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Kinetics and in Vitro Origin of the Temperature-Dependent Transition of the Estrogen Receptor Monomer†

Jeffrey C. Hansen‡ and Jack Gorski*
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin—Madison, 420 Henry Mall, Madison, Wisconsin 53706

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ABSTRACT: Partitioning of estrogen receptors in aqueous two-phase polymer systems has provided the basis for a detailed kinetic analysis of the effects of temperature on estrogen receptor (ER) structure in vitro. Exposure to temperatures of 0–30 °C increased the rate of change in ER partition coefficients by up to 100-fold but did not affect the final extent of the process. The temperature-dependent change in ER partition coefficients was characterized by a linear Arrhenius plot and an activation energy of 25 kcal/mol. The rate of the temperature-dependent ER transition (28 °C) was found to be unaffected by greater than 50-fold changes in receptor concentration, which indicates that the temperature-dependent change in partition coefficients reflects a first-order process. The partition coefficients of heated ER were unaffected by subsequent 18-h incubations at 0 °C, indicating that the temperature-dependent ER transition is irreversible in vitro. Direct heating of the unoccupied ER resulted in both a change in ER partition coefficients and a loss of ER binding sites. The temperature-dependent change in unoccupied ER partition coefficients was complete within 30 min at 28 °C and yielded a first-order rate constant that was the same as that obtained for heating the receptor–estradiol complex at 28 °C. In contrast, the loss of unoccupied ER binding sites that occurred during 28 °C incubations did not reach completion after 150 min of heating and was found to behave as a second-order process. Thus, the temperature-dependent change in unoccupied receptor structure is a separate process from the temperature-dependent loss of unoccupied ER binding sites. Taken together, these results indicate that the temperature-dependent change in ER partition coefficients represents an irreversible, hormone-independent change in the conformation of the ER monomer that originates entirely as a consequence of tissue homogenization and subsequent in vitro extraction of the receptor into the cytosol.

It is becoming increasingly clear that the estrogen receptor monomer[1] is a dynamic protein composed of multiple functional domains (Green et al., 1986; Krust et al., 1986; Kumar et al., 1986). In general, all steroid hormone receptors share the same organization of these functional domains, in which the hormone binding site is located in the hydrophobic carboxy-terminal region of the receptor, the nuclear association domains in the middle of the primary sequence, and a transcription "modulating" function near the amino terminus of the protein (Carlstedt-Duke et al., 1983; Giguere et al., 1986; Green & Chambon, 1986; Hollenberg et al., 1987). Recent mutational analyses of estrogen receptor (ER[2] and glucocorticoid receptor cDNAs indicate that hormone-dependent induction of gene transcription in vivo at least in part involves conformational rearrangement of steroid receptor monomers upon hormone binding (Green & Chambon, 1987; Godowski et al., 1987; Hollenberg et al., 1987). In vitro, it has been demonstrated that the structure of the ER monomer is influenced by both hormone binding (Hansen & Gorski, 1985, 1986) and exposure to elevated temperature (Bailly et al., 1980; Sakai & Gorski, 1984; Muller et al., 1985; Hansen & Gorski, 1986; Redeuilh et al., 1987). Hormone binding has also recently been demonstrated to alter the structure of progesterone (Moudgil & Hurd, 1987) and glucocorticoid (Moudgil et al., 1987) receptors in vitro, suggesting that a hormone-induced conformational change is a general property of all steriod receptors. Although temperature-dependent changes in steroid receptor structure have been routinely and reproducibly observed with virtually every experimental approach [see Gorski and Gannon (1976), Grody et al. (1982), Sherman and Stevens (1984), and Andreasen (1987) for reviews], the temperature-dependent transition of the ER monomer occurs in vitro in the absence of bound hormone (Hansen & Gorski, 1986), and thus it is not clear what biological role can be attributed to this hormone-independent phenomenon. Perhaps least understood is the origin of the temperature-dependent ER transition in vitro; i.e., why does temperature influence the structure of a protein recently isolated from the 37 °C in vivo environment?

In an attempt to better understand the nature and role of the in vitro effects of temperature on the structure of the ER

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*To whom correspondence should be addressed.
‡Present address: Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331.

[1] The estrogen receptor "monomer" is defined as the M_r 65 000 steroid binding protein encoded by the estrogen receptor gene. The ER monomer has been referred to elsewhere as the "ER steroid binding subunit" and the "4S estrogen receptor".

