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THERMAL INACTIVATION AND MOLECULAR FORMS OF THE ESTROGEN RECEPTOR : EFFECTS OF MOLYBDATE

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SUMMARY

Exposure at 40°C of low salt calf uterine cytosol leads to the "transformation" of the estradiol-receptor complexes in 5 min, immediately followed by the formation of thermostable > 12 S "aggregated" receptor forms. Molybdate prevents this phenomenon but does not reverse it. Molybdate has a protective effect against thermal inactivation of the 9 S non-aggregated form of the receptor in the absence of hormone. In the presence of molybdate, the inactivation rate of this 9 S receptor is the same with and without hormone, and follows a first order reaction ($t_{1/2}$ = 7-8 min). The biphasic kinetics of thermo-inactivation of estradiol-receptor complexes is ascribable to the relative amounts of non-aggregated and aggregated forms.

INTRODUCTION

Thermal inactivation of estradiol-receptor complexes in calf uterine cytosol demonstrates a biphasic kinetic, indicating that a fraction of the binding sites are thermo-resistant (1, 2). This is in contrast to the behavior of other steroid hormone receptors that all are inactivated rapidly. Molybdate prevents the spontaneous decrease in the glucocorticoid binding sites normally occuring with time in rat liver and L-cell cytosols and of progesterone receptor activity in chick oviduct cytosol (3-5). Molybdate also prevents the hormonal-dependent transformation of steroid-receptor complexes in cytosol induced by thermal treatment, 30 min at 25-28°C (4-6).

In this paper, we report data on thermal inactivation, assessed by the decrease in receptor binding activity resulting from exposure of cytosol to 40°C. The term "thermal inactivation" is used, in this report as elsewhere (1, 2), whatever the mechanism may be ; i.e. we do not exclude the contri-

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bution of proteolysis, precipitation... We report that molybdate has different effects on thermal stability of the estrogen receptor in calf uterine cytosol maintained at 40°C depending upon the presence or absence of estradiol. The data may provide an explanation for the thermo-resistance of a portion of the estrogen binding sites.

MATERIALS AND METHODS

Buffers. TE buffer : 50 mM Tris HCl, 1.5 mM EDTA ; TE-M buffer : TE buffer containing 20 mM sodium molybdate. All buffers were adjusted to $pH_{25°C}$ 7.4.

Preparation of calf uterine cytosol, determination of estrogen receptor binding activity, protein concentration measurements and sucrose gradient analysis were performed as previously described (7, 8). In cytosol, receptor binding activities and protein concentrations were 4-6 pmol/ml and 8-10 mg/ml respectively. The specific radioactivity of [^{3}H]-estradiol (CEA-France) was 55 Ci/mmol and the counting efficiency, 25-35 %.

The extent of receptor aggregation was evaluated by sucrose gradient analysis of samples treated with charcoal to remove unbound steroid. It was calculated from the radioactivity of fractions sedimenting faster than 12 S and found at the bottom of the centrifuge tubes. As previously described (9), the non-aggregated receptor sedimented at 8.6 S in TE buffer and at 9.2 S in TE-M buffer.

Thermal inactivation experiments were performed on receptor either or not complexed with the hormone. In the first case, cytosol was preincubated for 2 h at 0°C with 10 nM [^{3}H]-estradiol or 10 nM [^{3}H]-estradiol isotopically diluted with 3 µM non-radioactive estradiol. Aliquotes (800 µl) of the cytosol were distributed into tubes, and at time zero the tubes were transferred at 40°C for the times indicated. They were then chilled in ice and hormone-receptor complexes measured by charcoal adsorbtion (7). In the absence of hormone, the experiment was carried out in the same way, except that the incubations with the radioactive hormone or the radioactive hormone isotopically diluted were performed on samples cooled after the treatment at 40°C. Values were corrected for non-specific binding and the results expressed as percent of the binding value at zero time.

RESULTS AND DISCUSSION

Thermal inactivation of estradiol-receptor complexes

We have investigated the influence of molybdate on thermal inactivation of non-transformed estradiol-receptor complexes and of estradiol-receptor complexes transformed by treatment 30 min at 28°C in low-salt calf uterine cytosol.

