STEROID INDUCTION OF THE mRNA FOR HEPATIC TRYPTOPHAN OXYGENASE

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Abstract

Poly (A) containing RNA from rat liver, purified by cellulose chromatography directs the synthesis of subunits of tryptophan oxygenase in a heterologous cell-free protein synthesis system derived from Krebs ascites cells supplemented with reticulocyte initiation factors. The newly synthesized enzyme protein was identified by precipitation with monospecific antibodies prepared against the homogenous enzyme and subsequent sodium dodecylsulfate-polyacrylamide electrophoresis of the immunoprecipitate. The increased *in vivo* rate of synthesis of hepatic tryptophan oxygenase after glucocorticoid treatment is paralleled by an enhanced tissue level of the mRNA for tryptophan oxygenase. The intracellular accumulation of the mRNA is dependent on the dose administered and increases with time during the action of the hormone.

The mechanism of action of steroid hormones has been a very suitable model system for the study of gene regulation in higher organisms, since it has become increasingly apparent that steroid hormones elicit their physiological action by specifically modulating gene expression. The problem of control of genetic expression can be stated in the following way: Since all cells of a multicellular organism contain the same genetic information, the qualitative and quantitative differences in the set of structural and catalytic proteins at various developmental and physiological states must reflect differential expression of the constant set of genetic information. The fundamental action of steroid hormones is to induce specific macromolecular synthesis and we have used the induction of tryptophan oxygenase to obtain insight into the processes controlling gene expression.

The increase in tryptophan oxygenase activity after glucocorticoid treatment was the first demonstration of an inducible enzyme in a mammalian system (1). It was subsequently shown that this increased activity of tryptophan oxygenase activity is due to an elevated level of enzyme protein present (2), which is the result of an increased rate of synthesis of the protein (3).

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Two major modes of action can be visualized to explain the enhanced rate of synthesis of the tryptophan oxygenase protein. The hormone may enhance translation by a more efficient readout of the mRNA for tryptophan oxygenase, the concentration of which however, remains unchanged. Alternatively, the hormone may control the mRNA content itself. Distinction between these alternatives obviously requires the isolation of the mRNA in question, its faithful translation in a heterologous cell-free protein synthesis system and the assay of its intracellular concentration in such a system after hormonal induction of the enzyme.

The successful translation of the mRNA for rabbit globin and for chicken ovalbumin (4), encouraged us to attempt the cell-free synthesis of tryptophan oxygenase (5), even though tryptophan oxygenase represents only a minute fraction of total hepatic protein synthesis.

Results

Fig. 1 (left) shows the effect of rat liver poly (A) containing RNA, purified on cellulose, on protein synthesis in the 30,000 x g supernatant from Krebs ascites cells (S-30 preparation). 6 μg oft this mRNA fraction stimulated the incorporation of L-[^{3}H]leucine into total proteins five-fold. We have used S-30 preparations with a high basal incorporation rate, since they yielded increased synthesis of specific

