RESULTS

Effect of Exogenous Alkaline Phosphatase on Transformation of Estradiol Receptor Is Not Due to Phosphatase Activity. Analogous to what was described for the glucocorticoid receptor system (15, 16), commercial calf intestinal alkaline phosphatase (type I-S) added to uterine cytosol or to partially purified 8S estradiol receptor induces changes of the binding molecule similar to those observed when cytosol or partially purified 8S receptor are incubated at 20°C in the presence of estradiol; i.e., it decreases the sedimentation of the ^{3}H-containing peak from 8 to 4 S and the KCl concentration required to elute receptor from DEAE-cellulose from 200 mM to 50 mM. The effect of this enzyme occurs at 0°C, but not at higher temperature, and does not require estradiol (data not shown). We assayed *in vitro* nuclear binding of 8S receptor at 0°C in the presence of various concentrations of Sigma type I-S alkaline phosphatase (Fig. 1*A*). The greatest nuclear binding corresponded to 60% of the binding obtained by heating the 8S receptor in the presence of estradiol for 30 min at 20°C (17,393 cpm). This suggested that the transforming activity of exogenous alkaline phosphatase might not be due to the phosphatase activity of the enzyme. This conjecture became certainty when we used the Sigma type VII-S enzyme, which has a much higher specific activity than type I-S. Even though the specific activity of the type VII-S enzyme toward the synthetic substrate *p*-nitrophenyl phosphate was 163-fold greater, we had to use 20-fold more enzyme to obtain substantial transformation. A very high, nonphysiological concentration of exogenous calf intestinal alkaline phosphatase was required to enhance transformation of the glucocorticoid receptor (15). Superose 6B chromatography of the low-specific-activity, type I-S enzyme (Fig. 1*B*) showed that the transforming activity was separable from the phosphatase activity. No RNase, DNase, phosphoprotein phosphatase, or phosphodiesterase activity corresponded to the transforming-activity peak. However, caseinolytic activity was coincident with transforming activity (data not shown). The transforming activity of calf intestinal alkaline phosphatase was stimulated by SBTI (100 μg/ml), partially inhibited by 5 mM Trp-OMe, and unaffected by leupeptin,

