Progesterone Receptor Synthesis and Degradation in MCF-7 Human Breast Cancer Cells As Studied by Dense Amino Acid Incorporation

EVIDENCE FOR A NON-HORMONE BINDING RECEPTOR PRECURSOR*

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We have used the technique of density labeling of proteins by biosynthetic incorporation of $^2$H, $^{13}$C, $^{15}$N (dense) amino acids to study the synthesis and degradation rates of the progesterone receptor in MCF-7 human breast cancer cells. In cells grown in the absence of progesterin, sucrose gradient shift analyses reveal that it takes 17 h for the normal density progesterone receptor levels to be reduced to half the initial value, whereas in the presence of 10 nM of the synthetic progesterin [3H]R5020, the receptor turns over more rapidly, such that the normal density R5020-occupied progesterone receptor complexes are reduced to half in 12 h. The accelerated progesterone receptor turnover in the presence of [3H]R5020 reflects increased degradation rates of the progesterone receptor in steady-state conditions: cells incubated with medium containing normal $^{15}$N, $^{13}$C, $^3$H amino acids (NAA) are shifted to medium containing dense $^{15}$N, $^{13}$C, $^3$H amino acids (DAA); since the newly synthesized receptor is of a higher density than the pre-existing protein, it can be separated and quantitated by gradient centrifugation techniques (10, 11). We have found that the turnover of PR occupied with the high affinity progesterin R5020 is considerably faster than the turnover of unoccupied PR. In addition, a lag that characterizes the turnover kinetics of both occupied and unoccupied PR suggests that there is a non-hormone binding biosynthetic precursor to the receptor.

MATERIALS AND METHODS

Chemicals—Radioactive R5020 (promegestone; 17,21-dimethyl-19 nor-pregna-4,9-diene-3,20-dione-[17α-methyl-3H]), 37 Ci/mmol and radioinert R5020 (New England Nuclear Corp.; 17α-estradiol, progesterone, cortisol, leupeptin, phenylmethylsulfonyl fluoride, soybean trypsin inhibitor, Tria; EDTA, thiglotocrin Sigma. All chemicals for the SDS-polyacrylamide gel electrophoresis analysis were obtained from Bio-Rad. The Triton/xylene scintillation fluid contained 3 g/liter 2,5-diphenyloxazole, 0.2 g/liter p-bis-[2(5-phenyloxoyloxy)]-benzine, and 25% Triton X-114 in xylene.

Cells and Cell Culture Conditions—MCF-7 cells, obtained from Dr. Charles McGrath of the Michigan Cancer Foundation, were maintained in 37 °C in closed Corning T-75 flasks (Corning Glassworks, Corning, NY) and passaged in logarithmic growth phase. Growth medium was Eagle's minimal essential medium containing Hanks' balanced salts (Grand Island Biological Co. (Gibco), catalog number 410-1200), supplemented with 0.006 μg/ml insulin (Sigma), 10 μg/ml hydrocortisone (Sigma), 0.01 M HEPES buffer (Gibco), 50 μg/ml gentamicin (Schering), 100 units/ml penicillin (Gibco), 100 μg/ml streptomycin (Gibco), and 5% charcoal-dextran-treated calf serum (CDCS) prepared as described previously (12). At 4 days prior to use, 10 mM estradiol was added to media to increase PR levels. All density shift experiments were performed with near confluent cultures. For experiments, cells were grown in normal amino acid medium supplemented with 15% dialyzed charcoal-dextran-treated serum, 0.5% charcoal-dextran treated but nondialyzed serum, and 10 μM estradiol (NAA medium + estradiol). At times ranging from 1 to 36 h prior to

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The abbreviations used are: PR, progesterone receptor; DAA, dense amino acids; NAA, normal density amino acids; R5020, 17,21-dimethyl-19 nor-pregna-4,9-diene-3,20-dione; estradiol, 1.35(10)-estratriene-3,17β-diol; CDs, charcoal-dextran-treated calf serum; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.
harvesting the cells, the normal medium was replaced with medium containing dense amino acids.

**Dense Amino Acid Medium**—The composition and preparation of the dense amino acid medium was exactly as described previously (13, 14). The medium contained 0.5 mg/ml of $^{15}$N, $^{15}$N-$^{15}$H-amino acids (dense amino acids, DAA; Merck Sharp and Dohme). It was necessary to use dialyzed serum for these studies in order to remove amino acids present in the serum. However, in the presence of dialyzed CDCS (CDCS exhaustively dialyzed against Hanks’ balanced salt solution), cellular PR levels dropped substantially. Addition of 0.5% CDCS eliminated this problem. Thus 100 ml of DAA medium contained 15 ml of dialyzed CDCS plus 0.5 ml of CDCS.