[2] Abbreviations: ER, estrogen receptor(s); K_{obsd}, partition coefficient; PEG, poly(ethylene glycol); TED, 10 mM Tris-HCl, 1.5 mM EDTA, and 0.5 mM dithiothreitol.

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monomer, we have examined in detail the kinetic mechanism of the temperature-dependent change in ER partition coefficients that has been observed in aqueous two-phase polymer systems. These kinetic data reveal the origin of temperature effects on ER structure in vitro and together with previous studies suggest a relationship between temperature-dependent alterations in the structure of the ER monomer and temperature-dependent changes in ER aggregation that are observed in other experimental situations.

Experimental Procedures

Materials. Dextran (M_r 531 000) and poly(ethylene glycol) (M_r 8000) were purchased from Sigma. Highly purified poly(ethylene glycol) 8000 was purchased from Koch-Light (Colnbrook Bucks, England). 17β-[2,4,6,7-^{3}H]Estradiol (90.5 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Immature female rats (19 days old) were obtained from Holtzman (Madison, WI). Bio-Gel HT hydroxylapatite was purchased from Bio-Rad (Richmond, CA). All other chemicals were of reagent grade.

Phase Partitioning. Preparation of ER, preparation of phase systems, hydroxylapatite ER assay of phases, and calculation of ER partition coefficients were performed as described previously (Hansen & Gorski, 1985). Briefly, immature uteri were homogenized in 10 mM Tris-HCl, 1.5 mM EDTA, and 0.5 mM dithiothreitol (TED) buffer and centrifuged at 135000*g* for 1 h to obtain cytosol containing unoccupied ER. Hormone-bound ER were obtained by incubating unoccupied ER with 5–10 nM [^{3}H]estradiol for 2.5–3 h at 0 °C. Temperature-transformed receptors were obtained by incubating hormone-bound ER for various lengths of time at 4–30 °C. In all cases, parallel incubations included a 200-fold molar excess of unlabeled estradiol to determine nonspecific binding. Cytosol containing the different receptor forms was partitioned to equilibrium in phase systems composed of 5.4% (w/w) dextran, 5.4% (w/w) PEG, and 0.1 M Li_2SO_4, buffered with TED, pH 8.0. After phase partitioning, upper and lower phase samples were removed and assayed for ER content by using the hydroxylapatite ER assay. If the unoccupied receptor had been partitioned, phases were incubated with ligand for 2.5–3.0 h at 0 °C prior to hydroxylapatite receptor assay. All procedures were performed at 0–4 °C. Partition coefficients were calculated as the estrogen receptor concentration in the top and bottom phases, respectively. In studies reported here, upper and lower phase H_2O contents were 90.6 ± 0.4% H_2O and 80.4 ± 0.5% H_2O (SD, n = 14), respectively.

Measurement of Rate Constants at >23 °C. At temperatures >23 °C, the temperature-dependent change in ER partition coefficients was essentially complete within 90 min, and time points were taken at 1–5-min intervals. For determination of rates of ER partition change at these temperatures, the following experimental design was used: cytosols were incubated with 5 nM [^{3}H]estradiol for a combined total of 240 min at 0 and ≥23 °C. After the appropriate times at 0 °C (150–240 min), tubes containing hormone-bound estradiol–receptor complexes were preheated for 5 s at ≥23 °C and then placed in a water bath at the indicated temperature for 0–90 min. In this manner, all of the time points completed the ≥23 °C incubations at the same moment, at which point they were immediately transferred to a 0 °C ice–water bath. Phase partitioning was then immediately performed as described.

Measurement of Rate Constants at <20 °C. Since the temperature-dependent change in ER partition coefficients was only complete at ≥4 h of heating at temperatures ≤20 °C and time points cover 0.5–8-h intervals, a slightly different experimental design was used for experiments at these temperatures. For all time points, [^{3}H]estradiol–receptor complexes were first formed by incubating receptor with tritiated ligand for 150 min at 0 °C. The hormone-bound ER partition coefficient was determined immediately after these incubations. In addition, at this time, samples were exposed to ≤20 °C for 0.5–24 h. At the end of each individual time point, samples were transferred to 0 °C, and phase partitioning was performed as described above.