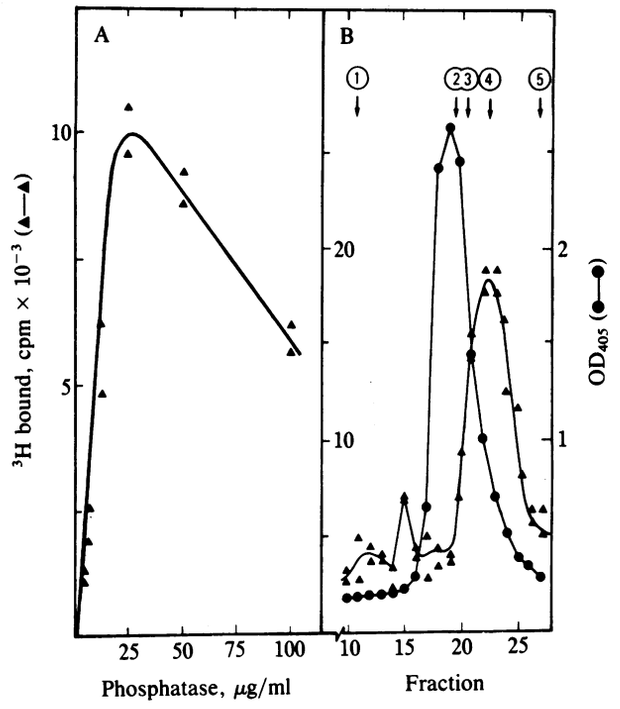

FIG. 1. (*A*) Nuclear binding of [^{3}H]estradiol–8S receptor complex in the presence of alkaline phosphatase. Aliquots of 8S receptor (binding capacity 95,000 cpm) were incubated in duplicate at 0°C with various concentrations of Sigma type I-S alkaline phosphatase, in a final volume of 0.8 ml in buffer A, containing 5 nM [^{3}H]estradiol. After 90 min, nuclei (120 μg of DNA) were added and incubation continued for an additional 60 min at 0°C. Nuclei were pelleted in a swinging bucket rotor at 3000 × g for 10 min and washed three times with 4 ml of buffer C. The final pellet was resuspended in 1 ml of buffer C and assayed for radioactivity. (*B*) Superose 6B chromatography of phosphatase and receptor-transforming activities of calf intestinal alkaline phosphatase. Sigma type I-S alkaline phosphatase (3 mg) was dissolved in 0.5 ml of buffer B and applied to a calibrated column (18.5 × 1.6 cm) of Superose 6B. The column was developed with buffer B, and 1.3-ml fractions were collected and analyzed for phosphatase activity, using *p*-nitrophenyl phosphate as substrate (●). Estradiol receptor-transforming activity (▲) was determined utilizing 8S receptor (binding capacity 156,000 cpm) incubated in duplicate at 0°C with 0.1 ml of each fraction in a final volume of 0.8 ml in buffer A containing 5 nM [^{3}H]estradiol. After 60 min, nuclei were added and incubation was continued for an additional 60 min at 0°C. [^{3}H]Estradiol–8S receptor complex bound to nuclei was measured as described for *A*. Arrows: 1, void volume; 2–4, elution volumes of bovine plasma albumin, ovalbumin, and myoglobin, respectively; 5, internal volume of the column.

pepstatin, or aprotinin (all at 100 μg/ml). This suggested that a chymotrypsin-like enzyme destroyed by a trypsin-like enzyme could be responsible for transformation. The coincidence of caseinolytic activity and the transforming activity of exogenous alkaline phosphatase was interesting, since the product of the exogenous phosphatase was structurally (for size and charge) and functionally (being able to bind to nuclei) similar to the product of the endogenous transforming system. We considered, therefore, that a specific protease might be involved in the hormone- and temperature-dependent transformation of the estradiol receptor.

Serine Proteinase Inhibitors and Substrates Inhibit Transformation of Estradiol Receptor, and Aprotinin Blocks Modifications Correlated with Transformation. None of the biologically active peptides we tried (antipain, pepstatin A, phosphoramidone, and leupeptin) had any effect either on the transformation of the 8S estradiol receptor, or on the binding of estradiol to the receptor, or on the nuclear binding of the transformed estradiol–receptor complex. Among the protease substrates, Trp-OMe, but not *N*-benzoyl-L-tyrosinamide, effectively reduced the nuclear binding of the estradiol–receptor complex if it was present during the 20°C preincubation; it did not work if added after the preincubation (Fig. 2*A*). Trp-OMe also affected the estradiol binding capacity of the receptor, but less than it did nuclear binding (Fig. 2*B*). Bz-Arg-OMe and Bz-Arg-OEt behaved similarly, although they were less effective. SBTI (60–600 μg/ml), ovomucoid (60 μg/ml), and α_1-antitrypsin (60 μg/ml) were without effect. When present during the 20°C preincubation, aprotinin (50 μg/ml) almost completely inhibited the nuclear binding of the estradiol–receptor complex. The inhibition was concentration-dependent and did not occur when aprotinin was added after the preincubation (Fig. 3). Fifty percent inhibition of transformation at 20°C occurred at about 3 μM aprotinin ($M_r \approx 6500$). Temperature and salt concentration had a striking effect on inhibition by aprotinin. Aprotinin was not effective under conditions where the transformation reaction was faster (i.e., at higher temperature or higher salt molarity). iPr_2P-F also inhibited transformation when present during the 20°C preincubation, but not when added after, giving 50% inhibition at 1.4 mM. Aprotinin blocked the hormone- and temperature-induced size changes (Fig. 4, closed circles) when incubation was carried out in the absence of salt, but not when transformation was stimulated by the presence of 0.15 M KCl (Fig. 4, open circles). At 3 mM, iPr_2P-F impeded the conversion of the M_r 240,000 native receptor into the lower M_r forms (data not shown). DEAE-cellulose chromatography showed that in the presence of aprotinin or iPr_2P-F, the temperature- and estradiol-dependent appearance of more basic estradiol–receptor complexes did not occur (data not shown).