**Preparation of Cytosol and Nuclear Extracts**—MCF-7 cells from a single confluent T-75 flask were incubated at 37 °C for the indicated times in NAA medium + estradiol or in DAA medium + estradiol. The medium was decanted and the cells were rinsed once with Hanks’ balanced salt solution containing 1 mM EDTA. The cells were centrifuged at 180,000 $\times$ g crude nuclear pellet was resuspended in 300 μl of TETG buffer containing protease inhibitors (soybean trypsin inhibitor, 5 mg/ml; leupeptin, 1 mg/ml; and phenylmethylsulfonyl fluoride 1 mg/ml) by 40 strokes of a Dounce homogenizer (B pestle). The homogenate was centrifuged at 800 x g for 10 min. The supernatant thus obtained was centrifuged at 180,000 x g for 30 min. A 300-μl aliquot of the high-speed supernatant (cytosol) was transferred to a minilype tube containing [3H]R5020 in 3 μl of absolute ethanol such that the final [3H]R5020 concentration was 10 nM and incubated for 2 h at 0–4 °C. Parallel tubes contained a 100-fold excess of radioinactive R5020. At the end of the 2-h incubation, charcoal-dextran slurry (5% acid washed Norit, and 0.5% dextran C in 10 mM Tris-HCl, pH 7.4, at 4 °C, containing 0.02% sodium azide) was added at 1 part slurry to 9 parts cytosol and incubated for 2 min at 0–4 °C followed by centrifugation for 2 min at 15,600 x g. An aliquot (50 μl) was removed for determination of recovery and 250 μl were layered onto linear 5–20% sucrose gradients containing medium with estradiol (NAA medium) at various times ranging from 6 to 36 h before cell harvest and fractionation. Cytosol was then prepared, labeled in vitro with [3H]R5020, and layered onto 5–20% sucrose gradients containing 0.4 M KCl, as described under “Materials and Methods.”

**Sucrose Gradient Centrifugation and Analysis of Gradient Profiles**

**Unoccupied PR**—To determine the rates of synthesis and degradation of the unoccupied PR, MCF-7 cells that had been treated with 10 nM estradiol for 4 days were incubated at 37 °C in normal amino acid-containing medium with estradiol (NAA + estradiol medium) for 36 h or until replacement with dense amino acid medium plus estradiol (DAA + estradiol medium) at various times ranging from 6 to 36 h before cell harvest and fractionation. Cytosol was then prepared, labeled in vitro with [3H]R5020, and layered onto 5–20% sucrose gradients containing 0.4 M KCl, as described under “Materials and Methods.”

**Results**

**Stimulation of PR Levels in MCF-7 Cells**—It is most convenient to apply the density shift technique to measure receptor turnover when the system is at steady-state with respect to PR synthesis and degradation rates. Therefore, we studied the time course of PR stimulation by treating MCF-7 cells with 10 nM estradiol for 1–6 days before determining PR levels. As seen in Fig. 1, PR levels rose from low basal levels in the controls (0.58 ± 0.01 pmol/mg DNA) to maximal PR levels which were achieved by day 2, and these levels (4.1 ± 0.1 pmol/mg DNA; 6.5–7.8-fold increase above the controls) remained constant for the remaining period assayed.

**Dense Amino Acid Labeling of Unoccupied PR**—To determine the rates of synthesis and degradation of the unoccupied PR, MCF-7 cells that had been treated with 10 nM estradiol for 6 days were incubated at 37 °C in normal amino acid-containing medium with estradiol (NAA + estradiol medium) for 36 h or until replacement with dense amino acid medium plus estradiol (DAA + estradiol medium) at various times ranging from 6 to 36 h before cell harvest and fractionation. Cytosol was then prepared, labeled in vitro with [3H]R5020, and layered onto 5–20% sucrose gradients containing 0.4 M KCl, as described under “Materials and Methods.”