Table I: Temperature-Dependent Change in ER Partition Coefficients Is Irreversible upon Return to 0 °C[a]

min at 24 °C	min at 0 °C	K_{obsd}[b]
0	30	0.46 ± 0.09
15	0	0.31 ± 0.06
	30	0.26 ± 0.02
70	0	0.09 ± 0.01
	15	0.10 ± 0.02
	30	0.09 ± 0.01
	1080	0.08 ± 0.03

[a] Estradiol–receptor complexes were formed at 0 °C as described under Experimental Procedures and then exposed to 24 °C as indicated. Receptors were then returned to 0 °C for the indicated times, followed immediately by phase partitioning. [b] ER partition coefficients were obtained in systems composed of 5.4% dextran, 5.4% PEG, and 0.1 M Li_2SO_4 and buffered with TED, pH 8.0. Values represent the mean ± SD of duplicate partitionings.

Data Analysis. Fraction of ER that had not undergone the temperature-dependent change in partition coefficients (fraction nontransformed) was calculated according to fraction nontransformed = $K^M_{obsd} - K^F_{obsd}/K^I_{obsd} - K^F_{obsd}$ where K^M_{obsd} is the measured partition coefficient after heating, K^I_{obsd} is the initial ER partition coefficient at 0 °C, and K^F_{obsd} is the final partition coefficient obtained after 60 min at 30 °C.

Initially, fraction ER nontransformed versus duration of heating was plotted as both semilogarithmic and reciprocal plots. In all cases, semilogarithmic plots appeared linear, whereas the corresponding reciprocal plots were nonlinear. Semilogarithmic plots were analyzed by a nonweighted method that determined the maximum and minimum slots using a fixed y intercept. The rate constants are expressed as the mean ± SD of the maximum and minimum slopes. In all cases, the rate constants derived from a least-squares linear regression (nonweighted, nonfixed y intercept) fell within the calculated range. The correlation coefficients of these linear regressions varied from 0.91 to 0.99.

Results

Temperature-Dependent Change in ER Partition Coefficients Is Irreversible. Table I lists the results of experiments designed to test whether the temperature-dependent change in ER partition coefficients is reversible. Estradiol–receptor complexes formed at 0 °C either were left at 0 °C or were exposed to 24 °C for 15 or 70 min. Samples were then returned to 0 °C and either partitioned immediately or allowed to sit at 0 °C for the indicated times. Results indicate that ER partition coefficients were unaffected by even 18-h incubations at 0 °C, following exposure to elevated temperature (Table I). Thus, the temperature-dependent change in ER partition coefficients is not reversible upon return to 0 °C. Furthermore, these data indicate that low temperature (0 °C) effectively "quenches" the progress of the reaction over at least a 30-min period at 0 °C. Taken together, the results in Table I indicate that temperature-dependent changes in the ER K_{obsd} reflect accurate determination of the progress of the temperature-dependent reaction as a function of time and temperature.

Effect of Temperature on the Extent of Change of ER Partition Coefficients. Depending on the molecular nature