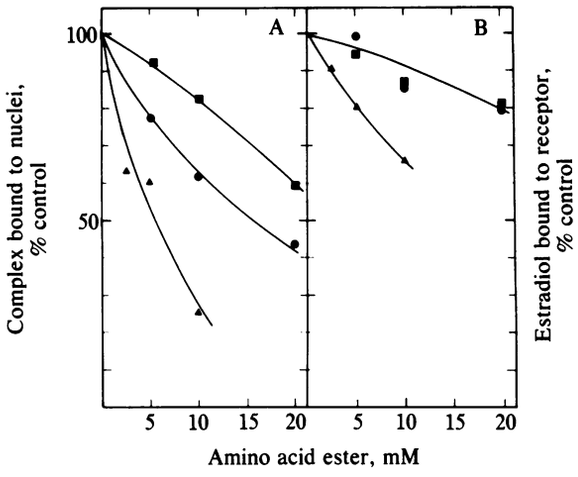

FIG. 2. Effect of amino acid esters on estradiol receptor transformation and estradiol-binding activity. (*A*) Aliquots of 8S receptor (binding capacity 76,000 cpm) were incubated in duplicate at 20°C in the presence or absence of Trp-OMe (▲), Bz-Arg-OMe (■), or Bz-Arg-OEt (●) in a final volume of 0.8 ml in buffer A containing 5 nM [^{3}H]estradiol. After 60 min, the samples were cooled to 0°C, nuclei were added, and incubation continued for an additional 60 min at 0°C. If amino acid esters were added after the incubation at 20°C, there was no effect on the nuclear binding of estradiol–receptor complex. Nuclear bound radioactivity measured as described in legend to Fig. 1, is presented as percentage of radioactivity (20,350 cpm) obtained in the absence of the esters. (*B*) Specific estradiol binding activity in the presence of Trp-OMe (▲), Bz-Arg-OMe (■), or Bz-Arg-OEt (●) was measured by incubating 60 min at 20°C and another 60 min at 0°C. Values are expressed as percentage of the radioactivity (76,000 cpm) obtained in the absence of the esters.

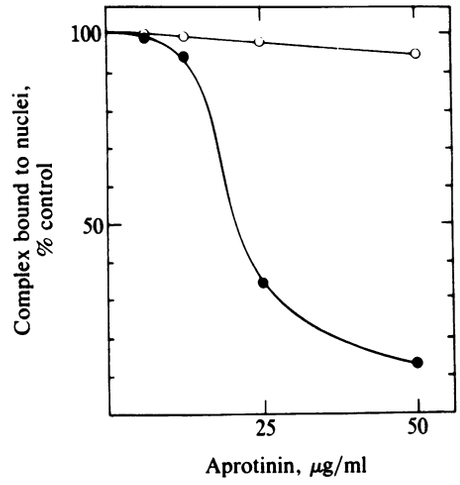

FIG. 3. Aprotinin inhibits estradiol receptor transformation. Aliquots of 8S receptor (binding capacity 110,000 cpm) were incubated in duplicate in a final volume of 0.8 ml in buffer A containing 5 nM [^{3}H]estradiol in the presence (●) or absence (○) of aprotinin as indicated. After 30 min at 20°C, the samples were cooled to 0°C, nuclei were added, aprotinin as indicated was added to those samples (○) that had been preincubated in its absence, and incubation was continued for an additional 60 min at 0°C. Nuclear bound [^{3}H]-estradiol–8S receptor complex was measured and is presented as percentage of radioactivity (33,280 cpm) obtained in the absence of aprotinin.