**Polyacrylamide Gel Electrophoresis**—Following SDS-polyacrylamide gel electrophoretic analysis was performed using tube gels and followed the Laemmli procedure (18). Photolyzed samples were diluted (1:1) in sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% mercaptoethanol), boiled for 2 min at 100 °C, and stored at −80 °C. Protein standards (myosin, Mᵦ 205,000; β-galactosidase, 116,000; phosphorylase b, 97,400; bovine serum albumin, 66,000; ovalbumin, 45,000; carbonic anhydrase, 29,000) were run on separate tube gels.

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**RESULTS**

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**Dense Amino Acid Labeling of Unoccupied PR**—To determine the rates of synthesis and degradation of the unoccupied PR, MCF-7 cells that had been treated with 10 nM estradiol for 6 days were incubated at 37 °C in normal amino acid-containing medium with estradiol (NAA + estradiol medium) for 36 h or until replacement with dense amino acid medium plus estradiol (DAA + estradiol medium) at various times ranging from 6 to 36 h before cell harvest and fractionation. Cytosol was then prepared, labeled in vitro with [3H]R5020, and layered onto 5–20% sucrose gradients containing 0.4 M KCl, as described under “Materials and Methods.”

**Fig. 2** shows that the “new-dense” (newly synthesized) receptor and the “old-light” (pre-existing) receptor are well

![Fig. 1. Estradiol-stimulated increase in the cellular PR content of MCF-7 cells.](image-url)
resolved by this velocity sedimentation technique. PR from cells grown in NAA + estradiol medium s as a single 4 S moiety. After 6 h of exposure to DAA + estradiol medium, there is little change in the [3H]R5020-labeled PR profiles on sucrose gradients. Thereafter, however, turnover appears to accelerate, such that by 12 h, nearly 35% of the total PR has turned over (and is thus represented by a progressive increase in the amount of dense receptor, with a concomitant and proportional decrease in the light receptor), and by 36 h, nearly all of the [3H]R5020-labeled receptor is seen at the position of the newly synthesized, dense receptor. Thus, there appears to be a lag in the appearance of newly synthesized receptor. This lag is more apparent in the kinetic plot (Fig. 5A), and the significance of this observation is discussed later.

As we fractionate MCF-7 cells, PR that we obtain in the cytosol fraction represents only about 30% of total PR in cells. Therefore, in order to examine whether turnover of total cell PR was the same as that for cytosolic PR, we performed a similar time course of dense amino acid labeling. Cells were incubated in NAA + estradiol medium with subsequent transfer to DAA + estradiol medium, but at 1 h prior to harvesting of the cells, [3H]R5020 was added to the growth medium to label total cellular PR. This total cell PR was then extracted with 0.6 M salt and analyzed on density gradients (Fig. 3). Fig. 3 shows that the rate of disappearance of normal density receptor and rate of appearance of dense receptor are virtually identical to that of the unoccupied cytosol PR (Fig. 2), suggesting a similar turnover for unoccupied receptor observed in the cytosolic or total cellular extract (cf. Fig. 5, A versus B).

It is of note that over the period of 36 h there was no significant decrease in total PR labeled in cells. Also, the newly synthesized, dense receptor was indistinguishable from the normal density receptor with respect to affinity for [3H]R5020 (data not shown). Thus, it was possible to quantitate relative amounts of the normal and dense receptors and thereby to determine the rate of decrease of the normal density receptor (see Fig. 5).

**Dense Amino Acid Labeling of PR Occupied by R5020—**

We next wished to determine whether occupancy of PR by the potent progesterin R5020 influenced receptor turnover. To perform these experiments, cells were treated with 10 nM [3H]R5020 for 24 h prior to DAA exposure. This concentration of hormone was found to be adequate to saturate the receptor, and it resulted in localization of over 90% of PR in the nucleus (19). Furthermore, 10 nM R5020 does not affect the rate of cell growth over this brief time, although it does reduce cell growth rate over a more prolonged time period. In addition, our measurement of total cell protein synthesis rate, as monitored by [3H]leucine incorporation, showed no alteration over a 72 h period of exposure to 10 nM R5020. With this concentration of R5020, cytosol receptors were depleted, and there was a corresponding increase in the level of nuclear receptor.
that remained elevated for at least 72 h.

Thus, to measure the turnover of R5020 occupied PR, cells treated with 10 nM estradiol for 4 days to elevate PR were exposed for 24 h to 10 nM [3H]R5020. At various times over the next 36-h interval, separate flasks of cells were transferred to DAA + estradiol medium also supplemented with 10 nM [3H]R5020 (DAA medium + estradiol + [3H]R5020). At the 36-h point, cells were harvested and fractionated, and nuclear extracts were prepared and analyzed on density gradients.