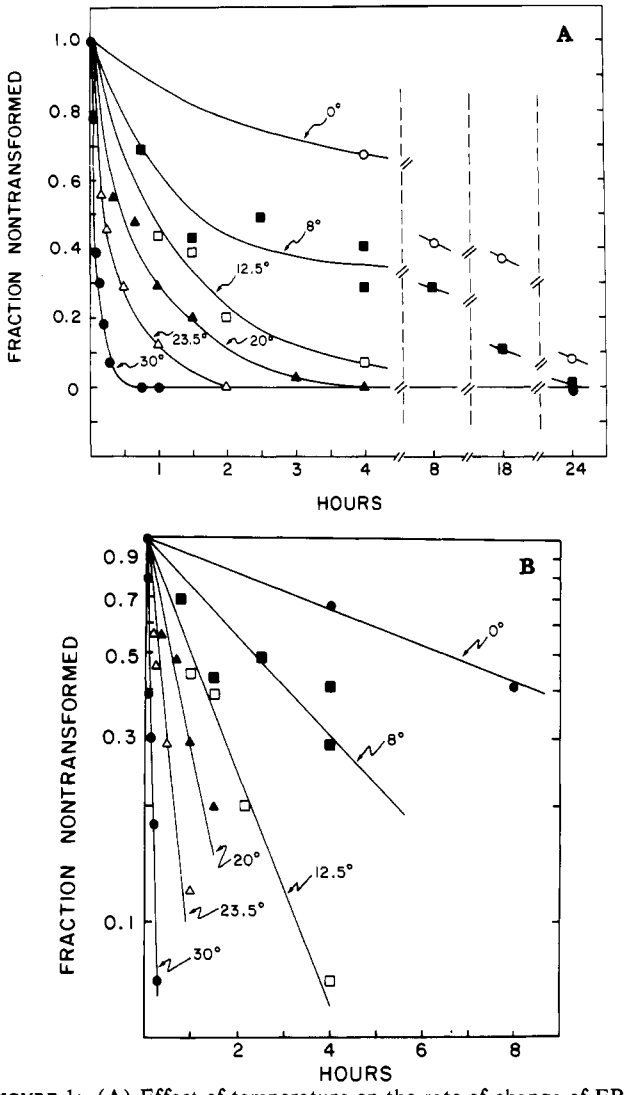

FIGURE 1: (A) Effect of temperature on the rate of change of ER partition coefficients. Estradiol–receptor complexes were formed at 0 °C as described under Experimental Procedures and then incubated at 0 °C (○), 8 °C (■), 12.5 °C (□), 20 °C (▲), 23.5 °C (△), or 30 °C (●) for the indicated times. Receptors were then immediately partitioned in phase systems composed of 5.4% (w/w) dextran, 5.4% (w/w) PEG, and 0.1 M Li_2SO_4 buffered with TED, pH 8.0. Data obtained at 4 and 28 °C are not shown. This figure represents the results of a numer of different experiments, and therefore the partition coefficients were normalized to fraction nontransformed ER, where fraction nontransformed is defined as the fraction of estradiol–receptor complexes that have not undergone the temperature-dependent ER transition. In these experiments, the partition coefficients of the unheated (nontransformed) (K^I) and temperature-transformed (K^F) ER ranged from 0.49 ± 0.09 to 0.68 ± 0.08 and from 0.10 ± 0.03 to 0.13 ± 0.02, respectively. (B) The data in (A) are replotted as logarithm fraction nontransformed versus time.

of the temperature-dependent process, there are two possible effects of exposure of ER to temperatures between 0 and 30 °C: (1) effect(s) on the rate of the process and (2) effect(s) on the extent of the process. The results in Table II indicate clearly that ER partition coefficients obtained after extended incubations at temperatures between 8 and 23 °C are identical with those obtained after 60–1410 min at 28 °C. Thus, exposure of occupied ER to very low temperatures for sufficient time results in the same final extent of receptor change as does exposure of receptors to 30 °C for ≥60 min.

Effects of Temperature on the Rate of ER Partition Change. Figure 1A illustrates the progress of the temperature-dependent change in occupied ER partition coefficients versus time, at temperatures in the range of 0–30 °C. These

Table II: Effect of Temperature on the Final Extent of ER Partition Change[a]

temp (°C)	time (min)	K_{obsd}[b]
0	0	0.62 ± 0.05
28	60	0.10 ± 0.02
28	1410	0.09 ± 0.02
23	120	0.10 ± 0.01
20	240	0.10 ± 0.01
12	240	0.13 ± 0.01
8	1410	0.10 ± 0.01

[a] Estradiol–receptor complexes were formed at 0 °C as described under Experimental Procedures. Receptors were then incubated at the indicated temperatures and times, followed immediately by phase partitioning. [b] Partitionings were performed in systems composed of 5.4% (w/w) dextran, 5.4% (w/w) PEG, and 0.1 M Li_2SO_4 buffered with TED, pH 8.0. Values represent the mean ± SD of duplicate partitionings.