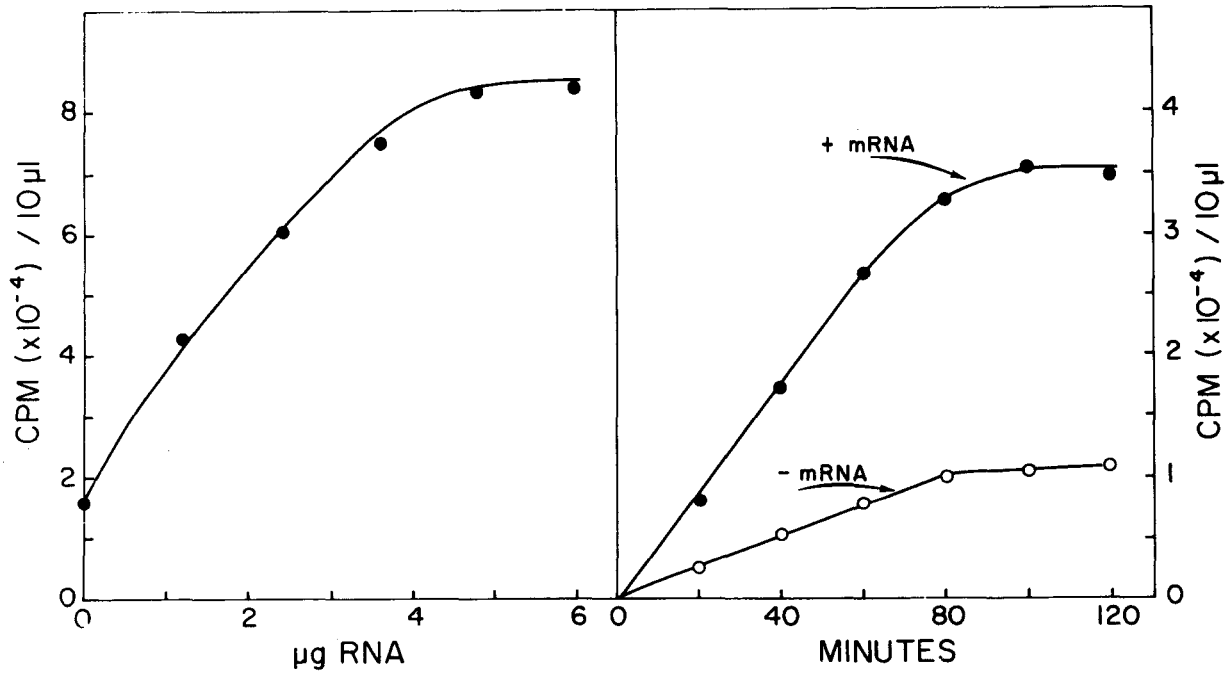

Fig. 1: Effect of rat liver poly (A) containing RNA, on protein synthesis in a Krebs ascites cell-free system.
Left panel: Following incubation for 60 min of 50 μl reaction mixtures with the indicated amounts of cellulose-purified rat liver RNA, the incorporation of L-[^{3}H]leucine was determined (5).
Right panel: Time course of protein synthesis in the absence (○ ——— ○) and the presence (● ——— ●) of 20 μg/ml of cellulose-purified RNA. Different S-30 preparations were used in these two experiments.

products, even though the same mRNA preparation stimulated the protein synthesis up to 15 fold in a S-30 preparation with a much lower background reaction. Protein synthesis in the endogenous as well as in the stimulated reaction is linear for at least one hour (Fig. 1, right).

The newly synthesized tryptophan oxygenase was identified by specific immunoprecipitation and subsequent SDS polyacrylamide electrophoresis of the immunoprecipitate. Since we estimated that only 0.01—0.1 % of the total hepatic protein synthesis could be tryptophan oxygenase, we took great care to obtain monospecific antibodies. Thus, enzyme, purified to homogeneity (6), was used for the preparation of the antibodies. The antibodies obtained yielded a single precipitation line with the purified enzyme as well as rat liver cytosol in an Ouchterlony diffusion analysis. When the purified enzyme is incorporated into the agar the precipitation line with the cytosol is eliminated.