Fig. 4 shows the PR profiles obtained. As before, PR extracted from cells that had been only in normal amino acid-containing medium (0 h), sedimented as a single 4 S species. However, in contrast to the situation seen with unoccupied PR (cf. Figs. 2 and 3), the [3H]R5020 occupied receptor showed a more rapid shift to the dense species. At 6 h, the newly synthesized receptor represented approximately 10% of the total PR; by 12 h, approximately 50% of the old, normal density receptor had been degraded and replaced by an equal amount of dense receptor, and by 18 h, this process was 90% complete. At 24 and 36 h (not shown), there was no change in the profile from that obtained at 18 h. Thus, exposure to 10 nM [3H]R5020 results in a more rapid turnover of PR in these cells. In addition, there again seems to be a lag before newly synthesized receptor is detected (cf. Fig. 5C). It is very unlikely this lag represents a problem in dense amino acid equilibration into amino acid pools, since studies in the same MCF-7 cells on estrogen receptor turnover indicated no lag before incorporation into estrogen receptors (13, 14, 20, 21).

Turnover of the M2 Perhaps the most obvious question is whether the turnover of both subunits A and B were being affected, and if so, whether to the same extent, since A and B subunits co-sediment on 0.4 M KCl-containing sucrose gradients (2). To address this issue, the subunit composition of the normal and dense receptor species, separated by sucrose gradient centrifugation, by covalent photolabeling with [3H]R5020, and electrophoretic separation of the A and B subunits on denaturing SDS gels. If there were a marked difference in the turnover rates of the two subunits, one would expect that their relative proportions in the normal and dense regions of the sucrose gradient would change with time. For example, if one subunit were turning over very rapidly in comparison with the other, it would disappear faster from the normal density region of the gradient and appear more rapidly in the dense region.

With the above rationale in mind, we exposed cells to 14 h of DAA medium + estradiol + [3H]R5020. (This relatively long time was chosen to accentuate potential differences in subunit turnover times, since the faster turning over subunit would be present largely as the newly synthesized, dense receptor and be very low in the normal density region of the sucrose gradient.) Cells in the control flask were incubated in NAA medium + estradiol + [3H]R5020. The cells were harvested and fractionated, and nuclear extracts were layered onto sucrose gradients containing 0.4 M KCl in buffered deuterium oxide. After centrifugation, the gradients were fractionated into minifuge tubes containing 10 μl of 10 mg/ml leupeptin to reduce the possibility of proteolysis. A small portion of each fraction was counted to determine the regions of the gradient that contained normal density and heavy PR (Fig. 6, upper panels).

As shown in Fig. 6A (top), fractions 20 and 21 from the gradient of the control experiment were selected and pooled.

![Fig. 4. Density shift of MCF-7 [3H]R5020 occupied PR labeled for various times with dense amino acids.](image-url)
to represent normal density, control receptor, and as is shown in Fig. 6B (top), from the 14-h density shift experiment, fractions 20 and 21 were pooled to represent the normal density PR, and fractions 26 and 27 were pooled to represent the dense receptor. These three samples were irradiated for 1 h at 0–4 °C at 350 nm to effect R5020-PR photocross-linking, and were then prepared for analysis on SDS-polyacrylamide gels. The SDS gel profiles (Fig. 6, lower panels) revealed that the B:A (Mr-115,000:85,000) ratio remained very similar during the course of PR turnover. Thus, assuming equal efficiency of photactivated cross-linking of R5020, we can conclude that the turnover of the two PR subunits occupied by R5020 is very nearly the same.

Degradation of PR in Cells Exposed to Cycloheximide—Because of the unusual turnover kinetics of PR determined by density labeling, we studied the rate of degradation of PR by a different, more widely used method, wherein biosynthetic inhibitors are used to reduce cellular protein synthesis to insignificant levels, and the rate of disappearance of pre-existent proteins is monitored. For these studies, MCF-7 cells were exposed to 10−4 M cycloheximide (a concentration which inhibited protein synthesis over 97%) at the indicated times prior to cell harvest and fractionation. A ½ of approximately 18 h was observed (Fig. 7). Interestingly, after cycloheximide treatment, no lag was seen in the degradation of PR.