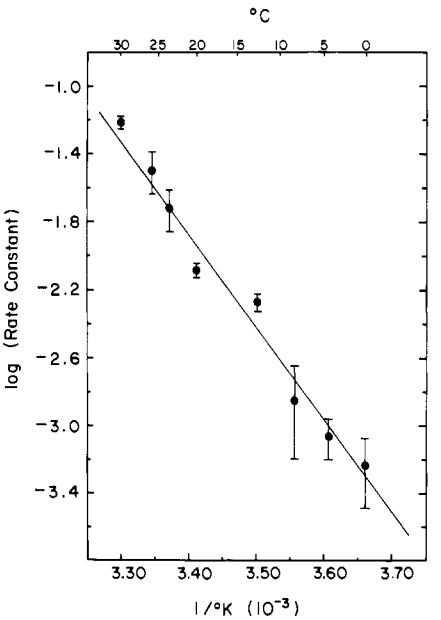

FIGURE 2: Arrhenius plot of the temperature-dependent change in ER partition coefficients. The line represents a least-squares linear regression ($r = 0.99$). The rate constants calculated from the data in Figure 1 were as follows: 30 °C, $(6.2 \times 10^{-2}) \pm (4.0 \times 10^{-3})$ min^{-1}; 28 °C, $(3.8 \times 10^{-2}) \pm (6.0 \times 10^{-3})$ min^{-1}; 23.5 °C, $(1.9 \times 10^{-2}) \pm (5.0 \times 10^{-3})$ min^{-1}; 20 °C, $(8.3 \times 10^{-3}) \pm (8.0 \times 10^{-4})$ min^{-1}; 12.5 °C, $(5.3 \times 10^{-3}) \pm (7.0 \times 10^{-4})$ min^{-1}; 8 °C, $(1.4 \times 10^{-3}) \pm (9.0 \times 10^{-4})$ min^{-1}; 4 °C, $(8.6 \times 10^{-4}) \pm (2.3 \times 10^{-4})$ min^{-1}; 0 °C, $(5.9 \times 10^{-4}) \pm (2.7 \times 10^{-4})$ min^{-1}.

data indicate that there is a dramatic effect of temperature on the rate of the reaction. Interestingly, the temperature-dependent ER transition is actually occurring at 0–4 °C, such that the process is essentially complete within 18–24 h at these temperatures. Semilogarithmic plots of the data in Figure 1A are linear at all temperatures (Figure 1B). The rate of the temperature-dependent change in ER partitioning varied from 5.9×10^{-4} min^{-1} at 0 °C to 6.2×10^{-2} min^{-1} at 30 °C. The rate constants determined from the data in Figure 1 were used to construct an Arrhenius plot of the temperature-dependent change in ER partition coefficients (Figure 2). A linear regression of the data yields an activation energy for the temperature-dependent ER transition of 25 kcal/mol.

Effect of ER Concentration on the Rate of the Temperature-Dependent ER Transition. All indications thus far suggest that the temperature-dependent change in ER partition coefficients is a simple first-order process (Figure 1). However, in order to rigorously define the order of the reaction, the effect of ER concentration on the rate of ER partition change at 28 °C was determined (Table III). Results indicate that both

Table III: Effect of ER Concentration on the Rate of the Temperature-Dependent ER Transition at 28 °C

[ER] (nM)[a]	k_{trans} ± SD (min^{-1})
3.5	0.027 ± 0.015
1.5	0.031 ± 0.006
0.2	0.044 ± 0.014
0.06	0.024 ± 0.006

[a] In these experiments, concentrated cytosol was diluted with TED immediately prior to addition of [^{3}H]estradiol. In this manner, both ER and total protein concentrations were changed prior to exposure to 28 °C.

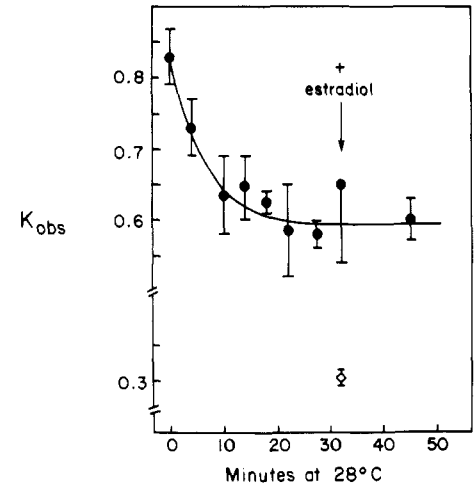

FIGURE 3: Kinetics of the change of unoccupied ER partition coefficients at 28 °C. Unoccupied ER was heated at 28 °C for the indicated times (●) followed immediately by phase partitioning. In addition, unoccupied receptor that had been heated at 28 °C for 32 min was incubated with 5 nM [^{3}H]estradiol for 2.5 h at 0 °C (◊) prior to phase partitioning. Receptors were partitioned in phase systems composed of 5.4% (w/w) dextran, 5.4% (w/w) PEG, and 0.1 M Li_2SO_4 buffered with TED, pH 8.0. The first-order rate constant for the temperature-dependent change in unoccupied ER partition coefficients at 28 °C was 0.049 ± 0.009 min^{-1}, as determined from the corresponding semilogarithmic plot.

the linear semilogarithmic plots (not shown) and the apparent first-order rate constants are unaffected by changes in ER concentration between at least 0.06 and 3.5 nM, confirming that the temperature-dependent process is first order.