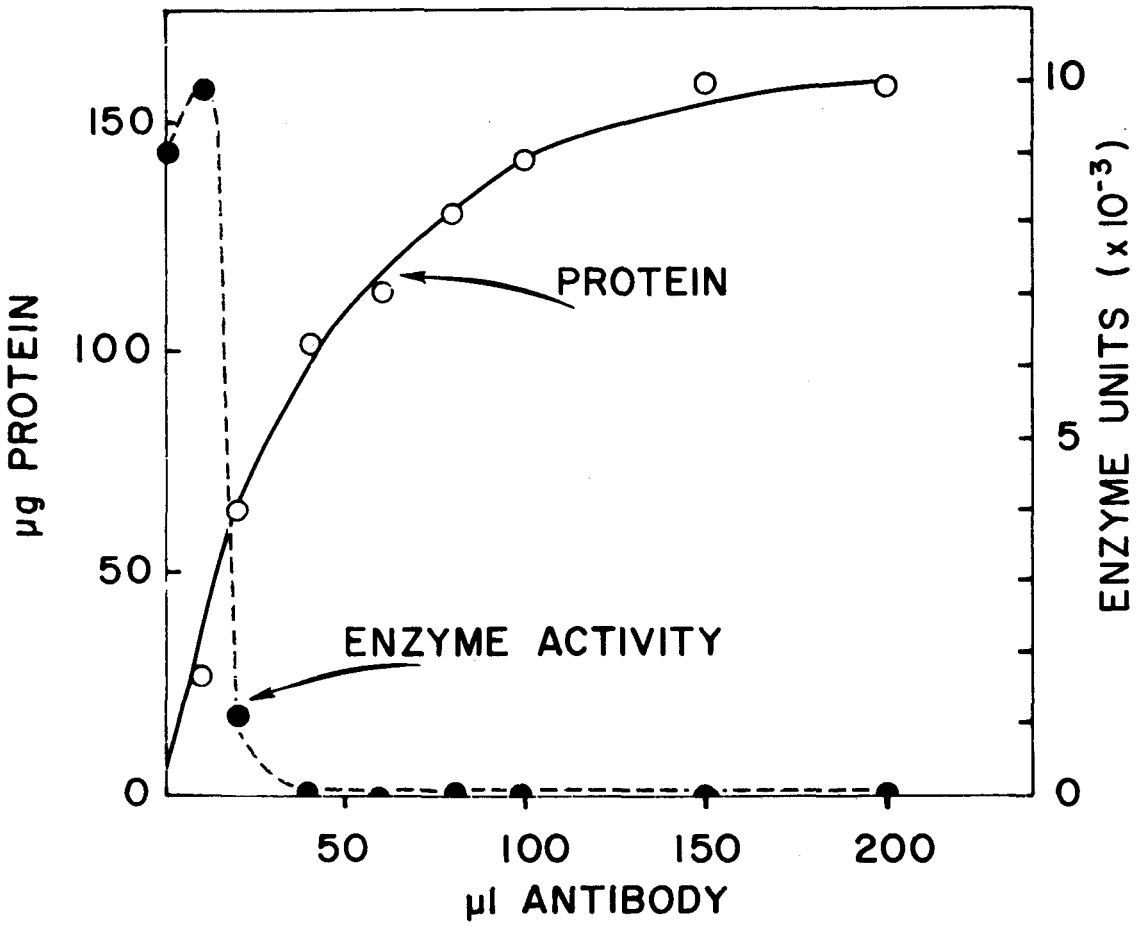

Fig. 2: Determinations of the equivalence point for immunoprecipitation of tryptophan oxygenase by anti-tryptophan oxygenase.
8 µg of tryptophan oxygenase were incubated with increasing amounts of the antibody. The protein content of the washed immunoprecipitates was determined; tryptophan oxygenase activity was measured in the supernatants after removal of the immunocomplexes.