DISCUSSION

Since the actions of progestins appear to be mediated via interaction with PR, there has been tremendous interest in understanding the basic biochemistry of this receptor and its role in regulating the function of reproductive tissues and the response of human breast and endometrial cancers to hormone treatment. PR measurements, along with estrogen receptor measurements, are now widely used in assessing the hormonal dependence of human breast cancer and in predicting the therapeutic utility of endocrine therapy and the disease-free survival of patients (22, 23).

As a step toward understanding some fundamental aspects of the regulation of PR levels in cells, we undertook a study to determine the rates of synthesis and degradation of this molecule and to study the factors that might influence these rates. We have used a powerful density shift technique to address the question of rates of synthesis and degradation of this protein. Cells were exposed to DAA-containing media such that any newly synthesized proteins incorporating the dense amino acids were distinguishable from the pre-existent pool of proteins by their faster sedimentation on sucrose gradients. The B:A ratio remained very nearly the same. As a result, we undertook a study to determine the rates of synthesis and degradation of this molecule and to study the factors that might influence these rates. We have used a powerful density shift technique to address the question of rates of synthesis and degradation of this protein. Cells were exposed to DAA-containing media such that any newly synthesized proteins incorporating the dense amino acids were distinguishable from the pre-existent pool of proteins by their faster sedimentation on sucrose gradients.
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gradients. PR labeled with tritiated ligand can thus be studied with respect to rates of synthesis (appearance of dense PR) or degradation (disappearance of normal density PR). This technique has the advantage of causing little perturbation to the system being studied when compared with other methods frequently employed (use of inhibitors of protein synthesis or transcription). We found that after DAA addition, the time required for the level of the unoccupied form of the PR to be reduced to half of the initial value is approximately 17 h, while this time is reduced to 12 h for PR occupied by the progestin R5020.

These observations of an effect of R5020 occupancy on receptor turnover brought us into a very interesting, although rather controversial subject in the field of PR studies, namely, whether the PR is constituted by one or two proteins and the relationship between the observed A and B subunits. Until recently, it was well accepted that PR was composed of two structurally and functionally dissimilar subunits; their molecular weights were estimated to be 79,900 and 108,000 on the basis of studies performed on the chick oviduct (2), and two subunits of similar or somewhat larger size were found in T47D human breast cancer cells (5). In contrast, work done on the rabbit PR system seems to indicate that a single Mr = 110,000 protein may function as the PR in this species (6). In our studies, we have always seen both Mr = 85,000 and 115,000 proteins during SDS-polyacrylamide gel electrophoretic analysis of R5020 binding to cytosolic or nuclear PR. (The Mr values we have obtained in 4 separate analyses are 85,000 ± 3,000 for the A unit and 113,500 ± 2,200 for the B unit). However, that does not rule out the possibility that the smaller species is being generated by proteolysis of the larger during cell fractionation, even though protease inhibitors (leupeptin, phenylmethylsulfonyl fluoride, and soybean trypsin inhibitor) are present in the homogenization and nuclear extraction buffers, and care is taken that the samples remain at 0-4 °C. Our studies show that both subunits have the same turnover rate, and that they are not linked in a precursor-product relationship in vivo. This would be consistent either with a model in which the subunits arise independently from distinct precursors, or a model in which there is in vivo only one form of PR (B) with A being generated only upon proteolysis during cell fractionation.

Of great interest is our finding of a pronounced lag in density shift kinetics observed in experiments employing dense amino acid labeling of the PR. This lag can best be appreciated in Fig. 5, which summarizes data from the experiments presented in Figs. 2-4 plus several additional experiments; these semi-logarithmic plots reveal that the decay of normal density receptor is clearly not first order, but has a pronounced lag, regardless of whether unoccupied or R5020-occupied PR is being observed. This lag in the decay profile would be consistent with the existence of a biosynthetic intermediate between amino acids and the ligand-binding form of the receptor that is assayed by this method. This precursor form would not bind hormone, but would be converted into the binding form by some activation process. The lag in the decay of the hormone binding form (receptor) after density shift would result from the fact that the normal density hormone binding form of the receptor continues to be produced for as long as the pool of normal density precursor persists; only as the pool of normal density precursor becomes depleted, does the decay rate of normal density receptor increase to maximum. It is extremely unlikely that this lag represents a problem in dense amino acid equilibration in amino acid pools, since studies in the same MCF-7 cells on estrogen receptor turnover indicated no lag before incorporation into estrogen receptors (13, 14, 29, 21). In addition, it is also unlikely that this lag represents a precursor-product relationship between the B and A subunits of PR. Our experiments analyzing the subunit composition of PR during the density shift (Fig. 6) indicate that the turnover rates of the B and A subunit are very similar. Also, a kinetic model involving a precursor-product relationship between subunits B and A would predict only a modest change in the slope of the normal density receptor decay curve, not a pronounced lag as we have observed.