Under Transforming Conditions, Estradiol Receptor Acquires a Serine Binding Site for Aprotinin. The estradiol–receptor complex was able to bind to aprotinin-agarose at 20°C but not at 0°C (Fig. 5*A*), unless it was preincubated at 20°C. The binding did not occur in the presence of 10 mM molybdate (Fig. 5*A*) but was accelerated by the presence of estradiol (Fig. 5*B*). Addition of molybdate after the 20°C preincubation had no effect (data not shown). Thus, the receptor recognizes aprotinin only after transformation. No binding of estradiol receptor to SBTI-agarose or to ovomucoid-agarose was detected, even though agarose substitution was 10-fold greater. Although protamine-agarose did bind estradiol receptor, this interaction was neither dependent on estradiol and temperature nor inhibited by 10 mM molybdate. iPr_2P-F inhibited the interaction between the estradiol receptor and aprotinin-agarose in a concentration-dependent (Fig. 6*A*) and competitive manner (Fig. 6*B*). Further, 10 mM iPr_2P-F was able to elute specifically the transformed estradiol–receptor complex that had been bound to aprotinin-agarose (Fig. 6 *Inset*). This indicates that the binding of transformed estradiol–receptor complex to aprotinin-agarose occurs through a site containing an active serine residue.

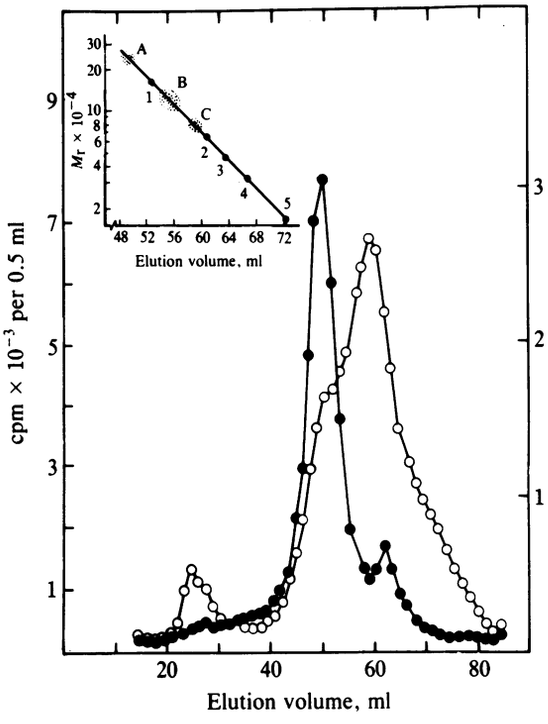

FIG. 4. Superose 6B chromatography of [^{3}H]estradiol–8S receptor complex incubated at 20°C in the presence of aprotinin plus or minus KCl. Aliquots of 8S receptor (binding capacity 977,760 cpm) were incubated at 20°C with 5 nM [^{3}H]estradiol in the presence of aprotinin (50 μg/ml) with (●) or without (○) 150 mM KCl in a final volume of 3 ml in buffer A. After 30 min, the samples were cooled to 0°C and incubation continued for an additional 30 min. Free hormone was eliminated by the DCC method (9). Samples were applied consecutively to a calibrated column (17.5 × 2.6 cm) of Superose 6B equilibrated in buffer B containing 200 mM KCl and were eluted with the same buffer. Fractions (1.45 ml) were collected and analyzed for radioactivity. (*Inset*) M_r vs. elution volumes: 1, human gamma globulin; 2, bovine plasma albumin; 3, ovalbumin; 4, half-mero hemoglobin; 5, myoglobin. Stippled areas represent untransformed receptor (A), intermediate shoulder (B), and transformed receptor (C) (see *Materials and Methods*). Void volume of the column was 24.9 ml; internal volume was 87.6 ml. Upward flow rate of 33 ml/hr was obtained with a peristaltic pump.