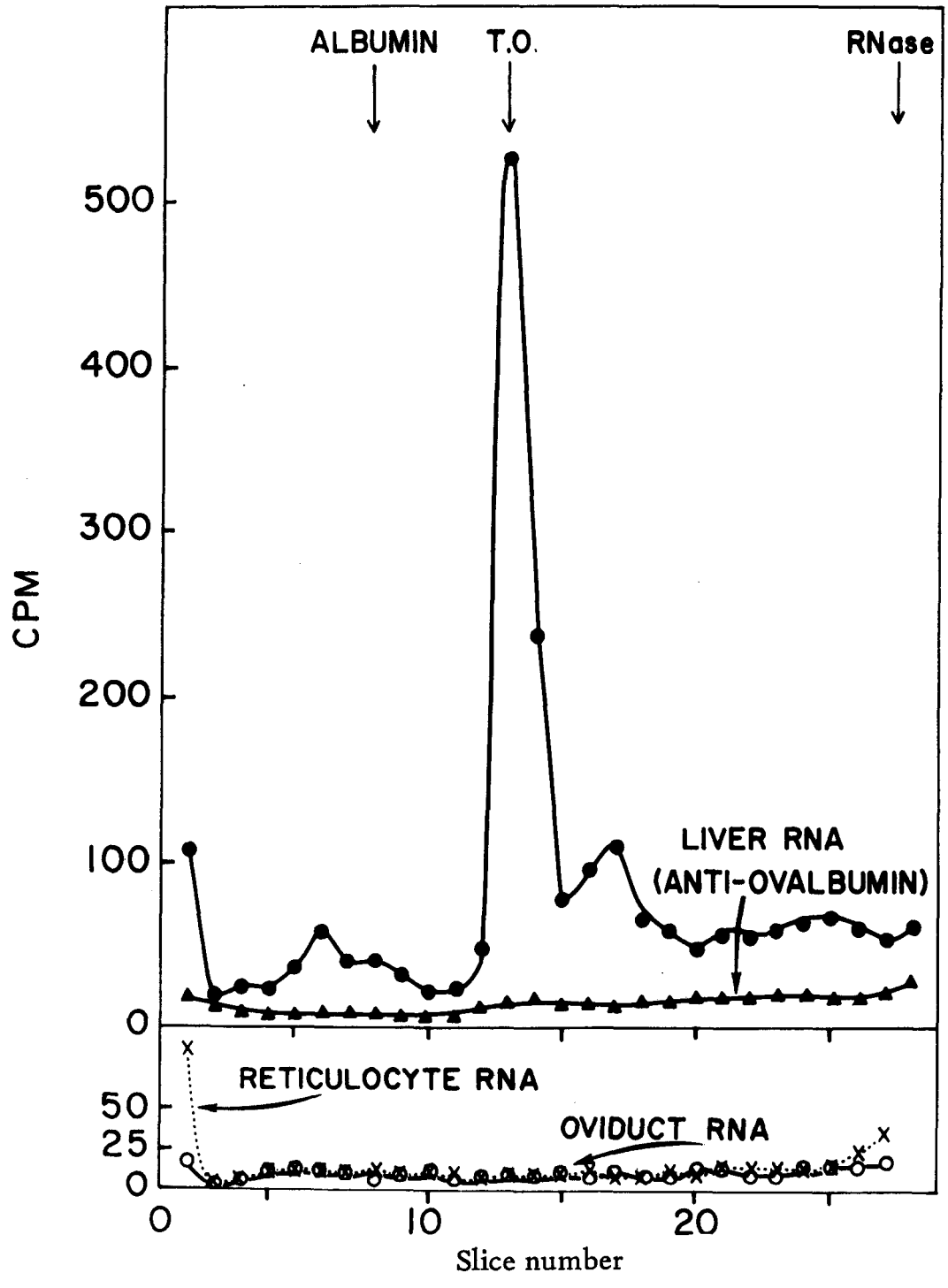

Fig. 3: Sodium dodecyl sulfate-polyacrylamide electrophoresis of *in vitro* synthesized tryptophan oxygenase.
Upper panel: Proteins, synthesized in response to 120 μg/ml of rat liver Poly (A) – containing RNA from animals that had received hydrocortisone were immunoprecipitated with carrier tryptophan oxygenase and anti-tryptophan oxygenase (●————●) and with ovalbumin and anti-ovalbumin (▲————▲). The washed immunoprecipitates were subjected to sodium dodecyl sulfate-polyacrylamide electrophoresis.
Lower panel: Proteins directed by 14 μg of cellulose purified RNA from rabbit reticulocyte polysomes (X - - - - - - - X) and by 50 μg of cellulose purified RNA from chicken (○————○) oviducts were treated with carrier tryptophan oxygenase and anti-tryptophan oxygenase.

In Figure 2, the determination of the equivalence point of the homogenous tryptophan oxygenase and anti-tryptophan oxygenase is shown. The antibody inactivates the enzyme and leads to its precipitation at the equivalence point.

To increase the specificity of our identification procedure, the immunologically isolated products were subjected to sodium dodecyl sulfate-polyacrylamide electrophoresis. Rat liver tryptophan oxygenase is a tetrameric enzyme ($\alpha_2\beta_2$), with two pair of subunits of identical molecular weight of 43,000 (6, 7); we therefore expected the same electrophoretic behavior of the newly synthesized enzyme components.

Figure 3 shows the results of an experiment which we believe represents the *in vitro* synthesis of tryptophan oxygenase. The major product which is synthesized in response to rat liver mRNA and immunoprecipitated with the specific antiserum comigrates with tryptophan oxygenase subunits upon sodium dodecyl sulfate–acrylamide gels. Two types of control experiments were performed to ensure the specificity of detection for *in vitro* synthesized tryptophan oxygenase.

a. The proteins synthesized in response to rabbit reticulocyte polysomal RNA and chicken oviduct RNA, processed with carrier tryptophan oxygenase and anti-tryptophan oxygenase, did not yield radioactivity above background b.
b. When products directed by rat liver RNA were treated with chicken ovalbumin and anti-ovalbumin (rather than with tryptophan oxyganese and anti-tryptophan oxygenase), the newly synthesized tryptophan oxygenase was not precipitated.

The rate of synthesis of tryptophan oxygenase is proportional to the amount of mRNA added and [^{3}H]leucine incoporation plateaus at the same concentration as the incorporation into total protein (unpublished results), suggesting non-preferential translation of the various liver mRNAs contained in our preparation. Table I compares the inctracellular concentrations of the mRNA for tryptophan oxygenase in livers from control and hormone treated animals. Equal amounts of the mRNA fractions from these livers, processed in identical fashion gave rise to the same incorporation of L-[^{3}H]leucine into total protein; the mRNA from the hormone treated animal leads to a three times higher incorporation of [^{3}H]leucine into tryptophan oxygenase components, suggesting an enrichment in the mRNA content for tryptophan oxygenase after hydrocortisone administration.

Figure 4 shows the accumulation of the mRNA activity for tryptophan oxygenase after hydrocortisone acetate administration for 2 and 4 hours. The observed increase in the mRNA activity, suggesting increase in mRNA levels is as expected from the Actinomycin D inhibition of the induction of tryptophan oxygenase (8).

A correlation exists between the increase in tryptophan oxygenase activity with the tissue level of the mRNA for tryptophan oxygenase after administration of various concentrations of hydrocortisone for 4 hours (data not shown), which again indicates that the increased rate of synthesis of the enzyme is a consequence of the accumulation of its mRNA.