Kinetic Analyses of the Effects of Temperature on the Properties of the Unoccupied Estrogen Receptor. The kinetics of the effect of elevated temperature on the partition coefficient of the unoccupied estrogen receptor are shown in Figure 3. Exposure of the unoccupied receptor to 28 °C resulted in a decrease in the ER partition coefficient that reached completion within 30 min. A semilogarithmic plot of the data in Figure 3 was linear through >90% of the reaction (data not shown) and yielded a first-order rate constant that was not significantly different than that obtained for the temperature-dependent transition of the estradiol–receptor complex at 28 °C (Figure 1). Consistent with previous observations (Hansen & Gorski, 1986), addition of estradiol to the temperature-"transformed" unoccupied receptor further lowered the partition coefficient to the range observed for ER that were heated *after* hormone binding at 0 °C (Figure 3).

In addition to the effects of temperature on unoccupied ER partition coefficients, exposure of the unoccupied receptor to 28 °C resulted in a loss of ER binding sites that continued to occur after 2.5 h of heating. In contrast to that observed for temperature-dependent changes in ER partition coefficients, a semilogarithmic plot of the kinetics of the temperature-dependent loss of ER binding sites was nonlinear, whereas a reciprocal plot of the same data was linear (Figure 4), indicating that the temperature-dependent loss of cytosolic ER binding sites behaves as a second-order process.

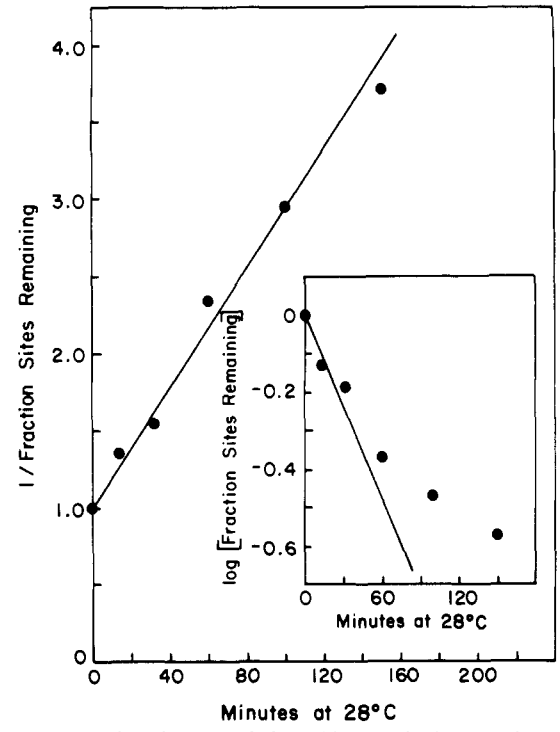

FIGURE 4: Kinetics of unoccupied ER binding site inactivation at 28 °C. The loss of ER binding sites that occurred during the experiment illustrated in Figure 3 was graphed as either a reciprocal or a semilogarithmic (inset) plot versus time. A least-squares linear regression of the reciprocal plot gave a y intercept of 1.0 (r = 0.99). The amount of unoccupied ER recovered after heating at 28 °C was as follows (cpm/25 μL of cytosol): 0 min, 4520.9; 14 min, 3358.5; 32 min, 2935.5; 60 min, 1961.6; 100 min, 1524.0; 150 min, 1240.5.

Discussion

The kinetic studies described here reveal that the temperature-dependent change in ER partition coefficients is hormone independent (Figure 3), irreversible (Table I), and occurs (albeit slowly) even at 0 °C (Figure 1). Furthermore, these data indicate that exposure of ER to elevated temperature in vitro only accelerates this ER conformational change and does *not* induce it as is almost universally presumed.[3] Taken together, these observations indicate that this structural change begins to occur in vitro immediately upon tissue homogenization and extraction of the receptor from the nucleus into cytosol. The observation that the temperature-dependent change in receptor partition coefficients is an irreversible, first-order process which occurs to the same extent at all temperatures (Figure 1) confirms that the partition change is due to a conformational change in the ER monomer.[4] A temperature-dependent structural change of the ER monomer has been shown previously to modulate receptor–DNA–cel-

[3] In virtually all studies of temperature effects on steroid receptor structure, the properties of receptors that have been warmed for 15–60 min are compared with those of receptors that have remained at 0–4 °C for the same time. Invariably, no change in receptor structure is observed at 0 °C, leading investigators to conclude that warming "induces" structural changes. However, the data in Table I and Figure 1 indicate clearly that 15–60 min at 0 °C is insufficient time to observe the structure change(s) that are actually occurring at these low temperatures.