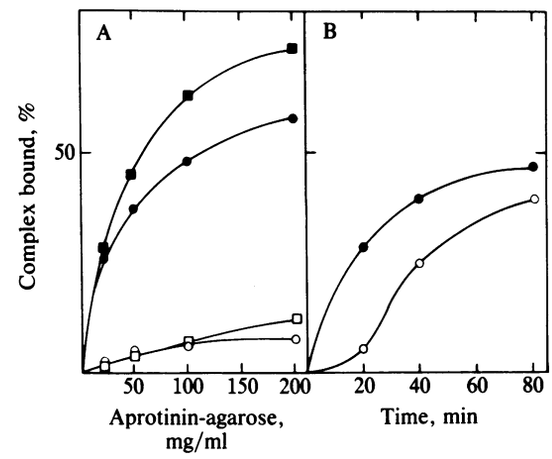

FIG. 5. (*A*) Effect of temperature on the binding of [^{3}H]estradiol–receptor complex to aprotinin-agarose. Aliquots of 8S receptor (binding capacity 120,000 cpm) were incubated with 5 nM [^{3}H]estradiol in the presence of various concentrations of aprotinin-agarose at 0°C (○), at 20°C (■), and at 20°C in the presence of 10 mM molybdate (□) in a final volume of 1 ml in buffer A containing 100 mM KCl. After 3 hr, the samples were cooled to 0°C and centrifuged at 3000 × *g* for 10 min, and the radioactivity in the supernatant was determined after elimination of free hormone by the DCC method. A parallel experiment was performed by incubating the 8S receptor with 5 nM [^{3}H]estradiol for 30 min at 20°C. The sample was then cooled to 0°C, and binding to various concentrations of aprotinin-agarose was determined after 60 min at 0°C (●). (*B*) Effect of estradiol on the binding of 8S receptor to aprotinin-agarose. Aliquots of 8S receptor (binding capacity 106,900 cpm) were incubated at 20°C with (●) or without (○) 5 nM [^{3}H]estradiol in a final volume of 1 ml of buffer A containing 100 mM KCl in the presence of 25 mg of aprotinin-agarose. At the indicated times, samples were cooled to 0°C and centrifuged at 3000 × *g* for 10 min. Supernatants were collected, and samples without estradiol were incubated with 5 nM [^{3}H]estradiol at 0°C for 90 min. Bound radioactivity, determined after elimination of free hormone by the DCC method, is expressed as percentage of the total [^{3}H]estradiol-8S receptor complex added.

DISCUSSION

The molecular mechanism of transformation of the steroid hormone receptors that confers to the receptor the capacity to bind to the nuclei is not understood. Several reports (17–19) have suggested that a dephosphorylation reaction is involved, since some inhibitors of phosphatase block transformation in cell-free systems. Steroid receptors have been shown to be phosphorylated proteins (20–22), and exogenous calf intestinal phosphatase was able to induce transformation (15, 16). Our results are not consistent with a dephosphorylation hypothesis for receptor transformation. The receptor transformation induced by the exogenous phosphatases appears to be due to a contaminating proteolytic enzyme rather than due to a dephosphorylation reaction.

It has been reported (23) that a serine protease(s) might be involved in the mechanism of activation of glucocorticoid–receptor complex into a nuclear form. The great sensitivity of steroid receptors to proteolytic attack is well-known (24). Even though limited proteolysis of receptor, due to endogenous or exogenous enzymes, may lead to functionally active forms capable of binding to nuclei or DNA (25–27), receptor proteolysis has been generally implicated in the degradation rather than in the transformation of receptor. One of the conceptual obstacles to accepting proteolysis as the activating step was the fact that no proteolytic enzyme so far studied (28–30) was able to distinguish between the occupied and the unoccupied receptor. Further, the role of nonreceptor molecules in the transformation process has been excluded repeatedly in recent publications. It has been reported that transformation can occur in highly purified receptor preparations (31, 32) and that the molecular weight of the steroid binding receptor subunit does not change upon transformation (31) or nuclear translocation (33, 34). The irreversibility of the transforming reaction may be explained by chemical changes that would render reassociation impossible.

Our results on the interaction of the transformed estradiol receptor with aprotinin and the effect of iPr_2P-F on this interaction indicate that a serine protease activity responsible for the transformation may be in the native receptor molecule itself. It is devoid of catalytic activity in the absence of the hormone but becomes activated as a consequence of the conformational change brought about by the hormone. The dissociation of the oligomeric native receptor would be a consequence and not the mechanism of this process, as it has been postulated (35). The initial, very specific cleavage of one or a few peptide bonds may also occur in a nonsteroid-binding component of the oligomeric native receptor (36) and thus render the process irreversible, without changing the molecular weight of the estradiol-binding subunit.