If the hormone-binding form of PR is derived from a non-hormone-binding precursor by an activation process, then analysis of the non-linear (lag-decay) kinetics requires a more complex kinetic model than is typically used to analyze the kinetics of protein turnover.

Simple Protein Turnover Model (First Order Decay)

\[ \text{Amino acids} \xrightarrow{k_0} R \xrightarrow{k_d} \]

Non-binding Precursor Model (Lag-Decay)

\[ \text{Amino acids} \xrightarrow{k_0} \text{pre-}R \xrightarrow{k_1} R \xrightarrow{k_d} \]

\[ \text{The ligand binding form is designated R (receptor), and the precursor form pre-R. The zero-order biosynthetic rate constant is } k_0, \text{ and } k_1, \text{ and } k_2 \text{ are the first order rate constants for the activation process and the degradation process, respectively. A complete mathematical presentation of this model is given in the Appendix; a summarized version will be given here, together with the conclusions that can be drawn by the application of this model to the density-shift turnover kinetics we have determined for PR.} \]

Just prior to the shift to dense amino acids, the system is in a steady state, i.e. the concentration of pre-R and R are constant, since each is being formed and degraded at the same rate. Mathematically, this initial state can be expressed as:

\[ k_0 = k_1 \cdot \text{pre-R}_o = k_2 \cdot R_o \]

where \( \text{pre-R}_o \) and \( R_o \) are the concentrations of the receptor precursor and receptor at time 0. To express this proportionality relationship, it is convenient to define a proportionality constant \( \alpha \) (Equation 2), as the ratio of the precursor to receptor concentrations, or, what is equivalent, the ratio of the rate constants of degradation to activation.

\[ \alpha = \frac{\text{pre-R}_o}{R_o} = \frac{k_2}{k_1} \]

Very soon after the normal density amino acids are replaced by heavy amino acids, the biosynthesis of the normal density forms of the receptor ceases (\( k_0 \) goes to 0). The following differential equations then describe the decay kinetics of the normal density species pre-R and R.

\[ \frac{d(\text{pre-R})}{dt} = -k_1 \cdot (\text{pre-R}) \]

\[ \frac{d(R)}{dt} = k_1 \cdot (\text{pre-R}) - k_2 \cdot (R) \]

The first differential equation is easily integrated (Equation
cases must be considered:  

\( (\text{pre-R}) = (\text{pre-R}_o) e^{-k t} \)  \hspace{1cm} (5)  

\[ \frac{d(R)}{dt} = k_1 (\text{pre-R}_o) e^{-k t} - k_2 (R) \]  \hspace{1cm} (6)  

This form of the differential equation for \( R \) (Equation 6) can be integrated using Laplace transforms (as described in the Appendix), and the integrated form can be expressed solely in terms of \( R_o, k_1, \) and the proportionality factor \( \alpha. \)  

\[ \frac{R(t)}{R_o} = \frac{1}{1 - \alpha} \left[ e^{-k t} - \alpha e^{-k t}/\alpha \right] \]  \hspace{1cm} (\( \alpha \neq 1 \)) \hspace{1cm} (7)  

\[ \frac{R(t)}{R_o} = e^{-k t} (1 + k t_2) \]  \hspace{1cm} (\( \alpha = 1 \)) \hspace{1cm} (8)  

Different integrated rate equations are used to describe the situations where \( \alpha \neq 1 \) (precursor and receptor pool sizes different; Equation 7) and \( \alpha = 1 \) (precursor and receptor pool sizes the same; Equation 8). It is interesting that because of the initial steady-state boundary condition (Equation 1), the decay kinetics of the system are fully defined by specifying only two terms, the degradation rate constant \( (k_2) \) and the steady-state proportionality constant \( (\alpha) \). The initial concentration of \( R \) is determined by direct measurement, and the other terms \( (\text{pre-R}_o, k_1, \) and \( k_2) \) can be derived from \( k_2, \alpha, \) and \( R_o \) using Equations 1 and 2.  