The thermal inactivation curve of the non-transformed estradiol-receptor complexes in cytosol was biphasic (Fig. 1A). Initially, a rapid

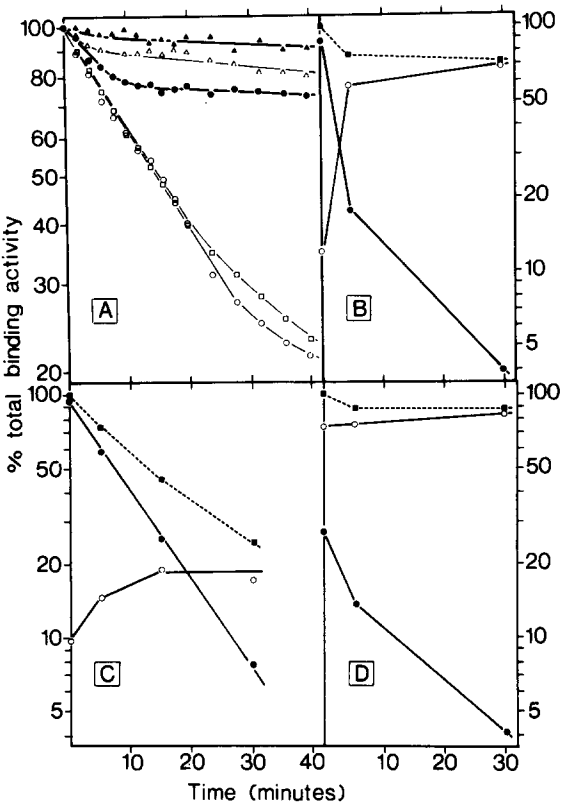

Figure 1. Treatment at 40°C of the estradiol-receptor complexes.
A. Thermal inactivation kinetics of the estradiol-receptor complexes. Cytosol (●——●), cytosol containing molybdate (○——○), cytosol treated for 30 min at 28°C (transformed receptor) (▲——▲), cytosol containing transformed receptor, readjusted to 20 mM molybdate (△——△), cytosol treated for 30 min at 28°C in the presence of molybdate (□——□).
B, C, D. Distribution patterns of aggregated (○——○) and non-aggregated (●——●) receptor during treatment at 40°C, calculated from ultracentrifugation experiments, B : cytosol, C : cytosol containing molybdate and D : cytosol treated for 30 min at 28°C. (■---■) total amount of receptor calculated from aggregated and non-aggregated receptor. All experiments were performed on the same batch of cytosol. The binding activities at zero time were the same regardless of the nature of the cytosol treatment (177,600 cpm/ml).

decrease of the 8.6 S receptor form, simultaneous with an increase of aggregated receptor was observed. However, after 5 min at 40°C, 80 % of the binding sites remained present, corresponding to an aggregated thermostable receptor form (Fig. 1B).

In cytosol containing 20 mM molybdate, receptor binding activity decreased rapidly and more extensively (Fig. 1A). The disappearance of the 9.2 S receptor form followed an apparent first order reaction ($t_{1/2}$ = 8 min), and aggregation occured to a much lesser extent (Fig. 1C).

The 40°C treatment of previously transformed estradiol-receptor complexes indicated thermostability, whether or not molybdate was present during the incubation at 40°C (Fig. 1A). In low salt medium, the treatment for 30 min at 28°C resulted in the formation of the thermostable aggregated receptor form (Fig. 1D). Secondary addition of molybdate did not change this pattern. However when molybdate had been previously added during this 30 min at 28°C, a condition in which receptor transformation does not occur (6, 9), receptor aggregation was prevented. The thermal inactivation curve was identical to that obtained for non-transformed receptor complexes in cytosol.

These data demonstrate that, at 40°C, receptor transformation occurs rapidly (5 min), and results in the formation of thermostable aggregated receptor, explaining the biphasic curve obtained for thermal inactivation of estrogen receptor in low-salt calf uterine cytosol. Molybdate blocks this process, prevents aggregation, and leads to an apparent decrease in receptor thermostability and monophasic inactivation kinetics.