[4] Temperature-dependent dissociation of the receptor monomer from an oligomer would also be a first-order process. However, if this were the case, the partition change would be reversible upon return to 0 °C and would occur to intermediate extents at intermediate temperatures, neither of which is observed experimentally.

lulose interactions (Bailly et al., 1980), expose hydrophobic patches on the receptor surface (Hansen & Gorski, 1986), and decrease the rate of estradiol dissociation (Sakai & Gorski, 1984; Redeuilh et al., 1987). Redeuilh et al. (1987) have also reported that the temperature-dependent decrease in the rate of estradiol dissociation cannot be reversed by low temperatures but can be reversed by addition of exogenous dithiothreitol, suggesting that the in vitro effect of temperature on the structure of the ER monomer involves changes in the ER redox state. Consistent with our results, such a mechanism would be characterized kinetically as an irreversible, temperature-dependent first-order process. However, given that under the conditions of this study, the in vitro effect of temperature on ER structure is hormone independent and originates as a consequence of cellular fractionation, the long presumed importance of events associated with in vitro temperature-dependent receptor "transformation" must be reevaluated. The need to reexamine the biological role of the in vitro effects of temperature on receptor structure is also supported by the recent observations that hormone binding in and of itself modulates the structure of a number of functional and structural domains on the ER surface (Hansen & Gorski, 1985, 1986; Skafar & Notides, 1985; Hutchens et al., 1985).

What is (are) the relationship(s) between the temperature-dependent change in conformation of the ER monomer and the temperature-dependent changes in the states of ER aggregation that have been observed in other experimental circumstances? The Arrhenius activation energy for the monomeric conformational change (25 kcal/mol) (Figure 2) is very similar to the values reported for the temperature-dependent processes of 4S → 5S ER dimerization (Notides et al., 1975), modulation of glucocorticoid receptor interactions with nuclei in vitro (Atger & Milgrom, 1976), and 9S → 4S dissociation of the glucocorticoid receptor (Luttge & Densmore, 1984), consistent with a common temperature-dependent mechanism in all cases. These apparently diverse in vitro temperature-dependent phenomena also share the characteristic of occurring at a significant rate at low temperatures and in many cases are also irreversible (Andreasen, 1987). Therefore, it seems likely, as first proposed by Milgrom and co-workers (Bailly et al., 1980), that the temperature-dependent change in conformation of the ER monomer directly mediates the temperature-dependent changes in ER aggregation observed with hydrodynamic techniques (e.g., 9S → 4S dissociation, 4S → 5S dimerization). If the temperature-dependent transition of the ER monomer is indeed the rate-limiting step for subsequent receptor interactions with other macromolecules in vitro, then for the reasons discussed above the validity of models based on these changes in interactions must be reexamined. In this respect, while it has been demonstrated that the temperature-dependent 9S → 4S dissociation of the progesterone receptor (PR) is hormone independent (Moudgil et al., 1985; Renoir et al., 1986) whereas the analogous temperature-dependent dissociation of the glucocorticoid receptor (GR) requires steroid (Moudgil et al., 1986), 9S → 4S dissociation per se of both PR and GR does not depend on bound hormone in vitro (Andreasen, 1987).

In the past, interpretation of the in vitro effects of temperature on the conformation of the unoccupied ER has been complicated by the loss of receptor binding activity that occurs at elevated temperature (Katzenellenbogen et al., 1973; Hansen & Gorski, 1986). However, we have been able to demonstrate rigorously that the temperature-dependent change in unoccupied ER partition coefficients (Figure 3) is a distinct and independent phenomenon from the temperature-dependent loss of ER binding sites (Figure 4). The temperature-dependent change in unoccupied receptor partitioning is a first-order process that occurs at the same rate (at 28 °C) as that of the estradiol–receptor complex. In constrast, the temperature-dependent loss of ER binding sites behaves as a second-order process (Figure 4) and does not occur when either 17β-estradiol or 4-OH-tamoxifen is bound to the receptor (Hansen & Gorski, 1986). Thus, unlike the temperature-dependent change in ER partition coefficients, the loss of binding sites appears to involve additional cytosolic factors and is consistent with thermally accelerated enzymatic modification of a population of unoccupied receptors to a nonhormone binding form [see Grody et al. (1982) and Dahmer et al. (1984) for reviews].

