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PROTECTION OF STEROID HORMONE RECEPTORS BY PROTEASE INHIBITORS

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SUMMARY: Steroid hormone receptors are proteolyzed by different types of enzymes present in target tissues. Effective protease inhibitors protecting steroid hormone receptors in various target tissues were investigated. Progesterone receptor (PR) in hen oviduct and estrogen receptor (ER) in cow uterus were specifically protected by relatively low concentrations (0.5 mM) of leupeptin or antipain (inhibitors of serine and thiol proteases). It was indicated that two different types of enzymes which modify native glucocorticoid receptor (GR) are present in rat liver. One was inhibited by 1 mM leupeptin or 1 mM antipain, while the other was inhibited by 1 mM phosphoramidon (inhibitor of thermolysin like proteases) or 10 mM sodium molybdate. Native PR, ER, and GR were shown to have similar Stokes radii (44 Å).

INTRODUCTION: Purification and characterization of native hormone receptors are essential to a correct understanding of the action mechanism of steroid hormones. We have recently described the analysis of the molecules comprising the ER system of the cytosol of cow uterus (1-3). Protection of native ER from proteolysis by utilizing antipain (4), a protease inhibitor, contributed significantly to the study (1-3). Different target tissues of steroid hormones might contain different proteolytic enzymes which can effect the modification of native receptors.

We thoroughly examined the possibilities for protecting native ER of cow uterus, PR of hen oviduct and GR of rat liver by utilization of appropriate protease inhibitors. The present paper describes the successful protection of native hormone receptors in these different target tissues.

Abbreviations : ER, estrogen receptor; PR, progesterone receptor; GR, glucocorticoid receptor; TEM buffer, 10 mM Tris-HCl, 1.5 mM EDTA, 1.5 mM 2-mercaptoethanol, pH 8.0 at 2°C; TESH buffer, 10 mM Tris-HCl, 1.5 mM EDTA, 10 mM thioglycerol, pH 8.0 at 2°C.

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MATERIALS AND METHODS: Antipain (4), leupeptin (4), chymostatin (4), elastatinal (4), pepstatin (4), phosphoramidon (4), and bestatin (5) were a gift from Dr. T. Aoyagi, Institute of Microbial Chemistry. Trypsin inhibitors from soybean (Type 11-S), chicken egg white (Type 11-O), and beef pancreas (Type 1-P) from Sigma Chemical Company; diisopropylfluorophosphate from Calbiochem; [2,4,6,7,17-^{3}H]estradiol-17β(140 Ci/mmol), [1,2,6,7-^{3}H] progesterone (110 Ci/mmol), [1,2 (n)-^{3}H]dexamethasone (30 Ci/mmol) and iodo[2-^{14}C]acetic acid (57 Ci/mmol) from Radiochemical Centre, Amersham, were used for these experiments. Cow uterine cytosol was prepared in TEM buffer as described previously (1). Cytosol of hen oviduct was prepared by a similar method in TESH buffer. Liver cytosol was prepared in TEM buffer from perfused livers of male Wister rats (180 g) which were adrenalectomized 7 days before the experiment. The Stokes radii of native and proteolyzed receptors were determined according to Siegel and Monty (6). Stokes radius values for the standard proteins were from the literature (6,7). All other chemicals and procedures have been described previously (1).

RESULTS: When the cytosol of hen oviduct labeled with [^{3}H]progesterone was subjected to gel filtration on a Sephadex G-150 column in hypertonic (0.4 M KCl) buffer, PR eluted in the fractions (fraction I, Fig. 1A) with a Stokes radius of approximately 35 Å. The Stokes radius value obtained was similar to that of partially proteolyzed ER (1). When the gel filtration was carried out in the presence of 0.5 mM antipain, PR eluted in the fractions (fraction II, Fig. 1A) with a Stokes radius of approximately 44 Å. This Stokes radius value is similar to that of native ER (1). These results indicated that the proteolytic modification of native PR took place during the process of gel filtration in the absence of antipain. Relatively low concentration (0.5 mM) of leupeptin or antipain protected native PR completely from the proteolytic modification. Trypsin inhibitor from beef pancreas (Type 1-P) was weakly effective, showing 50 % inhibition of the proteolysis at 1 mg/ml. The other protease inhibitors tested (see the materials and methods section) did not show significant inhibitory effect.

Protective effects of the inhibitors on native PR and ER were parallel, suggesting that the receptor-modifying enzymes in uteri and oviducts are similar. Thus, utilization of antipain or leupeptin is recommended for the study of steroid hormone receptors in uteri and oviducts.