If the hormone is the allosteric effector of the serine protease responsible for the transformation process, it is necessary that the hormone binding site and the catalytic site be near each other (37). The effect of serine protease substrates and inhibitors on the hormone binding of all steroid receptors (38–41) and the analogy of the transforming

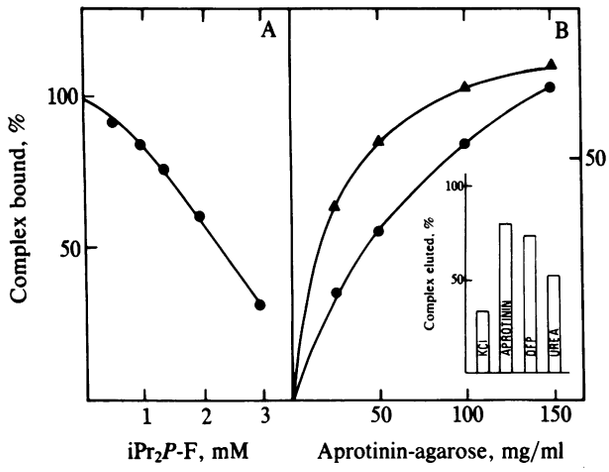

FIG. 6. iPr_2P-F inhibits interaction of estradiol receptor with aprotinin-agarose. (*A*) Aliquots of 8S receptor (binding capacity 112,000 cpm) were incubated with 5 nM [^{3}H]estradiol in a final volume of 1 ml in buffer A containing 100 mM KCl, 20 mg of aprotinin-agarose, and iPr_2P-F as indicated. After 60 min at 20°C, samples were cooled to 0°C and centrifuged at 3000 × *g* for 10 min. Supernatants were collected, and bound radioactivity was measured after elimination of free estradiol by the DCC method. Experimental values are presented as percentage of the amount of complex bound to aprotinin-agarose in the absence of iPr_2P-F. (*B*) In a parallel experiment aliquots (120,000 cpm) containing 8S receptor plus 5 nM [^{3}H]estradiol were incubated for 60 min at 20°C with various concentrations of aprotinin-agarose with (●) or without (▲) 1 mM iPr_2P-F in a final volume of 1 ml in buffer A containing 100 mM KCl. Values are expressed as percentage of the total ^{3}H added. (*Inset*) Elution of estradiol–receptor complex from aprotinin-agarose. 8S receptor (binding capacity 1.6×10^6 cpm) was incubated with 400 mg of aprotinin-agarose in a final volume of 5 ml in buffer A containing 0.4 M KCl and 5 nM [^{3}H]estradiol. After 80 min at 20°C, the sample was centrifuged at 3000 × *g* for 10 min. The agarose was washed by centrifugation two more times with the same buffer and then divided into four aliquots that were individually incubated for 15 min at 0°C with 2 ml of 2 M KCl, 3-mg/ml aprotinin solution, 10 mM iPr_2P-F (DFP), or 4 M urea. Radioactivity released in the supernatant by each of the eluents is expressed as percentage of the [^{3}H]estradiol-8S receptor complex bound to aprotinin-agarose (574,000 cpm).

process, both in terms of conditions (hormone requirement, effect of ionic strength, nucleotides, dilution, aging, molybdate, chelators, and sulfhydryl reagents) and in terms of molecular changes (altered mobility on DEAE-cellulose and decrease in size) (1–3), suggest a common mechanism for steroid receptor transformation. The fact that, until now, proteolysis has been proposed as a mechanism only for the glucocorticoid receptor (23) can be explained because the putative intrinsic proteolytic activity of the receptor may be highly specific and resistant to the common protease inhibitors. Aprotinin, which in our case is the best inhibitor, has no effect when used in crude cytosol or when transformation in partially purified 8S receptor preparations is accelerated by temperature and ionic strength.

Whether the proteolytic activity of the estrogen receptor is required only for the transformation process or whether it also participates in the modification of other functions and structures at the various cellular levels where the receptor acts remains to be established.

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