The combined results of these kinetics studies and our previous analyses of ER partitioning (Hansen & Gorski, 1985, 1986) suggest that the hormone-dependent and temperature-dependent receptor transition originate in different domains of the protein. From recent analyses of steroid receptor cDNAs, we know that a majority of the carboxy-terminal 240 amino acid residues (region E) of all steroid receptors are hydrophobic and ultimately fold into a domain that contains the steroid binding site (Giguere et al., 1986; Green & Chambon, 1986). It has also been widely observed that native steroid receptors can be proteolyzed into highly globular fragments that contain the steroid binding site and are composed of about 200 amino acids (Carlstedt-Duke et al., 1983; Sherman & Stevens, 1984), suggesting that the carboxy-terminal segment of the receptor folds into a globular substructure. The hormone-dependent change in receptor structure observed in aqueous two-phase systems involves the specific hormone binding site, abolishes in vitro binding site inactivation, and buries hydrophobic domain(s) on the receptor surface (Hansen & Gorski, 1986), all of which suggest that the conformational change induced by hormone binding occurs at least in part in the folded, globular carboxy-terminal (region E) receptor domain. Similarly, recent studies of hormone-dependent transcriptional activation by translation products of mutated steroid receptor cDNAs (Green & Chambon, 1987; Godowski et al., 1987; Hollenberg et al., 1987) suggest that structural rearrangement of the hormone binding (region E) and transcriptional modulating (region C) domains occurs after hormone binding to steroid receptors in vivo. Given that the temperature-dependent change in ER structure occurs in vitro without influencing either binding site inactivation (Figure 4) or the hormone-induced conformational change (Figure 3; Hansen & Gorski, 1986), we feel that the temperature-dependent ER transition (receptor "transformation") most likely originates in the amino-terminal domain (region A/B of the native receptor). Interestingly, on the basis of phase partitioning of native and proteolyzed glucocorticoid receptors, Andreasen has concluded previously that temperature-dependent "transformation" of the glucocorticoid receptor also occurs in the chymotrypsin-sensitive amino-terminal domain of the native protein (Andreasen, 1983, 1987).

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Complexation of Fibronectin with Tissue Transglutaminase†

P. M. Turner and L. Lorand*
Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60208
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ABSTRACT: Previous work [Lorand, L., Dailey, J. E., & Turner, P. M. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1057–1059] showed that fibronectin might serve as a specific carrier for transglutaminases accidentally discharged from erythrocytes or other cells into plasma. In the present study we examined the association of these proteins in purified systems. Complexation was readily demonstrable by nondenaturing electrophoresis, using dansylcadaverine-dependent activity staining as well as immunoblotting procedures, and also by HPLC gel filtration. The results indicate a stoichiometry of 2:1 for the binding of the human erythrocyte transglutaminase (80K) to human plasma fibronectin (440K). The attachment is noncovalent in nature and does not involve cross-linking of the proteins either to themselves or to each other. Binding occurs in the absence of Ca^{2+}, suggesting that a domain on the transglutaminase molecule other than the catalytic site is needed for complexation with fibronectin. Limited proteolysis with chymotrypsin for delineating the relevant region in fibronectin yielded two gelatin- (collagen) binding fragments (56K and 46K), each displaying affinity for transglutaminase. Moreover, these fragments—like intact fibronectin—bound erythrocyte transglutaminase and gelatin simultaneously in ternary complexes.

In terms of homeostatic regulation, the binding of erythrocyte transglutaminase to fibronectin in plasma (Lorand et al., 1988) may be analogous to the association of hemoglobin with haptoglobin [for a review, see Putnam (1975) and Lowe and Ashwell (1982)]. In intravascular hemolysis, for instance, both transglutaminase and hemoglobin would be released from red cells and would have to be cleared from the fluid phase of blood by processes in which the respective carrier proteins can be assumed to play important roles. Being widely distributed in tissues, transglutaminase could also be discharged as a result

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