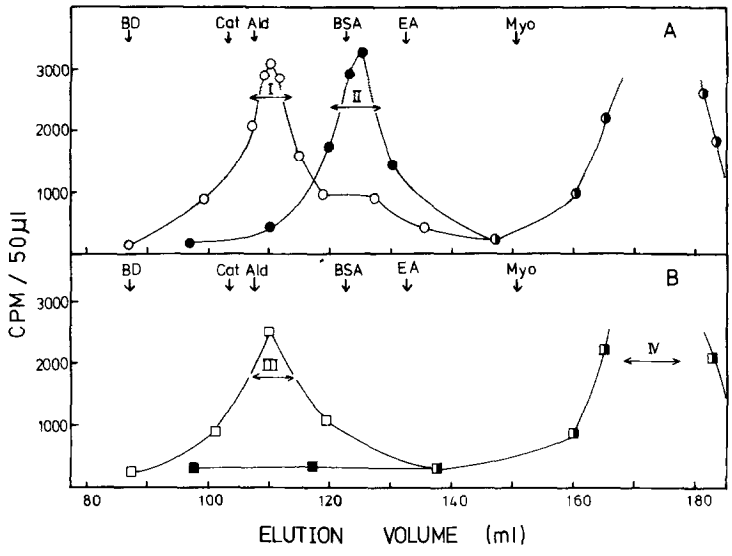

Fig. 1. Elution profiles of native and partially proteolyzed receptors from a Sephadex G-150 column (1.8 x 110). A. Hen oviudct cytosol labeled with [^{3}H]estrogen was subjected to gel filtration in the presence (○) or absence (●) of 0.5 mM antipain (or 0.5 mM leupeptin) in TESH buffer containing 0.4 M KCl. B. Rat liver cytosol labeled with [^{3}H]dexamethasone was subjected to gel filtration in the presence (□) or absence (■) of 1 mM antipain (or 1 mM leupeptin) and 1 mM phosphoramidon (or 10 mM sodium molybdate) in TEM buffer containing 0.4 M KCl. The arrows indicate the peaks of elution for Blue Dextran (BD), catalase (Cat), aldolase (Ald), bovine serum albumin (BSA), egg albumin (EA) and myoglobulin (Myo).

When rat liver cytosol labeled with [^{3}H]dexamethasone was subjected to sucrose gradient centrifugation under hypotonic conditions, GR sedimented at 2.8 - 3.2S (Fig. 2). We have previously shown that 8S ER is a 1:1 complex between native ER (Stokes radius 44 Å, sedimentation coefficient 4.5S) and a cytoplasmic component [8S estrogen receptor-forming factor, ("8S"ER)-FF](Stokes radius 51 Å, sedimentation coefficient 6.9S)(2.3). Native ER lost its ability to bind with ("8S"ER)-FF through Ca^{++}-independent proteolysis (2). Absence of 8S GR peak under hypotonic conditions suggested that proteolysis of native GR took place during the process of cytosol preparation or subsequent sucrose gradient centrifugation. In the presence of 0.25 mM leupeptin or 0.25 mM antipain, approximately 30 % of the GR sedimented at around 8 - 9S, while the rest of the GR sedimented at 2.8 - 4.5S (Fig. 2). With the further

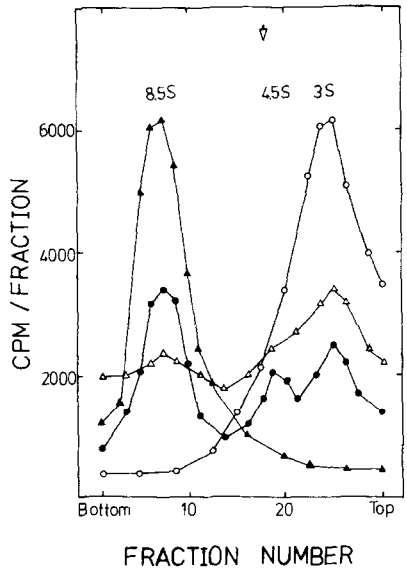

Fig. 2. Sedimentation analysis of native and proteolyzed GR. Cytosol preparation, labeling with [^{3}H]dexamethasone, and sucrose gradient centrifugation were carried out in TEM buffer (○). Parallel procedures were carried out in the presence of 0.25 mM leupeptin (or 0.25 mM antipain)(●), in the presence of 1 mM phosphoramidon (or 10 mM sodium molybdate)(△), or in the presence of 0.25 mM leupeptin (or 0.25 mM antipain) and 1 mM phosphoramidon (or 10 mM sodium molybdate) (▲). The arrows mark the peak of ^{14}C-labeled bovine serum albumin (4.6S).

addition of 1mM phosphoramidon, all of the GR activity sedimented at around 8 - 9S (Fig. 2). Sodium molybdate (10 mM) could be substituted for phosphoramidon (Fig. 2). Phosphoramidon or sodium molybdate alone did not completely protect native GR (Fig. 2) from enzymatic modification. These results indicate the presence of a common receptor-modifying protease in uterus, oviduct and liver which is inhibited by antipain or leupeptin. The presence of another receptor-modifying enzyme in rat liver, which is inhibited by phosphoramidon or sodium molybdate is further indicated.

When the liver cytosol labeled with [^{3}H]dexamethasone was subjected to gel filtration in hypertonic buffer, most of the applied radioactivity was recovered as free dexamethasone (fraction IV, Fig. 1B) due to the inactivation of GR. When the gel filtration was carried out in the presence of 1 mM antipain (or 1 mM leupeptin) and 1 mM phosphoramidon

(or 10 mM sodium molybdate), GR eluted in the fractions (fraction III, Fig. 1B) with a Stokes radius of approximately 44 Å. The Stokes radius value obtained is similar to that of native ER (1) and of native PR described above.

DISCUSSION: Both native PR and GR were shown to have identical Stokes radius values to that of native ER reported previously (1). The Ca^{++}-activated protease which hydrolyzes the steroid hormone receptors has been shown to be present in calf uterus (8,9) and hen oviduct (10,11). The Stokes radius of the protease has been reported to be 43 Å - 45 Å (9,11), which is very similar to that of the native steroid hormone receptors described above. The results presented in this paper and previous papers (1-3) show that the enzyme proteolyzes native receptors also in the absence of Ca^{++} during gel filtration. We have recently shown the presence in cow uteri of an inhibitor (Stokes radius 51 Å) of the protease (2,3). During the processes of purification or characterization of native receptors, the cytoplasmic protease inhibitor may be removed, leaving the receptor and the protease unseparated. Utilization of appropriate protease inhibitors would then be indispensable for the study of steroid regulation elements.

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