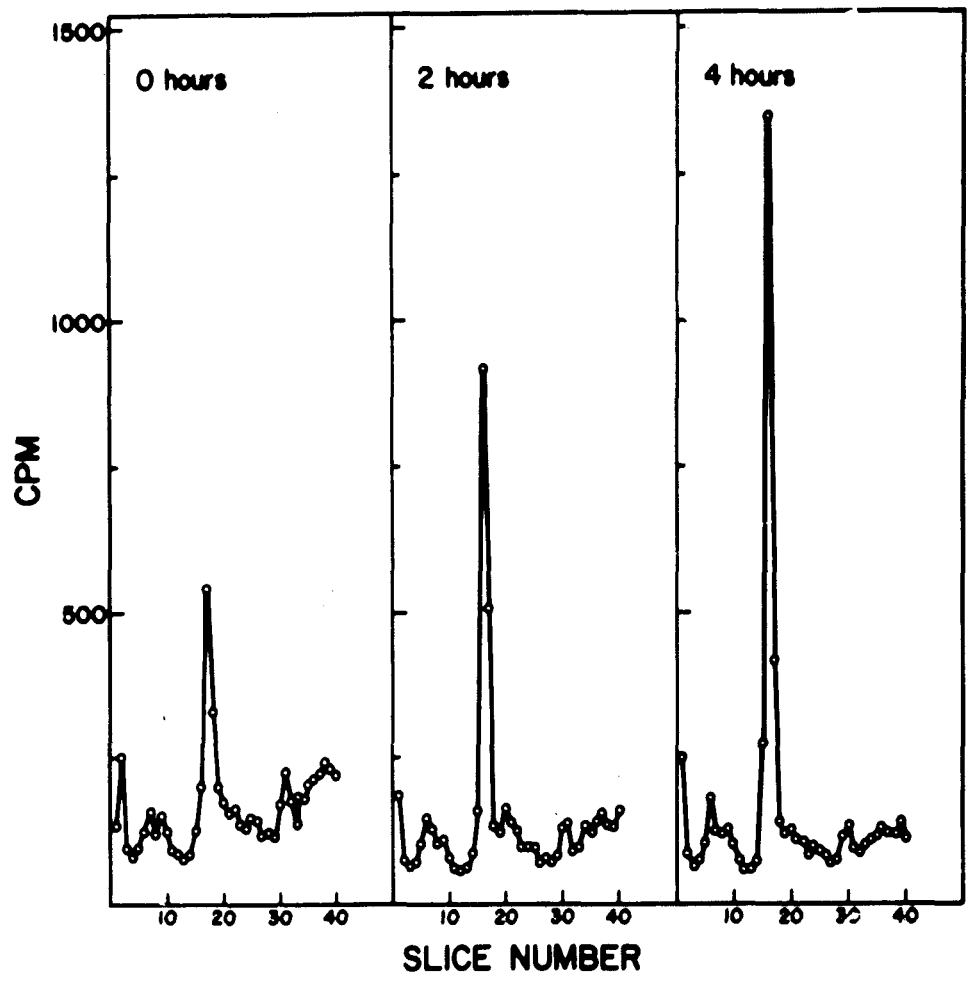

Fig. 4: Accumulation of the mRNA for tryptophan oxygenase after hydrocortisone acetate. Rat liver RNA, extracted from animals that had received no hydrocortisone, and hydrocortisone for 2 and 4 hours respectively were processed as described (5). The released newly synthesized proteins directed by equal amounts of these RNA fractions were analyzed for tryptophan oxygenase (5).

Effect of Hydrocortisone Acetate on the Intracellular Concentration of the mRNA for Tryptophan Oxygenase

	Amount assayed (μg/ml)	cpm incorporated into total proteins	cpm incorporated into released proteins	cpm in tryptophan oxygenase*
Control	120	4.4×10^6	1.25×10^6	290
Hydrocortisone Acetate (3 HRS.)	120	4.2×10^6	1.18×10^6	940

* Represented by the area of tryptophan oxygenase subunits on SDS-polyacrylamide gels.

Conclusion

The increased hepatic concentration of the mRNA for tryptophan oxygenase and its parallelism with the rate of synthesis of the enzyme protein *in vivo* argues in favor of the hypothesis that the accumulation of the specific mRNA activity accounts for the induction of the enzyme. It is at present unknown as to whether this accumulation results from an enhanced rate of transcription of the tryptophan oxygenase gene or from stabilization of pre-existing mRNA, or by activation of the mRNA. It seems that the answer to this question will require the purification of the mRNA for tryptophan oxygenase.

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