The general character of these functions (that is, the shape of the decay curves for \( \text{pre-R} \) and \( R \) as a function of \( \alpha \), for a fixed value of \( R_o \) and \( k_2) \) is explored in greater detail in the Appendix. Either a non-linear curve fitting procedure or a graphical analysis method (that utilizes the measurement of limiting slopes and intercepts) can be used to extract values of \( k_2 \) and \( \alpha \) from our experimental kinetic data (Fig. 5). The results of this analysis are given in Table I.  

As is explained in greater detail in the Appendix, three cases must be considered:  

**Case I \( (\alpha < 1) \):**  

\[ \ln \left[ \frac{R(t)}{R_o} \right] = -k_2 t + \left[ \ln \left[ 1 - e^{-w \left( \frac{\text{le} \left( \frac{\text{le}}{2} \right) }{\text{le} \left( \frac{\text{le}}{2} \right) } \right) } - \ln \left( 1 - \alpha \right) \right] \]  \hspace{1cm} (9)  

**Table I**  

**Determination of kinetic parameters for progesterone receptor synthesis, activation, and degradation in the unoccupied and R5020 occupied state from kinetic modeling analyses**  

Values for \( k_2 \) and \( \alpha \) have been determined from the data presented in Fig. 5, using the graphical analysis method described in the Appendix. The standard error of the mean for the measurement of \( R_o \) is given in the Table \( (n = 6) \). Hence the coefficient of variation at the 95% confidence limit for the measurement of \( R_o \) (unoccupied) is 10% and for \( R_o \) (R5020 occupied) is 14%. Error (not calculated) is also introduced in determining the limiting slope and intercept from the lag-decay profiles and is propagated into the calculations of the other kinetic parameters. Therefore the values in the Table are given to 2 significant figures only.  

| Kinetic parameters | Unoccupied (Fig. 5B) | R5020 occupied (Fig. 5C) |
|-------------------|----------------------|-------------------------|
|                   | Case I \( (\alpha < 1) \) | Case II \( (\alpha > 1) \) | Case I \( (\alpha < 1) \) | Case II \( (\alpha > 1) \) |
| \( k_2 \) (pmol/mg DNA h) | 0.35 | 0.61 | 0.80 | 0.96 |
| \( \text{pre-R}_o \) (pmol/mg DNA) | 2.5 | 7.1 | 3.3 | 4.8 |
| \( k_1 \) (h \( ^{-1} \)) \( (\text{le} \text{le} \text{le}) \) | 0.15 [4.5] | 0.087 [8.0] | 0.24 [2.9] | 0.20 [2.5] |
| \( R_o \) (pmol/mg DNA) | 4.0 ± 0.2 | 4.0 ± 0.2 | 4.0 ± 0.3 | 4.0 ± 0.3 |
| \( k_1 \) (h \( ^{-1} \)) \( (\text{le} \text{le} \text{le}) \) | 0.087 [8.0] | 0.15 [4.5] | 0.20 [2.5] | 0.24 [2.9] |
| \( \alpha \) | 0.57 | 1.8 | 0.83 | 1.2 |

**Case II \( (\alpha > 1) \):**  

\[ \ln \left[ \frac{R(t)}{R_o} \right] = \frac{k_2 t}{\alpha} + \left[ \ln \left[ \frac{\alpha - e^{-w \left( \frac{\text{le} \left( \frac{\text{le}}{2} \right) }{\text{le} \left( \frac{\text{le}}{2} \right) } \right) } - \ln \left( \frac{\alpha - 1}{\alpha} \right) \right] \]  \hspace{1cm} (10)  

and for each case, there is a convenient form of the integrated rate expression (Equations 9–11). Kinetic analysis of the lag-decay profile alone does not permit one to distinguish between Cases I and II (cf. Appendix); independent methods are needed to determine whether the precursor pool is larger or smaller than the receptor pool. Therefore, two sets of estimates for the pool sizes and kinetic rate constants, that correspond to Cases I and II, are presented in Table I. We are justified in not considering Case III \( (\alpha = 1) \), since in all cases the values estimated for \( \alpha \) differ substantially from 1.  

There is a reciprocity in the equations that describe Cases I and II, such that analysis of the same data by the two expressions produce values for \( \alpha \) that are reciprocals of one another. As a result, as can be seen in Table I, the kinetic rate constants \( k_1 \) and \( k_2 \) determined according to Case I, become the values for \( k_2 \) and \( k_1 \), respectively, determined in Case II. The pool size for \( R \) (\( R_o \)) is the same for both cases, since it is determined independently, by assay of the receptor content at time 0. However, estimates for the pool size of \( \text{pre-R} \) (\( \text{pre-R}_o \)) and for the biosynthetic rate constant \( (k_0) \) differ, as these both depend on the value of \( \alpha \).  