Thermal inactivation of estrogen receptor in the absence of hormone

Thermal inactivation of estrogen receptor in low-salt cytosol was biphasic, with a major first component (apparent $t_{1/2}$ = 3 and 35 min for the first and second components, respectively) (Fig. 2A). The second component was due to the presence of aggregated receptor present in the cytosol at zero time. It varied quantitatively with different batches. The aggregates are destroyed more slowly than the 8.6 S receptor form (Fig. 2B).

If molybdate was added during thermal treatment, the inactivation curve was monophasic (Fig. 2A). Most of the receptor was 9.2 S, and its decrease followed a first order reaction ($t_{1/2}$ = 7 min). The amount of aggregated receptor remained low and constant over time (Fig. 2C).

These data demonstrate a protective effect of molybdate against thermal inactivation of estradiol-receptor in the absence of hormone. In the presence of molybdate therefore, the inactivation rate of the receptor at

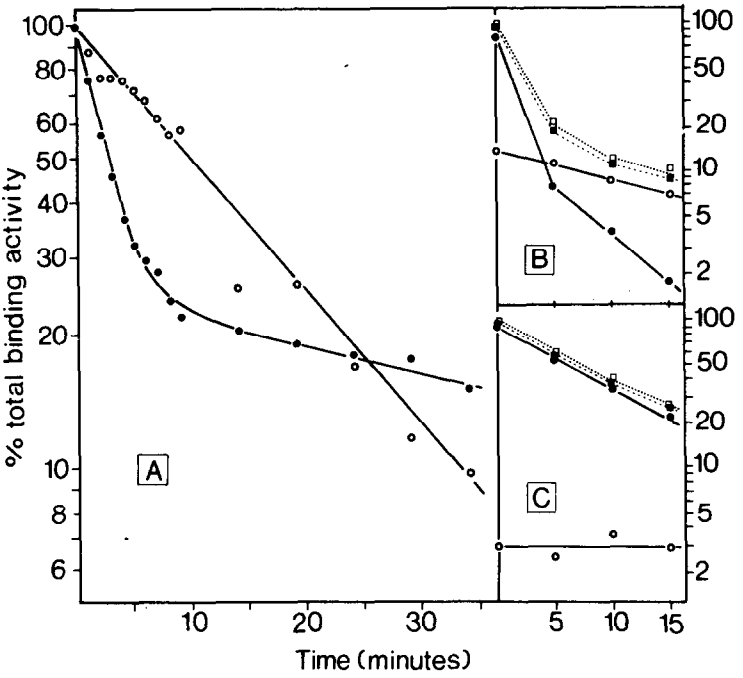

Figure 2. Treatment at 40°C of the estrogen receptor in the absence of hormone.

A. Thermal inactivation kinetics of the estradiol receptor. Cytosol (●—●) cytosol containing molybdate (○—○).

B, C. Distribution patterns of aggregated (○—○) and non-aggregated (●—●) receptor, during treatment at 40°C, calculated from ultracentrifugation experiments. B : cytosol, C : cytosol containing molybdate. The total amount of receptor was either calculated from the amounts of aggregated and non-aggregated receptor (■- - -■) or measured by charcoal adsorbtion (□·····□). Two different cytosol batches were used. Binding activities at zero time : A : 154,500 cpm/ml, B and C : 276,000 cpm/ml.

40°C was of the same order of magnitude whether or not the receptor was complexed to the hormone.

In conclusion, the difference of molybdate effects on thermal stability of the receptor in the presence or absence of the hormone is related to two distinct events : 1) it increases thermal stability of the receptor in the absence of hormone ; 2) however, by blocking the transformation of hormone-receptor complexes occuring during thermal treatment, it prevents the formation of the thermostable aggregated receptor form. The dissection of the effect of molybdate ions permits a better understanding of the biphasic pattern of thermal inactivation kinetics of estradiol-receptor complexes, which can be explained on the basis of the relative thermostability of > 12 S sedimenting "aggregated" forms.

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