It is apparent from the \( k_2 \) values (Table I) that R5020 occupied receptor is degraded more than 50% faster than unoccupied receptor; the rate constant for precursor activation \( (k_1) \) and for overall biosynthesis \( (k_0) \) for R5020 occupied receptor are also larger, but the precursor pool size \( (\text{pre-R}_o) \) is smaller. Hence, it is of note that R5020 accelerates PR turnover. Ligand binding has also been shown to accelerate turnover of the glucocorticoid receptor and thyroid hormone receptor (24, 25), although we observed little effect of estrogen on the rapid rate of turnover \( (t_2 = 3–4 \text{ h}) \) of the estrogen receptor in MCF-7 cells (13, 26).  

Furthermore, it is of note that the turnover rates of steroid and thyroid hormone receptors cover a considerable range, having half-lives in the unoccupied state from 3–4 h for estrogen receptors (13, 14, 20, 21, 26) and androgen receptors (27), and 5 h for thyroid hormone receptors (25), to approximately 19 h for glucocorticoid receptors (24). From this study, which is the first report on turnover of the PR using density shift analyses, we find rather rapid rates of degradation of the hormone binding form of PR \( (t_2 = 4.5 \text{ or } 8.0 \text{ h}), \) with conversion of prereceptor to the hormone binding form occurring with a \( t_2 \) of 8.0 or 4.5 h, depending upon estimates of the precursor pool size.  

It is of note that no lag is observed in the degradation of PR in MCF-7 cells when protein synthesis is blocked by the use of cycloheximide and that a longer apparent half-life for PR \( (18 \text{ h}) \) is then estimated. The hypothesized precursor pool thus appears no longer capable of generating hormone binding moieties. This would be explicable on the basis of the presence of a rapidly turning over activating enzyme: inhibition of protein synthesis would cause a dramatic decrease in the enzymatic activity, thus precluding receptor activation to a hormone binding form. In addition, degradation of PR could be slowed if a rapidly turning over enzyme is involved in the PR degradation. Thus, the \( t_2 \) for PR observed in the studies using cycloheximide is considerably longer \( (18 \text{ h}) \) than that.
measured by the density shift procedure. In T47D human breast cancer cells, the use of cycloheximide also revealed an apparent half-life of 16 h for the PR (28). Hence, cycloheximide results in what is most likely an artifactually prolonged half-life for PR. Such an effect of cycloheximide on degradation has been observed for other proteins (29, 30), including the estrogen receptor in these MCF-7 cells and in uterine cells (21).

The observation that some cells produce immunoreactive nor-hormone binding proteins (presumably receptor-related forms), that specifically react with highly purified antireceptor monoclonal antibodies, may be particularly relevant to our findings. Several groups have reported recently on the presence of immunoreactive 94,000 molecular weight material (the same molecular weight as the normal hormone binding glucocorticoid receptor) that fails to bind hormone in some mutant rat hepatoma (HTC) and mouse lymphoma (S49 and WEHI7) cells (31–33). Interestingly, both wild type and mutant (nuclear transfer deficient and “receptorless”) S49 cells contain significantly more immunoreactive material than hormone binding activity. Indeed, the receptorless mutant, which virtually lacks hormone binding activity, nevertheless produces 20–50% as much 94,000 molecular weight immunoreactive material as the wild type parent (31–33). In addition, the recent use of cDNA probes for the glucocorticoid receptor have predicted the occurrence of hormone-binding and non-hormone binding glucocorticoid receptor forms in the same cell (34). In the case of the PR, monoclonal antibodies generated to the subunit B protein from chick oviduct (35) have been able to detect antibody-antigen interactions only under denaturing conditions, and it has been concluded at this point that the antibodies react either with a denatured conformation of receptor that does not bind hormone or with a modified, possibly precursor form of receptor unable to bind hormone.

In conclusion, the turnover of PR in MCF-7 cells, as we have studied it by the density shift technique, appears to be complex, with a pronounced lag in decay kinetics being consistent with the existence of a non-hormone binding biosynthetic precursor to receptor. Further biochemical studies and the use of antireceptor immunochemical probes should enable us to learn more about these progesterone receptor precursors.

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